The dietary ionic effects on sex ratios in animal models and its use in the prevention of X-linked disorders

by

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Declaration

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Abstract

X-linked disorders are more expressed in male offspring and prevention of these hereditary diseases is the only recourse to date. Influencing conception towards female offspring can circumvent this problem; however sex ratio adjustment remains highly contentious. Treatment of genetic disorders through sex ratio adjustments has been examined and adopted as acceptable, easier, cheaper, safer and legal. Historically, society has been rife with allegations that diet does influence sex ratios though this has not been proven fully. The dietary chemical compositions have been claimed to act as modulators that affect the electrical charges or potential of the membranes of the oocytes and cause allosteric modification or electrotactism through a process referred to as galvanotropism and cause selective attraction towards either of the male gametes and subsequently influence the gender of the conceptus.

This study was performed in an attempt to address the various questions, allegations and speculations that have been rife in many societies concerning interplay between diet, fertilization and sex ratios so as to verify the validity of these social claims by taking them to the laboratory for experimental verification.

Swiss Webster mice study

In a double-blind fashion, nine double groups were set up comprising of 144 families of Swiss (Webster) mice, each receiving different ionic formulations in their drinking water: 1) water and 2) glucose as controls; high serum concentrations of single elements of 3) sodium, 4) potassium, 5) calcium and 6) magnesium; combined double elements 7) sodium + potassium and 8) calcium + magnesium; and finally a cocktail of the four elements 9) sodium + potassium + calcium + magnesium.

Tests included the perinatal mortality rate; the relationship between high chemical composition diet and serum levels; the effects of the study chemicals on weight gains of the study models; the effects of birth order on sex ratios and the effects of seasonal variations on sex ratios.

There were 1528 deliveries with 13,040 (6,348 females and 6,692 males) pups at 8.5 pups on average per litter.

a) Glucose, sodium, potassium and sodium + potassium supplementation influenced the sex ratios towards male progeny ($p<0.001$). Calcium ($p<0.014$), magnesium ($p<0.008$) and calcium + magnesium ($p<0.001$) supplementation influenced sex ratios towards the female progeny. The water ($p>0.61$) and cocktail solutions ($p>0.0609$) had no influence.
b) The perinatal mortality rate was 32/1000 and was female biased among the magnesium ($p<0.005$) and combined calcium + magnesium ($p<0.044$) groups only.
c) Normal serum levels were observed in the control groups ($p>0.165$), while significant elevated serum levels were observed among the experimental groups ($p<0.0001$).
d) The total mean weight gains were 11.12g and 10.55g among the females and males respectively. The weight trends were used to track the general wellbeing of the animal models.
e) The mean litter size was 8.5 per delivery in all the groups and generations, while no influence due to birth order were detected.
f) Seasons affect the litter size, in particular the rainy season, but not the gender ratios ($p>0.061$).

Cat fish study

Parallel double blind studies looking at the dietary chemical ionic effects on the oocyte membrane electrical potential were done utilising a cat fish model (n=108). The study sought to find out effects of the following solutions on the oocyte electrical charges: 1) plain electrolyte solution, 2) glucose solution 3) sodium solution 4) potassium solution 5) calcium solution 6) magnesium solution 7) sodium + potassium solution 8) calcium + magnesium solution and the 9) cocktail solution of the four elements combined.

The results revealed that oocytes retrieved from the two control groups had baseline oval polar attraction significantly more towards the positive than the negative pole ($p<0.0003$).

There was however more significant oocyte polar attraction towards the positive electrode among the oocytes retrieved from the sodium, potassium and the combined sodium + potassium solutions ($p<0.0001$).

Oocytes retrieved from calcium, magnesium and combined calcium + magnesium solutions had significant affinity towards the negative electrode and minimal affinity towards the positive pole ($p<0.0001$).

Oocytes harvested from the solutions constituted with all the salts demonstrated dual attraction with more attraction to the positive electrode than the negative electrode but of no statistical significance ($p>0.0530$).

These study findings do confirm the social allegations that a positive relationship does exist between dietary components and sex ratios. The chemicals acted as the dietary modulators that ultimately influenced the electrical cellular gametal charges and subsequently the resulting progeny.

Our platform of comfort is unlike artificial sex ratio adjustment methods; the natural sex ratio adjustment methods that include the dietary method under scrutiny in this study are practiced always at the comfort of many people's homes and are difficult to quantify or have legislation on. However, this study shows that their long term effects conform to the Fishers principle of evolution towards 1:1 sex ratios and therefore do not have significant social gender skewing on a long term basis.

The study clearly explains the molecular basis upon which ions of single valency attracts the Y-bearing sperm leading to a male conceptus and how cations of double valency attracts the X-bearing sperm leading to a female conceptus despite being positively charged.

The study further reaffirms the natural feminine supremacy by demonstrating that it is the ova and by extension the woman who determines the sex of the conceptus.

The study ultimately confirms that the dietary ionic effects on sex ratios can be used for prevention of X-linked disorders.

Opsomming

X-gekoppelde afwykings is meer uitgesproke in die manlike nageslag en voorkoming van hierdie oorerflike siektes is die enigste behandelingsopsie tot op datum. Deur bevrugting te beïnvloed om aanleiding te gee tot ‘n vroulike nageslag kan hierdie probleem omseil; maar geslagsverhouding aanpassing bly hoogs omstrede. Behandeling van genetiese afwykings deur geslagsverhouding aanpassings was ondersoek en aangeneem as aanvaarbaar, makliker, goedkoper, veiliger en wettig. Histories was die samelewing deurspek met bewerings dat dieet geslagsverhoudings kan beïnvloed, alhoewel dit nog nie ten volle bewys kon word nie. Daar was beweer dat die chemiese samestelling van die dieet as modulators kan optree wat die elektriese ladings of potensiaal van die oösiete se membrane kan affekteer; dit veroorsaak allosteriese modifikasie of elektrotaktisme deur ʼn proses waarna verwys word as galvanotropisme en veroorsaak selektiewe aantrekking van twee verskillende tipes manlike gamete wat dus die geslag van die embrio beïnvloed.

Hierdie studie was uitgevoer in ʼn poging om die verskillende vrae, bewerings en bespiegelings aan te spreek wat algemeen in verskeie gemeenskappe voorkom met betrekking tot die interaksie tussen dieet, bevrugting en geslagsverhoudings ten einde die geldigheid van hierdie sosiale aansprake te verifieer deur dit in die laboratorium na te vors vir eksperimentele verifikasie.

Switserse Webster muis-studie

Tydens ʼn dubbel-blinde studie is nege groepe bestaande uit 144 gesinne van Switserse (Webster) muise opgestel; elke groep het verskillende ioniese formulerings in hulle drinkwater ontvang: 1) water en 2) glukose as kontroles; hoë serum konsentrasies van enkele elemente van 3) natrium, 4) kalium, 5) kalsium en 6) magnesium; gekombineerde dubbele elemente, 7) natrium + kalium en 8) kalsium + magnesium; en uiteindelik 'n mengsel van al vier die elemente 9) natrium + kalium + kalsium + magnesium.

Toetse het ingesluit die bepaling van die perinatale sterftesyfer; die verhouding tussen ʼn hoë chemiese samestelling dieet en serum vlakke; die gevolge van die bestudeerde chemikalieë op die gewigstoename van die studie modelle; die gevolge van geboorte-orde op geslagsverhoudings en die gevolge van seisoenale variasies op geslagsverhoudings.

Daar was 1528 geboortes met 13040 (6348 vroulik en 6692 manlik) kleintjies met gemiddeld 8.5 kleintjies per werpsel.

a) Glukose, natrium, kalium en natrium + kalium aanvullings het die geslagsverhoudings oorwegend na manlike nageslag beïnvloed ($p<0.001$). Kalsium ($p<0.014$), magnesium ($p<0.008$) en kalsium + magnesium ($p<0.001$) aanvullings het die geslagsverhoudings oorwegend na vroulike nageslag beïnvloed. Die water ($p>0.61$) en die gekombineerde mengsel oplossing ($p>0.0609$) het geen invloed getoon nie.
b) Die perinatale sterftesyfer was 32/1000 en was vroulik bevooroordeeld onder die magnesium ($p<0.005$) en gekombineerde kalsium + magnesium ($p<0.044$) groepe alleenlik.
c) Normale serum vlakke was waargeneem in die kontrole groepe ($p>0.165$), terwyl aansienlike verhoogde serum vlakke onder die eksperimentele groepe ($p<0.0001$) waargeneem was.
d) Die totale gemiddelde gewig aanwins was 11.12g en 10.55g onder onderskeidelik die vroulike en manlike muise. Die gewig tendense is gebruik om die algemene welsyn van die dier-modelle te monitor.
e) Die gemiddelde werpselgrootte was 8.5 per geboorte vir al die groepe en generasies, terwyl geboorte-orde geen invloed getoon het nie.
f) Seisoene beïnvloed die werpselgrootte, in besonder die reënseisoen, maar nie die geslagsverhoudings nie ($p>0.061$).

Katvis studie

Parallelle dubbel-blinde studies is gebruik om die chemiese ioniese uitwerking van die dieet op die oösiet membraan se elektriese potensiaal te bepaal deur gebruik te maak van ‘n katvis model (n=108). Die doel van die studie was om die uitwerking van die volgende oplossings op die oösiet se elektriese ladings te bepaal: 1) gewone elektroliet oplossing, 2) glukose oplossing 3) natrium oplossing 4) kalium oplossing 5) kalsium oplossing 6) magnesium oplossing 7) natrium + kalium oplossing 8) kalsium + magnesium oplossing en die 9) mengsel van die vier elemente gekombineer.

Die resultate het aangetoon dat oösiete wat van die twee kontrolegroepe ingewin is basislyn ovaalmembraan polêre aantrekkingskrag het wat betekenisvol meer neig na die positiewe as die negatiewe pool ($p<0.0003$).

Daar was egter meer betekenisvolle polêre oösiet aantrekkingskragte na die positiewe elektrode onder die oösiete wat ingewin is vanuit die natrium, kalium en die gekombineerde natrium + kalium oplossingsgroepe ($p<0.0001$).

Oösiete ingewin vanuit die kalsium, magnesium en gekombineerde kalsium + magnesium oplossingsgroepe het ŉ beduidende verhoogde affiniteit teenoor die negatiewe elektrode getoon en minimale affiniteit teenoor die positiewe pool ($p<0.0001$).

Oösiete wat geoes was vanuit die oplossingsgroepe wat saamgestel was met al die soute het ŉ tweeledige aantrekkingskrag gedemonstreer met meer aantrekking na die positiewe elektrode as die negatiewe elektrode, maar was van geen statistiese belang nie ($p>0.0530$).

Die bevindinge in hierdie studie bevestig die sosiale bewerings dat daar wel ŉ positiewe verhouding bestaan tussen dieet komponente en geslagsverhoudings. Die chemikalieë het opgetree as die dieet modulators wat uiteindelik ŉ invloed gehad het op die elektriese sellulêre gameet ladings en die gevolglike nageslag.

Ons platform van gerief is in teenstelling met kunsmatige geslagsverhouding aanpassing metodes; die natuurlike geslagsverhouding aanpassing metodes wat die dieet metode in hierdie studie onder die soeklig plaas, word altyd uitgevoer in die gerief van talle mense se huise en is moeilik om te kwantifiseer of wetgewing oor te hê. Hierdie studie toon dat hulle langtermyn-effekte ooreenstem met die Fischer beginsel van evolusie wat neig na ŉ 1:1 geslagverhouding en dus nie 'n beduidende sosiale geslags-skeefheid op 'n langtermyn basis toon nie.

Die studie omskryf duidelik die molekulêre basis waarop ione van enkele valensie die Y-draende sperm aantrek wat lei tot 'n manlike embrio en hoe katione van dubbele valensie die X-draende sperm aantrek wat lei tot 'n vroulike embrio ten spyte daarvan dat dit positief gelaai is.

Die studie bevestig verder die natuurlike vroulike oorheersing deur aan te toon dat dit die eierselle en dus by implikasie die vrou is wat die geslag van die embrio bepaal.

Die studie bevestig dus uiteindelik dat die dieet ioniese uitwerking op geslagsverhoudings gebruik kan word vir die voorkoming van X-gekoppelde afwykings.

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Dedications

To my parents, Lucas Linge and Teresia Kaute, whom I watched desperately trying to conceive a daughter during almost their entire married lives. Ngina eventually graced our family briefly, but passed on pushing my parents back to the drawing board. As time waits for no one, the biological clock was against their endeavours leaving a gap in our family with endless questions and aspirations that we still struggle with in our wish baskets.

May God bless them.

To my sickle cell affected small age mates who were my playmates, schoolmates, friends and church choir mates whom we shared our earlier life journeys as we went to school, church, played together, accompanied some to the clinics, visited others in the hospitals and to the unfortunate attended their funerals is this work too dedicated to them.

May God bless them.

To my wife Prof Teresia Koki Kavoo Linge for her tireless support, my children Mark Mutuku Kavoo, Eunice Kaute Kavoo and Joseph Mutual Kavoo for their unwavering patience, tolerance, encouragement and understanding. Thank you for making the journey manageable.

May God bless them too.

This is my story as it unfolded and shaped my life.

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The journey has been long since 2001, since when I took up the first step and the candle was lit towards this destiny. It has been coupled with many road blocks, bottlenecks, red tape, financial and ethical challenges to face, address and overcome. The candle has kept burning despite the winds and storms that have threatened it all along. A lot of appreciation goes to the stewards who supported me and worked with me all along. My special thanks go to the usual suspects, first foremost and above all my promoters Professor SS du Plessis of Stellenbosch University and Dr Kimwele of University of Nairobi who not only stood by me, but also walked with me through the proposal and the study. Without them it would have been impossible to overcome the many challenges that crossed my path to the current premise.

My special thanks go to the institutions that contributed to this study in various ways including the much needed operational platforms. At its inception, I must thank mater, Nazareth and St Mary's Hospitals for work done on natural family planning methods, Stellenbosch University the host University, Nairobi University and Kenyatta National Hospital where mice studies were done, Institute of Primate Research (IPR) and Sagana Fisheries Research Station where aspects of the study on fish models were carried out and consultative services with the International Livestock Research Institute (ILRI) offered on the DNA studies.

Special tribute goes to Professors Kigondu for her advice at the inceptional stages of the study on research methods, Dr S Karanja on natural family planning methods, Professors Kioi, Mbugua and Patel for helping me put up the initial bits and pieces of the dietary study together on mice in the Physiology Department, University of Nairobi.

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I am greatly indebted to my wife Professor Koki Kavoo Linge for her immense contributions to this work, my children Mark Mutuku Kavoo, Eunice Kaute Kavoo and Joseph Mutual Kavoo for their unwavering love, encouragement and understanding.

Without the collective inputs of the above individuals, institutions, and many other stewards and supporters whom though unnamed are among the unsung heroes that I shared or worked with, and who without them, this work would not have been possible, I once again salute them. Appreciated in advance are those that have shared or will share the same research premise that I am standing on today and in times to come. May the almighty God bless them all.

General structure of the thesis

The thesis has seven chapters summarized as follows:-

The first chapter focuses on the general background and orientation of the study and begins by linking the study with the past experiences, passions, aspirations and social allegations. It proceeds to discuss the statement of the problem, research questions, ethical aspects, objectives, and the importance of the study and concludes by looking at the scope of the study, demarcation and definition of terms.

Chapter two Gives formal introduction of the subject and discusses the literature review focusing on themes found in social allegations, beliefs and practices through evolution of sex ratio adjustments. These are captured and conceptualised among traditional, natural and artificial modes of sex ratio adjustment methods that form the basis of this study.

Chapter three elaborates the theoretical and conceptual framework of the study touching on earlier beliefs and their reasons based on the level of knowledge and practices. It further dissects the various aspects of the study as it gives an elaborate account of the chemicals used, animal models under study and various milestones on certain aspects of the subject of sex ratio adjustment.

Chapter four traces the journey of the dietary study towards the search for research methods, designs, data collection and the statistical measurement tools used to test the various study hypotheses. It also includes results presentations, tabulations and analysis using the relevant statistical methods and programs.

Chapter five traces the journey of the oocyte membrane electrical potential study towards the search for research methods, designs, data collection and the statistical measurement tools used to test the various study hypotheses. It includes by discussing result presentations and analysis using the relevant statistical methods and programs.

Chapter six is focused on the discussions of the findings of the study, their theoretical aspects and practical applications.

Chapter seven sums up the new contributions of the study to society, conclusions, recommendations and suggestions of the future direction.

List of abbreviations

ART	Assisted reproductive technology
CVS	Chorionic villus sampling
DNA	Deoxy ribonucleic acid
FCM	Flow cytometry
FISH	Fluorescent in situ hybridization
FSH	Follicle stimulating hormone
GH	Growth hormone
GnRF	Gonadotrophin releasing factor
HCG	Human chorionic gonadotrophins
LH	Luteinizing hormone
ICS	Intracytoplasmic sperm injection
ISS	Incidental sex selection
IVF	In vitro fertilization
IVF-ET	In vitro fertilization and embryo transfer
NFP	Natural family planning
PRL	Prolactin
PGD	Pre-implantation genetic diagnosis
PSS	Pre-conceptional sex selection
PISS	Pre-implantation sex selection

Chapter 1

Orientation

1.1 Historical note

Everyone has a past that has shaped his life and the only difference among us all as my English friend puts it lies between who tells his story and who keeps his under wraps. However, in as much as I would like to keep my story out of the public domain the temptation to open Pandora's can literally beats me and here I am spilling the beans. Shame on me but do please allow me to share my little stories with you so that you may understand where I am coming from, the platform that I stand, the path that I take, its challenges and destiny. This story began many years ago while I was young as I watched my parents spent almost their entire married life trying to have a baby girl. Ngina eventually graced our family, but died soon after; leaving us with many questions and wishes that we still struggle with to date. Thereafter, my parents embarked on a replacement mission of the same but the natural clock was against them and their wishes still remain till today. My mother's last question on this was how, despite my education, I could not help her in her important mission, a challenge I am still working on. I respect my brothers (Christopher Mwau, Ambrose Ngui and Mathias Mwania) who always stepped in place of a daughter to help our parents in the many unnoticed, unspoken and probably unappreciated things that daughters do and represent and stand for in families.

Many are events that are life changers born of real life, defining and shaping times and moments that happens and set platforms of life long wishes and quest for answers to questions and concerns that arise from them. Even despite absence of past triggers in our lives, there are still many things that we harbour great passions for due to various reasons best known to ourselves. Deep in our conscience lie many questions, wishes, struggles and inner conflicts that come in the course of our life journeys. It is universally known without exception that some of our life moments define our lifetime endeavours, dreams, missions and goals as the ageless saying goes that it is only the shoe wearer who knows where the shoe pinches most and so do we know the things that matter and affect our lives most. The diversity of humanity and our opinions on various issues affecting our lives are mirrored in our backgrounds, attitudes, associates, judgments and deeds whose standards of judgement are amorphous.

Absence or scarcity of anything escalates its necessity and value and so does absence of a particular gender in a family. This comes with many other silent imbalances in all family activities right through people's lives. As children, teenagers, adults and even old age, there is always a place for a sister or a brother that if absent always remain missing, void or in the wish list basket. Our family lived both in the countryside and the major towns and our mother had no formal employment and so we rarely needed to have a house help. While young as boys, our main challenges were household chores such as cooking, fetching water, cleaning and washing. These mainly arose when our mother was either out visiting or unwell. As teenagers and young adults, the most challenging aspect of our lives was dating in absence of a sister.

Though gender imbalance may be insignificant to many, in our family I could appreciate as a child and even as an adult how our parents sincerely and dearly missed a daughter and the feminine touch and so did we miss a sister too. While young I did not appreciate the disadvantages of social gender influence or selection but as I became of age I understood the many social ills and evils associated with it and became its opponent that I am today. Though paradoxical, it is with this gesture that this work is dedicated to my parents and the many other couples in the world who share the agony, pains and struggles of gender imbalance in their families.

While young, among the places my father worked was the western part of Kenya where sickle cell disease was and is still prevalent till today. Then, many stories were rife over the importance of knowing the family lineages in regard to marriage and how families kept secret from the public domain and even to their children of their genetic disease predisposition.

As I grew up I got exposed to the difficulties, agonies, pains, tears and fatalities of genetic diseases as some of my friends, classmates and neighbours were affected. I therefore got to understand genetic diseases quite early in my life and prayed and wished that something could be done so that my little classmates, friends and playmates didn't get sick, suffer or even die from them.

This exposure to sickle cell affected community in the western part of Kenya became one of my key life shaping moments that happened to me in my early formative stages of life as we lived with this community. Among my contacts, Jimmy stands out vividly in my memoirs, though affected was very bright and talented in many ways and was the darling of the class. Many are the stories and conversations that went on in class though obviously many have been lost in our distant past, but Jimmy's story still echoes through my mind as he lamented on our way to hospital as I quote, "Here I am growing up with a disease that is a consequence of my descent, I am a victim of sickle cell disease that I cannot pretend to understand. I am innocent and cannot do anything to change my destiny even the doctors whom we look up to for help are helpless. My destiny is naturally defined by Mother Nature and my journey towards it is fairly predictable".

"I learned of my condition in the hospital bed which I shared with another victim who died as I left the hospital. I had never been told by my parents of my condition and when I complained to my father he promised to tell me how I could change the destiny of my next generation, but he succumbed to the same disease that threatened my life even before I became of age." Jimmy grew up echoing his father's sentiments that there was a way to change the destiny of the affected children and he kept on quoting his late father's promise "yes son I will tell you and possibly show you how when you are a little grown." Unfortunately that day never came to be.

Jane too came from a family of sicklers with three affected brothers and didn't quite conceptualize why the rest of the children in the neighbourhood were healthy, bouncy and bubbling with vitality of life. Jane lost her two brothers in their youth and was left with one brother who was always in and out of hospital. Fortunately Jane knew of her predicament and wanted to marry from a different tribe in an attempt to avoid her children from getting the disease but her parents insisted on her marrying from her tribe. Jane married Jimmy who was of her tribe, a carrier who kept it a secret. Their three sons were sicklers and all died young and were survived with two daughters. Their two daughters got well educated and so did the aged

Jimmy learn of what his late father could possibly have wanted him to know. All the available information, practices and technologies to circumvent their disease predicament were not only sought but applied to the letter. Their families are healthy and sound.

Jimmy, Jane and many others that I had a chance of sharing with, expressed their desperation, hopelessness and even outright bitterness as they lamented that they were handed over lives of uncertainty and felt like convicts waiting for the hangman's noose with occasional episodes of torture as they fell sick and were hospitalized. They complained bitterly that nature had been unfair to them having been given a life sentence for crimes they did not commit and were unwillingly serving and blamed this to the cruel and uncaring hand of Mother Nature.

Many families keep their disease patterns as secrets in an effort to avoid being ostracized or shunned by society. Cultural values, secrecy, and reluctance towards medical tests and in particular genetic disease help propagate the spread of these disorders. Due to the consequences of these genetic disease patterns, many ailments and deaths do affect some families. Often times due to the ignorance of the disease patterns in these families, the society associates these family predicaments to bad omen or evil spirits and shun such family trees. With increasing modernization, education and advent of medical tests this secrecy is being unearthed and rightful measures sought and applied.

Sex ratio adjustments for medical reasons and in particular for genetic diseases is permissible towards alleviation of human suffering from genetic diseases. (Burke, 2002; Savulescu, 2002; Schroeder, 1994; Vanden Veyver, 1995). It is in this regard that this study has been undertaken in support of this noble cause. Unlike the social sex ratio adjustments that are majorly skewed towards the male gender, this work supports the female gender that is preferentially favoured in genetic diseases (Scott et al., 1999; Vallejoj et al., 1996). It is also upon this same context and premise that this work is dedicated to my former, schoolmates, playmates and other couples and families affected by genetic disorders.

1.2 The study history and preparation

The study concept started in 2001 following many discussions in the family radio and television stations in Nairobi and has evolved over the years in phases coupled with many leaps and bounds. It has taken a while due to its wide scope, numerous variables, ethical issues and various logistical dynamics encountered in different stages.

Natural gender influencing methods were studied with the help of Drs. Kiura and Karanja both of whom are experts on the subject between 2001 and 2003. This method was abandoned due to numerous uncontrollable variables.

Traditional gender influencing methods by the use of herbs were explored between 2003 and 2004 with the help of Dr Kofi of KEMRI (Kenya Medical Research Institute) whose contributions in particular regarding the use of herbs were of much use. Contributions of the same were obtained from traditional practitioners from various provinces of Kenya who practice this method by the use of indigenous herbs through the coordination of Linet Oduor. Considerable contributions were obtained from eastern province through the guidance of Tabitha Musau who is a renowned traditional herbalist. These methods were abandoned mainly due to the suspicious relationships between the traditional practitioners and the modern health providers in particular failure to get the rightful information from the herbalists which_was cited as the biggest challenge among other uncontrollable variables.

Modern gender influencing and selection methods were explored during 2003 with consultations of Prof Kioy and Dr Mbugua both of the Medical Physiology Department, University of Nairobi. Of the numerous methods that had been advocated, diet was picked up as interesting, feasible, with fewer ethical challenges and with many academic gaps due to inadequate research. A concept of bridging society's beliefs and norms with modern science was conceived as the guiding principal of the study.

In 2003, in the animal house this study was set up, operations and upkeep of the animal models was started with valuable contributions given by Mr. Maloba of Kenyatta National Hospital and Ndungu of Chiromo Campus Medical Physiology Department. Details and fine tuning of the study setup was done in consultation with Dr Iirag of ILRI. The mice study was adopted and trials started culminating into a pilot study done in 2004/2005.

The results of the pilot study were presented at the Stellenbosch University in 2006 and formal application done. The fish studies were incorporated to it under the supervision of Professor Stefan du Plessis reciprocating with Dr Kimwele of the University of Nairobi and ultimately admission granted in 2008.

The dietary aspect of the main study was undertaken between August 2008 and September 2010 and additional aspects were included in the study. The IPR and Sagana Fisheries Research Station were incorporated in the study for the fish studies.

DNA extraction, polymerase chain reaction (PCR) and running of gels for gender determination were done in consultation with ILRI and Kenyatta National Hospital. The reagents were ordered from Belgium through the former institute by Primer Procurement Agents who offer these services for the institute.

1.3 Statement of the problem

The study concept attempts to define and highlight that:-

- Sex linked diseases is a medical challenge that requires solutions through scientific evidence.
- Sex linked diseases are silent but a growing global challenge.
- Sex linked diseases have remained a big challenge despite the progress made in other fields of medicine.
- They will become more prevalent as the other diseases decline through effective preventative and curative interventions.
- Sex ratio influence, as a preventative intervention, is still vital and relevant to date as the hope of finding cure still remains elusive.
- Anecdotal evidence for sex ratio adjustment methods needs verification.

- Allegations of dietary sex ratio influence has been rife in society since time immemorial and if true may offer some practical possibilities of prevention of these conditions due to their simplicity in costs, acceptability and application.
- Electrical charges have been confirmed on the cellular membranes and their functions are as yet fully established.
- In this study, it is hypothesized that at the molecular level, the electrical charges on the gametes are influenced by diets through the process of galvanotropism.
- Some factors that modulate the oocyte membrane electrical charges have been postulated and are under scrutiny in this study.
- Most earlier studies are linked to commercial firms and are short trials with small sample sizes that are difficult to draw sound analytical conclusions.
- Genetic disorders to date are still better addressed ethically by simple sex ratio adjustment methods rather than other unorthodox methods.

1.4 Research Questions

The research questions of this study are as old as mankind and have been driven to great depths and heights by societal quest to know the truth on how to achieve their desired results. In this regard questions have been asked, issues raised, concerns expressed and attempts made to get solutions over the years. In an attempt to get answers man has devised many ways towards the solutions and where there was failure to get concrete answers mankind often times resulted to unconfirmed claims, allegations, speculations, myths and even tales. Over the years, scientific questions have arisen from research reviews, enquiries and presentations over whether diet has anything to do sex ratios but have not been adequately addressed.

There has been a lot of debate, speculation, reviews and arguments over dietary effects on sex ratios but little long term serious studies carried out over significant periods of time. This has undoubtedly left an obvious knowledge gap that has often been exploited by some members of society. In this day and age we need to avoid further protracted verbal and theoretical controversies on this subject and serious studies be done to address this human enquiry. In this study, attempts have been made to enhance the human understanding of causal effects of various parameters on sex ratios through answering the various questions related to this subject such as:-

1 a) The effects of high dietary sodium, potassium, calcium and magnesium as single elements on sex ratios.

b) The effects of double dietary study elements on sex ratios.

c) The effects of multiple dietary study elements on sex ratios.

2) Gender determination by PCR (DNA) and physical methods and trends of perinatal mortality.
3) Relationship between high dietary chemical intake and serum concentrations or dynamics.
4) The effects of birth order on sex ratios.
5) The effects of seasonal variations on sex ratios.
6) The relationship between dietary elements and the oocyte membrane electrical charges.
7) The relationship between fertilization and electrical membrane charges on the gametes.

1.5 Objectives of the study

General Objective

The general aim of the study is to unravel the anecdotal evidence posed by societal allegations of dietary influence on fertilization and subsequently sex ratios.

Through experimentation this study attempts to:-

1) Establish whether there is clear relationship between high dietary chemical composition and sex ratios.
2) Determine the perinatal mortality by early gender determination by PCR before physical determination is feasible.
3) Establish the relationship between high dietary chemical composition and serum levels.
4) Establish effects of the study chemicals on weight trends of the study models.
5) Evaluate the effects of birth order on sex ratios.
6) Evaluate the effects of seasonal variations on sex ratios.
7) Determine chemical formulations effects on the oval electrical membrane potential.

Specific aims

1 a) To examine the effects of high dietary chemical elements namely sodium, potassium, calcium and magnesium on sex ratios.

b) To assess the effects of high dietary combined sodium with potassium and calcium with magnesium on sex ratios.

c) To evaluate the effects of the cocktail of all the above chemical combinations on sex ratios.

2) To evaluate early gender determination by PCR before physical methods are feasible.

3) To determine the serum biochemical levels of the respective elements.

4) To establish the study weight trends among the study groups.

5) To verify effects of delivery sequence order on sex ratios.

6) To determine the effects of seasonal variations on sex ratios.

7) To establish the effects of these chemical formulations on the fish oval membrane potential.

1.6 Importance of the study

The study will help in highlighting the global silent advancing challenges of sex linked disorders.

With confirmed effective preventive measure, the increasing trend of sex linked disorders can be reversed.

Confirming the allegations will add another feather in the basket of preventive measures or tools of sex linked disorders.

Confirming these allegations will put to rest the many unconfirmed questions, allegations and speculations.

Dietary methods may offer practical preventive solutions due to their simplicity in application in dealing with prevention of X linked disorders.

The study will offer more reliable data as it examines the entire life spans of the study animal models compared to the earlier short run studies.

If the allegations are found untrue then society will be saved from adopting wrong practices.

If the allegations are true then the electrical aspects of fertilization will have been confirmed.

Factors or agents that are postulated to modulate the oval membrane potential will have been identified.

This study will reaffirm the importance of putting social allegations, beliefs and myths into scientific scrutiny and open up new forums and avenues for more to follow.

The study is likely to open up an interesting and controversial frontier by throwing some light as to who between man and woman determines the gender of the conceptus.

Dietary effects at the molecular or cellular level have been wanting and therefore this study will offer an opportunity for this cause effect to be studied.

Concerns surrounding sex ratio adjustment methods and -X- linked diseases are numerous and therefore, objective and verifiable data obtained through research would avoid unsound practices based on anecdotal evidence.

Affected couples who are usually faced with difficult choices and consequences will have a choice to opt for prevention through sex ratio adjustment rather than other unorthodox practices.

To get rightful information regarding this subject that has remained unresolved in our social midst from time immemorial.

To set records straight for reference purposes so that couples affected don't get taken advantage of by people who purport to offer social gender balancing services.

It will add another preventive instrument to the few existing arsenal of sex linked disease management modalities.

To alert the possibility of future challenges between personal rights in relation to family ratio balancing versus social rights or legislature and which take precedence when gender imbalance presents.

1.7 The ethical considerations

Sex ratio adjustment as a subject is highly emotive as it does not only touch on the core of humanity but the blue print of life and can easily make us start thinking with our hearts and not with our heads. As a topic, it has often times been relegated to the academic no go zone due to the many ethical questions, intrigues and red tape.

In order to address the various challenging human ethical *qurmare*, Swiss (Webster) animal models have been used for the dietary study and the cat fish for the oocyte membrane electrical potential studies.

The study:-

- Only uses animal models.
- Is designed to look for solutions towards prevention of X - linked diseases only.
- Does not concern itself with any other non-medical aspects of sex ratio adjustments.
- Is not affiliated to any of the firms that advocate any of the social sex adjustment methods.

The entire research team does not support sex ratio adjustments for social reasons.

It is ethical as it seeks to support sex ratio adjustment methods for medical reasons only through promoting the female gender progeny for purposes of disease prevention.

It is in conformity with the recommendations of human rights and biomedicine which states that sex selection is permitted only for prevention of hereditary diseases or any other relevant medical challenges.

Should the hypotheses be found true**,** only then the dietary method of sex ratio adjustment would appear to hold good promise for future application as it is non-invasive, inexpensive and does not require high technological approach for prevention of hereditary disorders.

Whereas the ethical question on direct sex selection methods are in no doubt an unwelcome debate, questions still linger as to where to place the grey areas of indirect natural gender influencing practices that are still in practice today and yet difficult to enforce legislation on.

Future debate will undoubtedly be focused on personal rights verses social rights and the legality of natural indirect natural gender influencing methods.

The housing and experiments involving the animals were conducted following ethical approval granted by the Ministry of Livestock and Fisheries (Kenyan Government) to ensure that ethical requirements comply with the Kenyan regulations.

1.8 The challenges

The mention of sex ratios even without details as a subject is highly emotive and stirs controversies even before one has heard your story. However being the blue print of our lives, it is not surprising to get this kind of reactions which at times take us up like the whirlwind and make us start thinking with our hearts rather that our brains.

It takes a real leap of imagination, courage, patience and endurance to venture into new horizons and probably more in particular areas with a good measure of controversies. As the old saying goes that no one discovers new oceans unless he has the courage to lose sight of the shore. Others say that some things will only happen when the seas come to the shore though one wonders whether the seas have any reason to come to the shore as they are always living by the side.

Like a story burning in someone urging the individual to spill it out, but lacking the right avenues, are some life changing moments and events that affect our lives and the passion, aspiration and the zeal to share and if possible get answers to them. Everyone has a story to tell, a song to sing or something to offer to the world among the many roles that we play and should not shy away or shelf anything that may benefit humanity. Many a times, forums, platforms and the means to channel our thoughts, aspirations, wishes, our past, passions and even fears go missing. Through this work I have told my story as part of my signature and I must salute Stellenbosch University for giving me a platform and a vuvuzella to tell it to the world. The findings of the study are laid on the table and let the world find a useful and not a destructive place for these findings I beg.

Besides the specific controversies pertaining to this study, the study shares the similar challenges that are universal to most others. Among the chief five challenges were finances, ethical issues, time constraints, long distant learning and lack of sponsorship. Like any activity finance is always a challenge but having the passion for the subject I got the courage to go on to this far and I still have the humph to soldier on and further the study to post-doctoral level before I hang up my boots and gloves on this subject.

Ethical issues do present varying challenges in many research programs involving reproduction and in particular issues concerning sex ratios but the animal models and adherence to the laid down legislation that allows research on sex ratios pertaining to sex linked disorders saw us safely through.

Time constraint has proved a major challenge to the investigators as they are spread far and wide and are also involved in their own routine busy schedules of social, domestic and official engagements. This challenge has been a major contributor towards some delays during the study.

Long distance learning (though a demerit) has come with its benefits of meeting new people, experiences and places and can only be said to be more of a blessing than a challenge.

1.9 Scope of the study

The study covers most aspects of sex ratio influence and selection in a holistic approach. This offers a wide scope as it seeks to address the many questions pertaining to relationships between diet, birth order, seasonal variations, fertilization and sex ratios. It attempts to fill in the many academic and social knowledge gaps that have remained in our midst since time immemorial.

Many areas concerning this important subject were put under scrutiny with interesting findings. The dietary chemical ionic effects of sodium, potassium, calcium and magnesium either as single elements, in double combinations or all-inclusive on sex ratios were exhaustively examined analysed and relevant conclusions made. The area of dietary glucose and gender influence has been a subject dominating the social debate and was also given reasonable attention, time and space with interesting results. The effects of high dietary chemical composition on the serum concentrations has had its share of controversies among scientists and was given some considerable attention and relevant conclusions made. The effects of birth order and sex ratios have had its share of speculations with little research works dedicated to it and it too was given prominence with interesting findings. The effects of seasonal variations on sex ratios as a subject too has had its social touch and input though has had little attention on the gender bias and was given its time share too with good results that will have their positive contribution to this whole debate. The dietary chemical ionic effects on the oocyte membrane electrical charges appears to be the spark of this study that stands out above all other findings of this study and if our direction of thought is right will see rewriting of books as to who determines the gender of a baby whether the man or the woman.

The study attempts to stay within its ethical confines and attempts to steer within scientific guidelines. The study stands out as the only one that has been carried out for the longest time and with the most study subjects and where many aspects were collectively examined. One of the most unique, rare and interesting aspect of this study is, it examines the study models in their entire life spans due to the long ample span of time the study was given. This aspect of the study allows confident analysis of data without fear that some later life spans of the models that are not taken into consideration may affect the results.

The study evolved from the natural family planning to herbal medicine and finally to the modern methods using the dietary methods of gender influencing. The study therefore encompassed various institutions which offered these different contributions. In the formative stages of the study, Nazareth hospital, Matter hospital and St Marys were the institutions that the study was initiated because they practice natural family planning methods. Kenya medical research institute came into play while addressing the herbal contributions to the subject. Kenyatta National Hospital offered the initial logistics on animal upkeep and further advice on the same was given by the University of Nairobi's Physiology Department which also provided the mice experimentation on dietary studies. The Veterinary Physiology Department of the University of Nairobi and the ILRI offered advisory logistics on the DNA work. The reagents of the latter work were outsourced from Belgium. The IPR and Sagana Fisheries Research Station were involved in the fish oocyte studies while Stellenbosch University hosted the study.

1.10 Demarcation

This study differs from others in many ways in that whereas there are many gynaecological treatments that are already established and ongoing that offer invaluable contribution to mankind, this study is advocating a non-invasive and an ethical mode of management of genetic diseases. Amongst the old age conservative treatments include, routine open gynaecological operations and the relatively new minimal invasive laparoscopic and laser surgeries. Of the newly emerging areas of gynaecologic care are the various forms of fertility treatments, immunological and new modalities of genetic disease treatments. As the permanent methods of genetic disease treatment are still being sought, the current study compliments the preventive aspects of the genetic disease management through sex ratio influence. Many methods have been advocated but most still remain in the speculative stage with little data due to short run studies coupled with few study subjects.

Of all the natural gender influencing methods, only natural sex ratio influencing has received its due share of attention with long run studies and with many study subjects. In this study, the shortfalls of the previous studies have been addressed with long run studies over the years involving many study animal models over their entire life spans and their subsequent progenies. Similarly, long run studies were done on fish oocyte membrane charges to give credible results and further studies are still on going.

This study further diverts from the many others in that it takes societal allegations and translates them into a laboratory experiment in an effort to confirm or refute these allegations. This study should open up a new concept where more societal allegations and even myths can be subjected to experimentation for further scrutiny and verification.

The study concept goes further and challenges the common norm as to who determines the sex of a child whether it is the man or the woman through examining the eventual events that determine fertilization. This challenge comes in mind as we try to conceptualize the fact that it is the oval charge that eventually determines which sperm either the negatively charged or positively charged to accept. This emerging concept challenges or overrides the old theory that it is the man who determines the sex of the conceptus and makes this study and others along this line of thought as important drivers towards getting to the eventual answers to this interesting debate.

1.11 Personal reflection and triumphs

Like every story has its flip side, so does this study have its smooth and rough_stretches of its journey. I had excellent support from my promoters; infact I am the one as the candidate who has been lagging behind which is the very contrast of the common stories that are rife among many students.

Despite lack of good sponsorship, I always had some money to put in the project. Interestingly I considered this project as one of my hobbies and pastimes. Whereas many people spent huge sums of money in leisure activities and other pass times I chose to use my leisure money and time on this project and in this way I considered it a worthwhile social investment.

Despite its huge cost due to its diverse aspects, the attitude that I developed towards it and the passion that I have had, have provided enough rightful platforms and driving forces to see the study through to its end.

Like any play, has its downside and side shows and so did my study for it exposed me to many new people, friends and places that without it would not have been possible.

Hopefully, through this study I will attain my goals of a PhD and possibly a Post-doctoral as the arrangements are feasible and practical.

Of the flip side, one of the lessons to carry home is the benefit of carrying out studies to their conclusion while relatively young because then one has the opportunity to make full use of the benefits of being relatively free. This hopefully will be a topic of one of my social lecturers that I intent to give some day so as to pass this message to young students including my own children. It is interesting how life changes with changing needs and expectations, this is because while doing my fellowship in the Carolinska University - Stockholm Sweden in the nineties I was offered a PhD sponsored programme that I declined and went back home to Nairobi as I was already qualified as a gynaecologist and did not foresee the benefits of a PhD, after all I had a masters in one of the most competitive fields of medicine. Little did I know then that some day later in life I would eventually not only go for the PhD but would sponsor myself.

I am comforted that my curiosity was satisfied too and just like the cat, I survived the whole process without facing significant ethical issues. I must admit I was apprehensive as I started the work and even feared someday I would have people rioting outside my office. However due to my rightful intentions I can assure my readers and audience that this did not and will not happen. This work will always stand out in my life as my greatest test of patience, perseverance, tolerance and endurance. I am however glad that the social myths, allegations, questions and concerns have reasonably been answered satisfactorily.

I am satisfied by the turn out of the work piece as it has turned out better that I ever thought and would love to advance it to a higher level and discover more levels of knowledge higher up the ladder.

It is my sincere hope and wish that this study will spur thoughts in the right direction towards the possible benefits and that the findings will find a useful and profitable place in society in days to come.

1.12 Definition of terms

ART assisted reproductive technologies include all fertility treatments where both the oocytes and sperms are manipulated for various reasons where medical indication exist (Shushan et al., 1993).

Genetic disorders are a large group of hereditary disorders resulting from either minor or major mutations in the genetic composition or alterations in chromosomal numbers. They are classified into single-gene, chromosomal, multifactorial and mitochondrial disorders (Adam., 2004; Arrayed, 1999; El-Hazmi., 1999).

X-linked diseases are conditions that are expressed in situations where - X chromosome exist alone such as in the male chromosomal status of -XY- as oppose to the double - XX - female chromosomal status thus making it more prevalent among the males than the females (Adam., 2004; College., 2002).

Y-linked disorders are caused by mutations on the Y chromosome. They only affect males and therefore all the sons are affected. Since the Y chromosome is small, most Y- linked disorders majorly cause infertility (Adam, 2007; El-Hazmi., 1999).

A***utosomal disorders*** are conditions arising from mutations of the non-sex chromosomes or the autosomes) (Owain et al., 2012).

Single-gene disorders - Results from a mutation in or around a single gene. So far over 6000 disorders have been identified (El-Hazmi, 1999).

Chromosomal disorders are conditions that involve either an increase or decrease in the total number of chromosomes or abnormalities in their structure. Down syndrome was the first to be recognized in 1959 among these disorders (Kumar et al., 2010).

Electromagnetic fields consist of both electric and magnetic components and are created when electrically charged particles are put in motion or accelerated. Electromagnetic fields: (Vivijalaxmi et al., 2005).

Sex ratio is the proportion of males to females in a given population, usually expressed as the number of males per 100 females (Hammound, 1965).

Sex ratio adjustment modalities involve either natural or artificial practices that lead to either gender influence or selection (Ericsson, 1994; Shettles, 1970).

Natural gender influencing practices are wilful use of natural body’s or gametal physiological characteristics in an effort to predetermine or influence sex ratio outcome (Shettles, 1970).

Artificial gender selection is wilful use of artificial body's or gamete manipulating techniques in an effort to predetermine sex ratio outcome. (Ericsson, 1994).

Thermotaxis is cellular movement directed by temperature gradients found among bacteria and human cells (Bahat, 2006).

Photootaxis is directional movement either cellular or of an organism in response to a light source (Richard et al., 2009).

Chemotaxis is induced directional cell movement towards or away from an attractant or repellent that is secreted by another cell or its immediate environs (Dina Ralt et al., 1994; Sun et al., 2003).

Chemokinesis is modulation of swimming speeds in response to external chemical stimuli (Dina Ralt et al., 1994, Fei Sun et al., 2003).

Galvanotropism is directional movement either cellular or of an organism in response to an electric current (Richard et al., 2009).

***Electric field* -** is the space surrounding a charged particle or a region characterized by the existence of a force generated by electric charge (The American Heritage Dictionary, 2000.

Magnetic field - is a field of force surrounding a magnet or a moving charged particle (Vivijalaxmi et al., 2005).

Electromagnetic field - consists of both electric and magnetic components and is generated when electrically charged particles are put in motion or accelerated (Vivijalaxmi et al., 2005).

Voltage is an electrical gradient created by the difference of the electrical charges between the inside and the outside of a cell (Raffaele, 2004).

Resting potential (R) is the stored potential energy created by the voltage difference across the cell membrane i.e. the difference between the internal and the external membrane voltages creates a store of potential energy (Raffaele, 2004).

Karyotype is the number and appearance of chromosomes in the nucleus of a cell or the complete set of chromosomes in a species or an individual organism (King et al., 2006).

Mitosis is a process whereby a cell divides into two identical cells each with the same number of chromosomes and genetic material as the parent cell. It is characterized by four phases, i.e. prophase, metaphase, anaphase and telophase (Yu Fan et al., 2004).

Meiosis is the cell division whereby a nucleus divides into four nuclei each containing half the chromosome number of the parent nucleus thus reducing the gametes or spores from diploid state to haploid status. The first meiotic division is subdivided into five stages:- The prophase stage which is further characterized by leptotene, zygotene, patchytene, diplotene and diakinesis subdivisions **(**Yu Fan et al., 2004., Rovere., 2006).

Valency is the ability of a chemical element to combine with other elements measured in relation to the hydrogen element **(**The American Heritage Dictionary, 2010).

Atomic weight is the average mass of an element expressed in relation to carbon 12 which is referred to the 12 atomic mass unit (The American Heritage Dictionary, 2010).

Ion is an atom that has lost or gained one or more electrons resulting in a net electric charge that is either positive (cations) or negative (anions) (Valera et al., 1994).

Ion channels were first described by Peter Agre and MacKinnon, of John Hopkins University and the Rockefeller University, New York respectively 1998 as proteins or protein complexes through the cell membrane that form channels to facilitate the movement of ions through the membrane as per their electrochemical gradient. These can be open, closed or gated. The latter channels include voltage, chemical, ligand or mechanically gated channels among others (Valera et al., 1994).

Ligand is a molecule, ion, atom or a co-factor e.g. haemoglobin that combines to specific sites, competitively and reversely to another to form a complex for particular biological function (Valera et al., 1994).

Single blind study is a study in which the administrators of a test product don't inform the subjects that they are being subjected to a test or a control treatment neither the product under scrutiny so as to circumvent bias (The American Heritage Dictionary, 2007).

Double blind study is a study whereby both the investigators and participants are unaware of the test or control treatments being used by the study subjects to enhance objectivity of the study (The American Heritage Dictionary 2007).

Action potential is an event that rapidly makes the electrical membrane potential rise and fall briefly among the excitable cells that include neurons, muscle cells and endocrine cells. This is propagated through depolarization a process that makes the cell membrane more positive and a reverse process called hyperpolarization (Stephanova et al., 2012; Wang et al., 2001).

Depolarization - a change in cell membrane potential towards the positive caused by influx of sodium cations through the sodium ion channels (Wang et al., 2001).

Hyperpolarization is a change in cell membrane potential towards the negative often caused by efflux of the potassium cations through the potassium ion channels or influx of the chloride through the chloride ion channels (Stephanova et al., 2012).

Tricaine mesylate (Tricaine methanesulfonate, TMS, MS222) is a fish anaesthetic in powder form used for sedation of fish for experimental purposes (Cater et al., 2011).

Polymerase chain reaction (PCR) was developed by Kary Mullis in the 1980s is based on the ability of the enzyme DNA polymerase to synthesise new DNA complementary strands on addition of the necessary nucleotides with the assistance of primers (Pfaffl, 2004; MacClive et al., 2001).

Operational sex ratio (ORS) is the ratio of actively sexually competing males ready to mate with actively sexually competing females (Nuno et al., 2012).

Physical sex ratio is the ratio of all males to females both sexually active or inactive and competitive and non-competitive (Nuno et al., 2012).

Sexual selection - the forces determined by mate choice that act to cause one genotype to mate more frequently than another genotype (Nuno et al., 2012).

Batemans principle states that the male reproductive success increases with multiplicity of mating unlike the females (Brown et al., 2013).

Trivers-Willard hypothesis - In 1973, a biologist Robert Trivers and a mathematician Dan Willard in Harvard University expounded an evolutionary theory that mothers in better living conditions produce relatively more sons while those in poorer living conditions tend to produce relatively more daughters (Bradbury et al., 1998; Elissa et al., 1999).

Fishers principle of evolution - An important milestone that was described in 1930 by Fisher that sexually reproducing species have their sex ratios around 1:1 proportions and this inclines towards equal sex proportions. This is due to natural sex selection by the accumulation of autosomal alleles that direct the parental reproduction effort toward the rare sex (Cavalho et al., 1998).

A new life begins when the sperm meets the ova and initiates a series of processes that lead to sperm penetration sperm oocyte fusion and zygote division (Bahat et al., 2006). This describes fertilization which can be said to be the ***bridge between generations***.

Egg is the Latin name of ovum (Jaffe et al., 2004, Rovere, 2006).

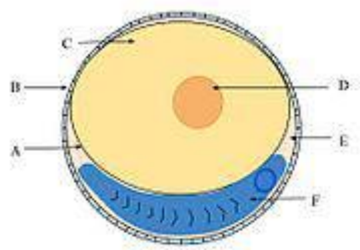


Figure 1.1 The structure of fish egg

a) A - Vitelline membrane, B – chorion, C – Yolk, D - oil globule, E - perivitelline space, F - embryo
b) Size - average size is generally 1 ml
c) Colour

Oviparity describes the most common form of fish reproduction where the female eggs are laid unfertilized and are then fertilized externally by the male. This is found in over 90% of fish species (Crocco et al., 2008).

Ovo-viviparity describes internal fertilization where the eggs are laid already fertilised (often found among sharks). These ova have no connection with the host fish and therefore receive no form of support (Crocco et al., 2008; Surget et al., 2001).

Viviparity - where the eggs are fertilised and nourished in the body. These have a placenta analogous to that found in mammals and is found among reef sharks (Groba et al., 2001).

Roe is the ripe mass of oocytes in the ovaries of fish usually considered as a delicacy in various countries of the world (Shirai et al., 2006).

Oology - study of eggs (Collins English Dictionary - 2009; (Jaffe et al., 2004).

Life begins when the sperm meets the ova and initiates a series of processes that lead to sperm penetration sperm oocyte fusion and zygote division (Anat et al., 2006).

Cloning describes the processes used to create an exact genetic replica of another cell, tissue or organism. The copied material, which has the same genetic make-up as the original, is referred to as a clone (Popa et al., 2003; American medical association 2013).

Reproductive rights involves an individual's voluntary process of decision making to marry, make a family, have the number of children of his choice, with voluntary timing and spacing and further grants the right to access information and a free choice to exercise the aforesaid (Baird et al., 1993).

Perinatal mortality - the World Health Organization defines the human perinatal mortality as the "number of stillbirths and deaths in the first week of life per 1,000 live births, the perinatal period commences at 22 completed weeks (154 days) of gestation and ends seven completed days after birth (1994 Global health for all database, Geneva: WHO).

Plasticity in phenology is a mechanism in which individuals buffer themselves from the impacts of climate change (Telemeco et al., 2013).

Charnov – Bull model is defined as temperature dependant sex determination (TSD) which is also referred to as environmental sex determination that is dependent on the temperatures prevailing during certain critical embryonic development most commonly observed among the reptiles. The critical embryonic is defined as the thermosensitive period during which sex differentiation of the gonads is determined which is usually during the middle third of their embryonic development (Warner et al., 2005).

Darwin theory - Described in 1871, is a biological evolutionary explanation of how species develop given by Charles Darwin with others that explain how all species of organisms develop through the natural selection of various inherited qualities that increase individuals' ability to compete, survive and reproduce (Ayala, 2009). This explains human sexual differences that evolved based on their sexual selection of certain physical, mental or psychological traits that evolved in order to aid competition amongst individuals so as to access preferred mates or traits that enhance traits that help attract mates (Paul, 2002).

Choosing a mate is an important milestone in one's life and demands various investments towards it that differ among the sexes. In this regard, whereas men's successful competitiveness involves money, power and status, the females' competiveness involves attractiveness, physique and skills of life (Ayala, 2009; Bull, 1981; Buunke et al., 2002; Rommel, 2002).

Study summaries

1.13 Introduction/Background

X-linked diseases majorly manifest in males while the females present as carriers but disease free (Arrayed, 1999). Despite the immense medical advances over the years, there is still no proven cure of these diseases except their control through preventive measures (El-Hazmi, 1999). Prevention through sex ratio influence and selection where possible, has been shown to offer some valuable solution as the curative treatment modalities are being sought (Seguy et al., 1975). Among the affected couples therefore, having female children can circumvent the agony of life long affected male siblings (Barron, 1996).

Prevention of genetic disorders through sex ratio adjustments has been examined and adopted as an acceptable and practical preventive tool for genetic diseases as this is easier, safer and cheaper than the current curative treatment modalities (Burke, 2002). Many sex ratio adjustment methods have come on board over the years but most of them have scanty conclusive evidence over their efficacy (Hewit, 1987). These include traditional, natural and some modern methods (College, 2002; Barron et al., 1996; Folk wisdom, 2002). Dietary method is among the methods that have been flouted to offer some practical solutions among the natural preventive methods though not fully proven and is under scrutiny in this study (Papa, 1983; Stolkowski, 1982).

1.14 The study concept

The study concept is based on the social allegations, myths and beliefs on various aspects and modes of gender ratio influence. In an effort to ascertain and put to rest the various social allegations pertaining to this, several study components examining the relationship between dietary chemical components and sex ratios were carried out so as to compare these allegations with the accruing facts, statistics and real time events.

The study concept simply involves taking a social allegation to the laboratory, designing appropriate experiments for testing and verification so as encourage the allegation or to disapprove the claim and lays it to rest. The concept was however wide and involved various diverse components that required various approaches that were adopted. With new concepts on board and many inadequacies of the prior studies, various trials were done so as to come up with appropriate study instruments, tools and designs of the various study arms that culminated into the various pilot studies.

Details of the initial trials and their results were not reported but some brief summaries of the preparatory works are included together with the pilot study results. Please allow theses inclusions because like meat that adds value to the pie so will these inclusions of the early preparatory works add value to the study and shed more highlights to the journey towards its eventual destination.

In the main study, the dietary method was exhaustively studied using various parameters and their effects on sex ratios analysed. These included the dietary chemical ionic influence on sex ratio outcome, perinatal mortality by the use of early gender determination using PCR, determination of the blood serum chemical concentration levels of the respective dietary chemical ingredients, effects of birth order on sex ratios, seasonal influence on sex ratios and their ionic effects on the oocyte membrane electrical potential.

The animal models weight dynamics were used to monitor their wellbeing. These various aspects were studied exhaustively and the results used to address most of the pertinent questions that frequently arise pertaining to this emotive subject. Presented below therefore are the abstracts of the various aspects of the study.

The study was carried out majorly in two parts:-

1) Ionic dietary study on Swiss mice.

a) Pilot study b) Main study.

2) Ionic effects on the oval electrical membrane potential in catfish.

b) Pilot study b) Main study.

1.15 Dietary chemical Ionic effects on sex ratios - Pilot study

A study aimed at examining the sex ratio influence of sodium, potassium, calcium and magnesium either as single or combined dietary elements was carried out using Swiss (Webster) breed mice animal models. The rationale of the study was based on the fact that dietary chemical influence on sex ratio outcomes has been advocated from time immemorial though not fully proven as yet.

Methods: A double blind study using 128 families of Swiss (Webster) breed were fed with sodium, potassium, calcium and magnesium as single elements, combined sodium with potassium and calcium with magnesium and others with all-inclusive chemicals (cocktail) in their drinking water. Two equivalent control groups were set up of plain drinking water and a second similar group with glucose added to it. They were followed up till each group had two generations of deliveries and sexing of their progeny performed using applicable methods.

Results: The mean number of pups per delivery was 5.6. No statistical significant difference in sex ratio was found in the control groups (water, $p=0.17$; glucose, $p=0.383$). Sodium, potassium and sodium + potassium supplementation influenced the sex ratios towards male progeny ($p<0.001$). Calcium ($p<0.014$), magnesium ($p<0.008$) and calcium + magnesium ($p<0.001$) influenced sex ratios towards the female progeny.

Conclusion: From the accrued data ample evidence does exist that show that dietary chemical contents do influence sex ratios. Further studies including oocyte membrane potential was subsequently carried out in support these findings.

1.16 Dietary chemical Ionic effects on sex ratios - Main study

A similar double blind study aimed at examining the chemical ionic influence of sodium potassium calcium and magnesium was carried using mice animal models as follows.

Methods: 144 families of Swiss (Webster) breed were randomly divided into 9 groups receiving different ionic formulations in their drinking water. They were followed up through their life spans of between 2 – 3 years and sexing of their progeny performed using applicable statistics.

Results: There were a total of 1528 deliveries with 13,040 pups delivered comprising of 6,348 female pups and 6,692 male pups. The mean pups per delivery were 8.5/delivery. There was no significant statistical difference in sex ratio found in the control water group, $p=0.61$ while, glucose, Sodium, potassium and sodium + potassium supplementation influenced the sex ratios towards male progeny ($p<0.001$). Calcium, magnesium and calcium + magnesium ($p<0.001$) influenced sex ratios towards the female progeny. The cocktail of all the combined elements together had no significant influence towards either genders ($p>0.0609$).

Conclusion: This study does confirm that dietary chemical contents do influence sex ratios through their ionic influence along gender lines and that glucose seems to have similar influence on sex ratios towards the male gender.

1.17 Perinatal mortality determination by early gender determination using PCR/DNA

Perinatal mortality has been used as good measure of the health status in humans and by extension can equally be extended to animal health. In order to determine perinatal mortality and sex ratios, gender determination was done using either physical makers or the PCR. To avoid losing data, counting of the pups and cutting off tail (DNA) material was done within 24 hours of delivery. Sex determination by physical means was done based on the anal - genital distance which is about 1mm in the females and twice this distance in the males. The optimal time for this determination is about three weeks of age. The pups whose gender could not be determined physically because they either died before attaining this age or were eaten by the adults had their DNA extracts subjected to PCR.

Results: A total of 415 pups out of 13,040 perished before gender could be physically ascertained and their DNA materials subjected to PCR analysis. This constituted approximately 3.1% of the total pups delivered with means of 1,449 pups delivered per group and 46 demised pups per group respectively.

No significant statistical gender difference in sex ratios was found among the demised pups in the water ($p>0.66$), glucose ($p>0.166$), sodium ($p>0.49$), potassium ($p>0.339$), calcium ($p>0.612$), combined sodium + potassium ($p>0.104$), and all-inclusive group ($p>0.7995$), signifying absence of gender influence among the demised pups. There was significant sex ratio difference towards female progeny among the demised pups in the magnesium ($p<0.005$) and combined calcium + magnesium ($p<0.044$) groups towards the female progeny.

Conclusion: The perinatal mortality rate was found to be 32/1000 which is comparable to the countries with high human perinatal mortality rates. The study did show evidence of gender female bias among the demised pups unlike human studies that show some slight higher male skewed mortality (1994 Global health for all database, Geneva: WHO).

1.18 Serum chemical concentrations study

To determine the effects of high dietary chemical compositions on biochemical serum concentrations of the respective chemicals.

A study aimed at examining the relationship between high dietary chemical supplementation and the relevant serum chemical concentrations was carried out. It sought to examine whether the respective high chemical element concentrations in the various feeding dietary solutions were reflected systemically. The rational was based on the fact that high dietary chemical components in our diets have been advocated to influence the sex ratio outcome through their systemic effects. These chemicals are absorbed from the intestines via the liver to the blood stream and subsequently to the extra cellular space where cells including gametes are bathed in this fluid. With the presence of increased amounts of chemicals in the extra cellular fluid, various cellular reactive responses take place through osmosis, passive diffusion and active ion channel movements. These adjustments attempt to maintain the extracellular and intracellular equilibrium state of the relevant chemicals by their charges and in the process of the cellular ionic dynamics create cell surface charges that by extension of their immediate environs such as the neighbouring, surrounding or attached proteins influence their cellular activities including fertilization.

Methods*:* Determination of the serum biochemical levels of the respective elements under investigation was done in all the nine groups. Blood samples of 0.5 ml were drawn through nipping off the tail and intra orbital routes. Using the standard automated systems the biochemical analysis was done on weekly basis.

Results*:* Normal levels of sodium, potassium, calcium and magnesium were found among the control groups with means of 140 mg/ml, 7 mg/ml, 2.4 meq/l and 1.7 mg/ml respectively. No statistical significant differences were found between the concentrations of these four chemicals between the two control groups with sodium ($p>0.165$), potassium ($p>0.818$), calcium ($p>0.878$) and magnesium ($p>0.116$). Significant statistical differences were found between the corresponding elements in the water control group and the experimental groups of sodium, potassium, calcium, magnesium, sodium + potassium, calcium + magnesium group and all-inclusive ($p>0.0001$) except between calcium and magnesium in group one and that of the all-inclusive (cocktail) group with ($p>0.9603$) and ($p>0.455$) respectively.

Conclusions*:* Normal serum levels were observed in the control groups while elevated serum levels were observed among the experimental groups except in the calcium group and magnesium element in the all-inclusive group.

1.19 The weight trends as a measure of the animal model wellbeing and study follow

This study was done with an aim of examining the effects of various chemical formulations on the weight trends on the animal models. The rationale was based on the fact that weight trends have been used before as a good measure of the wellbeing of the animal models and success of the study. This was based on the background of the study since its inception, where dissolved in tap water, the various chemicals under study were fed to the animals and their weight trends examined. In the initial stages, the animal models in groups four, five, six and eight that were constituted with potassium, calcium and magnesium had poor feeding habits as some were refusing to feed and were continually losing weight while others even starved an aspect that persisted despite various dilutions till glucose was added.

Methods*:* To study the effects of the various chemical formulations on the weights of the animal models, six pairs of animal models were selected from each of the nine groups and their weights taken on a weekly basis for a period of six months. The models were introduced to the study at the age of six weeks in line with the rest of the study's other aspects. The chemical formulations, constitutions and applications were done as stipulated later.

Results*:* The total mean weight gains among the female and male animal models were 11.12g and 10.55g respectively representing mean monthly weight gains of 1.85g and 1.76g respectively. The maximum weight attained among the females was 28.19dg and 27.73dg among the males which are within the average adult weight ranges. There was general weight gain and absence of recorded weight loss.

Conclusion*:* The general or universal weight gain among all the animal models reaffirmed general wellbeing of the animal models, intake of the study chemicals and validity of the results.

1.20 Effects of birth order and sex ratios

A study aimed at examining the relationship between birth order and sex ratios was carried out using the data accrued. This study sought to know whether birth order had significant influence on sex ratios of the progeny. The rational was based on the many unconfirmed allegations in society that sex ratios may be influenced by birth order.

Methods: Data on all the deliveries of nine groups each with sixteen families were collected over their entire life spans of the animal models of between two and three years. Data on all deliveries were recorded and analysed. The delivery order was designated as generation in that the first delivery was referred to as generation one and the second delivery as generation two and so on.

Results*:* Among the 9 groups with sixteen families each, there were 1528 deliveries with a total of 13,040 pups comprising of 6,348 Female pups and 6,692 Male pups. The mean litter size per family was 8.5 per delivery. The mean female pups per family per delivery were 4.15 and the mean male pups delivered per family whereas 4.38.

The average pups delivered among the 1 – 9 generations (deliveries) were between 8 - 9 and dropped from the 10th delivery (generation) to 6 pups per delivery and down to eventual 2 in the last 12th delivery. The sex ratio influence towards the female gender among groups 5, 6 and 8 was maintained and so was male gender inclination maintained in groups 2, 3, 4 and 7. Absence of gender influence was maintained in groups 1 & 9.

Conclusions: The mean litter size was 8.5pups per delivery in all the groups and generations. The litter size was not found to be influenced by birth order and was universally similar in all the groups and families in particular among the first ten deliveries with an average litter size of 8.5 pups per delivery. Birth order was found to decline in numbers as from the 10, 11th & 12 deliveries declining within the range of 6 - 2 pups per delivery. In all the nine groups, birth order was not found to influence sex ratios except declining litter sizes from the 10th delivery onwards.

1.21 Effects of seasonal variations on sex ratio

A study aimed at examining the relationship between seasonal variations and sex ratios was carried out using the accumulated data. The study sough to find out if food, water and the environment were held relatively constant, whether there could be any sex ratio influence in their progeny. The rational was based on the fact that studies in the animal kingdom in their natural habitats have shown sex ratio influence related to the availability of food as well as seasonal variations.

Methods: Data on all the deliveries of the nine groups each with sixteen families was collected over their entire life spans of between two and half years. The number of deliveries were analysed against the seasons during which the study was carried out. The seasons considered were those prevailing in our local region. These include the hot, cold and the rainy seasons. The local hot seasons run in the months of January, February, March, August and September, and the cold seasons prevails in the months of June and July. The rainy seasons are represented by the months of April, May, October, November and December.

Results: Seasonal variations had similar sex ratio results of absence of gender influence in groups one and nine towards neither gender with the control water group, $p>0.61$ and group nine with the cocktail of all the combined elements $p>0.0609$ while, glucose, Sodium, potassium and sodium + potassium supplementation groups influenced the sex ratios towards male progeny ($p<0.001$). Calcium, magnesium and calcium + magnesium ($p<0.001$) influenced sex ratios towards the female progeny. In all the nine groups showed significant influence on more pups delivered during the rainy season than the other seasons.

Conclusions: From the studies, there is enough evidence that suggest that seasons appear to mainly affect the litter size in particular the rainy season but not the gender ratios. The gender difference is mainly attributed to the ionic effects of the dietary components without the effects of seasons overriding the dietary ionic effects.

1.22 Chemical ionic effects on the oocyte membrane electrical potential

A study aimed at examining the relationship between dietary ionic supplementation, oocyte membrane electrical potential and sex ratios was carried out. The rational was based on the fact that the oocyte membrane may have alternating membrane electrical charges that are influenced by dietary ionic effects. These ions exert allosteric modification or electrotactism that allows selective gamete attraction and subsequent gender outcome.

Methods: A double blind control study with 108 female cat fish that were randomly divided into 9 groups each receiving different ionic formulations in their swimming solutions. Each group comprised of twelve female catfish that were exposed to the respective chemical formulation in their swimming water and followed up at a time for a period of one week. Ova were retrieved 24 hours following ovulation induction by Ovaprim 0.4 ml/kg given intramuscularly. The harvested oocytes from the respective groups were subjected to electrical fields and their behavioural patterns studied. This procedure was repeatedly done twelve times in each group and the findings subjected to the relevant analysis.

Results: Oocytes retrieved from the two control groups had no significant oval polar attraction to either the negative or positive electrodes. There was however significant oocyte polar attraction towards the positive electrode among the ova retrieved from the sodium, potassium and the combined sodium and potassium solutions.

Oocytes retrieved from calcium, magnesium and combined calcium + magnesium solutions had affinity towards the negative electrode and no significant affinity towards the positive pole.

Oocytes harvested from the solutions constituted with all the salts demonstrated dual attraction with some ova being attracted towards the positive pole and others towards the negative pole. There was however more attraction noted on the positive pole than the negative pole though not statistically significant.

Majority of oocytes are not attracted to either electrode and are therefore most likely not electrogenic,

Conclusion: Solutions constituted with sodium and potassium elements appear to have skewed ionic influence over the oocyte membrane electrical charge towards the negative charge hence their attraction towards the positive pole. Their common electrical factor is their valency one (+). Practically if the oval electrical charge is influenced towards the negative then during fertilization it will selectively favour the positively charged gamete which is the Y bearing sperm resulting to a male conceptus.

Similarly, Solutions constituted with calcium and magnesium appears to have skewed ionic influence over the oocyte membrane electrical charge towards the positive charge influencing their attraction towards the negative pole. Their common electrical factor is their valency two (++). Practically if the oval electrical charge is influenced towards the positive charge then during fertilization it will selectively favour the negatively charged gamete which is the X bearing sperm resulting to a female conceptus.

Failure to achieve close to 100% results in sex ratio influence using natural methods conforms to the Fishers principle which predicts evolution evolves towards the 1:1 female/male proportions. This serves as a natural safeguard against species extinction or over production of any particular gender.

All experiments were done with the approval of the Kenyan Animal Ethics Committee.

Chapter 2

Literature review

2.1 Introduction/Background

To alleviate suffering and improve life, mankind has historically desired to find cure for all his ailments (Barabasi et al., 2011). In the process, a lot has been achieved however, hereditary diseases such as –X- linked disorders have remained a big challenge without eminent hope of finding complete cure except prevention which has remained as the only recourse to date (El-Hazmi, 1999).

Acquired diseases have significantly reduced over the recent past due to improved economic status, better nutrition, better medical care and expansion of screening services (El-Hazmi, 1999). This has shifted the overall disease prevalence thus making genetic diseases a major cause of morbidity and mortality (Arrayed, 1999). If this trend is not arrested by finding more preventive or curative measures, genetic diseases are headed to be more prevent years to come (Arrayed, 1999; Owain et al., 2012).

-X- Linked diseases are conditions that are expressed mainly in situations where the X chromosome exist in single status as in the male chromosomal expression of -XY- as opposed to the double -XX- female chromosomal representation (Adam, 2004). The double -X- chromosome acts as a preventive tool against these conditions and they are therefore more prevalent among the males than the females. Over 350 have so far been reported (College, 2002; El- Hazmi, 1999). Females are mosaics with two cell lines of maternal X chromosomes and the paternal -X- chromosomes. In normal circumstances one X chromosome is randomly inactivated in early embryonic life and stays permanently inactivated (Orstavik, 1999; Wutz, 2007). In circumstances where there is X linked diseases, there is preferential or skewed X inactivation where he mutant X chromosome or the affected chromosome is inactivated (Orstavik, 2006).

When a couple is affected by an -X- linked disease and the foetus is a female, there is a high probability that the child will not be affected although a carrier status may present (Barron-

Vallejo et al., 1996). However, if the child is a male, the odds are such that the child will have a 1:2 chance of being affected (Robertson, 2001; Young, 1991). Subsequently, influencing conception towards predominantly female offspring can therefore circumvent the social, financial and emotional costs that go with bearing affected progeny (Seguy, 1975).

The practice of sex ratio adjustment precedes the written history (Hewit, 1987). The first documented gender selection was presumably done by God when after creating Adam, He went further to create Eve as a balancing act. The rest was left to mankind as he was commissioned them to go forth and fill the earth (Genesis 1: 27 - 28; Quran chapter 23, verse 12-14). Man has since been preoccupied by the desire to influence the sex ratios of his off springs since creation (Hewit, 1987). Failure to achieve the aspired or desired family gender distribution has led to many sex ratio adjustment methods that span from the ancient cultural myths and practices to the modern day technological methods (College, 2002; Folk wisdom, 2002).

There are three main principles of sex ratio adjustment that have so far been advocated and practiced (College, 2002). Evolution-wise, these include the traditional practices, natural indirect influencing methods and the direct artificial sex ratio selection methods (Folk wisdom, 2002; Seguy et al., 1975). Their differences are reflected in the times of discovery, advocacy and practice in human history (Savulescu, 1999; Schenker, 2002).

The initial traditional sex ratio adjustment methods were based on either long term observations or rudimentary scientific knowledge and many have already fallen out of use (Folk wisdom, 2002). Over the years, these methods translated into the indirect natural methods of sex ratio influencing that exploits the natural physical properties of the gametes or the body's physiological properties reflecting increasing understanding of the human anatomy, physiology and their clinical applications (Carvalho et al., 1998; Edwards, 2000; Screenay, 1993). The latest artificial sex ratio adjustment method is based on direct manipulation of the gametes or the conceptus and defines direct sex selection and the latest understanding of human reproduction (Burke, 2002; Scott et al., 1999; Shushan et al., 1993).

Most of the traditional gender ratio adjustment methods have fallen into disuse and out of favour and therefore remain as myths or tales that hold little value and use. To date, the majority of sex ratio adjustments methods that are practiced fall in the category of natural or indirect sex ratio influence. These include billings, smart stork and dietary methods (Billings, 1972; Odebla., 1997; Screenay., 1993; Shettles, 1970; Whelan, 1985). Among the direct sex ratio selection methods include Erickson method that exploit the oocyte kinetic or density differential and the oocyte membrane electrical potential properties (Erickson, 1994; Vidal et al., 1998).

The dietary method is among the various indirect natural gender influencing methods that have been advocated (Stolkowski, 1982). The rational is based on the fact that the oocyte membrane may have alternating membrane electric potential to negative, positive or neutral status (Papa et al., 1983). This electrical potential may be influenced by modulators in the diet that are under scrutiny in this study. It is postulated that the oocyte membrane in its negative state, attracts the androsperm while at its positive state, attracts the gymnosperm while at its neutral status accepts either gamete at a 50:50 chance. (Celik et al., 2003; College, 2002). This phenomenon has been described as galvanotropism (Collin et al., 2005, Wong et al., 2010).

Galvanotropism is a concept whereby natural cells are attracted to one another due to their inherent membrane electrical charges (Collin et al., 2005). The attraction is proportional to the electrical membrane potential generated between them (Wong et al., 2010). Gametes are electrogenic and function through the transmembrane ionic transport and channel systems that allow various ion currents to mediate various biologic and physiological events during cell growth, maturation, division and fertilization (Tosti et al., 2004).

The dietary method is based on this concept (Celik et al., 2003). Through ionic influence, the chemical contents of the diets act on the receptor sites or some proteins on the oval membrane that the spermatozoa bind as it fertilizes the ova and exert some specific allosteric modification or electrotactism that allows selective attraction towards either gamete and subsequently influence the gender of the conceptus (Celik et al., 2003; Rajan, 1999; Wong et al., 2010).

In the animal kingdom, studies have shown that breeding patterns can be influenced by various factors such as the prevailing climatic conditions, dietary intake, age and the adult sex ratio (Chenyi et al., 2003; Elissa et al., 1999; Sven et al., 1989; Westber, 2001). Among the crocodiles, sex differentiation can be pre-determined by the temperature at which the eggs are incubated (College, 2002; Telemeco et al., 2013; Warner et al., 2005).

Whereas the ethical question on direct sex selection methods are in no doubt an unwelcome debate, questions still linger as to where to place the grey aspects of the indirect natural gender influence practices that are in practice today and difficult to enforce legislation on (Billings, 1972; Ericson, 1994; Guerrero, 1971; Kalaca et al., 1985; Robertson, 2001; Shettles, 1970; Simcock, 1985).

2.2 Genetic disorders are a large group of disorders resulting from either minor or major mutations in the genetic composition or alterations in chromosomal numbers. They are classified into single-gene, chromosomal, multifactorial and mitochondrial disorders (Adam, 2004; Arrayed, 1999; El-Hazmi, 1999; Levin et al., 1995; Owain et al., 2012).

2.3 Single-gene disorders were first recognized by Garod when he described alkaptonuria as a genetic defect which results from a mutation in or around a single gene. Over 6000 such disorders have been identified (El-Hazmi, 1999). ***Chromosomal disorders***, involve either an increase or decrease in the total number of chromosomes or abnormalities in their structure. Down syndrome was the first among these disorders to be recognized in 1959 (El-Hazmi, 1999, Kumar et al., 2010). ***Multifactorial disorders*** comprise of the most common congenital malformations such as the cleft lip and adult life diseases such as diabetes mellitus or hypertension. They result from the interplay of the genetic make-up and other multiple environmental factors (El-Hazmi, 1999; Lalit et al., 2006).

Mitochondrial disorders have been described as maternal because only egg cells contribute mitochondria to the developing embryo and therefore only females can pass these conditions to their children (El-Hazmi, 1999).

Mammalian genetic disorders are passed on to subsequent generations in various predetermined ways, either as ***autosomal disorders*** which *are* conditions arising from mutations of the non-sex chromosomes (autosomes) or as ***sex linked disorders***, caused by mutations in genes on the sex chromosomes either the X or Y chromosome referred to as X-linked or Y-linked respectively (Arrayed, 1997, Orstavik, 2006).

2.4 X-linked disorders arise from the X chromosome defects and usually differentially affect the progeny due to the variation in their modes of transmission (Arrayed, 1997; Orstavik, 2006; Wutz, 2007). In human, the male progeny of a male parent with an ***X-linked recessive disorder*** will not be affected while his female progeny will be carriers (Owain et al., 2012). With each pregnancy, a female parent who carries an X-linked recessive disorder has a 50% chance of having sons who are affected and a 50% chance of having daughters who are carriers (El-Hazmi, 1999). Some examples of these conditions include Haemophilia and certain variants of Colour blindness (Adam, 2004; College, 2002).

2.5 X-linked dominant disorders are caused by mutations in genes on the X chromosome that affect males more than females and their mode of transmission differs between men and women (El-Hazmi.,1999). The sons of a man are not affected while all the daughters do inherit the condition while a female with this condition has a 50% chance of having an affected daughter or son with each pregnancy. Some of these conditions are fatal to males but not in females such as Klinefelters syndrome (El-Hazmi., 1999).

2.6 Y-linked disorders are caused by mutations on the Y chromosome. Only males can get them, and all of the sons of an affected father are affected. Since the Y chromosome is small, Y-linked disorders mainly cause infertility (Adam, 2007).

Genetic disorders are chronic, incurable and require lifelong care and management strategies (El-Hazmi, 1999). They require a multi-level management approach through primary care which is aimed at prevention, secondary care which is geared towards treatment of the conditions and finally tertiary care which targets the consequences (Barron et al., 1996). Despite advances in medical care, prevention of hereditary disorders still remains the mainstay management

(Levin et al.,1995). Sex ratio adjustment therefore still plays a key role in managing some of these conditions (Barron et al., 1996; El-Hazmi, 1999).

2.7 Prevention of X-linked disorders through sex ratio adjustment methods

In humans, sex ratio adjustment methods evolved from traditional myths (Folk wisdom, 2002) to the natural influencing methods (Carvalho et al., 1998; Edwards, 2000; Screenay, 1993) and finally to the current artificial sex selection methods (Vidal et al., 1998; Wang, 2006). In all these methods, sex ratio adjustment process is achieved either before or after conception through influencing the natural characteristics of the body, the gametes or through direct manipulation of the gametes (Carvalho et al., 1998; Edwards, 2000; France 1981 Screenay 1993, Shettles, 1970).

The first documented gender selection was presumably done by God when after creating Adam; he went further to create Eve as a balancing act.The rest was left to mankind as he was commissioned to go forth and fill the earth (Genesis,1: 29). The first documented human assisted reproductive technology started around 400 BC and remained traditional until Charles Darwin gave an account of the descent of man. His theory was further advanced by Carl Dusing in 1883 (Edwards, 2000).

In the 1920s the human physiology was fairly well understood with more light being shed in the 1930s when Fisher described the theory behind sex ratios (Carvalho et al., 1998; France 1981, Morishita 1879, Screenay 1993). With the advent of the pill in the late fifties and early sixties, Man was able to control his reproduction better (Brown, 1981; Burkman, 2011; Odeblad, 1997).

Post conceptional sex ratio adjustment methods came into practice in the sixties but were proscribed due to ethical issues and artificial laboratory methods of sperm manipulation adopted (Manger et al., 1997). The latter culminated in *in vitro* fertilization procedures in the seventies. Through the latter, baby Louise Brown was born in 1978 work pioneered by Lord Roberts Winston in London (Fugger et al., 1998; Vidal et al., 1998).

Human cloning took centre stage in the nineties with Stillman reporting the first human cloning in the United States in 1994. His work was improved by the Raelian religious group that reported the first human clone in December 2002 (Burley 1999, Havstad 2010, Jaenisch, 2004; Rhind et al., 2003). Currently, through genetic engineering, more understanding of reproductive dynamics has been achieved and therefore more characteristics other than gender influence or selection can now be addressed (College, 2002; Strong 2005).

Studies in ***the animal kingdom***, both domesticated and the wild-types, have shown possibilities of sex ratio adjustment through the prevailing climatic conditions, dietary intake, age and the adult sex ratio (Chenyi et al., 2003; Elissa et al., 1999). Among the zebras and horses, the Trivers-Willard hypothesis has been found to hold true whereby mothers in better living conditions produce relatively more sons while those in poorer living conditions tend to produce relatively more daughters. Some studies conducted in the dairy industry have generated mixed results (Bradbury et al., 1998; Elissa et al., 1999; Hossain et al., 1998). Among the reptiles such as the crocodiles and the tortoise species, sex differentiation can be pre-determined by the temperature at which the eggs are incubated (Allsop et al., 2006; Telemeco et al., 2013; Warner et al., 2005).

2.8 Natural Methods

Natural methods of sex ratio adjustment are based on thorough understanding of the menstrual cycle (Billings, 1972; France, 1981). They exploit physiological changes that prevail during the fertile phase by timing ovulation and synchronizing it with the kinetics and other physical properties of the sperms. These achieve conception of the aspired gender at an overall success rate of 70 - 80% (Whelan, 1985; Screenay, 1993).

Cervical mucus consistency and its pH as well as body temperature changes are the main demonstrable physiological changes of the body that mirror the different phases of the menstrual cycle (Flynn et al., 1976; James, 1981; Morishita et al., 1979). Gametes function optimally in different states of the cervical mucus (Billings, 1972; Odeblad, 1997). At ovulation, the cervical mucus is alkaline, an environment that favours the androsperm hence the basis of advising mothers to try and achieve a male conceptus at this time. Conversely, before ovulation, it is acidic and this favours the gynosperm and female conceptus (Billings, 1972; Shettles, 1970). The cervical mucus pH changes have been translated into the douching methods which adjust the vaginal environment to either acidic or basic states. This method has been associated with an overall success rate of 55-65% (Guerrero R, 1971; Shettles, 1970).

Shettles, 1970, described the androsperm as lighter and faster but with shorter life span than the gymnosperm. If intercourse is therefore timed at ovulation, the androsperm would get to the egg first resulting in a male conceptus. Conversely, if intercourse occurs several days prior to ovulation, the gynosperm would outlive the androsperm (Shettles, 1970).

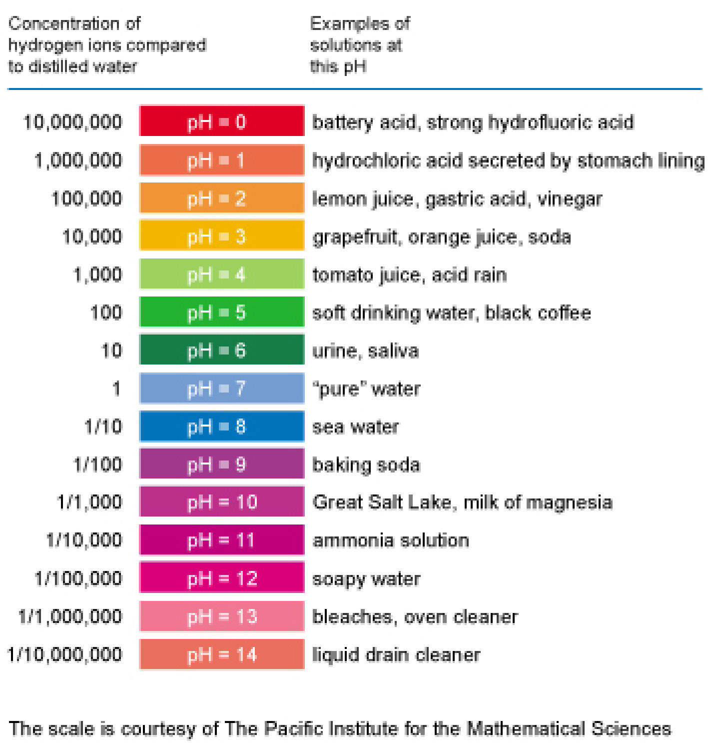


Figure 2.1 Different solutions and their associated pH

2.9 Artificial Methods

Kinetic differential

The kinetic differential in swimming abilities of the gametes was first described by Shettles in 1972 and is exploited in the ***modified swim*** up technique and the ***Erickson's method*** (Erickson, 1994; Erickson et al., 1992). In these methods, the semen sample is centrifuged leading to sedimentation. Culture media is then overlaid on the semen sample and the sperms allowed to swim-up through the media for a fixed period of time. The androsperms swim up the sorting apparatus faster and creates enriched sperm samples of up to 75-80% (Erickson, 1994).

2.10 Density differential

The Centrifuge or cytometric separation exploits the density differential concept (Erickson, 1994). This is done by spinning the gametes at very high speeds in a test tube. It makes the slightly light androsperms rise to the top leaving the heavier gynosperms at the bottom. This achieves enriched groups of up to 85% of the desired gender (Erickson et al., 1992). The density differential between the X and Y bearing sperms was first reported by Johnstone in 1993 to be 2.8% difference between the total DNA content (Fugger et al., 1998).

2.11 Concepts of electric potential differentials

Koltzoff and Shroder, both Russian scientists found out that it was possible to separate the sperms with -Y- or -X- bearing chromosomes by passing them through an electrical field in 1958. They observed that the gynosperms are attracted to the positive charge and therefore bearing a negative charge while the androsperms are attracted to the negative charge and therefore bearing a positive charge (College, 2002).

2.12 Microsort is a method that makes use of the different electrical charges on the gametal membranes and separates the gymnosperms from the androsperms (Fugger et al., 1998). This separation is done by passing them through flow cytometry (FCM) and DNA staining known as fluorescent in situ hybridization (FISH) (Manger et al., 1997). This process gives the gynosperm about 85-90% chance of success and the androsperm about 75% chance of achieving a pregnancy of the desired gender (Vidal et al., 1998).

2.13 Dietary Ionic effects

The idea of dietary influence on the sex of the conceptus originated from German studies in the 1940s which looked at the environmental influence on reproduction of worms (Stolkowski et al., 1982). Studies conducted in humans reported an overall success rate of 80% (Papa et al., 1983; Stolkowski et al., 1982).

The diets presumably exert ionic effects on the oval membranes which preferentially attract the fertilizing sperm and subsequently influence the gender of the conceptus (Stolkowski et al., 1982). The attraction is mediated through some specific allosteric modification or electrotactism on the membrane through the receptor sites which the spermatozoa bind as it fertilizes the ova or some membrane proteins on the cell surfaces (Rajan, 1999; Celik et al., 2003).

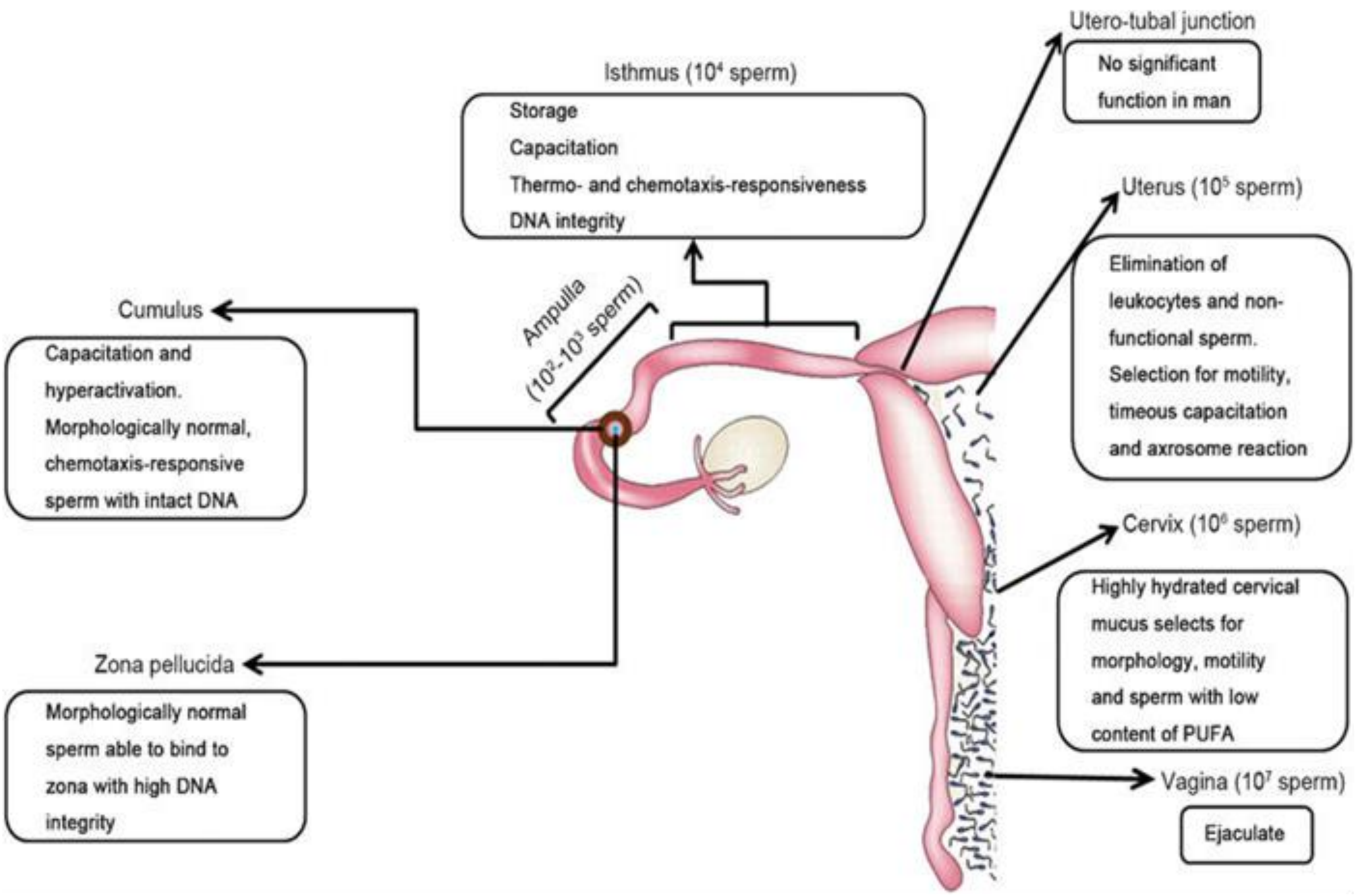
The importance of the electrical control of cell physiology became apparent following the famous experiments of Galvani in the epic frog muscle studies in 1700 (Collin et al., 2005). Since the advent of the Galvani studies, many more studies have demonstrated evidence that suggest that living cells both somatic or germ cells are electrogenic and that they do respond to electrical fields depending on their electrical potential (Mikhail., 2001). All cells are produced with specific set of functions that are executed through well-orchestrated set of events (Tosti et al., 2011). Some of these involve ionic movements outside the cells, across the cell membranes and inside the cells that generate electrical potential that can be converted into mechanical energy for various cell functions including fertilization (Valic et al., 2003).

Whereas somatic cells are produced through mitotic divisions, germ cells are produced through meiosis a process that brings in sexual diversity in reproduction. The process starts off with diploid chromosomes and ends up with tetraploid chromosomes. It is divided into prophase 1 & 11, Metaphase 1 & 11, Telophase 1 & 11 and anaphase 1 & 11. The oocytes are arrested at prophase 1 till hormonal presence that triggers resumption of meiosis and get re-arrested in metaphase II till fertilization when it is completed (Tosti et al., 2011).

2.14 Forces and sperm selection sites involved in fertilization

The process of fertilization remains as among the many complex physiological dynamics that are as yet fully understood (Miller, 1985). Proceeded by mating, which acts as an external mechanical aid that gets the male gametes into the female reproductive system, the rest of the task is left to the intrinsic properties of the gametes and the inherent properties of their pathways to aid them to find each other culminating into the process of physical cell interaction characterized by gamete recognition, selection, binding, and fusion (Anat Bahat et al., 2006; Tosti et al., 2011).

Following sperm deposition in the female genital tract, the process of sperm selection begins and is carried out efficiently at various sites along the genital tract through mechanical, biochemical and biophysical methods (Eisenbach, 1999). These sites include the cervix, uterus, uterotubal junction the fallopian tube, cumulus oophorus and the zona pellucida (Miller, 1985).



With such a long distance to cover between the gametes, there must be some form of ordered and structured way of getting to each other and not by chance as nature leaves nothing to chance (Miller, 1985). Without proper guidance, the prospects of the gametes getting to each other are minimal (Anat Bahat et al., 2006; Ralt et al., 1991). Sperm guidance has therefore been confirmed and found useful not only among the species with internal fertilization but also among externally fertilized oocytes in particular among the marine animals where the gametes are laid into the sea (Eisenbach, 1999; Miller 1985).

Over the years, more understanding of human fertilization has been attained though more understanding is still awaited to further explain the puzzle of the sperms navigational prowess from the cervico-vaginal depository site to its reservoirs at the uteri isthmic junction and finally to the isthmic ampullary fertilization site (Anat Bahat et al., 2006). The large numbers of spermatozoa that are released into the depositary sites in the reproductive system aid in taking care of the sperm loss on the way to their eventual destination (Anat et al., 2006). The current knowledge clearly elaborates some of the extrinsic forces that come in aid of the navigational mechanism that are involved in the combined mechanisms that come into play (Eisenbach, 1999). The mechanical aids that play part of this exercise include inherent sperm motility, uterine and oviduct contractile or peristaltic properties (Nature med. vol. 9 number 2 Feb. 2003, Anat et al., 1995).

Besides the intrinsic mechanical forces of the gametes and the pathways, there is further evidence that suggest that there is still further clear communication between the sperms and the oocytes. This communication appears to be mediated by some factors that operate at different sites and locations. Some appear to operate at a distant, others within the immediate environs and finally intra-gametal (Eisenbach, 1999, Miller, 1985; Tetsuya et al., 2002). It is only a fraction of the sperms 10% of the sperms that undergo capacitation and ultimately become responsive to dynamic forces that propel them to their eventual fertilization sites (Anat et al., 2006).

The various operational forces include the distant, communication factors that appear to be mediated through thermotaxis which control the sperms and direct them to a closer range. Chemotaxis appear to be the guiding factor around the immediate environs of the gametes (Anat Bahat et al., 2006; Heini et al., 1998), then finally galvonotropism that operates at the closest range and selectively influences the sperm that ultimately fertilizes the ova (Richard et al., 2009). This presumably appears to be the order of events along the journey of a sperm to its final destination. All natural events are in some well-orchestrated form of order or control and don't happen haphazardly and the events leading to fertilization are no exception (Fei Sun et al., 2003). Thermotaxis is a long range directional cellular movement effected by a temperature gradient expressed in the animal, bacteria and plants species. In humans, thermotaxis seems to play a significant role among sperms that are deposited in the cooler areas and directs them to the warmer fertilization areas (Anat et al., 2006). Majority of the sperms are non-capacitated and are therefore not responsive to this phenomenon. Through thermotaxis, capacitated sperms are therefore guided from the cooler isthmus sperm reservoirs or storages sites to the warmer ampullar fertilization sites (Bahat et al., 2006).

Chemotaxis is the attraction of cells to chemo-attractants produced by other cells or their surroundings (Eisenbach, 1999). A phenomenon observed in both plant and various animal species that include marine species, amphibians and mammals. The phenomenon has however not been found to be species specific (Fei Sun et al., 2003). Some chemo-attractants have been identified from the oocytes and the cumulus (Fei sun et al., 2005) include progesterone from follicular fluid (Bijay et al., 1999) and various chemokines such as rantes (Tetsuya et al., 2002). Some other areas where chemo-attractants and chemokines are involved are in states of inflammation and wound healing where there is induced directional cell movement (Heini et al., 1998).

Galvanotropism has since been described as a unique feature among living cells in which their movements are influenced by external electrical fields (Hebbel, 1936; Hyma et al., 1922; Ogawa et al., 2006, Richard et al., 2009). Though the mechanism is not fully understood, it is thought to involve the effects of the electrical charges or fields on the cell membrane, receptors on the membrane or some proteins on the immediate environs of the cell surfaces (Hardy et al., 1997, Tosti et al., 2011). These charged cell membranes, receptors or surface proteins may experience electrophoretic or chemotactic forces attracting them towards the oppositely charged cells, electrodes or fields (Hardy et al., 1997).

Gametes being electrogenic function through the transmembrane ion transport and channel systems, allowing various ion currents that mediate various biologic and physiological events during cell division, maturation and fertilization (Tosti, 2004). Using various electrophysiological techniques ion currents involving calcium, sodium, potassium, magnesium and others have been shown to play vital role in meiosis, fertilization and normal embryo development (Richard et al., 2009; Tosti et al., 2011).

Cell to cell communication is a common phenomenon that has been demonstrated in the cumulus oocyte complex through gap junctional contacts and bidirectional coupling through electrophysiological testing between the cumulus cells and the oocytes (Norton et al., 1978; Ralt et al., 1991). The latest findings do suggest that the female may have a gender biasing mechanism manifested through the oviducts that act as biological sensors that screens either X or Y bearing sperms by modifying the tubal micro environment (Alminana et al., 2014).

Among the interesting observations in reproduction are the vast numbers of sperms produced for a single fertilization. A glaring example is found in humans where only a small fraction of spermatozoa 2 - 10% respond to chemo-attractants most probably the functionally mature sperms among other factors (Anat et al., 1995).

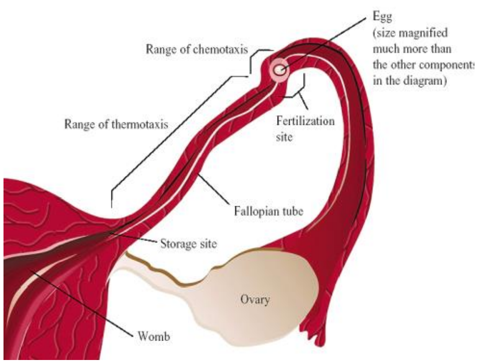
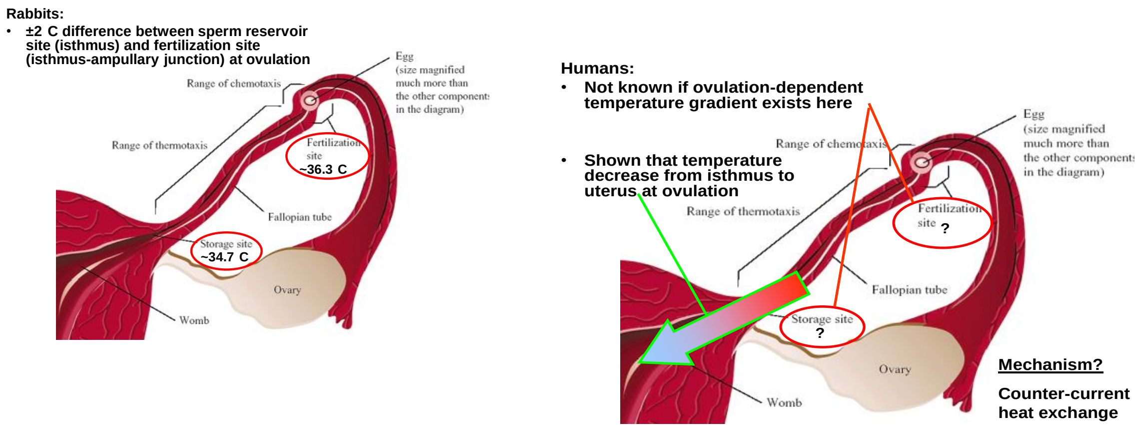


Figure 2.3 The various forces acting between the sperms and the ova that aid in fertilization that have been postulated.



F***igure 2.4*** *Temperature gradients along the fallopian tube*

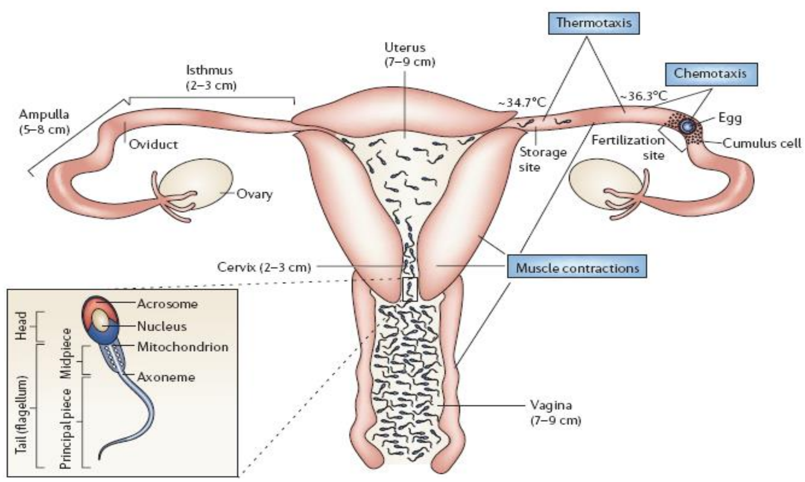


Figure 2.5 *Summary of the various processes involved in fertilization.*

2.15 Summary - Post-conceptional sex ratio adjustments

Most sex ratio adjustment methods are pre-conceptional as described above. Post-conceptional gender selection methods include, pre-implantation genetic diagnosis (PGD) in which gametes are fertilised producing several embryos. The gender of each embryo is determined and those of the desired sex are implanted (Shushan et al., 1993). Whereas sex selection for social reasons is associated with controversies, gender selection for purposes of prevention of genetic diseases is universally accepted (Baron al., 1996). Other methods include chorionic villus sampling, ultrasonic gender determination with or without termination of pregnancy but these methods, along with eugenic practices, have been found wanting due to their unethical principles (Eugenic watch, 2000; Gejman et al., 2002; Popa., 2003). Adoption is one of the oldest, modest and acceptable form through which sex ratio adjustment is practised among some families worldwide (Line, 2002).

Chapter 3

Theoretical foundation

3.1 Introduction

The subject of sex ratio influence is multidisciplinary, multifaceted and complex in itself (Schroeder, 1994; Vallejo 1996; Vidal et al., 1996). It is better conceptualized as supported by a whole matrix of all the other aspects of humanity and not as a standalone topic (Savulescu 1999; Schenkeri, 2002). The practice of sex ratio influence is majorly based on four concepts based on traditions, folk or astrology, the natural or indirect influence (Billings 1994) and the direct sex selection methods (Erickson, 1994). These various modalities of gender selection have come on board over the years with increasing knowledge of the physiology of the human body and reproduction (Flynn 1976; Odeblad, 1997; Whelan EM).

These can be traced from the pure traditions of pure folk, herbal or astrology, to the natural methods through the evolving and unfolding physiological knowledge and contribution (Savulescu 1999). Lately the direct sex selection methods have taken precedence through direct gamete manipulation in the latest in vitro fertility enhancement methods that have grown substantially through remarkable leaps and bounds (Fugger 1998; Wang 2006).

The earlier approaches that include astrology, traditional and natural modalities are usually exploited synergistically so as to achieve better results. However the latest methods work independently of each other but still achieve better success rates (Guerrero, 1971; Villamor et al., 2010).

X-Linked diseases are genetically inherited through the -X- chromosome (El-Hazmi 1999). They manifest in situations whereby the -X- chromosome exist alone like in the -XY- chromosomal status in the male as opposed to the double -XX- female chromosomal status. The second X chromosome in the females offers protection against these diseases. These conditions therefore manifested as diseases mainly in the males while the females present as carriers (Seguy 1975).

The odd ratios are such that if a couple is affected by an - X - linked disease. The odds are such that if the child is a male, the child has a 1:2 chance of being affected with the disease (Barron et al 1996). However if the foetus is a female, the odds are overwhelming that the child will not suffer from the disease but carrier status may present (Robertson, 2001, Schroeder, 1994; Young, 1991).

For remedial purposes: It follows therefore that if an affected couple can have a way of getting predominantly female children then they can circumvent the agony of having affected children with the subsequent social, financial and emotional responsibilities that go with it (Hewit, 1987; Seguy, 1975).

Difficult choices therefore face the affected couples today due to the frustrations and dilemmas posed by these diseases (Benagiano et al., 1999). Some affected couples today opt for selective termination of pregnancy should they discover that they are expecting a baby boy, others choose to have no children while others opt to nullify their marriages while only a paltry few leave it to chance (Campbell, 2002). Sex ratio adjustment therefore in particular towards female gender would go a long way in alleviating the suffering of these couples (Robertson, 2001).

3.2 Types of gender determination

There are four described aspects of gender determination. The first is based on genetic constitution that was described in the ***Fishers principle of evolution based on the X and Y gametes*** in 1930 by Fisher that sexually reproducing species have their sex ratios around 1 : 1 proportions and this inclines towards equal sex proportions. This is due to natural sex selection by the accumulation of autosomal alleles that direct the parental reproduction effort toward the rare sex (Cavalho et al., 1998).

The genetic predisposition works in conformity with the hormones in gender determination and this defines the second method of gender determination. In early embryonic stages presents the undifferentiated stage with both the paramesonephric and the mesonephric ducts that develops into female and the male genitalia respectively. The SRY gene located on the Y chromosome directs the development of the testis. The latter three hormones that directs the development of the male genitalia namely testosterone, dihydrotestosterone(DHT) and anti-Müllerian hormone. The latter causes the paramesonephric duct to regress and the former two cause the development of both the inyernal and external male genitalia including testicular descent (Harrisons, 2008; Kucinskas et al., 2005).

The third type of gender determination was described in the ***Charnov – Bull model*** referred to as temperature dependant sex determination (TSD). This is also referred to as environmental sex determination that is dependent on the temperatures prevalent during certain critical embryonic development most commonly observed among the reptiles. This occurs during certain critical embryonic periods defined as thermosensitive period during which sex differentiation of the gonads is determined. This is usually during the middle third of their embryonic development (Drickamer 1990; Warner et al., 2005).

The fourth gender determination was observed to be through maternal influence over the gender of the offspring depending on her eventual investment interest on the progeny. This is largely influenced by the prevailing environmental circumstances as defined by the ***Trivers-Willard hypothesis.*** The latter was expounded 1973 when Robert Trivers a biologist and Dan Willard a mathematician in Harvard university explained an evolutionary theory that mothers in better living conditions produce relatively more sons while those in poorer living conditions tend to produce relatively more daughters (Bradbury et al., 1998; Elissa et al.,1999).

3.3 Theoretical basis of the evolution of sex ratio adjustment methods

Assisted reproductive technologies the state of the art that is wilfully done to influences man's reproduction towards or close to his wishes and involves all fertility treatments in which certain physiological aspects of the body or both the sperms and the ova are manipulated (Burke, 2002; Scott et al., 1999; Shushan et al., 1993). It presumably started as early as 400 B.C. and has been sustained by man's curiosity and inability to achieve the aspired family size with the desired gender balance. It was not until in the 1920s and 30s when the human physiology was fairly understood and in particular when Fisher described the sex ratio concept of fertilization that the natural methods came on board (Edwards, 2000, Folk wisdom, 2002, Savulescu, 1999).

Sex ratio influencing was initially, done through certain cultural practices which differed from place to place (Savulescu, 1999; Schenker, 2002). However, the understanding of the female and the male body and in particular the menstrual cycle revealed that there were fertile and infertile phases of the menstrual cycle and through manipulating these, it was possible to either achieve or delay pregnancy (France et al., 1992). Further evidence came when studies showed that through similar manipulation of the menstrual cycle gender ratio adjustments could be achieved (Seguy, 1975).

More light was shed when man discovered in the late fifties and early sixties how to influence the reproductive system by the use of the contraceptive pill (Fraser, 2000). This enabled the woman to be able to achieve or postpone conception and further enabled her to postpone or stop periods temporarily (Burkman et al., 2011).

With further improvements of the scientific knowledge, it became possible to determine the sex of the foetus and if it turned to be the undesired gender then this could undergo selective termination of the pregnancy (Pembrey, 2002). The issue of termination of pregnancies brought up a lot of ethical concerns coupled with a lot of condemnations by the religious groups (Benagiano, 1999; Scott, 1999; Shushan, 1993).

Due to the strong opposition of the wilful termination of pregnancies together with the arising ethical and moral issues, the scientist was forced to go back to the drawing board to the laboratory where he could isolate and manipulate the gametes directly at will and subsequently either deposit them into the woman's reproductive system through artificial insemination (Ericson, 1994; Guerrero, 1971).

Prenatal diagnosis and selective termination of pregnancy that came into play in the sixties raised a lot of ethical and moral concerns forcing the scientist to look for other methods. Further advancement of sperm separation methods were introduced in the seventies and used for either artificial insemination or in vitro fertilization. By the latter method, baby Louise Brown was born on the 25th July 1978 in Oldham general hospital in London Britain through work pioneered by lord Roberts Edward, London (Fugger et al., 1998, Vidal et al., 1998). To date she is 36 years old with one baby and expecting another and millions of others have benefitted from this technology after three decades of clinical innovation and technological advancement (Wang et al., 2006).

Human cloning took centre stage in the nineties with Stillman reporting the first human cloning in the united states in 1994 (Jaenisch, 2004). Further advances were made by Dr. Ian in Scotland when he helped produce the first cloned big animal sheep named Dolly in 1997 (Moore, 1997). This work was improved by the Raelian religious group that reported the first attempts towards human cloning in December 2002 (Human cloning). Further advances towards this have been made despite ethical and genetic challenges that have contributed towards conflict of liberties or failure of human cloning debate (Burley, 1999, Havstad 2010; Rhind et al., 2003). The long expected and awaited breakthrough of human cloning is the cloning of stemcells from cloned embryo which is reportedly in the offspring. This is expected to offer potential benefits and possible solutions towards this goal (Havstad, 2010).

Further advances have been made lately on more characteristics other than gender adjustments through genetic understanding, manipulation and engineering (Strong, 2005). The other ongoing methods of gender adjustment methods though rarely given prominence include adoption and eugenics of which the latter has had its considerable share of controversies (Gejman et al., 2002; Scully et al., 2006, Strong, 2008).

In as much as folk and myths sound unreal, fascinating and even strange in modern times, in earlier times, they appeared real as they were based on rudimentary scientific foundations. They have however interestingly existed in all civilizational stages as they have not been disapproved scientifically but have been passed on as part of our cultural inheritance (Folk wisdom, 2002). Though disjointed and fragmented from place to place in line with different cultures, they still reflect some degree of rudimentary knowledge, operational tools and modes of application (Savulescu, 2002). The earlier rudimentary communication skills is also reflected in the high degree of disconnect among the players which also played a major role in hampering progress of sex ratio adjustment methods.
(Folk wisdom, 2002).

All modern scientific knowledge evolved from very basic archaic stages and moments but has been improved over the years (Savulescu 2002). These old sex ratio influencing practices are based on rudimentary knowledge of the human body anatomy, physiology, biochemistry and their operational interactions. The absence of plant physiology and biochemistry knowledge in relation to the human body is clearly reflected in some of the past allegations (Savulescu 2002). Over the years as more understanding was achieved, more insights were added to the information basket thus changing the whole scenario of sex ratio adjustments among other scientific knowledge (Carvalho et al., 1998; Edwards, 2000).

The link between statistics and our past especially in regard to long term practices, outcomes and modern day science is interestingly and clearly reflected in many allegations that are based on clear results accrued on long term observational practices. Where there was no link between sex ratios and the human body anatomy, physiology, biochemistry, plant or animal diets or long term observational practices, then astrology completed the puzzle (Ho ED et al., 1991; Villamor et al., 2010).

It is of interest to note that all traditional methods that were earlier advocated were accompanied by an explanation befitting their times despite their rudimentary status. One interesting allegation was the belief that female sperms originated from the left testis and that lying on the right during coitus would facilitate those (Savulescu et al., 2002). From this earlier understanding it is therefore clear how scientific knowledge has come of age and also how many folk myths have dropped by the wayside as new discoveries come on board. Scientific evolutionary evidence has proved some folk beliefs as unfounded and has set records straight by clarifying many grey areas that have existed in the past. Probably allegations should not be ignored because on occasions, some have been proved right (Savulescu, 2002).

Despite scientific advancement, there are still some folk practices still championed in various societies. These are mainly propagated by their proponents and advocates who base their arguments on long term observations or astrology (Schenker, 2002). Some claim that their beliefs have worked over the years and therefore have passed the test of time prompting people to believe in them through generations (Folk wisdom, 2002). The desperation of man to actualize his family gender balance has pushed man to try and device many ways of influencing sex ratios and even to believe and practice some unfounded claims (Liu, 1996). Unfortunately, there are many instances when the scientists have engaged each other on long prorated arguments and left the society engage in their own practices due to lack of misguidance (Schenker., 2002). This study attempts to answer many speculations beliefs or simply allegations that have preoccupied man's thoughts since creation.

3.4 Social inputs on sex ratio subject

In earlier days, man went forth with a lot of zeal in an effort to fill the earth as commissioned, an act that led to global overpopulation (Butler, 1994). This was countered by population reduction methods but just like each cloud has its silver lining and so are these family planning methods for as the couples used them they were confounded with the inability to achieve the aspired gender distribution within their families (Miller et al., 2007). It is this delicate balance of achieving small families and gender balance that ignited the social reasons why many seek the services of sex ratio influencing methods (Liu, 1996). Many of these methods of gender selection that have been advocated are still in use today depending on the place in question (Pembrey, 2002). As this subject majorly dominates the social aspects of gender influence and is outside the scope of our study, it will not be explored further.

3.5 Religious inputs to the sex ratio debate

There are two arguments pertaining to human creation or existence namely those that believe in God's creation and those that don't. For those that believe then the area of creation unquestionably belongs to God and the moment man ventures into this territory is like playing God or breaching the earlier covenants as stated in (Genesis 1 : 27-28, Psalms 139:13-14), which states that God created man in his own image, in the image of God he created him male and female. Following this God blessed them and told them to be fruitful and increase in number and fill the earth (Genesis 1:28).

In the Muslim version, The Holy Quran describes the development of an embryo into full human person. "We created (Khalaqna) man of an extraction of clay then we set him a drop in a safe lodging, then we created of the drop of a clot, then we created of the clot a tissue, then we created of the tissue bones, then we covered the bones in flesh, therefore we produced it as another creature so blessed be God, the best of creators". (Chapter 23, verse 12-14).

God therefore, presumably did the first gender balance when after creating Adam, he created Eve as a balancing act (Genesis 1: 27). After this balancing the rest was left to mankind when he was commissioned to go forth and fill the earth (Genesis 1: 27). Man set forth with a lot of zeal to accomplish his God given right. This resulted in creation of large families where gender balance was not an issue but globally led to over population and hence depletion of resources leading to poverty, diseases and other social ills (Butler, 1994; Scott et al., 1999). Man was therefore forced to reduce the population through small family sizes. As he achieved small family sizes he was faced with the problem of achieving the desired gender family balance (Butler, 1994). He therefore devised ways of manipulating gender outcome an art he called sex selection or assisted reproductive technology (Campbell, 2002; Savulescu, 1999; Schroeder, 1994).

Majority of sex ratio adjustments methods that are practiced fall in the category of natural or indirect sex ratio influence. These include billings, smart stork and dietary methods (Billings, 1972; Odeblad, 1997; Screenay, 1993; Shettles, 1970; Whelan, 1985). The direct sex ratio selection methods exploit the oocyte kinetic or density differential and the oocyte membrane electrical potential properties (Erickson, 1994; Vidal et al., 1998).

3.6 Individual factors and sex ratios

Gender ratios in humanity do have a lot to do with individual wishes, aspirations and origins as well (Baird, 1993). Some of these are consequences of relational dynamics that involve age, colour, race, education, socio economic places of origin (Buunk et al., 2002). These are some common factors that are shared within the animal kingdom as all animals gravitate around youth, good health, plenty of resources, status, fertility and other individual qualities like appearance for mate choices which are qualities that may even be used for competition or to deter rivals (Roccas et al., 2002). Other fine qualities that still play a remote part are good manners such as warmth, kindness, respect, sympathy, benevolence, peacefulness and good social or relational skills (Miller, 2007; Paul, 2002; Roccas et al., 2002; Rommel et al., 2002). In the animal kingdom it has been shown that mothers will invest in females in hard times and males during times of plenty in the Trivers Willard hypothesis (Bradbury et al., 1998; Elissa et al., 1999).

Other factors that may contribute to progeny gender choice is individuals backgrounds, upbringing and their living environs (Paul, 2002). For those that come from a gender challenged family, with one gender only, the wish for the other gender is natural. There are usual many questions that anyone would possibly ask, but are beyond the scope of this work. Naturally due to my youth and origins, I did not appreciate the social ills associated with the social sex ratio adjustment but as I grew up and learnt more on how this can be used to harm the society through the unborn or the young innocent victims, I changed my stand and became not only convinced but comfortably converted against the vice. My current conviction stands without any intention of looking back for any possible reconsideration.

Environments can be quite game changers in our lives and lead us into different life pursuits (Baird P., 1994; Rommel et al., 2002). In my youth as well I grew up in a sickle cell challenged society, we lived together, played and went to the same schools with my young age mates who suffered from this disease. We prayed in the same churches with and for them, agonized and cried together. We visited them in the hospitals and often times sadly attended funerals for some of them. These memories still linger in my mind and therefore I have good humble reasons in engaging myself in this noble task towards the prevention of these disorders.

3.7 Animal models in reproductive research

The mouse is a mammal in the class of *Rodentia* originally from Europe (Elizabeth et al., 2009; Leighton et al., 1977). The laboratory mouse is called *Mus musculus* and is most commonly used animal model for experimentation due to its easy breeding, availability of inbred strains, short generation time, refined map of the genome and an extensive knowledge of biological and immunological properties (Elizabeth et al., 2009, Krackow et al., 1989).

Its lifespan approximately ranges between 2 - 3 years with a potential of up to 4 years. The main causes of deaths are infections, fighting among themselves, tumour development and unexplained causes (Elizabeth et al., 1966).

Sexual maturity is usually attained between 30 - 50 days and their reproductive span extends up to two years. Its reproductive potential is high because it is polyoestrus with cycles of 4 – 5 days and spontaneous ovulation (Krackow et al., 1989). It can produce up to more than 10 ova per single cycle giving rise to average litter sizes of 8 – 12 but the inbred mice generally have less numbers of litters. Its gestation ranges between 19 – 21 days and weans after 21 – 28days (Krackow et al., 1989). The average birth weight is 0.5-1.8g per pub. The average adult female weight is 18 – 35g and 20 – 30g among the males (Greehami.et al., 1977).

3.8 Future trends

Among the areas under scrutiny include, special diaphragms that are designed to fit over the cervix and be able to selectively allow either an X sperm or a Y sperm. A pill or an injection is yet to be formulated which would preferentially stop production of either X or Y sperms. In both of these circumstances the couple would therefore be able to select the gender of their choice (College, 2002).

Pathogenesis whereby the ovum begins dividing without fertilization in asexual reproduction among many invertebrate species still remains to be exploited (College, 2002).

Controversies concerning cloning are as yet sorted out (Strong, 2005). Among these include the creation of humans beings with special traits, the possibility of eliminating the male factor in reproduction, idea of transgenic animals for organ transplant, establishment of genetic banks and cloning bone tissues (Human cloning, 2002).

The recent advances in the understanding of the molecular and cellular basis of diseases coupled with availability of tools to manipulate genetic diseases offer possibilities of new modes of treatment. Gene therapies have shown potential usefulness among animal models, but still await successful transition into the mainstream clinical medicine (Carlo et al., 2006; Levin, 1995). The need for screening programmes is becoming more apparent as genetic diseases become more prevalent (Arrayed, 1999).

3.9 Justification of the study

Sex linked diseases

- Sex linked diseases are a silent growing global challenge.
- They have remained a big challenge despite the progress made in other fields of medicine.
- They will become more prevalent as the other diseases decline through effective preventative and curative interventions.
- The search for preventative measures need to be intensified as these conditions are incurable, expensive and lifelong.
- Sex ratio influence, as a preventive intervention, is vital as the hope of finding cure still remains elusive.
- Sex ratio influencing methods have been associated with many unproven claims that need verification.
- Dietary methods may offer practical possibilities due to their simplicity in application.
- Most earlier studies are linked to commercial firms and are short trials with small samples that make it difficult to draw sound conclusions.
- Concerns surrounding sex ratio adjustment methods and -X- linked diseases are numerous and therefore, objective and verifiable data obtained through research would avoid unsound practices based on anecdotal evidence.

Affected couples are usually faced with difficulty choices and consequences. Many are actively opting for prevention through sex ratio adjustment methods instead of selective termination of pregnancy. Alternatively, some choose to have no children or resort to nullifying their marriages. Only a paltry few leave it to fate or chance.

- A further look at dietary claims at the molecular level is wanting and therefore this study is based on this concept. It has been shown that there are electrical charges on the gametes and further demonstrated that a halo of electrical reaction occurs as the gametes come into contact.

- Factors or agents that modulate the oval membrane potential have been postulated and are under scrutiny in this study.

The study concept defines a real medical problem that requires a solution through scientific verification.

- It is ethical as it seeks to support sex ratio adjustment methods for :-
- Medical reasons only.
- Its focus is on promoting the female gender for purposes of disease control only.
- It is in line with the recommendations of human rights and biomedicine which states that sex selection is permitted only to prevent hereditary diseases or any other medical reasons.

If the hypotheses is found true, the dietary method of sex ratio influence appears to hold good promise for future application as it is non-invasive, inexpensive and does not require high technological approach. The outcome of this study may have some practical uses where applicable in the field of genetic diseases whose treatment challenges have yet to be surmounted.

Study hypothesis

3.10 Predictions

1) Dietary chemical compositions do influence sex ratios.
2) Perinatal mortality trends vary with different chemical formulations.
3) Dietary chemical concentrations are mirrored in the serum chemical concentrations.
4) Animal model body-weights are a good measure of their wellbeing and study progress.
5) Birth order does affect sex ratios.
6) Seasonal variations do influence sex ratios.
7) Dietary chemical elements do influence oocyte membrane electrical potential.

3.11 Study objectives

Broad objectives

The broad objective is to study anecdotal evidence posed by societal allegations of possible sex ratio influence by dietary chemical contents, birth order and seasonal variations.

Specific objectives

1a) To study the effects of high dietary sodium, potassium, calcium, magnesium individual elements on sex ratios.

b) To assess the effects of high combined dietary sodium with potassium and calcium with magnesium elements on sex ratios.

c) To study the effects of all these chemical combinations (all-inclusive of cocktail) on sex ratios.

2) To assess the perinatal mortality by the use of PCR - (DNA) for early gender determination.

3) To determine the effects of high dietary chemical components on serum biochemical levels of the respective elements.

4) To establish the effects of delivery sequence (birth order) on sex ratios.

5) To evaluate the weight trends as a measure of the wellbeing animal models and the study progress.

6) To assess effects of seasonal variations on sex ratios

7) To evaluate effects of the chemical formulations on the oocyte membrane electrical potential.

Chapter 4

4.1 Dietary study research designs

Search for research designs for the dietary chemical studies:

Aim:

1) To design a study to examine the effects of high dietary sodium, potassium, calcium and magnesium as individual elements on sex ratios.
2) To design a model of determing the effects of combined high dietary
   a) Sodium and potassium.
   b) Calcium and magnesium elements on sex ratios.
3) To design ways of examining the effect of all combined four elements on sex ratios.

4.2 Search for research design

In an effort to come up with an acceptable study that would look into aspects of sex ratio influence in relation to genetic diseases, various preparatory approaches were adopted towards the development of a study strategy that would be applicable and with measurable parameters. As the foundation of the study is based on the society's allegations many of the claims proved difficult to quantify or with various parameters that were difficult to verify as some for instance were based only on beliefs alongside other road blocks, bottlenecks, red tape, coupled with financial and ethical challenges that had to be faced and addressed.

In search of study design three basic pre pilot studies on folk, herbal and natural methods of family planning were done in Kenya medical research institute, mater hospital, St Mary's Hospital, Nazareth Hospital and the University of Nairobi family planning clinics.

The following gender influencing methods were explored and either dropped or adopted and justifications given on:-

a) Folk allegations.
b) Traditional herbal based methods.

c) Natural gender influencing methods.
d) Modern gender influence methods.

4.2a Folk and myths were extensively explored and appreciated to have existed in all civilizational stages but this direction of the study was abandoned because they were found to be disjointed and fragmented from place to place and in different cultures. It was further glaringly clear that their rudimentary knowledge, operational tools, their applications and the disconnect among them and their players would pose additional challenges that would complicate the study interpretations and conclusions.

4.2b Traditional gender influencing methods were explored between 2003 and 2004 with the help of Kenya Medical Research Institute (KEMRI) whose contributions in particular regarding the use of herbs were of much use. Contributions of the same were obtained from traditional practitioners from various provinces of Kenya who practice gender influencing by the use of indigenous herbs. This arm of the study was abandoned mainly due to various uncontrollable variables that included suspicious relationships between the traditional practitioners and the modern health providers that would lead to a major challenge in particular failure to get the rightful information.

4.2c Natural gender influencing methods were studied with the help of experts from the Catholic Church through visiting their facilities that practice these methods. Among the clinics that were used were Nazareth hospital, Mater hospital, St Mary's hospital and their natural family planning clinics. In these facilities, the billings method of family planning was principally practiced. Of particular interests were ethical questions and concerns touching on sex ratios in relation to these methods of family planning. After thorough examination of these methods, there were abandoned due to numerous uncontrollable variables, the use of human subjects and difficulties in getting a good animal model for the studies.

The three aspects of gender adjustments approaches were however dropped following failure to meet the basic quantifiable parameters of modern research and in particular their rudimentary knowledge, operational tools, applications and disconnect in their practices. Following in-depth discussions of the data that was collected on the above three arms of this study each was found wanting and not suitable for our study and was abandoned. The data on theses pre-pilot studies is therefore not presented in this manuscript.

4.2d Modern sex ratio influencing and selection methods were explored with consultations with experts from the Medical Physiology Department, University of Nairobi and interest to carry out the work in their department expressed. It was mutually agreed that diet study was feasible, with fewer ethical challenges and with many academic gaps due to previous inadequate research. The dietary study therefore met the set requisites for the study and was adopted with an overriding concept of bridging society's beliefs and norms with modern science as the guiding principal of the study.

Various diets were sampled with consultations with experts from the Kenya Medical Research Institute but dropped due to difficulties in quantification of their various ingredients. The use of the chemicals in the diets was finally adopted as measurable and ideal for the study.

4.3 Animal model selection

The Swiss mice was used due to its easy availability, fast multiplication with short generational time, closeness of its genome to that of the humans, its well-known biological and immunological systems and its small size that makes the cost of maintaining a sizeable number affordable.

4.4 Pilot study

In lieu of the fact that this was a new research approach to this study various aspects of it were borrowed from the aforementioned searches in the other areas mentioned above then planned and designed into a pilot study that was carried out aimed at looking at:-

a) Dietary influence over the subsequent progeny of the study subjects.
b) Feeding patterns and chemicals dosaging.
c) Behavioural patterns.
d) Sexing the litter.

e) Beddings.

f) Temperature

Through a double blind study, eight groups of Swiss (Webster) breed each group comprising of 16 pairs of mice (families) were fed with sodium, potassium, calcium and magnesium as single elements, and combined sodium with potassium and calcium with magnesium in their drinking water. Two equivalent control groups were set up of plain drinking water and a second similar group with glucose added to it. They were followed up through two generations and sexing of their progeny performed using applicable methods.

4.5 The sampling methods and sample size

All studies are double blinded studies where the principal researcher knew the contents and not his assistants on the ground.

Calculation of sample size for the study was based on the following formula:-

$$N = \frac{(r+1)\ (Z_{\alpha/2} + Z_{1-B})^{2}\ \eth^{2}}{rd^{2}}$$

where

- Z =normal standard distribution.
- Alfa = Type 1 error usually 0.05.
- d - Study sampling design
- The minimum number of 2 deliveries per
- Beta = type 11 error between 0.05 - 0.20.
- r - Sample size ratio
- Group will be required.

4.6 Animal maintenance

The mouse is a good animal model for reproductive research due to its closeness to the human genome and its relative fast multiplication.

Cat fish is used for the oocyte electrical charges study is used for its resilience in different conditions such as being able to survive outside water due to its ability to breathe and ability to provide eggs externally for *in vitro* fertilization.

The maintenance of the animal models was based on the standards of operating procedures in accordance with internationally accepted standards (SOPS) for animal experimentation and care. All sample collections were performed following approved Institutional Review Committee (IRC) protocols.

4.7 Feeding and chemical formulations

The first biggest challenge encountered was how to feed the animal models with the chemicals and after many trials it was found that they were either feeding poorly or refusing to feed on the chemicals. After many trials glucose was found to solve this challenge. It was further found that, on the average, a mouse feeds 5g of dry food and 5 ml of fluids per day. From these findings, it was possible to calculate the total intake of the various elements in both the dry food and in solution.

In order to get the elements into solution, the chloride form was used. Using the atomic weights of the elements, the salts that are needed to be dissolved in water were calculated using the conversion factors of 58/23 for sodium chloride, 74/39 for potassium chloride, 110/40 for calcium chloride and 94/24 for magnesium chloride. The amount of each element in the dry or factory food was calculated based on the percentage of the element in it as specified by the manufacturers. The total intake was therefore the sum of the elements in the constituted solution and the dry food.

Table 4.1 The relative atomic weights of the various elements

Element	**Mr**
Sodium	23
Magnesium	24
Calcium	40
Potassium	39
Chlorine	35

Table 4.2 The average single element contents in tap water in Nairobi

ELEMENT	**/1000 ml**	**/500 ml**	**/250 ml**	**/125 ml**
Sodium	4.4 mg	2.2 mg	1.10 mg	0.55 mg
Potassium	1.5 mg	0.75 mg	0.38 mg	0.18 mg
Calcium	4.4 mg	2.2 mg	1.10 mg	0.55 mg
Magnesium	2.4 mg	1.2 mg	0.60 mg	0.30 mg

Table 4.3 The average single element contents in the dry (factory) food

Element	**% element in the dry food**	**Amount/kg**	**Amount/5g**	**Amount/g**
Sodium	0.49	4.9 g	24.5 mg	4.9 mg
Potassium	0.292	1.46 g	14.6 mg	1.46 mg
Calcium	0.996	9.96 g	49.8 mg	9.96 mg
Magnesium	0.08	0.80 g	4.0 mg	0.80 mg

Table 4.4 The constitution of the average chemical contents in the feeding solution

Element	**Atomic Weight**	**Chemical content**	**Conversion factor**	**Salt /1000 ml**	**Salt /500 ml**	**Salt /250 ml**	**Salt /125 ml**
Sodium	23	36.25	58/23	18.28 g	9.14 g	4.57 g	2.23 g
Potassium	39	42.3	74/39	16 g	8.03 g	4.01 g	2.01 g
Calcium	40	41.8	110/40	22.99 g	11.5 g	5.75 g	2.87 g
Magnesium	24	14	94/24	10.96 g	5.48 g	2.74 g	1.37 g
Chlorine	35						

Table 4.5 The daily element requirements of the total food (dry & solution) taken mg/day normal requirement (X) and one and half dose (1.5X)

	% Dry food intake	Amount of dry food	Amount solution	Mg/day dose x normal intake	1.5 x taken per day
Sodium	0.81	24.5 mg	36.25 mg	40.5 mg	60.75 mg
Potassium	0.75	14.6 mg	42.30 mg	38 mg	57.0 mg
Calcium	1.22	49.8 mg	41.80 mg	38 mg	91.6 mg
Magnesium	0.24	4.0 mg	14.00 mg	12 mg	18.0 mg

The initial difficulties that were experienced by the animal models in the formative years almost brought the study to a complete stop as some animal models were even dying following refusal to feed on the chemicals especially calcium element. Despite various dosage adjustments this *status quo* still persisted till glucose was added to the all feeding solutions except the first control group with water only. Since the inclusion of glucose in the feeding solutions, we have never looked back. Following various dosage adjustments, the starting optimal dosage of the relevant chemical was found to be one and half times the daily intake.

4.8 Location and infrastructure

The dietary work was done in the University of Nairobi Medical Physiology Department in consultations with Veterinary Department, Kenyatta National Hospital and ILRI. These institutes have relevant experts on animal research and animal houses with the necessary infrastructure for animal experimentation. The mice were outsourced from Kenyatta National Hospital animal house where valuable advice on their upkeep was provided by the chief overseer Mr Maloba. The materials for DNA work were outsourced from Belgium through ILRI who also provided valuable information on mice experimentation and DNA work by Dr Fuard of ILRI.

Animal house

The following basic infrastructure were provided in the animal house:

Experimentation room.

Quarantine room.

Food storage room.

Wash room.

General store.

The experimentation room had the following facilities:

Running water.

Air suction system.

Electric air heaters.

Operating tables.

Information on animal experimentation and ethics.

Photo 4.1 Air suction and water systems in the experimental room/animal house.

Photo 4. 2 Holding rack cage

4.9 Holding rack cages

There were four big holding racks each with six shelves as seen in photo 4.3. Each shelf could accommodate a total of 9 small experimental mice cages thus giving a total of 54 small mice cages per holding rack. Of these four big holding rack cages, three were used to accommodate the animal models and the fourth was used for storing equipment and excess animal models.

Photo 4.3 Experimental mice cage

4.10 Experimental Mice cages

Two types of experimental mice cages were used for this work. The big and the small mice cages that were either plastic or metallic. Both had similar features and only differed in sizes. A complete cage has the main body or framework, a grill, drinking bottle, a base where sawdust is place, food chamber and drinking water chamber. The metallic type had its base modified to accommodate a slot by the lateral aspect of its base; a flat sheet of metal could be slotted in so as to collect the waste. This modification was done to make the waste disposal easy by removing the mice then pulling off the modified base by the side with the waste or used beddings.

The big holding mice cage as seen in the photo was used for holding the mice before they were assigned to their respective experimentation. It was also used to keep the excess mice that were distributed to various departments for various experimentations. The small experimental mice cage was extensively used for this work and its components are demonstrated below.

Photo 4.4 The grill with the food compartment, drinking water bottle slot and the cage cover.

Photo 4.5 Small mice experimental cage complete with a grill. Small mice experimental cage complete with a grill, drinking bottle, sawdust or beddings, drinking bottle, food chamber with adult mice and its litter.

A big holding mice cage used for keeping excess mice before they were assigned for respective experimentation or deployed to various departments for various research work.

4.11 Pilot study, graphic presentation structure and chemical dosage

The pilot study using eight groups (N_1 –N_8) each with 16 families (NF_{1-16}, N_2F_{1-16}, N_3F_{1-16}, N_4F_{1-16}, N_5F_{1-16}, N_6 F-$_{1-16}$, N_7F_{1-16} and N_8F_{1-16}) was undertaken to identify both experimental and non-experimental variables. The pilot study consisted of eight groups while group nine was added later in the main study.

1) The eight groups of mice were exposed to the following chemical formulations:-

A) Control groups:-

n_1 Water only.

n_2 Glucose solution only .

B) Single chemicals with x 1.5 dosage formulation.

n_3 Sodium/Glucose.

n_4 Potassium/Glucose.

n_5 Calcium/Glucose.

n_6 Magnesium/Glucose.

C) Double chemicals with x 1.5 dosage formulations

n_7 Sodium/Potassium/Glucose..

n_8 Calcium/Magnesium/Glucose.

4.12 Graphic presentation of the group, family, birth order and the numbering pattern

N_X F_X G_X

In each group ($N_{1.}$ – N_9), there were16 family pairs (F_1 – F_{16}) that were allowed to have as many deliveries as possible. A total of 144 experimental animal model cages were placed in four lockable mobile racks with shelves. The numbering system that was used for identification purposes were $N_{x,}$ represented the group number, F_X, the family within the group and G_x represented the birth order or generation within the family ($N_XF_XG_X$). For example $N_1F_{12}G_2$ or {1 .12 .10} stood for group one (N_1), family twelve F_{12} and the 2^{nd} delivery (G_2).

Table 4.6 Graphic presentation of the group, family, birth order and the numbering pattern

F1 F2 F3 F4 F5 F6 F7 F8 F-9 F10 F11 F12 F13 ……F16
N_1 G_1-x G_1- x G_1- x G_1- x G_1- x G_{11}-5 G_1- x G_1- x. G_1- x G_1- x G_1- x G_1- x
N_2 „ „ „ „ „ „ „ „
N_3 „ „ „ „ „ „ „ „
N_4 „ „ „ „ „ „ „ „
N_5 „ „ „ „ „ „ „ „
N_6 „ „ „ „ „ „ „ „
N_7 „ „ „ „ „ „ „ „
N_8 „ „ „ „ „ „ „ „
N_9 G_{1-x} G_{1-x} G_{1-x} G_{1-x}. G_{1-x}. G_{1-x} G_5. G_{1-x} G_{1-x} G_{1-x} G_{1-x} G_{1-x} G_{1-x} G_{1-x} G_{1-x}

Amounts used among the single element study groups three, four.

Sodium chloride - 1.6g/L

Potassium chloride - 3.8 g/L

Chemical amounts used in study groups five and six.

Calcium chloride - 3.1 g/L

Magnesium chloride - 0.6 g/L

Dosages used among the double elements groups seven and eight mice study groups.

Sodium chloride - 0.8 g/L

Potassium chloride - 1.9 g/L

Calcium chloride - 3.1 g/L

Magnesium chloride - 0.6 g/L

Amounts used among the all-inclusive elements cocktail group nine study.

Sodium chloride - 0.40g/L

Potassium chloride - 0.95 g/L

Calcium chloride - 1.55 g/L

Magnesium chloride - 0.30 g/L

Sex ratio determination Procedures

4.13 Physical sex ratio determination

Sex ratio determination was done by using either physical makers or the PCR. To avoid losing data counting of the pups and cutting (DNA) tail material was done within 24 hours of delivery.

Sex determination by physical means was done using the physical makers based on the difference between the male and female anal-genital distance and was initially done on day two assisted by a magnifying glass to help highlight in differentiating male and female features. Thereafter these measurements were repeated at about three weeks at the weaning time. This distance in a female is about 1mm and in the male is twice this distance. The optimal time for physical gender determination is three weeks of age. Only those pups whose gender could not be determined physically were subjected to PCR. For further reaffirmation of the physical determination method, a random sampling of 50 samples was done and these same were subjected to DNA which confirmed the same results that had been done by physical means.

4.14 Sex ratio determination using PCR/DNA methods

Determination of the gender of the pups formed the back-borne of the dietary study and was done in the same infrastructures with assistance from Kenyatta National Hospital, ILRI and Invitrogen Company for the DNA extraction, PCR and running of gels for sex determination. The primers were imported from Belgium.

4.15 Preparation of the lysates

As it is not possible to know early which pups require PCR and those that don't, a 2 - 3mm piece of the tail of each pup was cut aseptically and put in a small polythene sachet containing an 8 molar urea solution of local anaesthetic and Flagyl antibiotic before and immediately after cutting.

Photo 4.6 Lysate packaging. **Photo 4.7** Single lysate

4.16 Preparation of 8 molar urea

8 g of urea is dissolved in 1000 ml of distilled water in a hot water bath and stirred till the urea crystals are fully dissolved. The cut tail pieces were preserved in 2 ml of the latter solution a small polythene sachets and kept in room temperature.

4.17 Lysate packaging

The lysates were kept in small polythene bags that accommodated each a delivery for example the above represents eight group third family and third delivery with eight pups per that delivery. Each sachet represents one lysate and therefore was eight sachets in the bigger sachet.

4.18 Lysate storage and handling

The lysates are stored in room temperature in small polythene sachets each representing one pup as shown above. All were clearly marked according to table 4.6 showing the graphic presentation of the group, family, birth order and the numbering pattern. Routine handling with routine care is required.

4.19 Procurement

Ordering method of the reagents was done through Segolip Primer Procurement Services agents who offer these services for the international research institute among others. An example of an invoice is attached where upon payment the relevant primers were ordered as follows:-.

Invoice No: Your Order No: **000092**

International Livestock **institute - ILRI.**

Date: 17-April-2012

PO. Box. 30709, 00100. Nairobi. Kenya.

Tpl254 20422 3000 1;1': 254 204223001

I-mail: ilri-kenya@cgiar.org www.ilri.org

ATT: DR. KAVOO LINGE

University of Nairobi / Stellenbosch

P.O. BOX 73554 - 00200

NAIROBI

Details

Being Payment Of:

Primer Procurement Services offered by Segolip in the month of April 2012

The primer sequence was ordered as follows:-

The primer ILR

Primer sequence (5' to 3)

TCATGAGACTGCCAACCACAG 21

CATGACCACCACCACCACCAA 21

TTACGTCCATCGTGGACAGC 20

TGGGCTGGGTGTTAGTCTTA 20

In normal circumstances, a full DNA strand has the following nucleotides A C G T adenine cystine, glutamine and thymine. These combine in the following pairing manner. Adenine to thymine and cystine to glutamine:- A - T C - G.

4.20 Extraction of DNA from 8M urea lysates using phenol:chroloforom method

When the tail cut pieces are put in the urea the whole solution is described as a lysate and the preparation of the DNA from it is done using phenol:chroloforom method which is carried out as follows:-

1. 600 µl of tail tissues in 8 M urea lysate is transferred into 600 µl phenol/chloroform/iso-amyl-alcohol in an Eppendorf 1.5 ml tube.
2. Mixed GENTLY for 1 – 2 minutes.
3. Microfuged for 5 minutes.
4. The upper layer collected into a new Eppendorf with 600 µl ph/chl/iso and alc.
5. This extraction is repeated 3 times.
6. 4th time, the upper layer is removed and extract once with chloroform only.
7. 1/10 volume 3 M Na acetate and 2.5 volumes absolute ethanol is added.
8. Mixing is done GENTLY by inverting 10 – 20 times then allowing it to stand for 5 mins.
9. The precipitated DNA is transferred to a new Eppendorf and 500 µl 70% ethanol added.

10. Again GENTLE mixing is done by inverting 10 – 20 times and allowed to stand for 5 mins.
11. 70% ethanol is removed as much as possible and the DNA dried under vacuum for 5 minutes.
12. 300µl sterile TE tris EDTA (Buffer) containing 20ug/ml RNAse is added.
13. GENTLE mixing is done overnight on rowing machine.
14. Measurements for concentration and purity are done on OD_{260}/OD_{280} of a 10 µl in 1 ml aliquot.

4.21 Extraction of DNA from mouse tail tissue using the Proteinase-K digestion method

1. About 2 cm mouse tail is sliced into approximately 2 mm pieces into a Falcon tube.

2. 2 ml of the Digestion buffer is added into the tube and vigorously vortexed for 2 min. to clean the tissue off any foreign matter.

3. This liquid is then pipetted out and discarded

4. 1 ml of Digestion buffer is again added to the preparation.

5. To the preparation is then added 50 µl of Proteinase-K 20 mg/ml.

6. This is mixed gently by swirling several times.

7. It is then incubated overnight at 60^{O}C in a shaker water bath.

8. Transfer to 5 ml Cryotube and allow cooling to RT.

9. Add an equal volume of Phenol**:**Chloroform**:**Isoamyl alcohol (25**:**24**:**1)

10. Mix gently on wing rollers for 30 mins.

11. Spin 2800rpm 10mins 4^{o}C (SORVALL Centrifuge).

12. Carefully pipette the upper clear aqueous phase into a new 5 ml cryotube.

13. Repeat 10-13.

14. Add approximately equal volume of chloroform and repeat 10-13.

15. Precipitate DNA: add 1/10 volume 3 M Na Acetate (pH5.2) and 2.5 vols. abs. ethanol.

16. Wash precipitated DNA in 3 ml 70% ethanol.

17. Remove 70% ethanol and dry DNA under vacuum for ≈5min.

18. Dissolve in TE/RNAase and leave on rollers at RT O/N. *TE/RNAase: To 10 ml TE add 10 µl (20 mg/ml) boiled RNAase.

4.22 Buffers

Proteinase - K reconstitution buffer

This is used for protein digestion and the stock is sold packaged as 100 mg lyophilized enzyme vial.

The enzyme is used at a concentration of 20 mg/ml that is prepared by dissolving 100 mg in 5 ml of the reconstitution buffer that is made as follows**:**

1M Tris.HCl pH 7.5	50 µl	to final conc. 10 mM
1M $CaCl_2$	100 µl	to final conc. 20 mM
Glycerol 100%	2.5 ml	to final conc. 50%
Add ddH_2O	5.0 ml	

This is dissolved in lyophilized enzyme in this buffer and stored at –20ºC up to for over 2 months without any noticeable deterioration.

Digestion buffer

This is reconstituted enzyme used for protein digestion in a specified buffer that is prepared as follows**:-**

1M NaCl	50 ml
1M Tris.HCl pH 8.0	5 ml
0.5 M EDTA pH 8.0	25 ml
10% SDS	50 ml
Add ddH_2O to	500 ml.

To 10 ml sterile TE (Tris EDTA) is added to 10µl RNAse powder at 20 mg/ml.

4.23 Purity of DNA & interpretation of the gel images - PCR condition

i) **The purity of the DNA extract** is assessed as shown below at optical density of 260nm/280nm. This ratio should be between 1.8 and 2.0. In case of contamination the value is normally less.

For running the gels on the agarose gel after PCR you use 1% in the 1X TAE Buffer.

The preferred density is between and if below refers to little DNA material and if above refers to excess DNA material. In both circumstances, the specimens should be considered for exclusion from the study.

Table 4.7 Distribution of optical densities

Module:	Nucleic	Path	10 mm
Software:	3.7.1 Firmware.		

Sample ID	User ID	Date	Time	ng/ul	A260	A280	260/280	260/230	Constant	Cursor Pos.	Cursor abs.	340 raw	Measurement Type	Serial #	Config.
G8_1	Default	27/04/2012	09:17	965.18	19.304	9.83	1.96	2.17	50	230	8.884	0.047	Measure	USB2G37441BH682	0.964108/0.02/96/16
G8_2	Default	27/04/2012	09:18	647.36	12.947	6.544	1.98	2.15	50	230	6.035	0.077	Measure	USB2G37441BH682	0.964108/0.02/96/16
G8_3	Default	27/04/2012	09:19	61.36	1.227	0.712	1.72	2.22	50	230	0.553	0.01	Measure	USB2G37441BH682	0.964108/0.02/96/16
G8_4	Default	27/04/2012	09:20	349.46	6.989	3.761	1.86	2.4	50	230	2.91	0.056	Measure	USB2G37441BH682	0.964108/0.02/96/16
G8_5	Default	27/04/2012	09:20	385.44	7.709	4.407	1.75	2.22	50	230	3.475	0.091	Measure	USB2G37441BH682	0.964108/0.02/96/16
G8_6	Default	27/04/2012	09:21	38.31	0.766	0.452	1.7	2.13	50	230	0.36	0.028	Measure	USB2G37441BH682	0.964108/0.02/96/16
G8_7	Default	27/04/2012	09:22	283.11	5.662	3.065	1.85	2.24	50	230	2.528	0.092	Measure	USB2G37441BH682	0.964108/0.02/96/16
G8_8	Default	27/04/2012	09:22	25.12	0.502	0.328	1.53	2.34	50	230	0.215	0.024	Measure	USB2G37441BH682	0.964108/0.02/96/16
G8_9	Default	27/04/2012	09:23	1639.91	32.798	16.833	1.95	2.23	50	230	14.723	0.189	Measure	USB2G37441BH682	0.964108/0.02/96/16
G8_10	Default	27/04/2012	09:24	1101.02	22.02	11.437	1.93	2.04	50	230	10.793	0.235	Measure	USB2G37441BH682	0.964108/0.02/96/16
G8_11	Default	27/04/2012	09:24	1018.78	20.375	10.56	1.93	2	50	230	10.187	0.183	Measure	USB2G37441BH682	0.964108/0.02/96/16
G8_12	Default	27/04/2012	09:25	6.2	0.124	0.095	1.3	5.52	50	230	0.022	-0.002	Measure	USB2G37441BH682	0.964108/0.02/96/16
G8_13	Default	27/04/2012	09:25	18.65	0.373	0.242	1.54	1.99	50	230	0.187	0.022	Measure	USB2G37441BH682	0.964108/0.02/96/16
G8_14	Default	27/04/2012	09:26	303.43	6.069	3.283	1.85	2.37	50	230	2.563	0.296	Measure	USB2G37441BH682	0.964108/0.02/96/16
G8_15	Default	27/04/2012	09:28	423.13	8.463	4.944	1.71	2.11	50	230	4.006	0.329	Measure	USB2G37441BH682	0.964108/0.02/96/16
G8_16	Default	27/04/2012	09:30	24.98	0.5	0.306	1.64	1.97	50	230	0.254	0.222	Measure	USB2G37441BH682	0.964108/0.02/96/16
G8_17	Default	27/04/2012	09:30	45.7	0.914	0.549	1.67	1.97	50	230	0.464	0.016	Measure	USB2G37441BH682	0.964108/0.02/96/16
G8_18	Default	27/04/2012	09:31	33.5	0.67	0.394	1.7	2.33	50	230	0.287	0.009	Measure	USB2G37441BH682	0.964108/0.02/96/16
G8_19	Default	27/04/2012	09:31	184.65	3.693	2.088	1.77	2.11	50	230	1.754	0.07	Measure	USB2G37441BH682	0.964108/0.02/96/16
G8_20	Default	27/04/2012	09:32	26.1	0.522	0.339	1.54	1.61	50	230	0.324	0.018	Measure	USB2G37441BH682	0.964108/0.02/96/16

.../Table 4.7

G8_21	Default	27/04/2012	09:33	61.64	1.233	0.809	1.52	2.33	50	230	0.529	-0.006	Measure	USB2G37441BH682	0.964108/0.02/96/16
G8_22	Default	27/04/2012	09:33	21.12	0.422	0.313	1.35	1.86	50	230	0.227	0.004	Measure	USB2G37441BH682	0.964108/0.02/96/16
G8_23	Default	27/04/2012	09:34	31.4	0.628	0.436	1.44	2	50	230	0.314	-0.022	Measure	USB2G37441BH682	0.964108/0.02/96/16
G8_24			09:34	166.61	3.332	2.096	1.59	2.04	50	230	1.631	0.009	Measure	USB2G37441BH682	0.964108/0.02/96/16

PCR Conditions DNA T template.

Table 4.8

5x Green Buffer	2 µl	100	2
MgCl 25mm	1 µl	50	1
DNTPS	0.2 µl	10	0.2
Primer Fwd	0.25 µl	12.5	0.25
Reverse primer	0.25 µl	12.5	0.25
Taq	0.05 µl	2.5	0.05
H_2O	5.25 µl	262.5	5.25

Table 4.9

Master mix	x 1	x160
Reddy mix	5 µl	800 µl
Primer Fwd	0.1 µl	16 µl
Primer Rev	0.1 µl	16 µl
Water	3.8 µl	608 µl

PCR Conditions

Proteinase - K Reconstruction

The stock is sold packaged as 100 mg lyophilized enzyme vial. This enzyme is supposed to be set at a concentration of 20 mg/ml. To attain this concentration, 100 mg is dissolved in 5 ml of reconstitution buffer solution prepared as follows:-

1 M Tris. HCL pH 7.5 50 µl to final concentration 10 mM

1 M $Cacl_2$ 100 µl to final concentration 20 mM

To the glycerol (100% diluted to a concentration of 50%) add water (5.0 ml) to dissolve the lyophilized enzymes in this buffer and store at - 20^0 C this can be stored for two months without significant changes.

1. 95^0C (Denaturing): This is the first step during the PCR process is where the double stranded DNA template is heated to make it single stranded so that it can bind to the complementary primer (oligonucleotide).
2. 50^0C to 60^0C (Annealing): This is a step where single stranded DNA template is cooled down so that it binds to the oligonucleotide. This is done with the help of the enzyme Taq polymerase.
3. 72^0C: This is the step which allows the pieces of DNA template which have not annealed to the complementary oligonucleotide to do so.

DNA extraction principles can be quoted from Maniatis Vol 3.

1. The phenol is used for deproteinization
2. Chloroform is used to remove any traces of phenol remaining in the solution.
3. Ethanol absolute is used for DNA precipitation.

1. 94^0C for 3min
2. 94^0C for 30 sec
3. 55^0C for 30sec
4. 72^0C for 30sec
5. 72^0C for 7min
6. 15^0C for infinity

Steps 2 - 4 are repeated 30cycles

ii) Interpretation of the gel images

Primer IL3 amplifies the X chromosome in the DNA for both male and female. Primer SY amplifies only for the male in the region of Y chromosome. Therefore in presence of both primers, if the DNA material is from the male it will amplify 2 bands as it will amplify the X in the IL3 marker and the Y in the SY marker but if it is from the female DNA material, it will amplify only one band in the IL3 marker and none in the SY marker. From the diagram, the markers are represented on the top lines as IL and SY and the adjacent two lines

Image 1 PCR gel image

4.24 Pilot study results

Presented below is data on the pilot study on groups one to eight (N_1 – N_8) including the various technical observations and challenges that were cited with some addressed and others solutions suggested. By the end of the pilot study, solutions to the technical challenges had been found before progressing to the main study.

Data analysis of Group 1

Table 4.10 The distribution of the number of pups per delivery

Family	F	M
1.00	2.00	3.00
2.00	2.00	0.00
3.00	5.00	4.00
4.00	0.00	0.00
5.00	5.00	4.00
6.00	4.00	4.00
7.00	0.00	0.00
8.00	1.00	1.00
9.00	2.00	1.00
10.00	0.00	0.00
11.00	5.00	6.00
12.00	3.00	3.00
13.00	1.00	1.00
14.00	3.00	1.00
15.00	4.00	4.00
16.00	2.00	2.00
Totals	39.00	34.00

In group one, 74 pups were delivered comprising of 39 females and 34males representing 53% and 47% respectively of all the total pups delivered.

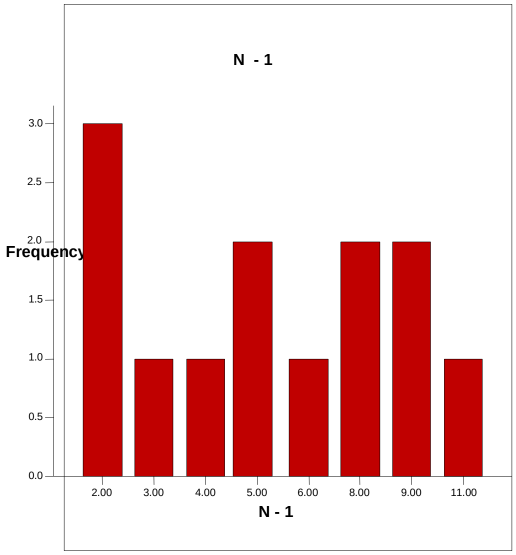


Figure 4.1 - The distribution of the total number of pups per delivery.

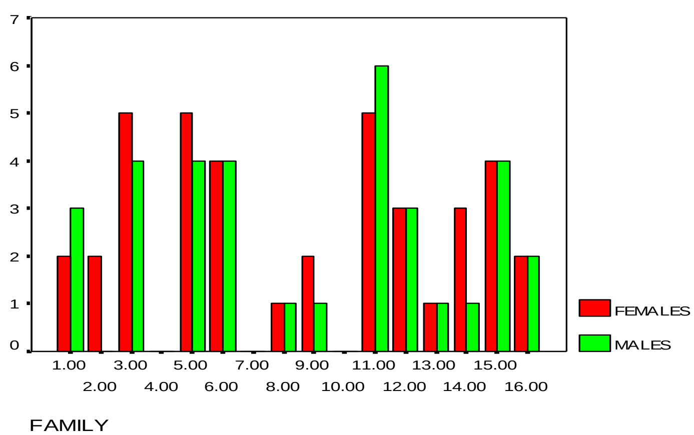


Figure 4.2 The distribution of the female and male pups per delivery.

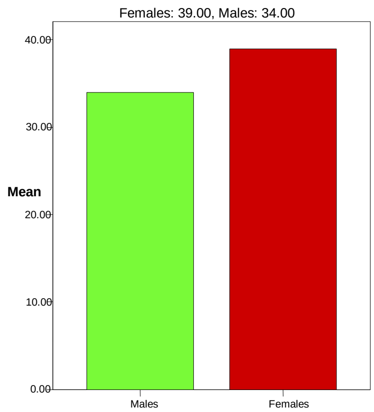


Figure 4.3 The sex ratio representation with 53% females and 47% males

Table 4.11 Summary statistics

Summary	Females	Males
Number	16.00	16.00
Sum	39.00	34.00
Mean	2.44	2.13
Standard deviation	1.79	1.89
Variance	3.20	3,58
%	53.00	47.00
Student's t-test p-value 0.63		

Conclusion

The sex ratio was within the normal range close to 1:1 with no inclination towards either gender. There was no statistical significant difference between the female and the male progeny in this group with two-tailed p-value of 0.63

Group 2

Table 4.12 The distribution of the number of pups per delivery

Family	Females	Males
1.00	5.00	3.00
2.00	0.00	0.00
3.00	0.00	0.00
4.00	0.00	0.00
5.00	0.00	0.00
6.00	1.00	1.00
7.00	1.00	1.00
8.00	6.00	4.00
9.00	4.00	3.00
10.00	2.00	3.00
11.00	4.00	4.00
12.00	2.00	3.00
13.00	3.00	3.00
14.00	.00	0.00
15.00	4.00	4.00
16.00	2.00	2.00
Total	34.00	31.00

In group two, there were 65 pups delivered constituting 34 females and 31 males delivered representing 52% and 48% respectively of the total pups.

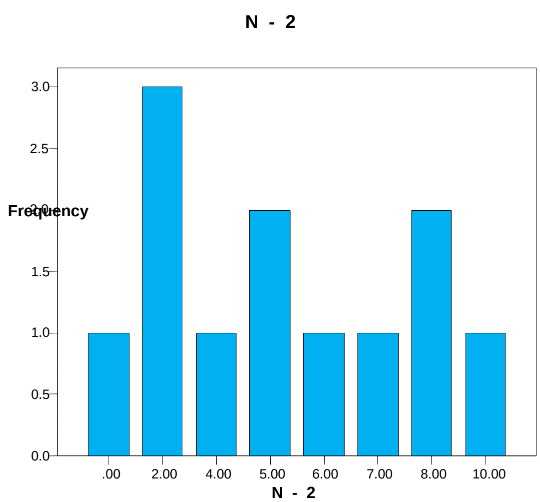


Figure 4.4 The distribution of the total number of pups per delivery

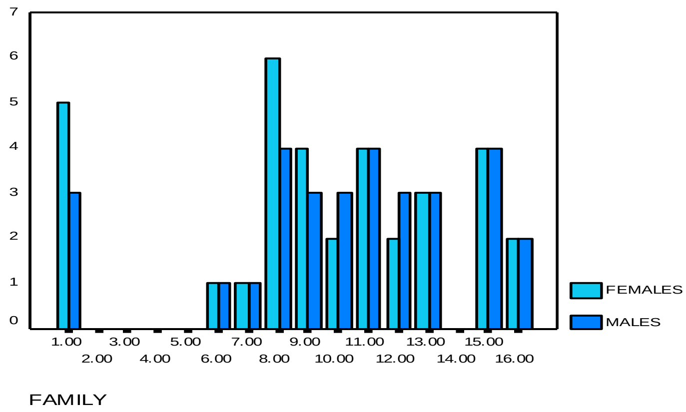


Figure 4.5 The distribution of the total number of female and male pups per delivery.

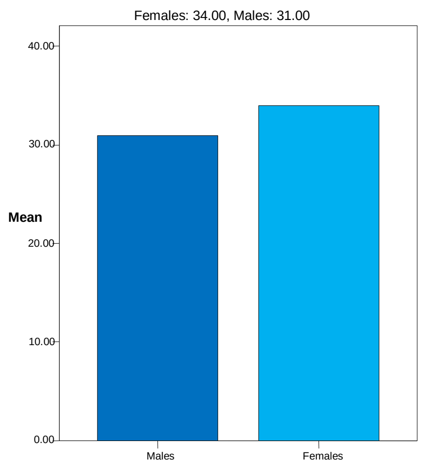


Figure 4.6 The sex ratio representation with 52% females and 48% males

Table 4.13 The summary statistics

Summary	Females	Males
Number	16.00	16.00
Sum	34.00	31.00
Mean	2.13	1.94

Standard deviation	2.00	1.61
Variance	3.98	2.61
%	52.00	48.00
Student's t-test p-value 0.77		

Conclusion

There was no sex ratio inclination towards either gender with a ratio close to 1: 1 and no statistical significant difference was found between the female and the male progeny in this group with a two-tailed p-value of 0.77.

Group 3

Table 4.14 The distribution of the number of pups per delivery

Family	Genneration.	F	m
1.00	2.00	0.00	0.00
2.00	2.00	3.00	5.00
3.00	2.00	2.00	6.00
4.00	2.00	2.00	4.00
5.00	2.00	1.00	2.00
6.00	2.00	.00	.00
7.00	2.00	3.00	5.00
8.00	2.00	.00	4.00
9.00	2.00	.00	3.00
10.00	2.00	1.00	8.00
11.00	2.00	2.00	8.00
12.00	2.00	.00	6.00
13.00	2.00	2.00	3.00
14.00	2.00	2.00	5.00
15.00	2.00	1.00	3.00
16.00	2.00	2.00	6.00
Total		21.00	68.00

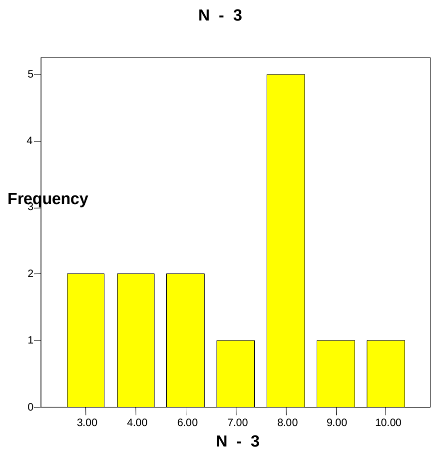


Figure 4.7 The distribution of the number of female and male pups per delivery

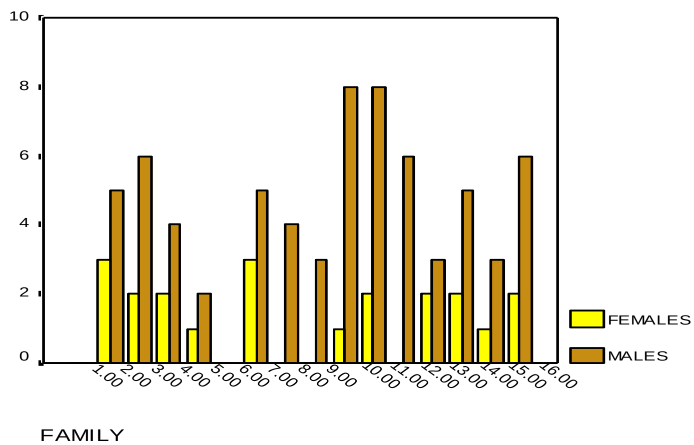


Figure 4.8 Graphic representation of the Sex ratios representing 22% females and 78% males

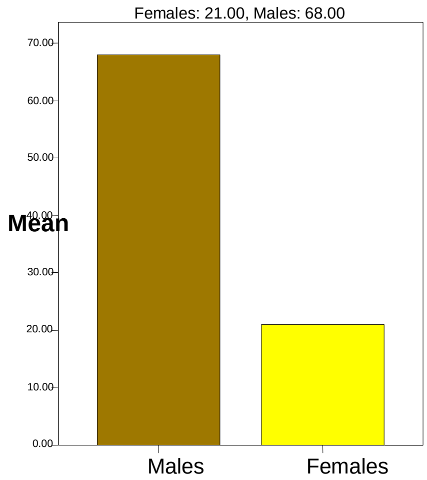


Figure 4.9 Graphic representation of the Sex ratios representing 22% females and 78% males

Table 4.15 The summary statistics

Summary	Females	Males
Number	16.00	16.00
Sum	21.00	68.00
Mean	1.31	4.25
Standard deviation	1.08	2.38
Variance	3.98	2.61
%	22.00	78.00
Student's t-test p-value 0.0001		

Conclusion

The paired Student's t-test p-value of 0.0001 indicates a skewed differential of sex ratio towards the male gender suggesting significant effect of sodium on the sex ratio outcome.

Data analysis for Group 4

Table 4.16 The distribution of the number of pups per delivery

Family	F	M
1.00	0.00	1.00
2.00	0.00	6.00
3.00	4.00	6.00
4.00	0.00	2.00
5.00	1.00	4.00
6.00	0.00	4.00
7.00	1.00	3.00
8.00	3.00	6.00
9.00	1.00	4.00
10.00	1.00	5.00
11.00	0.00	2.00
12.00	3.00	4.00
14.00	2.00	4.00
15.00	0.00	2.00
16.00	3.00	8.00
Total	21.00	61.00

Distribution of pups per delivery G – 4.

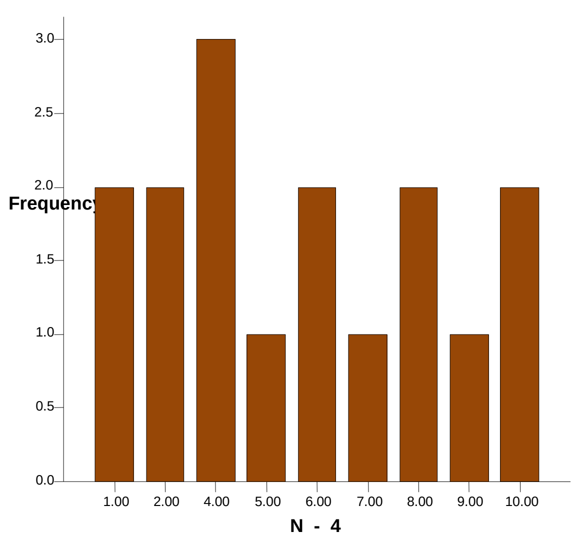


Figure 4.10 The distribution of the number of female and male pups per delivery

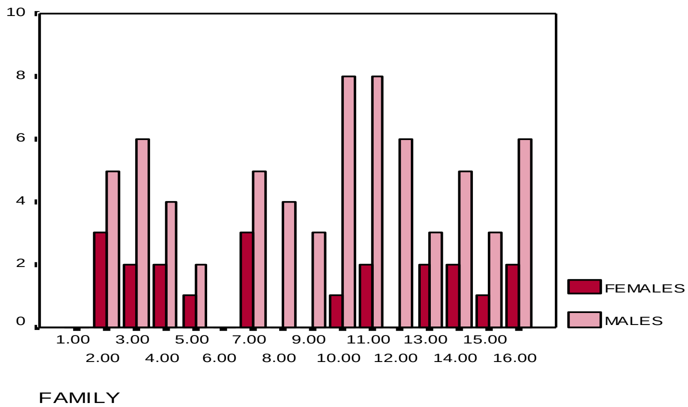


Figure 4.11 The distribution of the total number of female and male pups per delivery.

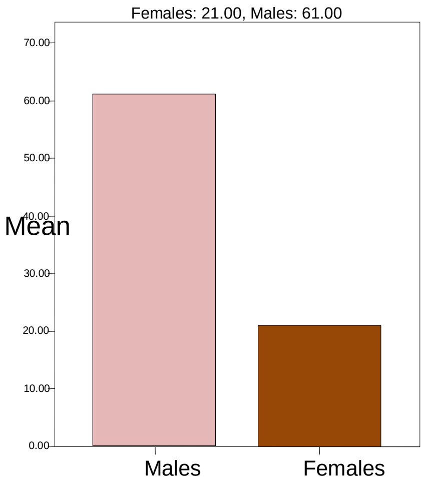


Figure 4.12 Graphic representation of the Sex ratios representing 22% females and 78% males.

Table 4.17 The summary statistics

Summary	Females	Males
Number	16.00	16.00
Sum	21.00	61.00
Mean	1.40	4.07
Standard deviation	1.35	1.91
Variance	1.83	3.64
%	26.00	74.00
Student's t-test p-value 0.019		

Conclusion

The paired Student's t-test of 0.019 indicates a significant influence on sex ratio outcome of potassium towards the male gender.

Data analysis for Group 5

Table 4.18 The distribution of the number of pups per delivery

Family	Generation	Females	Males
1.00	2.00	1.00	0.00
2.00	2.00	6.00	0.00
3.00	2.00	3.00	2.00
4.00	2.00	2.00	3.00
5.00	2.00	3.00	5.00
6.00	2.00	2.00	1.00
7.00	2.00	1.00	2.00
8.00	2.00	3.00	0.00
9.00	2.00	5.00	3.00
10.00	2.00	4.00	3.00
11.00	2.00	5.00	3.00
12.00	2.00	4.00	2.00
13.00	2.00	3.00	3.00
14.00	2.00	4.00	3.00
15.00	2.00	4.00	1.00
16.00	2.00	4.00	2.00

In group five, there were 54 females and 33 males delivered representing 62% and 38% respectively of the total pups delivered.

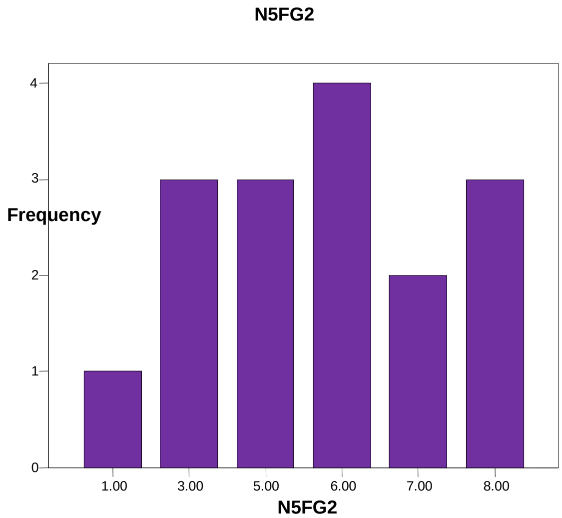


Figure 4.13 The distribution of the total pups per delivery.

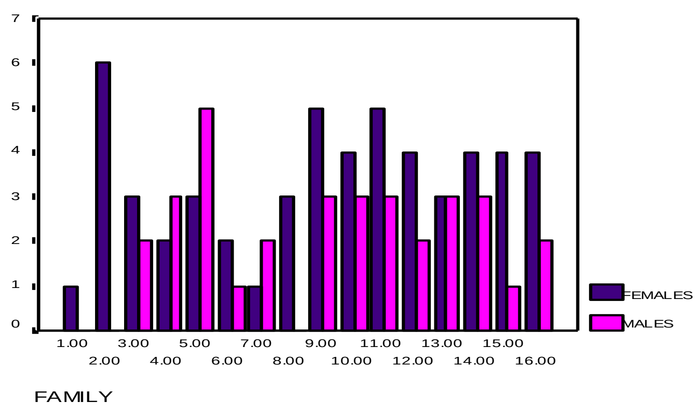


Figure 4.14 The distribution of the total number of female and male pups per family

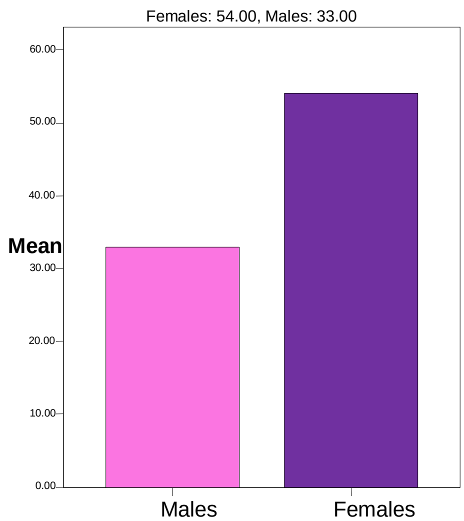


Figure 4.15 Graphic representation of the Sex ratios representing 22% females and 78% males.

Table 4.19 The summary statistics

Summary	Females	Males
Number	16.00	16.00
Sum	54.00	33.00
Mean	3.37	2.06
Standard deviation	1.41	1.39
Variance	1.98	1.93
%	62.00	28.00
Student's t-test p-value 0.013		

Conclusion

There was significant statistical difference between the females and the males in this group with a two-tailed p-value of 0.013 indicating possible dietary calcium influence over the progeny towards the female gender.

Data analysis for Group 6

Table 4.20 The distribution of the number of pups per delivery

Female	F	M	
1.00	2.00	2.00	0.00
2.00	2.00	1.00	3.00
3.00	2.00	4.00	1.00
4.00	2.00	3.00	4.00
5.00	2.00	3.00	2.00
6.00	2.00	5.00	2.00
7.00	2.00	0.00	0.00
8.00	2.00	2.00	1.00
9.00	2.00	0.00	0.00
10.00	2.00	0.00	0.00
11.00	2.00	4.00	1.00
12.00	2.00	5.00	1.00
13.00	2.00	5.00	2.00
14.00	2.00	4.00	3.00
15.00	2.00	3.00	2.00
16.00	2.00	8.00	2.00

In group six, there were 49 female and 24 male pups delivered representing 67% and 23% respectively of the total pups delivered.

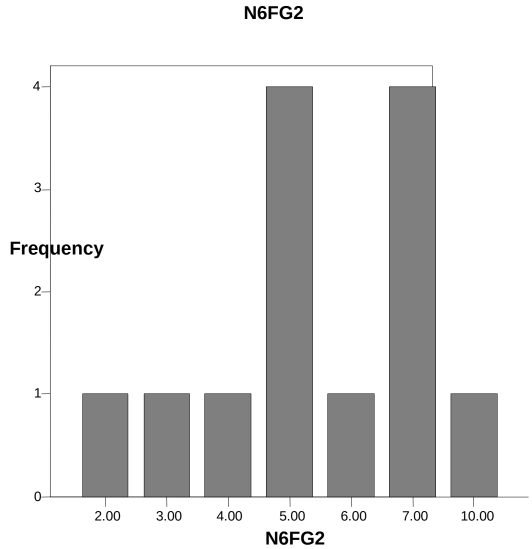


Figure 4.16 The distribution of the total pups per delivery.

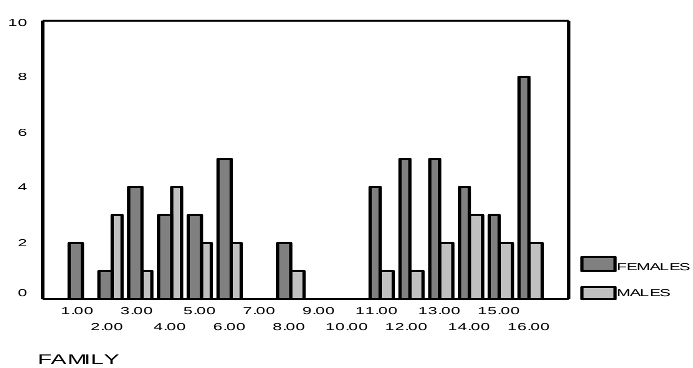


Figure 4.17 The distribution of the female and male pups delivered per family.

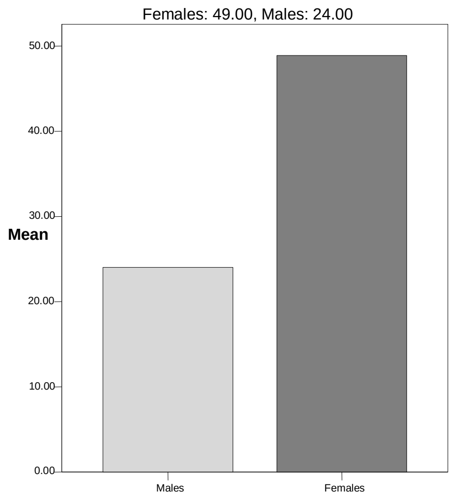


Figure 4.18 The sex ratios representing 67% females 23% Males

Table 4.21 Summary statistics

Summary	**Females**	**Males**
Number	16.00	16.00
Sum	49.00	24.00
Mean	3.06	1.50
Standard deviation	2.21	1.21
Variance	4.86	1.47
%	67.00	33.00
Student's t-test p-value 0.037		

Conclusion

There was statistical significant difference between the female and the male progeny in this group with a two-tailed p-value of 0.037 suggesting magnesium dietary influence towards the female gender.

Data analysis for Group 7

Table 4.22 The distribution of the number of pups per delivery

Family	F	M
1.00	0.00	4.00
2.00	2.00	6.00
3.00	3.00	4.00
4.00	3.00	3.00
5.00	2.00	6.00
6.00	3.00	6.00
7.00	0.00	0.00
8.00	0.00	0.00
9.00	0.00	4.00
10.00	0.00	1.00
11.00	0.00	2.00
12.00	0.00	0.00
13.00	3.00	6.00
14.00	0.00	0.00
15.00	1.00	7.00
16.00	0.00	4.00

In group seven, there were 53 male and 17 female pups delivered representing 76% and 24% respectively of the total pups delivered.

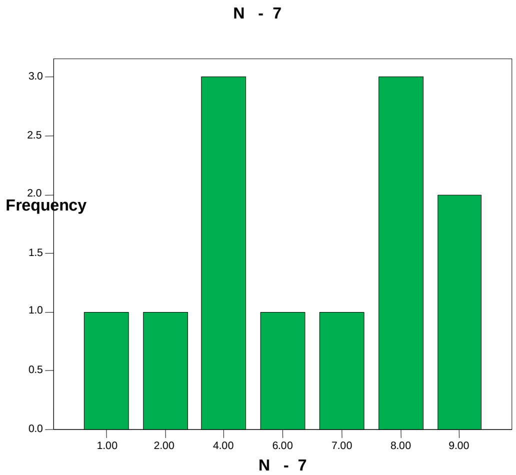


Figure 4.19 The distribution of the total pups per delivery

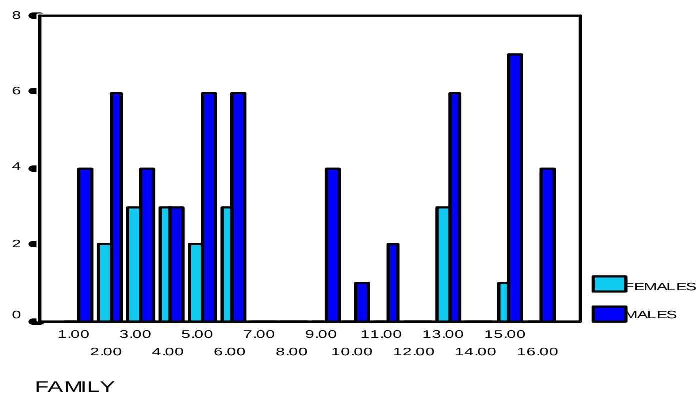


Figure 4.20 The distribution of female and male pups delivered per family.

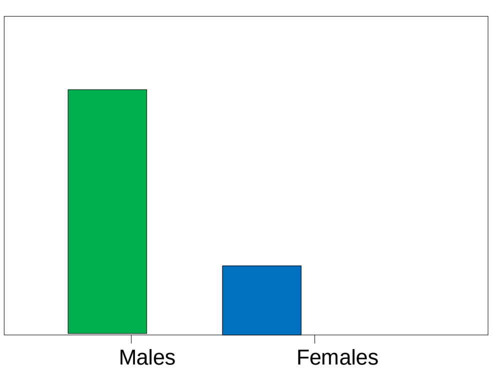


Figure 4.21 The sex ratios representing 24% females 76% Males

Table 4.23 Summary statistics

Summary	**Females**	**Males**
Number	16.00	16.00
Sum	17.00	53.00
Mean	1.06	3.31
Standard deviation	1.34	2.52
Variance	1.80	6.36
%	24.00	76.00
Student's t-test p-value 0.0037		

Conclusion

There was statistical significant difference between the female and the male progeny in this group with a two-tailed p-value of 0.0037

Data analysis for Group 8

Table 4.24 The distribution of the number of pups per delivery

Family	F	M
1.00	6.00	0.00
2.00	3.00	1.00
3.00	4.00	2.00
4.00	3.00	0.00
5.00	6.00	4.00
6.00	4.00	1.00
7.00	0.00	0.00
8.00	6.00	0.00
9.00	4.00	2.00
10.00	3.00	0.00
11.00	0.00	0.00
12.00	2.00	1.00
13.00	8.00	0.00
14.00	7.00	3.00
15.00	4.00	2.00
16.00	5.00	2.00
13.00	2.00	3.00

In group eight, there were 67 female and 21 male pups delivered representing 24% and 76% respectively of the total pups delivered.

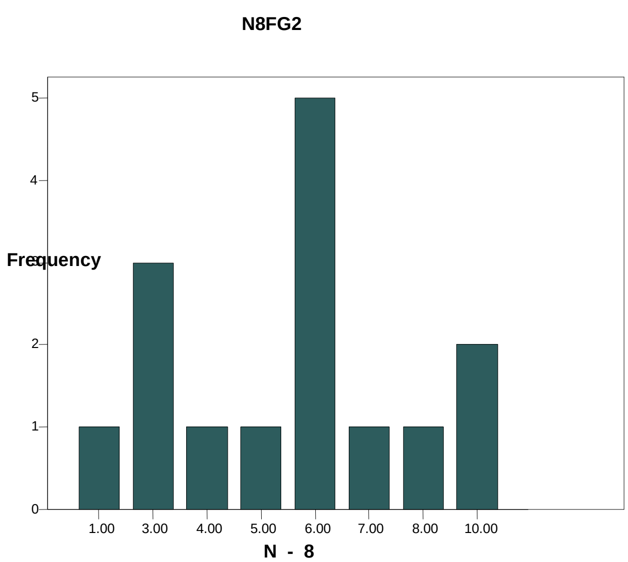


Figure 4.22 The distribution of the total pups per delivery.

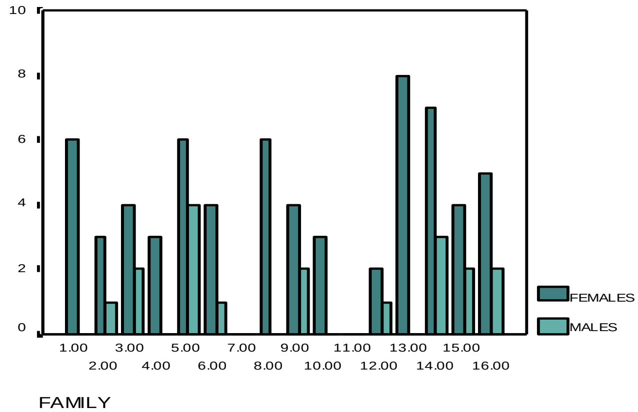


Figure 4.23 The distribution of female and male pups delivered per family

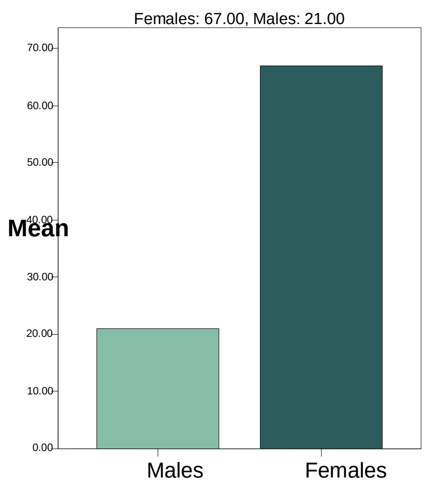


Figure 4.24 The sex ratios representing 24% females 76% Males

Table 4.25 Summary statistics

Summary	**Females**	**Males**
Number	16.00	16.00

Sum	67.00	21.00
Mean	3.94	1.24
Standard deviation	2.25	1.30
Variance	5.06	1.69
%	24.00	76.00
Student's t-test p-value 0.0002		

Conclusion

Statistical significant difference between the female and the male progeny in this group was observed with a two-tailed p-value of 0.0002 indicating dietary calcium and magnesium influence towards the female progeny.

Summary

Results for N1-8, F1 - 16 (Groups 1 - 8 and families 1 - 16).

Table 4.26

	Group 1	**Group 2**	**Group 3**	**Group 4**	**Group 5**	**Group 6**	**Group 7**	**Group 8**
Number	16	16	16	16	16	16	16	16
Mean	5.69	4.92	6.57	5.44	5.44	5.62	5.83	5.60
Std. Error of Mean	0.85	0.87	0.61	0.76	0.52	0.57	0.80	0.66
Median	5.00	5.00	7.50	5.50	6.00	5.00	6.50	6.00
Mode	2.00	2.00	8.00	4.00	6.00	5.00(a)	4.00(a)	6.00
Std. Deviation	3.07	3.03	2.28	3.05	2.06	2.06	2.76	2.56
Variance	9.397	9.174	5.187	9.329	4.263	4.256	7.606	6.543
Range	9.00	10.00	7.00	9.00	7.00	8.00	8.00	9.00
Minimum	2.00	0.00	3.00	1.00	1.00	2.00	1.00	1.00
Maximum	11.00	10.00	10.00	10.00	8.00	10.00	9.00	10.00
	Group 1	Group 2	Group 3	Group 4	Group 5	Group 6	Group 7	Group 8

Above table represents the deliveries among the 8 groups each with 16 families. N_{1-8}, F_{1-16}. The mean number of pups per delivery was 5.6. Details of the other measures of central tendency and dispersion are shown.

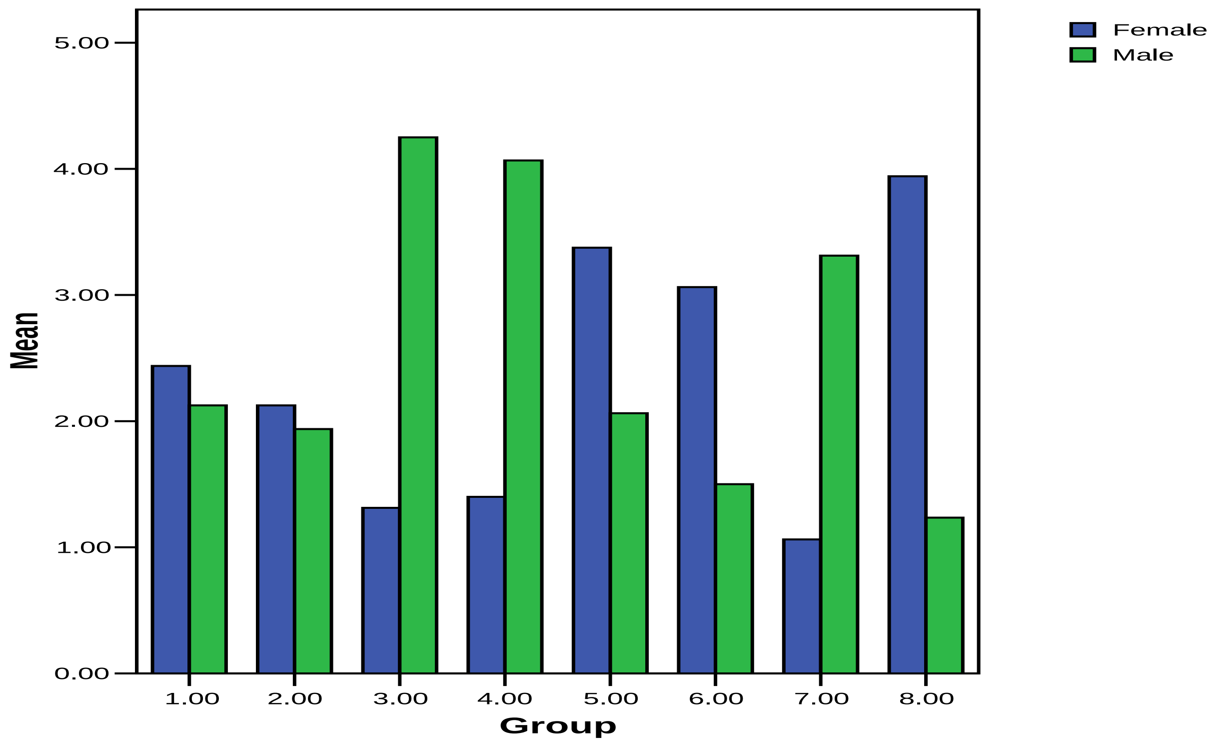


Figure 4.25 Graphic representation of the sex ratio outcome among the eight groups

Summary

- The mean number of pups per delivery was 5.6 with a standard deviation of 2.6.
- The control groups ***one*** and ***two*** ($N_{1\ \&\ 2}$) had similar sex ratio outcomes with no influence.
- Groups ***three, four and seven*** ($N_{3\ \&\ 4\ \&\ 7}$) had sex ratio skewed towards the male progeny.
- Groups ***five six and eight*** ($N_{5\ \&\ 6\ \&\ 8}$) had sex ratio skewed towards the female progeny.

Overall comment

Following the completion of the pilot study, the study group was well versed with the work as most of the practical issues had been addressed and therefore looking forward to the next phase of the study.

Conclusions

Summary of the pilot study

- The mean number of pups per delivery was 5.6 with a standard deviation of 2.
- The control groups ***one*** and ***two*** ($N_{1 \& 2}$) had similar sex ratio outcomes without influence over the sex ratios.
- Groups ***three, four and seven*** ($N_{3 \& 4 \& 7}$) had significant sex ratios skewed towards the male progeny.
- Groups ***five six and eight*** ($N_{5 \& 6 \& 8}$) had significant sex ratios skewed towards the female progeny.
-

General observations:

a) Behavioural patterns

Observation….. They keep to their familial tree and are hostile to outsiders.
They are well domesticated in the cages but get wild outside.

b) Feeding patterns

Observation….. Feeds most of the time on average 5 ml of fluids and 5 mg of food to double these amounts during the nursing period.

Comment .... This behaviour of feeding most of the time is good for the study as the chemical level will be kept high. Fluids are best changed after three days to avoid contamination and infection in the bottles.

Observation.… Feeding was poor among those on potassium, calcium and magnesium.

Solution..…..... After many formulations glucose was found to enhance the intake.

c) Breeding patterns

Observation…..Breeds from 0 weeks of age. Gestational period is three weeks. Litter ranges from 1–11. Can conceive immediately after 0 deliveries.
Weaning is done after three weeks.

Observation…. Parents can feed on the pups and sometimes can die without clear cause.

Comment……. Incidences of unexplained deaths of the pups can be reduced if male is separated from the nursing mother, raising the room temperature or reducing the dust in the beddings.

d) Sexing the litter

Observation…. Early physical sexing is prone to errors. Delaying the sexing increases the accuracy, but some litter may die and vital data may be lost.

Comment……. All DNA tail piece material from each pup is preserved but only those where sexing is not feasible through physical means were subjected to PCR.

e) Beddings

Comfortable with dry saw dust preferably with big chippings and less dust.

f) Temperature

The ideal temperature range is between 26 - 30^{o}C. The litter do not tolerate well temperatures outside these limits. The average humidity range is between 68-74. These results of the pilot study were presented at the Stellenbosch University in 2006 and formal application done. Following evaluations group nine and the oocyte membrane electrical study were included in the study and admission granted in 2008.

4.25 Dietary main study

The findings and experiences of the pilot study were translated into the dietary main study whereby a similar double blind study of nine groups of Swiss (Webster breed) each group comprising of 16 pairs of mice (families) were fed with sodium, potassium, calcium and magnesium as single elements, combined sodium with potassium and calcium with magnesium and all-inclusive cocktail of all these chemicals in their drinking water. Two equivalent control groups were set up of plain drinking water and a second group with glucose added to it. These were followed up through their entire lives and sexing of their progeny performed using applicable methods.

The work was done in the same institutions as the pilot study that included, University of Nairobi Medical Physiology Department in consultations with Veterinary Department, Kenyatta National Hospital and ILRI. These institutes have relevant experts on animal research and animal houses with the necessary infrastructure for animal experimentation. The mice were outsourced from Kenyatta National Hospital animal house where valuable advice on their upkeep was provided by the chief overseer Mr Maloba. The materials for DNA work were outsourced from Belgium through ILRI who also provided valuable information on mice experimentation and DNA work by Dr Fuard.

The key differences between the pilot and the main study designs were that the pilot study took 6 months while the main study examined the whole lifespan of the mice of about two and two and half years. Eight groups were used in the pilot study whereas group nine was included in the main study and comprised of the cocktail of all the chemicals under study.

The following are the various aspects of the dietary study that were carried out:-

1) a) Evaluation on the effects of high dietary sodium, potassium, calcium and magnesium as single elements and their ionic effects on sex ratios.
   b) Determination of the effects of combined high dietary
      i) Sodium with potassium elements.
      ii) Calcium with Magnesium elements and their ionic effects on sex ratios.
      iii) Determination of combined high dietary cocktail of all the above elements on sex ratios.
2) PCR/DNA method of early gender determination and perinatal mortality trends.
3) To determine the effects of high dietary chemical compositions on biochemical serum concentrations of the respective chemicals.
4) To assess the weight trends among the animal models as a monitoring tool of the study.
5) To determine the effects of delivery sequence on sex ratios.
6) To assess the effects of seasonal variations on sex ratios.

Methods

In a similar double blind study of nine groups of Swiss (Webster) breed each group comprising of 16 pairs of mice (families) were fed with sodium, potassium, calcium and magnesium as single elements, combined sodium with potassium and calcium with magnesium and all-inclusive cocktail of all these chemicals in their drinking water. Two equivalent control groups were set up of plain drinking water and a second group with glucose added to it. They were followed up through their entire lives and sexing of their progeny performed using applicable methods. The results and analysis is tabulated below.

Table 4.27 - Dietary main study Control group one - Water

1) Dietary chemical composition and their ionic effects on sex ratios

DATE	FEMALE		MALES		TOTAL	%	%	No Delivery
August 2008	33.00	33.00	31.00	31.00	64.00	52%	48%	8
September 2008	71.00	38.00	74.00	43.00	145.00	48%	52%	10
TOTAL		**71.00**		**74.00**	**145**	**48%**	**52%**	**18**

October 2008	165	94.00	158	84.00	323	51%	49%	22
November 2008	262	97.00	251	93.00	513	51%	49%	24
December 2008	339	67.00	323	72.00	662	51%	49%	17
TOTAL		**268.00**		**249.00**	**517**	**51.8%**	**48.2%**	**63**

January 2009	420	81.00	408	85.00	828	50%	50%	20
February 2009	445	25.00	437	29.00	882	50%	50%	6
March 2009	475	30.00	470	33.00	945	50%	50%	8
TOTAL		**136.00**		**147.00**	**283**	**48%**	**52%**	**34**

April 2009	502	27.00	493	23.00	995	50%	50%	6
May 2009	525	23.00	516	23.00	1041	50%	50%	6
TOTAL		**50.00**		**46.00**	**96**	**52%**	**48%**	**12**

June 2009	560	22.00	546	18.00	1106	50%	50%	5
July 2009	577	17.00	569	23.00	1146	50%	50%	5
TOTAL		**52.00**		**53.00**	**105**	**49.5%**	**50.5%**	**10**

August 2009	597	20.00	580	11.00	1177	51%	49%	4
September 2009	613	16.00	601	21.00	1214	50%	50%	5
TOTAL		**36.00**		**32.00**	**68**	**53%**	**47%**	**9**

October 2009	615	2.00	606	5.00	1221	50%	50%	1
November 2009	624	9.00	615	9.00	1239	50%	50%	2
December 2009	630	6.00	619	4.00	1249	50%	50%	1
TOTAL		**17.00**		**18.00**	**35**	**48.5%**	**51.5%**	**4**

January 2010	670	40.00	645	26.00	1315	51%	49%	8
February 2010	678	8.00	652	7.00	1330	51%	49%	2
March 2010	701	23.00	683	31.00	1384	51%	49%	7
TOTAL		**71.00**		**64.00**	**135**	**54.4%**	**45.6%**	**17**

April 2010	713	12.00	701	18.00	1414	51%	49%	4
May 2010	716	3.00	706	5.00	1422	51%	49%	1
TOTAL		**15.00**		**23.00**	**38**	**45.9%**	**54.1%**	**5**

June 2010	722	6.00	708	2.00	1430	51%	49%	1
July 2010	723	1.00	709	1.00	1432	51%	49%	1
TOTAL		**7.00**		**3.00**	**10**	**70%**	**30%**	**2**

.../Table 4.27

August 2010	723	0.00	711	2.00	1434	51%	49%	1
September 2010	724	1.00	712	1.00	1436	51%	49%	1
TOTAL		**1.00**		**3.00**	**4**	**25%**	**75%**	**2**

Table 4.28 - Dietary ionic effects on gender - Summary

Season	**Duration**	**Female**	**%**	**Male**	**%**	**Totals**	**%**	**No delivery**	**p/del**
Rainy season	10	347	47	336	47	683	47	84	8.13
Dry season	12	318	43	320	45	638	44	80	7.97
Cold season	4	59	10	56	8	115	9	12	9.58
Totals	**26**	**724**		**712**		**1436**		**176**	**8.15**

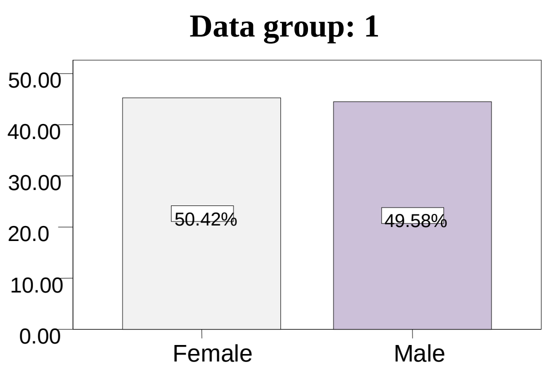


Figure 4.26 The gender representation with 50.42% females 49.48% Males

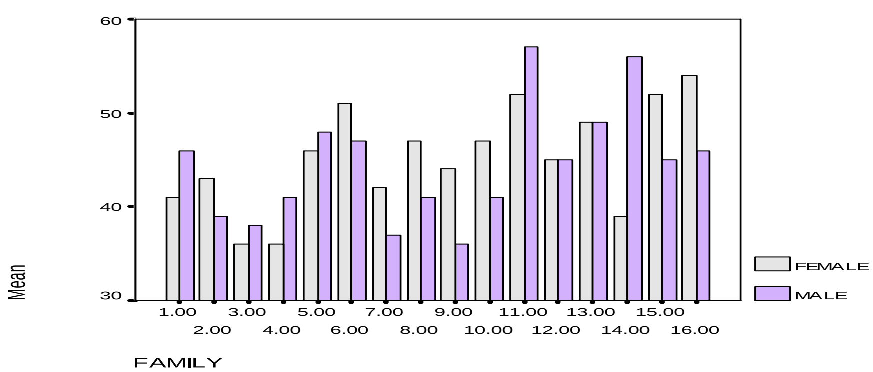


Figure 4.27 The female and male pups delivered per family

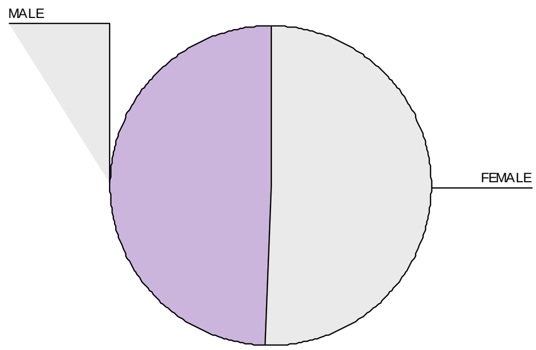


Figure 4.28 The female to male sex ratios 1:1

In group one, there were a total of 1436 pups delivered comprising of 724 females and 712 males representing 50.42% and 49.58% respectively at the rate of 8.15pups per delivery. There was no significant statistical gender difference between the female and male progeny p-value of 0.61.

Dietary study. Control group two - Glucose

Table 4.29 Dietary chemical composition and their ionic effects on sex ratios

DATE	FEMALE		MALES		TOTAL	%	%	No delivery
August 2008	21	21.00	17	17.00	38	55%	45%	4
September 2008	69	48.00	69	52.00	138	50%	50%	12
TOTAL		**69.00**		**69.00**	**138**	**50%**	**50%**	**16**
October 2008	192	123.00	208	139.00	400	48%	52%	32
November 2008	271	79.00	322	119.00	593	45%	55%	25
December 2008	339	68.00	413	91.00	742	45%	55%	19
TOTAL		**270.00**		**344.00**	**614**	**44%**	**56%**	**76**
January 2009	393	54.00	491	78.00	884	44%	56%	17
February 2009	415	22.00	526	25.00	941	44%	56%	5
March 2009	432	17.00	554	28.00	986	43%	57%	5
TOTAL		**93.00**		**131.00**	**224.00**	**40%**	**60%**	**27**
April 2009	452	20.00	589	35.00	1041	43%	57%	6
May 2009	467	15.00	612	23.00	1079	43%	57%	4
TOTAL		**35.00**		**58.00**	**93**	**38%**	**62%**	**10**
June 2009	480	13.00	625	13.00	1107	43%	57%	3
July 2009	509	29.00	650	25.00	1161	43%	57%	6
TOTAL		**42.00**		**38.00**	**80.00**	**52%**	**48%**	**9**
August 2009	529	20.00	676	26.00	1207	44%	56%	6
September 2009	540	11.00	694	18.00	1236	43%	57%	3
TOTAL		**31.00**		**44.00**	**75.00**	**41%**	**59%**	**8**
October 2009	544	4.00	701	7.00	1245	43%	57%	1
November 2009	548	4.00	710	9.00	1260	43%	57%	1
December 2009	550	2.00	722	12.00	1272	43%	57%	1
TOTAL		**10.00**		**28.00**	**38.00**	**26%**	**74%**	**3**
January 2010	560	10.00	735	13.00	1295	43%	57%	2
February 2010	574	14.00	753	18.00	1329	43%	57%	4
March 2010	599	25.00	784	31.00	1385	43%	57%	7
TOTAL		**49.00**		**62.00**	**111.00**	**44%**	**56%**	**13**
April 2010	610	11.00	803	9.00	1415	43%	57%	2
May 2010	612	2.00	806	3.00	1418	43%	57%	1
TOTAL		**13.00**		**12.00**	**25.00**	**52%**	**48%**	**3**
June 2010	614	2.00	809	3.00	1425	43%	57%	1
July 2010	616	2.00	812	3.00	1428	43%	57%	1
TOTAL		**4.00**		**6.00**	**10.00**	**40%**	**60%**	**2**
August 2010	621	5.00	817	5.00	1438	50%	50%	1
September 2010	625	4.00	821	4.00	1446	50%	50%	1
TOTAL		**9.00**		**9.00**	**12.00**	**50%**	**50%**	**2**

Table 4.30 The Dietary ionic effects on gender – Summary

Season	Duration	Female	%	Males	%	Totals	%	No delivery	p/del
Rainy season	10	330	53	458	56	788	54	92	8.5
Dry season	12	249	40	319	39	568	39	67	8.4
Cold season	4	46	7	44	5	90	7	11	8.18
Totals	**26**	**625**		**821**		**1446**		**170**	**8.50**

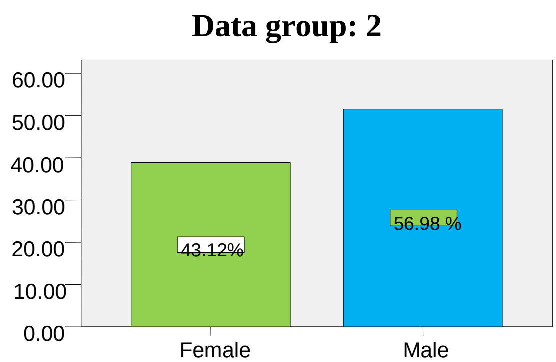


Figure 4.29 The gender representation with 43.12% females 56.98% Males

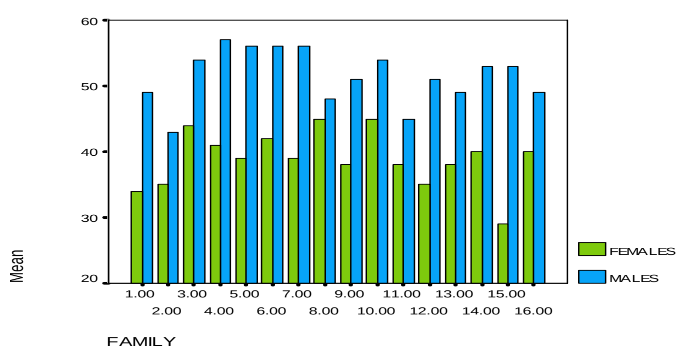


Figure 4.30 The female and male pups delivered per family

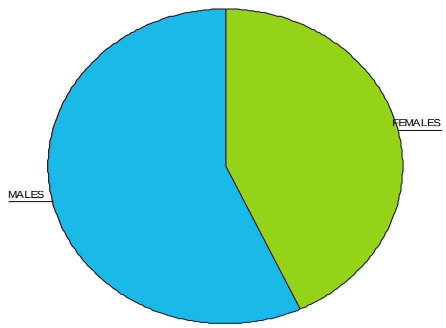


Figure 4.31 The female to male sex ratios 4:6

Conclusion

In group two, there were a total of 1446 pups delivered comprising of 622 females and 824 males representing 43.12% and 56.98% respectively at the rate of 8.5pups per delivery. The preponderance of the male gender was found to be of statistical significance $p<0.001$.

Dietary study. Study group three single elements - Sodium

Table 4.31 Dietary chemical composition and their ionic effects on sex ratios

DATE	FEMALE		MALES		TOTAL	%	%	No delivery
August 2008	21	21.00	32	32.00	53	39%	61%	6
September 2008	53	32.00	89	57.00	142	37%	63%	11
TOTAL		**53.00**		**89.00**	**142**	**37%**	**63%**	**17**
October 2008	124	71.00	246	157.00	370	33%	67%	27
November 2008	185	61.00	412	166.00	597	30%	70%	27
December 2008	251	66.00	499	87.00	750	33%	67%	19
TOTAL		**198.00**		**410.00**	**608**	**33%**	**67%**	**73**
January 2009	308	57.00	587	88.00	895	34%	66%	18
February 2009	335	27.00	610	23.00	955	35%	65%	6
March 2009	356	1.00	637	27.00	993	35%	65%	3
TOTAL		**105.00**		**138.00**	**243**	**43%**	**57%**	**27**
April 2009	382	26.00	667	30.00	1049	36%	64%	7
May 2009	402	20.00	692	25.00	1094	36%	64%	5
TOTAL		**46.00**		**55.00**	**101**	**46%**	**54%**	**12**
June 2009	416	14.00	709	17.00	1125	36%	64%	3
July 2009	435	19.00	738	29.00	1173	37%	63%	6
TOTAL		**33.00**		**46.00**	**79**	**42%**	**58%**	**9**

…/Table 4.31

August 2009	445	10.00	760	22.00	1205	36%	64%	4
September 2009	457	12.00	781	21.00	1238	36%	64%	4
TOTAL		**22.00**		**43.00**	**65**	**34%**	**66%**	**8**
October 2009	465	8.00	796	15.00	1261	36%	64%	2
November 2009	470	5.00	810	14.00	1280	37%	63%	2
December 2009	478	8.00	820	10.00	1298	37%	63%	2
TOTAL		**21.00**		**39.00**	**60**	**35%**	**65%**	**6**
January 2010	485	7.00	836	16.00	1321	36%	64%	3
February 2010	491	6.00	852	16.00	1343	36%	64%	2
March 2010	518	27.00	885	33.00	1402	37%	63%	7
TOTAL		**40.00**		**65.00**	**105**	**40%**	**60%**	**12**
April 2010	529	11.00	899	14.00	1427	37%	63%	3
May 2010	533	4.00	910	12.00	1443	37%	63%	1
TOTAL		**15.00**		**26.00**	**41**	**42%**	**58%**	**4**
June 2010	536	3.00	925	15.00	1461	37%	63%	2
July 2010	537	1.00	928	3.00	1465	37%	63%	1
TOTAL		**4.00**		**18.00**	**22**	**18%**	**82%**	**3**
August 2010	537	0.00	930	2.00	1467	37%	63%	1
September 2010	538	1.00	933	3.00	1471	37%	63%	1
TOTAL		**1.00**		**5.00**	**6**	**16%**	**84%**	**2**

Table 4.32 The Dietary ionic effects on gender - Summary.

Season	Duration	Female	%	Males	%	Totals	%	No delivery	p/del
Rainy season	10	280	52	530	57	810	55	95	8.52
Dry season	12	221	41	339	36	560	38	66	8.48
Cold season	4	37	7	64	7	101	7	12	8.41
Totals	**26**	**538**		**933**		**1471**		**173**	**8.50**

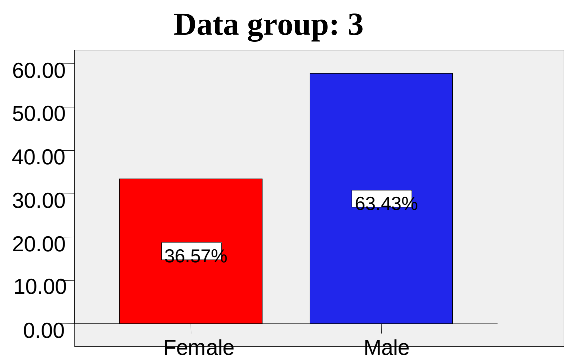


Figure 4.32 The gender representation with 35.57% females 63.43% Males

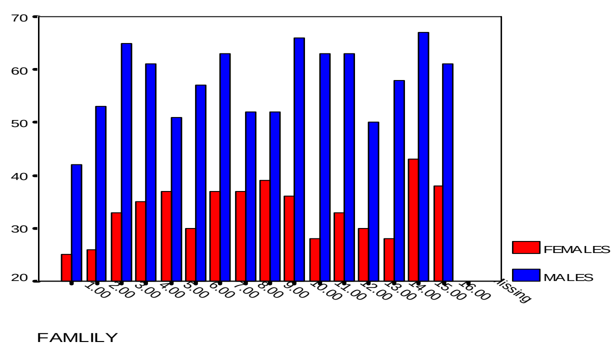


Figure 4.33 The female and male pups delivered per family

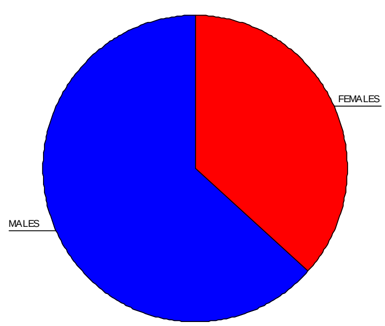


Figure 4.34 The female to male sex ratios 4 : 6

In group three, there were a total of 1471 pups delivered comprising of (538) females and 923 males representing 36.57% and 63.43%p respectively at p the p rate p of 8.5pups per delivery. There was significant statistical gender difference between the female and male progeny $p<0.0001$.

Dietary study. Study group four single element - Potassium

Table 4.33 Dietary chemical composition and their ionic effects on sex ratios

DATE	FEMALE		MALES		TOTAL	%	%	No delivery
August 2008	4	4.00	7	7.00	11	36%	64%	1
September 2008	39	35.00	72	65.00	111	35%	65%	12
T0TAL		**39.00**		**72.00**	**111**	**35%**	**65%**	**13**
October 2008	112	73.00	240	168.00	352	32%	68%	30
November 2008	190	78.00	392	152.00	582	32%	68%	28
December 2008	283	93.00	474	82.00	757	37%	63%	20
TOTAL		**244.00**		**402.00**	**646**	**38%**	**62%**	**78**
January 2009	346	63.00	557	83.00	903	38%	62%	18
February 2009	367	21.00	582	25.00	949	38%	62%	5
March 2009	390	23.00	609	27.00	999	39%	61%	6
TOTAL		**107.00**		**135.00**	**242**	**44%**	**56%**	**29**
April 2009	420	30.00	639	30.00	1059	39%	61%	7
May 2009	450	30.00	662	23.00	1112	40%	60%	6
TOTAL		**60.00**		**53.00**	**113**	**53%**	**47%**	**13**
June 2009	462	12.00	681	19.00	1143	40%	60%	3
July 2009	475	13.00	703	22.00	1178	40%	60%	4
TOTAL		**25.00**		**41.00**	**66**	**38%**	**62%**	**7**
August 2009	492	17.00	723	20.00	1215	41%	59%	4
September 2009	504	12.00	743	20.00	1247	40%	60%	4
TOTAL		**29.00**		**40.00**	**69**	**42%**	**58%**	**8**
October 2009	508	4.00	750	7.00	1258	40%	60%	1
November 2009	510	2.00	759	9.00	1269	40%	60%	1
December 2009	520	10.00	769	10.00	1289	40%	60%	2
TOTAL		**16.00**		**26.00**	**42**	**38%**	**62%**	**4**
January 2010	528	8.00	777	8.00	1305	40%	60%	2
February 2010	543	15.00	814	7.00	1357	40%	60%	2
March 2010	564	21.00	855	41.00	1419	39%	61%	7
TOTAL		**44.00**		**86.00**	**130**	**34%**	**66%**	**11**
April 2010	570	6.00	876	21.00	1443	39%	61%	3
May 2010	573	3.00	886	10.00	1460	39%	61%	1
TOTAL		**9.00**		**30.00**	**41**	**24%**	**76%**	**4**
June 2010	577	4.00	900	4.00	1475	39%	61%	1
July 2010	579	2.00	908	8.00	1488	39%	61%	2
TOTAL		**6.00**		**22.00**	**28**	**21%**	**79%**	**3**
August 2010	580	1.00	913	5.00	1494	39%	61%	1
September 2010	581	1.00	919	6.00	1500	39%	61%	1
TOTAL		**2.00**		**11.00**	**13**	**27%**	**73%**	**2**

Table 4.34 The Dietary ionic effects on gender - Summary

Season	Duration	Female	%	Males	%	Totals	%	No delivery	p/del
Rainy season	10	329	57	513	56	842	56	99	8.50
Dry season	12	221	38	343	37	564	38	63	8.95
Cold season	4	31	5	63	7	94	6	10	9.40
Totals	**26**	**581**		**919**		**1500**		**172**	**8.72**

Graphic data presentation Group 4

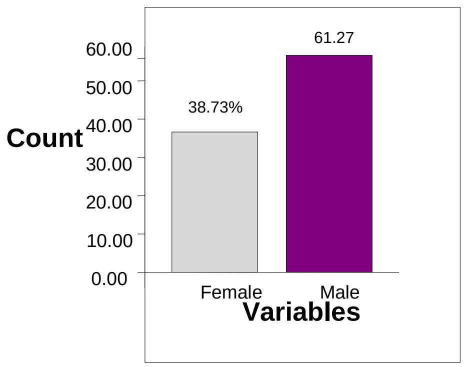


Figure 4.35 The gender representation with 35.57% female 63.43% Males

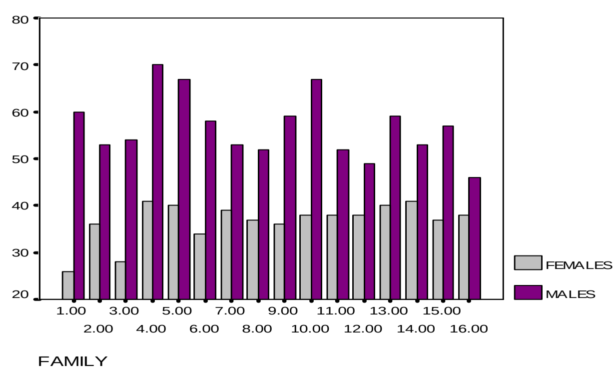


Figure 4.36 The female and male pups delivered per family

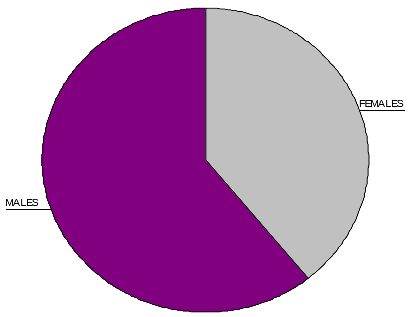


Figure 4.37 The female to male sex ratios 4:6

Conclusion

In group four, there were a total of 1500 pups comprising of 581 females and 919 males representing 38.73% and 61.27% respectively at the rate of 8.72pups per delivery. There was statistical significant gender difference between the female and male progenies ($p<0001$).

Dietary study. Study group five single element - Calcium

Table 4.35 Dietary chemical composition and their ionic effects on sex ratios

DATE	FEMALE		MALES		TOTAL	%	%	No delivery
August 2008	16	16.00	10	10.00	26	62%	38%	3
September 2008	70	54.00	43	33.00	113	61%	39%	10
TOTAL		**70.00**		**43.00**	**113**	**61%**	**39%**	**13**
October 2008	236	166.00	134	93.00	370	63%	37%	30
November 2008	385	149.00	222	88.00	607	63%	37%	27
December 2008	449	64.00	300	78.00	749	59%	41%	17
TOTAL		**373.00**		**257.00**	**636**	**59%**	**41%**	**74**
January 2009	525	76.00	349	49.00	874	60%	40%	15
February 2009	552	27.00	381	32.00	933	59%	41%	7
March 2009	582	30.00	401	20.00	983	59%	41%	6
TOTAL		**133.00**		**101.00**	**234**	**56%**	**44%**	**28**
April 2009	612	30.00	426	25.00	1038	58%	42%	6
May 2009	641	29.00	453	27.00	1094	58%	42%	7
TOTAL		**59.00**		**52.00**	**111**	**53%**	**47%**	**13**
June 2009	667	26.00	464	11.00	1121	59%	41%	4
July 2009	689	22.00	476	12.00	1155	60%	40%	4
TOTAL		**48.00**		**23.00**	**71**	**67%**	**33%**	**8**
August 2009	713	24.00	491	15.00	1194	59%	41%	4
September 2009	733	22.00	502	11.00	1225	60%	40%	4
TOTAL		**44.00**		**26.00**	**70**	**63%**	**37%**	**8**

…/Table 4.35

October 2009	743	10.00	506	4.00	1249	60%	40%	1
November 2009	751	8.00	508	2.00	1259	60%	40%	1
December 2009	761	10.00	518	10.00	1279	60%	40%	2
TOTAL		**28.00**		**16.00**	**44**	**63%**	**37%**	**4**

January 2010	781	20.00	536	18.00	1317	60%	40%	4
February 2010	808	27.00	546	10.00	1354	60%	40%	4
March 2010	839	31.00	567	21.00	1406	60%	40%	7
TOTAL		**78.00**	**49**	**49.00**	**127**	**63%**	**37%**	**15**

April 2010	852	23:00	579	12.00	1431	61%	39%	5
May 2010	862	10.00	580	11.00	1442	61%	39%	2
TOTAL		**33:00**		**23.00**	**56**	**60%**	**40%**	**7**

June 2010	871	9.00	582	2.00	1453	61%	39%	1
July 2010	877	6.00	584	2.00	1461	61%	39%	1
TOTAL		**15.00**		**4.00**	**19**	**79%**	**21%**	**2**

August 2010	880	3.00	587	3.00	1467	50%	50%	1
September 2010	885	5.00	588	1.00	1473	61%	39%	1
TOTAL		**8.00**		**4.00**	**12**	**89%**	**11%**	**2**

Table 4.36 The Dietary ionic effects on gender - Summary

Season	**Duration**	**Female**	**%**	**Males**	**%**	**Totals**	**%**	**No delivery**	**p/del**
Rainy season	10	489	55	338	57	827	56	98	8.40
Dry season	12	333	37	223	38	556	38	66	8.42
Cold season	4	63	8	27	5	90	6	10	9.00
Totals	**26**	**895**		**588**		**1473**		**174**	**8.46**

Graphic data presentation group: 5

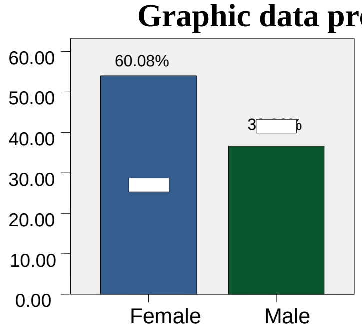


Figure 4.38 The gender representation with 35.57% females 63.43% Males

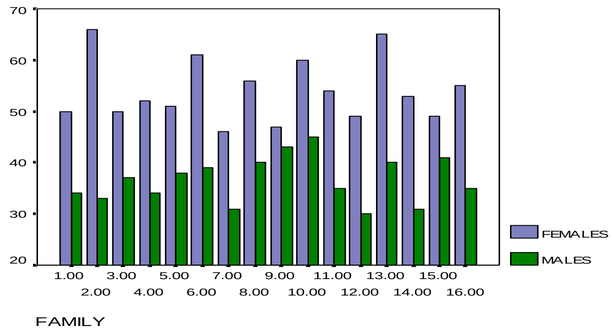


Figure 4.39 The female and male pups delivered per family

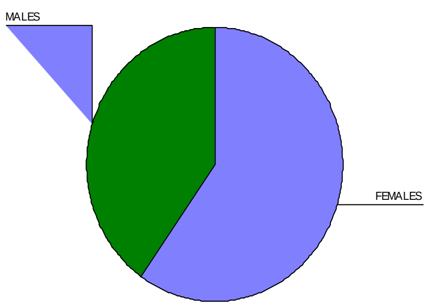


Figure 4.40 The female to male sex ratios 4:6

In group five, there were a total of 1473 pups comprising of 885 females and 588 males representing 60.08% and 39.92% respectively at the rate of 8.46 per delivery. There was a statistical significant gender difference between the female and male progeny p<0001.

Dietary study. Study group six single element - Magnesium

Table 4.37 Dietary chemical composition and their ionic effects on sex ratios

DATE	FEMALE		MALES		TOTAL	%	%	No delivery
August 2008	9	9.00	4	4.00	13	69%	31%	2
September 2008	59	50.00	37	33.00	96	61%	39%	10
Total		**59.00**		**37.00**	**96**	**61%**	**39%**	**12**
October 2008	227	68.00	150	113.00	377	60%	40%	24
November 2008	370	143.00	231	81.00	601	61%	39%	28
December 2008	441	71.00	327	96.00	768	57%	43%	22
Total		**382.00**		**290.00**	**672**	**56%**	**44%**	**74**
January 2009	539	98.00	374	47.00	913	59%	41%	20
February 2009	570	31.00	393	19.00	963	59%	41%	7
March 2009	601	31.00	412	19.00	1013	59%	41%	7
Total		**160.00**		**85.00**	**245**	**65%**	**35%**	**34**
April 2009	633	32.00	430	18.00	1063	59%	41%	6
May 2009	655	22.00	443	13.00	1098	59%	41%	5
Total		**54.00**		**31.00**	**85**	**63%**	**37%**	**11**
June 2009	671	16.00	452	9.00	1123	59%	41%	3
July 2009	706	35.00	468	16.00	1174	60%	40%	6
Total		**51.00**		**25.00**	**76.00**	**67%**	**33%**	**9**
August 2009	734	8.00	481	13.00	1215	61%	39%	2
September 2009	755	21.00	495	14.00	1250	60%	40%	4
Total		**49.00**		**27.00**	**76**	**64%**	**36%**	**6**
October 2009	765	10.00	498	3.00	1263	60%	40%	1
November 2009	773	8.00	500	2.00	1273	61%	39%	1
December 2009	783	10.00	520	20.00	1303	61%	39%	4
Total		**28.00**		**20.00**	**53**	**53%**	**47%**	**6**
January 2010	806	13.00	535	15.00	1341	61%	39%	3
February 2010	828	22.00	545	10.00	1373	60%	40%	4
March 2010	871	43.00	571	26.00	1442	60%	40%	8
Total		**88.00**		**51.00**	**139**	**63%**	**37%**	**15**
April 2010	888	17.00	582	11.00	1470	60%	40%	3
May 2010	891	3.00	584	2.00	1475	60%	40%	1
Total		**20.00**		**13.00**	**33**	**61%**	**39%**	**4**
June 2010	901	10.00	587	3.00	1488	60%	40%	2
July 2010	903	2.00	589	2.00	1492	60%	40%	1
Total		**12.00**		**5.00**	**17**	**70%**	**30%**	**3**
August 2010	904	1.00	589	0.00	1493	60%	40%	1
September 2010	906	2.00	590	1.00	1496	60%	40%	1
Total		**3.00**		**1.00**	**4**	**75%**	**25%**	**2**

Table 4.38 The Dietary ionic effects on gender – Summary

Season	Duration	Female	%	Males	%	Totals	%	No delivery	p/del
Rainy season	10	484	53	359	61	843	56	95	8.87
Dry season	12	359	40	201	36	560	37	69	8.11
Cold season	4	63	7	30	3	93	7	12	7.75
Totals	**26**	**906**		**590**		**1496**		**176**	**8.50**

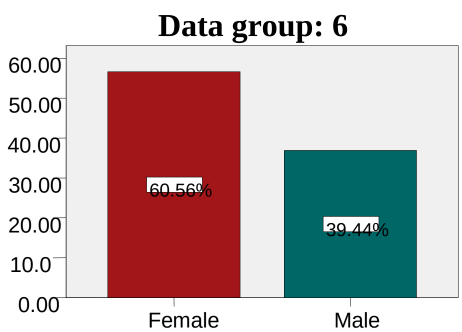


Figure 4.41 The gender representation with 35.57% females 63.43% males

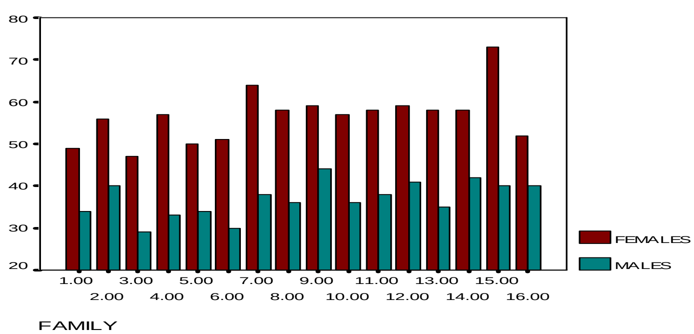


Figure 4.42 The female and male pups delivered per family

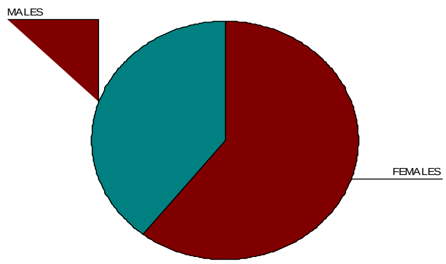


Figure 4.43 The female to male sex ratios 4:6

In group six, there were a total of 1496 pups comprising of 906 females and 590 males representing 60.56% and 39.44% respectively at the rate of 8.5 pups per delivery. There was a statistical significant gender difference between the female and male progeny p-value of 0001.

Dietary study. Study group seven double elements - Sodium/Potassium

Table 4.39 Dietary chemical composition and their ionic effects on sex ratios

DATE	FEMALE		MALES		TOTAL		F %	M %	No delivery
August 2008	8	8.00	14	14.00	22		36%	64%	2
September 2008	39	31.00	87	73.00	126		30%	70%	13
Total		**39.00**		**87.00**	**126**		**30%**	**70%**	**15**
October 2008	121	82.00	305	218.00	426	69.00	28%	72%	34
November 2008	171	50.00	456	151.00	627	31.00	27%	73%	23
December 2008	258	87.00	541	85.00	799	35.00	32%	68%	21
Total		**219**		**454**		**673**	**33%**	**67%**	**77**
January 2009	301	43.00	601	60.00	902	27.00	33%	67%	10
February 2009	333	32.00	626	25.00	959	57.00	34%	66%	7
March 2009	350	17.00	649	23.00	999	40.00	35%	65%	4
Total		**92**		**108**		**200**	**46%**	**54%**	**21**
April 2009	372	22.00	677	28.00	1049	50.00	35%	65%	6
May 2009	390	18.00	705	28.00	1095	46.00	35%	65%	5
Total		**40**		**56**		**96**	**42%**	**58%**	**11**
June 2009	396	6.00	716	11.00	1112	17.00	35%	65%	2
July 2009	414	18.00	756	40.00	1170	58.00	35%	65%	7
Total		**24**		**51**		**75**	**32%**	**68%**	**9**
August 2009	427	13.00	785	29.00	1212	42.00	34%	66%	6
September 2009	438	11.00	814	29.00	1252	40.00	34%	66%	5
Total		**14**		**58**		**82**	**19%**	**81%**	**11**

…/Table 4.39

October 2009	440	2.00	824	10.00	1264	12.00	34%	66%	1
November 2009	444	4.00	833	9.00	1277	13.00	34%	66%	1
December 2009	456	12.00	853	20.00	1309	32.00	34%	66%	4
Total		**18**		**39**		**57**	**32%**	**68%**	**6**

January 2010	462	6.00	906	53.00	1368	59.00	34%	66%	7
February 2010	478	16.00	912	6.00	1380	22.00	34%	66%	2
March 2010	498	20.00	966	54.00	1464	74.00	33%	67%	9
Total		**42**		**113**		**155**	**27%**	**73%**	**18**

April 2010	504	6.00	986	20.00	1490	26.00	33%	67%	3
May 2010	505	1.00	996	10.00	1501	11.00	33%	67%	1
Total		**7**		**30**		**37**	**16%**	**84%**	**4**

June 2010	507	2.00	1001	5.00	1508	7.00	33%	67%	1
July 2010	507	0.00	1004	3.00	1511	3.00	33%	67%	1
Total		**2**	**8**	**8**		**10**	**20%**	**80%**	**2**

August 2010	507	0.00	1006	2.00	1513	2.00	33%	67%	1
September 2010	508	1.00	1008	2.00	1516	3.00	33%	67%	1
Total		**1**		**4**		**5**	**20%**	**80%**	**2**

Table 4.40 The Dietary ionic effects on gender – Summary

Season	**Duration**	**Female**	**%**	**Males**	**%**	**Totals**	**%**	**No delivery**	**p/del**
Rainy season	10	289	56	581	58	870	57	98	8.8
Dry season	12	193	38	368	37	561	37	67	8.37
Cold season	4	26	6	59	5	85	6	11	7.72
Totals	**26**	**508**		**1008**		**1516**		**177**	**8.56**

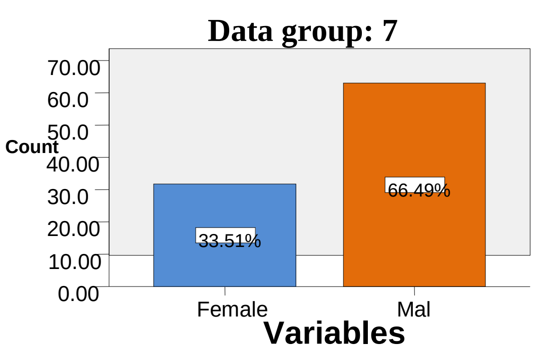


Figure 4.44 The gender representation with 35.57% females 63.43% males

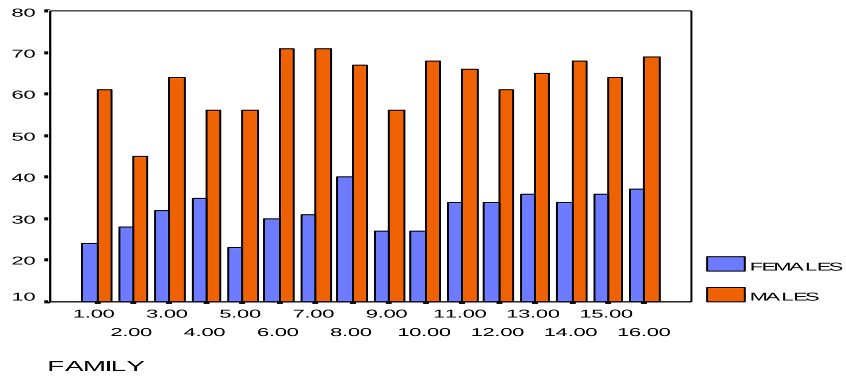


Figure 4.45 The female and male pups delivered per family

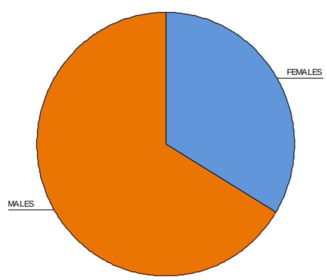


Figure 4.46 The female to male sex ratios 4:6

In group seven, there were a total of 1516 pups comprising of 508 females and 1008 males representing 33.51% and 66.49% respectively at the rate of 8.56 pups per delivery. There was a statistical gender difference between the female and the male progeny p<0001.

Dietary Study. Study group eight - Calcium and Magnesium solution

Table 4.41 Effect of seasonal variations on sex ratios

DATE	FEMALE		MALES		TOTAL		F %	M %	No delivery
August 2008	19	19.00	11	11.00	30		63%	37%	3
September 2008	80	61.00	40	29.00	120		66%	34%	11
Total		**80.00**		**40.00**	**120**		**66%**	**34%**	**14**
October 2008	301	221.00	124	84.00	425	77	70%	30%	35
November 2008	453	152.00	178	54.00	631	30	71%	29%	23
December 2008	532	79.00	255	77.00	787	44	67%	33%	16
Total		**452.00**		**215.00**		**667**	**67%**	**33%**	**74**
January 2009	601	69.00	313	58.00	914	34	65%	35%	12
February 2009	624	23.00	330	17.00	954	40	65%	35%	5
March 2009	655	31.00	349	19.00	985	50	66%	34%	5
Total		**123.00**		**94.00**		**217**	**56%**	**44%**	**22**
April 2009	692	37.00	369	20.00	1042	57	66%	34%	5
May 2009	718	26.00	384	15.00	1083	41	66%	34%	5
Total		**63.00**		**35.00**		**98**	**64%**	**36%**	**10**
June 2009	738	20.00	398	14.00	1117	34	66%	34%	4
July 2009	785	47.00	411	13.00	1196	60	66%	34%	7
Total		**67.00**		**27.00**		**94**	**71%**	**29%**	**11**
August 2009	809	24.00	421	10.00	1230	34	66%	34%	4
September 2009	837	28.00	436	15.00	1273	43	66%	34%	5
Total		**52.00**		**25.00**		**77**	**67%**	**33%**	**9**
October 2009	848	11.00	438	2.00	1286	13	66%	34%	1
November 2009	856	8.00	440	2.00	1296	10	67%	33%	1
December 2009	870	14.00	454	14.00	1324	28	67%	33%	3
Total		**33.00**		**18.00**		**51**	**64%**	**36%**	**5**
January 2010	916	46.00	462	8.00	1378	54	67%	33%	6
February 2010	939	23.00	471	9.00	1410	32	67%	33%	4
March 2010	975	36.00	472	21.00	1467	57	67%	33%	7
Total		**105.00**		**38.00**		**143**	**73%**	**27%**	**17**
April 2010	993	18.00	478	6.00	1491	24	67%	33%	3
May 2010	1002	9.00	493	8.00	1508	17	67%	33%	2
Total		**27.00**		**14.00**		**41**	**66%**	**34%**	**5**
June 2010	1009	7.00	501	15.00	1510	22	67%	33%	2
July 2010	1016	7.00	504	3.00	1520	10	67%	33%	1
Total		**14.00**		**18.00**		**32**	**43%**	**67%**	**3**
August 2010	1019	3.00	504	0.00	1523	3	67%	33%	1
September 2010	1021	2.00	505	1.00	1526	3	67%	33%	1
Total		**5.00**		**1.00**		**6**	**83%**	**17%**	**2**

Table 4.42 The Dietary ionic effects on gender - Summary

Season	Duration	Female	%	Males	%	Totals	%	No delivery	p/del
Rainy season	10	575	56	272	54	847	56	94	9.01
Dry season	12	365	36	188	37	553	36	64	8.64
Cold season	4	81	8	45	9	126	8	14	9.00
Totals	**26**	**1021**		**505**		**1526**		**172**	**8.87**

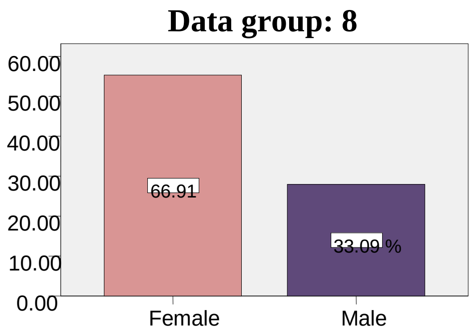


Figure 4.47 The gender representation with 35.57% females 63.43% males

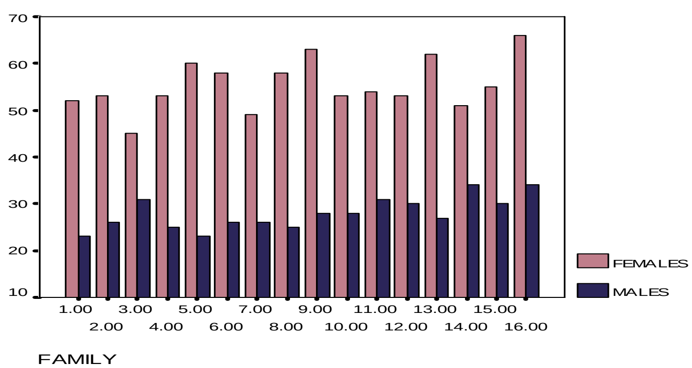


Figure 4.48 The female and male pups delivered per family

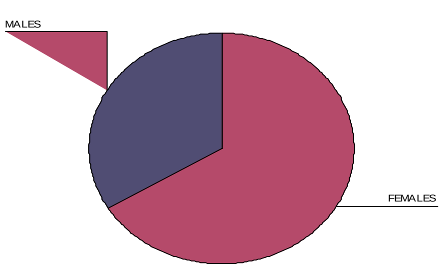


Figure 4.49 The female to male sex ratios 4:6

In group eight, there were a total of 1526 pups delivered comprising of 1021 females and 505 males representing 66.91% and 33.09% respectively at the rate of 8.97pups per delivery. There was a statistical significant gender difference between the female and the male offspring.

Dietary study. Study group nine - Cocktail of all the above elements

Table 4.43 Dietary chemical composition and their ionic effects on sex ratios

DATE	FEMALE		MALES		TOTAL	F %	M %	No delivery
August 2008	9	9.00	10	10.00	19	51%	49%	2
September 2008	38	29.00	35	25.00	73	52%	48%	6
Totals		**38.00**		**35.00**	**73**	**52%**	**48%**	**8**

October 2008	151	113.00	147	112.00	298	45.00	51%	49%	24
November 2008	228	77.00	229	82.00	457	29.00	50%	50%	20
December 2008	308	80.00	314	85.00	622	36.00	49%	51%	18
Totals		**270.00**		**279.00**	**549**		**49%**	**51%**	**62**

January 2009	347	39.00	374	60.00	721	45.00	48%	52%	15
February 2009	365	18.00	401	27.00	766	45.00	47%	53%	5
March 2009	386	21.00	425	24.00	811	45.00	47%	53%	5
Totals		**78.00**		**111.00**	**189**		**42%**	**58%**	**25**

April 2009	400	14.00	450	25.00	850	52.00	47%	53%	4
May 2009	415	15.00	470	20.00	885	48.00	47%	53%	5
Totals		**29.00**		**45.00**	**74**		**34%**	**66%**	**9**

June 2009	426	11.00	488	18.00	914	29.00	42%	58%	3
July 2009	458	32.00	504	16.00	962	48.00	43%	57%	6
Totals		**43.00**		**34.00**	**77**		**55%**	**45%**	**9**

August 2009	465	7.00	518	14.00	983	21.00	43%	57%	2
September 2009	477	12.00	527	9.00	1004	21.00	43%	57%	2
Totals		**19.00**		**23.00**	**42**		**45%**	**55%**	**4**

October 2009	480	3.00	531	4.00	1011	7.00	43%	57%	1
November 2009	484	4.00	534	3.00	1018	7.00	43%	57%	1
December 2009	504	20.00	554	20.00	1058	40.00	43%	57%	5
Totals		**27.00**		**27.00**	**54**		**61%**	**39%**	**7**

January 2010	509	5.00	559	5.00	1068	10.00	48%	52%	2
February 2010	515	6.00	566	7.00	1081	13.00	47%	53%	3
March 2010	522	7.00	572	6.00	1094	13.00	47%	53%	2
Totals		**18.00**		**18.00**	**36**		**50%**	**50%**	**7**

April 2010	532	10.00	583	11.00	1115	21.00	47%	53%	2
May 2010	535	3.00	594	11.00	1129	14.00	47%	53%	1
Totals		**13.00**		**22.00**	**35**		**37%**	**63%**	**3**

June 2010	539	4.00	601	7.00	1140	11.00	47%	53%	1
July 2010	549	10.00	603	2.00	1152	12.00	47%	53%	1
Totals		**14.00**		**9.00**	**23**		**60%**	**40%**	**2**

August 2010	556	7.00	612	9.00	1168	16.00	47%	53%	1
September 2010	560	4.00	616	4.00	1176	8.00	50%	50%	1
Totals		**11.00**		**13.00**	**24**		**47%**	**53%**	**2**

Table 4.44 Study group nine - Cocktail of all the above elements

Season	Duration	Female	%	Males	%	Totals	%	No delivery	p/del
Rainy season	10	338	60	378	61	716	61	81	8.83
Dry season	12	165	29	195	32	360	31	46	7.82
Cold season	4	57	11	43	7	100	8	11	9.09
Totals	**26**	**560**		**616**		**1176**		**138**	**8.52**

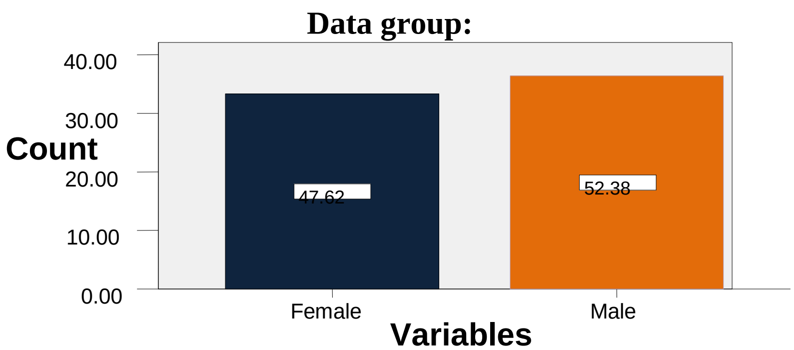


Figure 4.50 The gender representation with 35.57% females 63.43% males

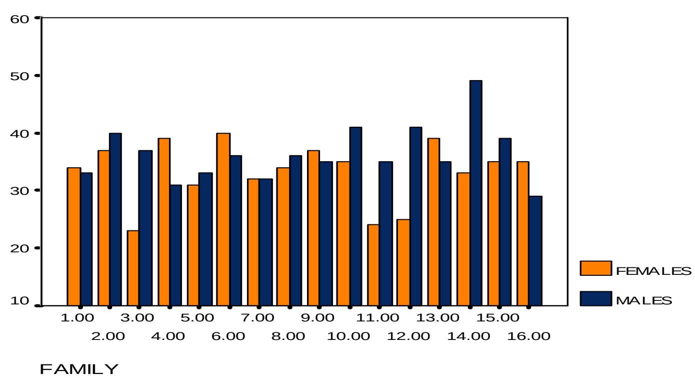


Figure 4.51 The female and male pups delivered per family

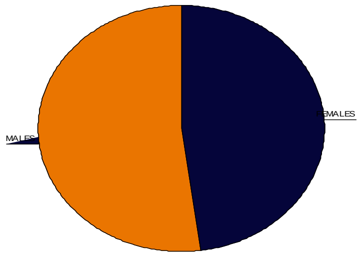


Figure 4.52 The female to male sex ratios 4:6

In group nine, there were a total of 1176 pups delivered comprising of 560 females and 616 males representing 47.62% and 52.38% respectively at the rate of 8.52 pups per delivery. There was no significant statistical gender difference between the female and male progeny with a two-tailed p-value of 0.0609 indicating balanced or absence of dietary ionic gender influence.

Table 4.45 Summary statistics of all the above elements

Groups.	**Total deliveries.**		**Total females**	**Total males**	**Total Pups/delivery**	**Females / delivery**	**Males / delivery**
1	176	1436	724	712	8.2	4.1	4.1
2	170	1446	622	824	8.5	3.7	4.8
3	173	1471	538	933	8.4	3.1	5.3
4	172	1500	581	919	7.8	3.1	4.7
5	174	1473	885	588	8.3	5.0	3.3
6	176	1496	906	590	8.5	5.1	3.4
7	177	1516	508	1008	8.5	2.8	5.7
8	172	1526	1021	505	8.0	5.0	3.0
9	138	1176	960	616	8.5	4.1	4.4
	1528	13,040	6,727	6,674			
			6855				

Total - 1528 - 13040/1528 - 8.5 pups per delivery.

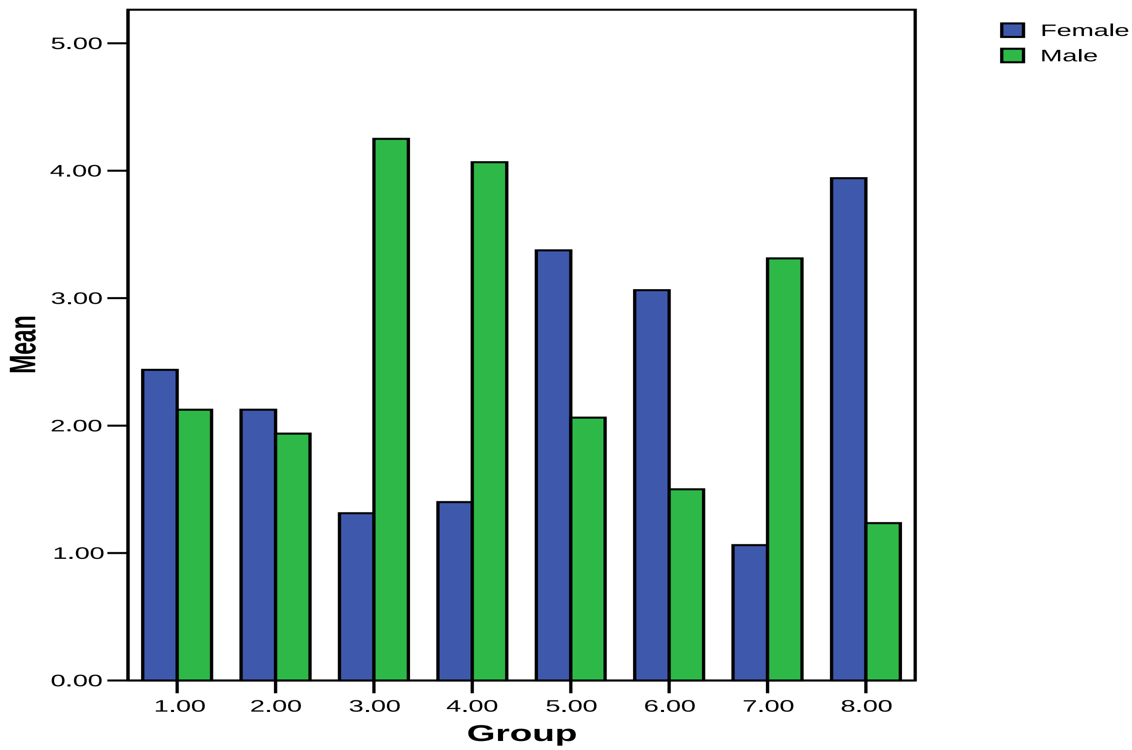


Figure 4.53 Summary of the eight pilot study groups

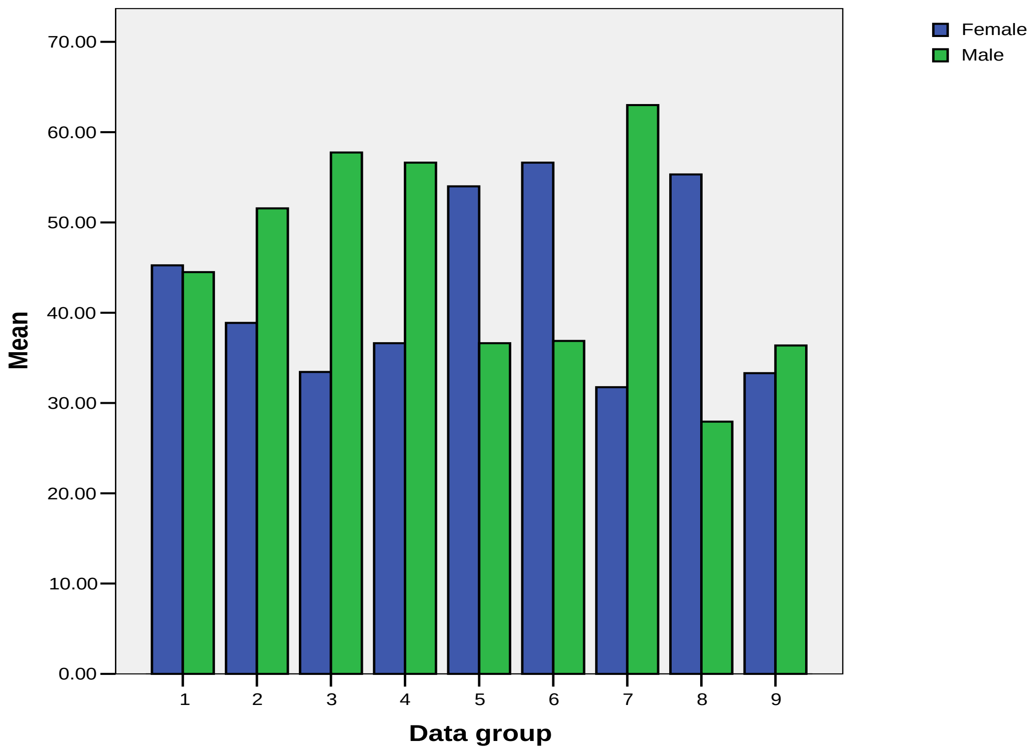


Figure 4.54 Summary of the nine main study groups

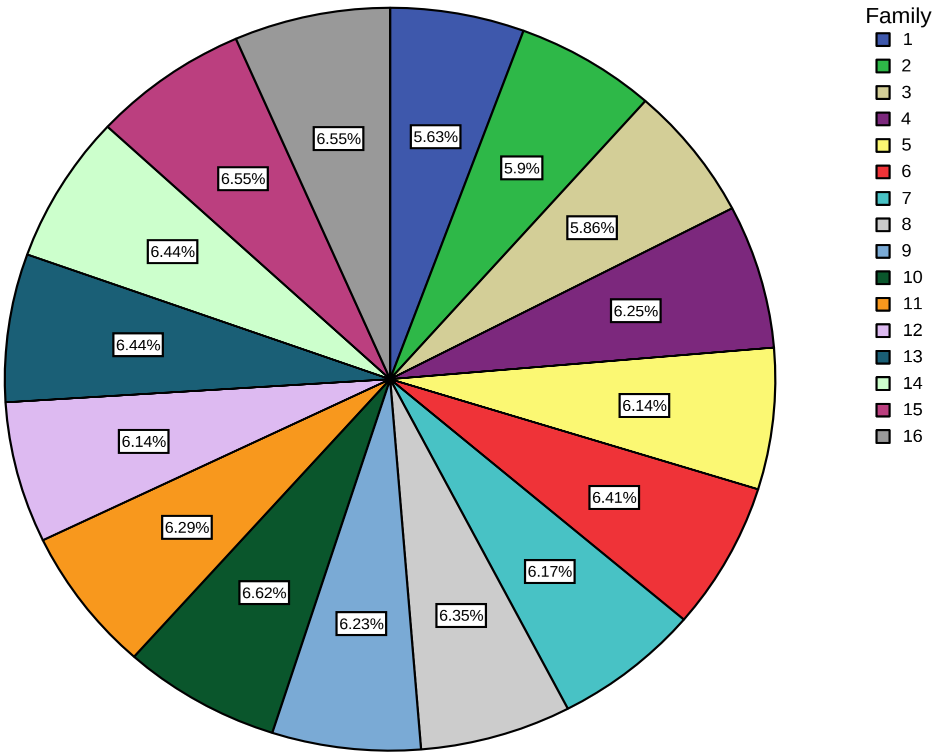


Figure 4.55 Percentage population distributions among the 16 families of the main study

Comparing the pilot and the main study:-

Control groups

- The control group ***one*** (N_1) had similar sex ratio outcomes without influence over the sex ratios.
- ***In contrast***
- The control group ***two*** with glucose (N_2) had influence over sex ratio outcomes towards the male progeny.

Combined or double groups.

The following *status quo* was maintained.

- Groups ***three, four and seven*** ($N_{3\ \&\ 4\ \&\ 7}$) had significant sex ratios skewed towards the male progeny. .
- Groups ***five six and eight*** ($N_{5\ \&\ 6\ \&\ 8}$) had significant sex ratios skewed towards the female progeny.

All-inclusive - Cocktail.

- Group ***nine*** (N_9) the cocktail of all the chemicals had no influence over sex ratio outcomes.

Comments

The study reaffirms the social allegations that there are factors in our diets that do influence sex ratio outcomes and that these factors could be chemically mediated.

It further reaffirms that:

Sodium and potassium do influence sex ratios towards the male gender.

Calcium and magnesium do influence sex ratios towards the female gender.

The new finding in the main study does go further to throw light into long standing allegations that sweet things do influence sex ratios towards the females but our study conveys the very opposite message that the influence with glucose is actually towards the male progeny.

The finding among the all-inclusive or cocktail does bring in an interesting natural aspect of sex ratio outcomes where the effects of various chemical elements may tend to cancel each other and modulate sex ratios towards 1:1 ratios.

The next second phase of the study looks into details as to how these chemicals do influence the gender outcomes.

The last and third phase of the study (Post-doctoral) will take the influenced oocytes of the second phase fertilize them and examine whether they will translate into the expected gender.

4.26 Dietary study

PCR/DNA method of early gender determination and early mortality trends. Sex ratio determination was done by using either physical makers or the PCR. To avoid losing data counting of the pups and cutting (DNA) tail materials were done within 24 hours of delivery.

Methods

To avoid losing data counting of all the pups and cutting (DNA) tail material was done within 24 hours of delivery. Sex determination by physical means was done using the genital anal distance in a female which is about 1 ml and in the male twice this distance. The optimal time for the physical gender determination is three weeks of age. As it is not possible to know early which pups require PCR and those that don't, a 2 – 3 mm piece of the tail of each pup was cut aseptically and put in a small polythene paper containing 8 molar urea solutions for preservation.

Sex determination by physical means was done using the physical makers based on the difference between the male and female anal-genital distance. This distance in a female is about 1mm while that of the male is twice this distance. The optimal time for physical sex determination is three weeks of age. Only those pups whose gender could not be determined physically because they died before attaining this age were subjected to PCR. For further reaffirmation of the physical determination method, a random sampling of 50 samples that had been determined by physical means was subjected to DNA which confirmed the same results that had been done by physical means.

DNA extraction, PCR and running of gels for gender determination were done with consultations of Institute of Primate Research (IPR, Nairobi), Department of Animal Physiology, University of Nairobi and ILRI. The reagents were ordered from Belgium through the latter institute. Ordering method of the reagents was done through primer procurement services agents who offer these services for the institute.

A total of 415 pups out of 13,040 perished before gender could be physically ascertained and their DNA materials subjected to PCR analysis. This constituted approximately 3.1% of the total pups delivered. The details and distribution of this is shown among the results presented below with coloured numbers. The females are represented by the traditional pink colour and the males by the blue colour. The samples from the demised pups from the respective groups were subjected to PCR for gender determination and their results are tabulated below. For those that were eaten up and had no DNA samples for testing their numbers were calculated as follows. All tails of all pups were cut and preserved and therefore deliveries with missing pups had all the samples subjected to DNA then routine physical sex determination was done. The difference constituted the missing pups.

Table 4.46 Dietary study. Group one - Control water solution

- PCR/DNA/ Physical methods of gender determination

Families	Physical gender determination (T : F : M)	PCR – Gender determination Females	PCR - Gender determination Males	Totals Demised	Total deliveries (T : F : M)
1	84:40:44	1	2	3	87:41:46
2	80:43:37	0	2	5	82:43:39
3	71:35:36	1	2	8	74:36:38
4	79:34:45	2	1	11	82:36:46
5	92:45:47	1	1	13	94:46:48
6	95:49:46	2	1	16	98:51:47
7	75:40:35	2	2	20	79:42:37
8	89:46:43	1	3	24	93:47:46
9	79:44:35	0	1	25	80:44:36
10	86:46:40	1	1	27	88:47:41
11	95:50:55	2	2	31	99:52:57
12	89:44:45	1	0	32	90:45:45
13	95:48:47	1	2	35	98:49:49
14	95:38:55	1	1	37	95:39:56
15	93:51:42	1	3	41	97:52:45
16	98:53:45	1	1	43	100:54:46
Totals	1393: 706 :687	18	25	43	1436: 724 :712

Gender determination per family done by physical means in black and by PCR in colour representing 18 females and 25 males.

Table 4.47 Dietary study. Group one - Control water solution

- PCR/DNA/Physical methods of gender determination

Generations	Physical gender determination (T : F : M)	PCR Females	PCR Males	Totals	Total deliveries (T : F : M)
1	158:75:83	2	0	2	160:77:83
2	134:73:63	1	2	3	139:74:65
3	119:61:58	3	1	4	123:64:59
4	144:74:70	2	1	3	148:75:73
5	147:75:72	0	1	1	148:75:73
6	132:62:70	3	3	6	138:65:73
7	135:63:72	0	3	3	138:63:75
8	128:69:59	1	2	3	131:70:61
9	121:58:63	3	0	3	124:61:63
10	92:54:38	3	2	5	97:57:40
11	55:29:26	0	6	6	61:29:32
12	25:14:11	0	4	4	29:14:15
	1393:706:687	18	25	43	1436:724:712

Gender distribution per generation done by physical means represented in black and by PCR in colour showcasing a range of between 2 - 6 demised pups among the generations.

Table 4.48 Dietary study - Control water solution

PCR/DNA/ Physical gender determination methods - Summary statistics.

	Group	Generation	Females	Males
Mean	1.0000	6.5000	1.5000	2.0833
Std. Deviation	0.00000	3.60555	1.31426	1.72986
Variance	0.00000	13.00000	1.72727	2.99242
Sum	12.00	78.00	18.00	25.00

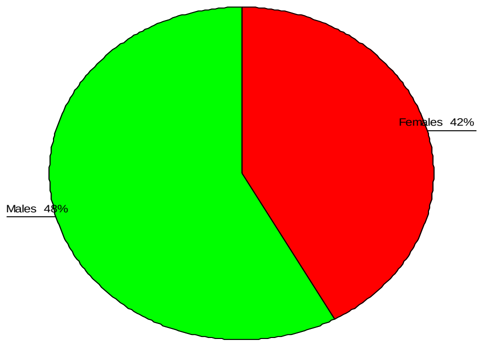


Figure 4.56 Gender distributions - 42% female and 48% males

In group one, there were a total of 1436 pups comprising of 724 females and 712 males representing 50.42% and 49.58% respectively. Represented in colour are 43 pups constituting 2.99% of the total that perished before physical gender determination could be done and PCR done. Of the demised were 18(42%) females and 25 (48%) males. Of the demised, no significant statistical gender difference was observed. p>0.66.

Table 4.49 Dietary study

Group two - Glucose solution - PCR/DNA/Physical methods of gender determination

Families	Physical gender determination (T : F : M)	Demised Females	Demised Males	Totals	Total deliveries (T : F : M)
1	82:33:49	1	0	1	83:34:49
2	75:34:41	1	3	5	79:35:44
3	97:44:53	0	1	6	98:44:54
4	97:41:57	0	1	7	98:41:57
5	92:38:54	1	2	10	95:39:56
6	97:41:56	1	0	11	98:42:56
7	91:37:54	2	2	15	95:39:56
8	89:43:46	1	1	17	91:44:47
9	86:37:49	1	2	20	89:38:51
10	97:45:52	0	2	22	99:45:54
11	80:37:43	1	2	25	83:38:45
12	86:36:50	0	1	26	87:36:51
13	83:37:46	1	3	30	87:38:49
14	92:39:53	1	0	31	93:40:53
15	81:29:52	0	1	32	82:29:53
16	89:40:49	0	0		89:40:49
	1436:724:712	11	21	32	1446:622:824

Gender determination by physical means in black and by PCR in colour among families.

Table 4.50 - Dietary study

Group two - Glucose solution - PCR/DNA/Physical methods of gender determination

Generation	Physical gender determination (T : F : M)	PCR Females	PCR Males	Totals	Total deliveries (T : F : M)
1	146:76:70	2	2	4	150:78:72
2	148:65:83	1	0	1	149:66:83
3	136:62:74	2	2	4	140:64:76
4	153:65:88	1	1	2	155:66:89
5	147:65:82	0	1	1	148:65:83
6	137:55:82	1	6	7	144:56:88
7	135:51:84	1	2	3	138:52:86
8	131:55:76	2	1	3	134:57:77
9	123:57:66	0	4	4	127:57:70
10	73:27:46	0	2	2	75:27:48
11	55:24:31	1	0	1	56:25:31
12	30:12:18	0	0	0	30:12:18
	1414:614:800	11	21	32	1446:625:821

Gender determination done by physical means shown in black and by PCR in colour among generations.

Table 4.51 Group two - Glucose solution.

PCR/DNA/ Physical gender determination methods - summary statistics

	Group	Generation	Females	Males
Mean	2.0000	6.5000	0.9167	1.7500
Std. Deviation	0.00000	3.60555	0.79296	1.76455
Variance	0.00000	13.00000	0.62879	3.11364
Sum		78.00	11.00	21.00

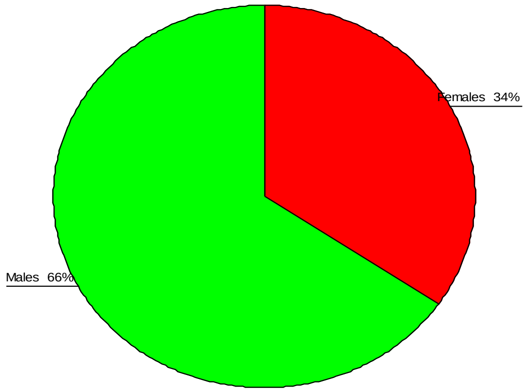


Figure 4.57 Gender distribution - 66% males and 34% females

In group two, there were a total of 1446 pups delivered comprising of 622 females and 824 males representing 43.02% and 56.98% respectively. Represented in colour are 32 pups comprising of 11 (34%) females and 21 (66%) males constituting 2.2% of the total pups that perished before physical gender determination could be done and PCR was carried out. No statistical significant gender difference was observed among the demised (p>0.166).

Table 4.52 Dietary study

Group three - Sodium solution. PCR/DNA/Physical methods of gender determination

Group	Physical gender determination (T : F : M)	Demised females PCR	Demised males PCR	Total	Total deliveries (T : F : M)
1	79:29:50	1	1	2	81:30:51
2	78:26:52	0	1	3	79:26:53
3	96:32:64	1	1	7	98:33:65
4	93:33:60	2	1	10	96:35:61
5	86:36:50	1	1	12	88:37:51
6	85:28:57	2	0	14	87:30:57
7	98:37:61	0	2	16	100:37:63
8	87:36:51	1	1	18	89:37:52
9	89:39:50	0	2	20	91:39:52
10	98:34:64	2	2	24	102:36:66
11	87:26:61	2	2	28	91:28:63
12	93:32:61	1	2	31	96:33:63
13	77:28:49	2	1	34	80:30:50
14	84:27:57	1	1	36	86:28:58
15	106:40:66	1	1	38	108:41:67
16	96:37:59	1	2	41	99:38:61
	1432:520:912	18	21	39	1471:538:933

Gender determination done by physical means in black and by PCR in colour among families.

Table 4.53 Dietary study

Group three - Sodium solution. PCR/DNA/Physical methods of early gender determination

Generation	Physical gender determination (T : F : M)	PCR Females	PCR Males	Totals	Total deliveries (T : F : M)
1	148:43:105	2	1	3	151:45:106
2	139:52:87	2	1	3	142:54:88
3	143:46:97	2	0	2	145:48:97
4	154:52:102	2	3	5	159:54:105
5	146:46:100	2	2	4	150:48:102
6	140:56:84	1	2	3	143:57:86
7	140:59:78	1	3	4	141:60:81
8	125:58:67	1	3	4	129:59:70
9	126:40:86	2	2	4	130:42:88
10	93:31:62	2	2	4	97:33:64
11	57:26:31	1	1	2	59:27:32
12	24:11:13	0	1	1	25:11:14
	1432:520:912	18	21	39	1471:538:933

Gender determination done by physical represented in black and by PCR in colour among generations.

Table 4.54 Dietary study Group 3 - Sodium solution

PCR/DNA/ Physical gender determination methods - summary statistics.

	Group	Generation	Females	Males
Mean	3.0000	6.5000	1.5000	1.7500
Std. Deviation	0.00000	3.60555	0.67420	0.96531
Variance	0.00000	13.00000	0.45455	0.93182
Sum	36.00	78.00	18.00	21.00

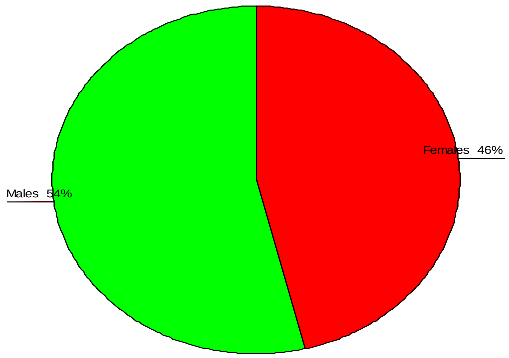


Figure 4.58 Gender distributions - 45% female and 55% males

In group three, there were a total of 1471 pups comprising of 538 females and 933 males representing 36.57% and 63.43% respectively. 39 pups constituting 2.65 % of the total pups perished before physical gender determination could be done and PCR was done. Of the demised were 18 (45%) females and 21 (55%) with no significant statistical gender difference (p>0.49).

Table 4.55 Dietary study

Group four - Potassium solution PCR/DNA/physical methods of gender determination.

Families	Physical gender determination (T : F : M)	Demised females	Demised males	Total	Total deliveries (T : F : M)
1	83: 24:59	2	1	3	86: 26:60
2	86:35:51	1	2	3	89:36:53
3	79:26:53	2	1	3	82:28:54
4	108:40:68	1	2	3	111:41:70
5	103:38:65	2	2	4	107:40:67
6	89:33:56	1	2	3	92:34:58
7	87:38:50	1	3		91:39:53
8	84:32:52	3	3	6	90:35:55
9	91:34:57	2	2	4	95:36:59
10	100:36:64	2	3	5	105:38:67
11	84:35:49	3	3	6	90:38:52
12	89:36:53	2	2	4	93:38:55
13	92:39:53	1	3	4	96:40:56
14	92:35:57	2	0	2	94:37:57
15	81:37:44	1	2	3	84:38:46
16	90:37:53	2	3	5	95:39:56
	1438 : 553 : 885	28	34	62	1500 : 581 : 919

Gender distribution by physical means in black and by PCR in colour among families.

Table 4.56 Dietary study

Group four - Potassium solution PCR/DNA/physical methods of gender determination.

Generation	**Physical gender determination (T : F : M)**	**PCR Females**	**PCR Males**	**Totals**	**Total deliveries (T : F : M)**
1	140:45:95	3	2	5	145:48:97
2	142:43:99	3	4	7	149:46:103
3	148:52:96	3	3	6	154:55:99
4	151:53:98	3	3	6	157:56:101
5	147:63:84	3	1	4	151:66:85
6	146:72:74	2	2	4	150:74:76
7	136:67:69	3	2	5	141:70:71
8	120:60:60	2	4	6	126:62:64
9	127:38:89	2	2	4	131:40:91
10	106:35:71	2	2	4	110:37:73
11	57:20:37	1	4	5	62:21:41
12	18:5:13	1	5	6	24:6:18
	1438:553:885	28	34	62	1500:581:919

Gender determination by physical means in black and by PCR in colour among generations

Table 4.57 Dietary study

1) PCR/DNA/ Physical Gender determination methods - summary statistics.

	Group	Generations	Females	Males
Mean	4.0000	6.5000	2.3333	2.8333
Std. Deviation	.00000	3.60555	.77850	1.19342
Variance	.00000	13.00000	.60606	1.42424
Sum	4.000	78.00	28.00	34.00

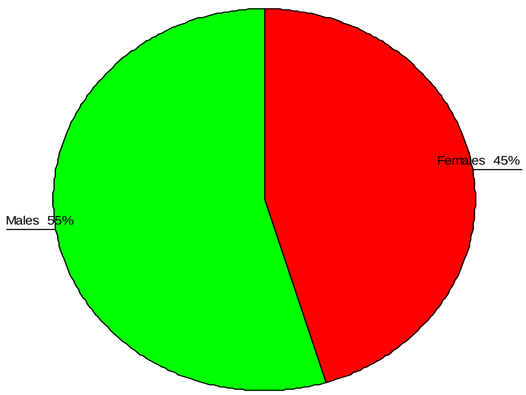


Figure 4.59 Gender distribution - 45% female and 55% males

In group four, there were a total of 1500 pups comprising of 581 females and 919 males representing 38.73% and 61.27 respectively. Sixty-two (4.14 %) pups perished before physical gender determination could be done and PCR was done. Of the demised were 28 (45%) females and 34 (55%) males with no significant statistical gender difference (p>0.339).

Table 4.58 Dietary study

Group five - Calcium solution. PCR/DNA/Physical methods of gender determination.

Deliveries	**Physical gender determination (T : F : M)**	**Demised females**	**Demised males**	**Totals**	**Total deliveries (T : F : M)**
1	81:49:32	1	2	3	84:50:34
2	98:66:32	0	1	1	99:66:33
3	85:49:36	1	1	2	87:50:37
4	81:49:32	3	2	5	86:52:34
5	87:50:37	1	1	2	89:51:38
6	97:60:37	1	2	3	100:61:39
7	72:43:29	3	2	5	77:46:31
8	95:56:39	0	1	1	96:56:40
9	89:47:42	0	1	1	90:47:43
10	101:58:43	2	2	4	105:60:45
11	86:53:33	1	2	3	89:54:35
12	76:47:29	2	1	3	79:49:30
13	102:64:38	1	2	3	105:65:40
14	89:55:34	2	1	3	92:57:35
15	94:53:41	1	1	2	96:54:42
16	99:67:32	0	0	0	99:67:32
	1432:866:566	19	22	41	1473:885:588

Gender distribution by physical means in black and by PCR in colour among families.

Table 4.59 Dietary study Group five - Calcium solution

Group five - Calcium solution PCR/DNA/physical methods of gender determination

Generation	Physical gender determination (T : F : M)	PCR Females	PCR Males	Totals	Total deliveries (T : F : M)
1	135:88:47	2	2	4	139:90:49
2	146:91:55	2	2	4	150:93:57
3	146:93:53	1	2	3	149:94:55
4	146:91:55	2	2	4	150:93:57
5	141:82:59	1	3	4	145:83:62
6	142:73:69	1	1	2	144:74:70
7	130:67:63	3	2	5	135:70:65
8	129:71:58	1	2	3	132:72:60
9	129:90:39	2	1	3	132:92:40
10	108:67:41	1	1	2	110:68:42
11	55:36:19	0	2	2	57:36:21
12	28:18:10	0	2	2	30:18:12
	1435:869:566	16	22	38	1473:885:588

Gender determination by physical means in black and by PCR in colour among generations.

Table 4.60 Dietary study

PCR/DNA/ Physical Gender determination methods - Summary statistics.

	Group	Generation	Females	Males
Mean	5.00	6.5000	1.5833	1.8333
Std. Deviation		3.60555	1.37895	0.57735
Variance		13.00000	1.90152	0.33333
Sum		78.00	19.00	22.00

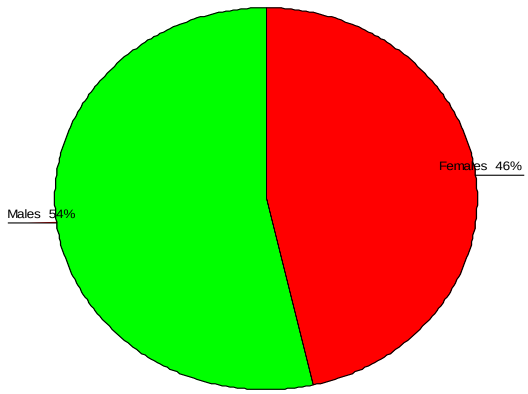


Figure 4.60 Gender distribution - 46% female and 54% males

In group five, there were a total of 1473 pups comprising of 885 females and 588 males representing 59.99% and 40.01% respectively. Represented in colour are 41 pups constituting 2.78% of the total pups who perished before physical gender determination could be done and PCR was done. Of the demised were 19 (46%) females and 22 (54%) males with no statistical significant gender difference (p>0.612).

Table 4.61 Dietary study

Group six - Magnesium solution. PCR/DNA/Physical methods of gender determination

Family	Physical gender determination (T : F : M)	Demised females	Demised males	Totals	Total deliveries (T : F : M)
1	82:48:34	1	0	1	83:49:34
2	93:54:39	2	1	3	96:56:40
3	73:46:27	1	2	3	76:47:29
4	87:55:32	2	1	3	90:57:33
5	80:48:32	2	2	4	84:50:34
6	76:48:28	3	2	5	81:51:30
7	99:62:37	2	1	3	102:64:38
8	93:58:35	0	1	1	94:58:36
9	98:56:42	3	2	5	103:59:44
10	91:55:36	2	0	2	93:57:36
11	94:57:37	1	1	2	96:58:38
12	96:57:39	2	2	4	100:59:41
13	92:57:35	1	0	1	93:58:35
14	97:56:41	2	1	3	100:58:42
15	110:72:38	1	2	3	113:73:40
16	91:51:40	1	0	1	92:52:40
	1452:880:572	26	18	44	1496:906:590

Gender determination per generation – Physical means shown in black and by PCR in colour.

Table 4.62 Dietary study

Group six - Magnesium solution PCR/DNA/physical methods of early gender determination

Generations	**Physical gender determination (T : F : M)**	**PCR gender determination**	**PCR gender determination**	**Totals**	**Total deliveries (T : F : M)**
1	137:82:55	3	2	5	142:85:57
2	138:84:54	2	1	3	141:86:55
3	152:89:63	3	2	5	157:92:65
4	150:89:61	2	0	2	152:91:61
5	147:82:65	3	3	6	153:85:68
6	146:83:63	2	2	4	150:85:65
7	132:86:46	2	2	4	136:88:48
8	127:84:43	3	2	5	132:87:45
9	116:79:37	2	1	3	119:81:38
10	113:64:49	2	2	4	117:66:51
11	67:42:25	1	1	2	69:43:26
12	27:16:11	1	0	1	28:17:11
Totals.	1452:880:572	26	18	44	1496:906:590

Gender determination by physical means in black and by PCR in colour among generations.

Table 4.63 Dietary study

PCR/DNA/ Physical Gender determination methods - Summary statistics.

	Group	Generation	Females	Males
Mean	6.0000	6.5000	2.1667	1.5000
Std. Deviation	0.00000	3.60555	0.71774	0.90453
Variance	0.00000	13.00000	0.51515	0.81818
Sum		78.00	26.00	18.00

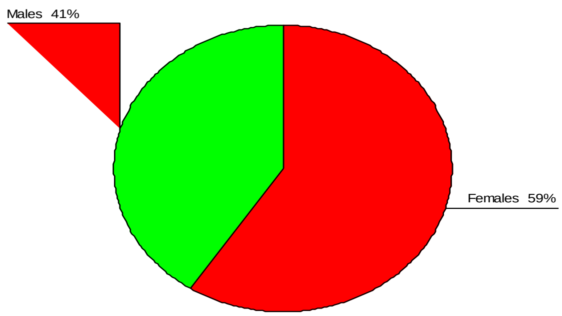


Figure 4.61 Gender distribution - 41% female and 59% male

In group six, there were a total of 1496 pups comprising of 906 females and 590 males representing 60.56% and 39.44% respectively. Represented in colour are 44 pups constituting 2.94% of the total pups who perished before physical gender determination could be done and PCR was done. Of the demised were 18 (46%) females and 26 (59%) males. Significant statistical difference was observed between the genders with a p-value of 0.005.

Table 4.64 Dietary study

Group seven - Sodium / Potassium solution. PCR/DNA/Physical methods of gender determination.

Generation	Physical gender determination (T : F : M)	PCR Females	PCR Males	Totals	Total deliveries (T : F : M)
1	136:39:97	1	3	4	140:40:100
2	123:33:90	4	3	7	130:37:93
3	157:46:111	1	1	2	159:47:112
4	148:49:99	1	4	5	153:50:103
5	143:46:97	3	2	5	148:49:99
6	147:74:73	2	3	5	152:76:76
7	131:57:74	4	3	7	138:61:77
8	131:47:84	1	1	2	133:48:85
9	129:37:92	2	3	5	134:39:95
10	117:34:83	1	3	4	121:35:86
11	79:19:60	1	2	3	82:20:62
12	25:6:19	0	1	1	26:6:20
	1466:487:979	21	29	50	1516:508:1008

Gender determination per family – Physical means are shown in black and by PCR in colour.

Table 4.65 Dietary study

Group seven - Sodium / Potassium solution. PCR/DNA/Physical methods of gender determination

Generation	Physical gender determination (T : F : M)	PCR Females	PCR Males	Totals	Total deliveries (T : F : M)
1	136:39:97	1	3	4	140:40:100
2	123:33:90	4	3	7	130:37:93
3	157:46:111	1	1	2	159:47:112
4	148:49:99	1	4	5	153:50:103
5	143:46:97	3	2	5	148:49:99
6	147:74:73	2	3	5	152:76:76
7	131:57:74	4	3	7	138:61:77
8	131:47:84	1	1	2	133:48:85
9	129:37:92	2	3	5	134:39:95
10	117:34:83	1	3	4	121:35:86
11	79:19:60	1	2	3	82:20:62
12	25:6:19	0	1	1	26:6:20
	1466:487:979	21	29	50	1516:508:1008

Gender determination per generation – Physical means are shown in black and by PCR in colour.

Table 4.66 Dietary study

PCR/DNA/ Physical Gender determination methods - Summary statistics

	Group	Generation	Females	Males
Mean	7.0000	6.5000	1.7500	2.4167
Std. Deviation	0.00000	3.60555	1.28806	0.99620
Variance	0.00000	13.00000	1.65909	0.99242
Sum		78.00	21.00	29.00

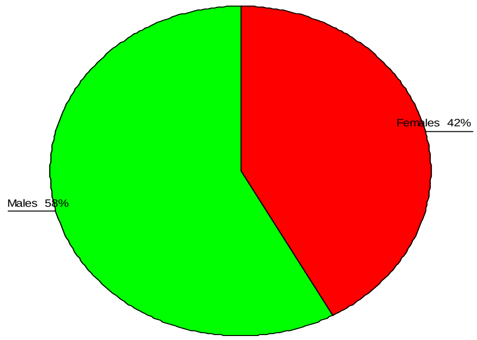


Figure 4. 62 Gender distribution - 42% female and 58% males

In group seven, there were a total of 1516 pups comprising of 508 females and 1008 males representing 33.51% and 66.49% respectively. Represented in colour are 50 pups constituting 3.298% of the total pups who perished before physical gender determination could be done and PCR was done. Of the demised 21 (42%) were females and 29 (58%) males. No significant statistical difference was observed between the genders with a p-value of 0.104.

Table 4.67 Dietary study

Group eight - Calcium / Magnesium solution. PCR/DNA/Physical methods of gender determination.

Family	Grand totals (T : F : M)	Demised females	Demised males	Totals	Total deliveries (T : F : M)
1	87:62:25	1	1	2	89 :63:26
2	99:67:32	3	0	3	102:70: 32
3	86:52:34	1	1	2	88:53:35
4	83:57:26	1	2	3	86:58:28
5	79:57:22	3	1	4	83:60:23
6	104:73:31	1	1	2	106:74:32
7	78:49:29	2	0	2	80:51:29
8	96:68:28	2	1	3	99:70:29
9	95:66:29	1	1	2	97:67:30
10	104:69:35	0	0	0	104:69:35
11	90:58:32	1	2	3	93:59:34
12	95:61:34	2	1	3	98:63:35
13	114:80:34	0	0	0	114:80;34
14	93:55:38	1	0	1	94:56:38
15	93:62:31	0	0	0	93:62:31
16	98:64:34	2	0	2	100:66:34
	1494 : 1000 : 494	21	11	32	1526 : 1021 : 505

Gender determination per family – Physical means are shown in black and by PCR in colour.

Table 4.68 Dietary study

Group eight - Calcium / Magnesium solution PCR/DNA/Physica method of gender determination.

Generations	**Totals (T : F : M)**	**PCR Females**	**PCR Males**	**Totals**	**Total deliveries (T : F : M)**
1	149:101:48	2	1	3	103:49
2	145:103:42	3	3	6	106:45
3	153:108:45	0	0	0	108:45
4	147;92:55	2	1	3	94:56
5	147:96:51	3	0	3	99:51
6	150:86:64	2	0	2	88:64
7	136:81:55	2	1	3	83:56
8	130:88:42	3	0	3	91:42
9	133:96:37	2	2	4	98:39
10	127:97:30	2	3	5	99:33
11	56:36:20	0	0	0	36:20
12	24:18:6	0	0	0	18:6
	1494:1000:494	21	11	32	1526:1021:505

Gender determination per generation – Physical means are shown in black and by PCR in colour.

Table 4.69 Dietary study

PCR/DNA/ Physical Gender determination methods - Summary statistics

	Group	Generation	Females	Males
Mean	8.0000	6.5000	1.7500	0.9167
Std. deviation	0.00000	3.60555	1.13818	1.16450
Variance	0.00000	13.00000	1.29545	1.35606
Sum		78.00	21.00	11.00

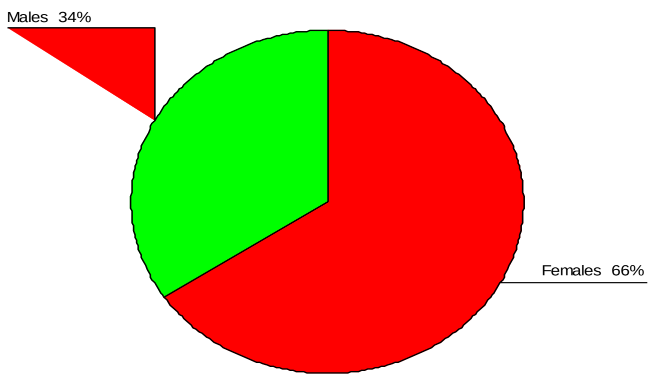


Figure 4.63 Gender distributions - 66% female and 34% males

In group eight, there were a total of 1526 pups comprising of 1021 females and 505 males representing 66.91% and 33.09% respectively. 32 (2.16%) of the total pups perished before physical gender determination could be done and PCR was done. Of the demised 21 (66%) were females and 11 (34%) males. Statistical significant gender difference was observed among these p>0.044.

Table 4.70 Dietary study

Group nine – All-inclusive solution. PCR/DNA/Physical methods of gender determination

Family	Total deliveries (T : F : M)	Demised females	Demised males	Totals	Total deliveries (T : F : M)
1	65:32:33	3	0	3	68:35:33
2	81:40:41	1	3	4	85:41:44
3	62:22:34	3	3	6	62:25:37
4	68:37:29	4	4	8	76:41:33
5	60:28:32	3	1	4	64:31:33
6	82:45:37	1	6	7	89:46:43
7	60:28:32	4	0	4	64:32:32
8	64:32:32	3	3	6	70:35:35
9	73:37:36	1	4	5	78:38:40
10	74:33:41	2	0	2	76:35:41
11	58:23:35	1	0	1	59:24:35
12	63:23:40	2	1	3	66:25:41
13	78:43:35	2	6	8	86:45:41
14	79:30:49	3	0	3	82:33:49
15	71 :32:39	3	0	3	74 :35:39
16	72:37:35	2	3	5	77:39:38
	1104:522:582	38	34	72	1176:560:616

Gender determination by physical means in black and PCR in colour per family.

Table 4.71 Dietary study

Group nine – All-inclusive solution. PCR/DNA/Physical methods of gender determination.

Generation	Physical gender determination (T : F : M)	PCR Females	PCR Males	Total
1	131:65:66	4	1	5
2	134:69:65	5	1	6
3	132:64:68	6	0	6
4	128:60:68	3	4	7
5	140:64:76	1	3	4
6	131:62:69	5	1	6
7	135:52:83	6	1	7
8	123:52:71	3	1	4
9	72:36:36	3	6	9
10	33:16:17	1	10	11
11	10:5:5	0	4	4
12	7:3:4	1	2	3
	1176:560:616	38	34	72

Gender determination done by physical means shown in black and by PCR in colour among generations.

Table 4.72 Dietary study

PCR/DNA/ Physical Gender determination methods - Summary statistics

	Group	Generation	Females	Males
Mean	9.0000	6.5000	3.1667	2.8333
Std. Deviation	0.00000	3.60555	2.08167	2.85509
Variance	0.00000	13.00000	4.33333	8.15152
Sum		78.00	38.00	34.00

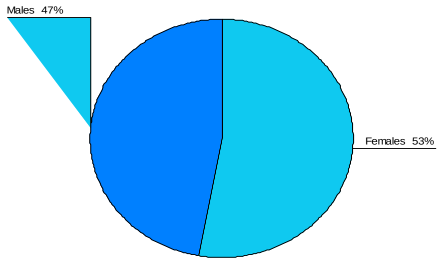


Figure 4.64 Gender distributions - 53% female and 47% male

In group nine, there were a total of 1176 pups comprising of 560 females and 616 males representing 47.62% and 52.38% respectively. 72(6.12%) pups perished before physical gender determination could be done and PCR was done. Of the demised 38 (52.78%) were females and 34 (47.22%) males with no significant statistical gender difference observed among these p>0.7995.

Table 4.73 Dietary study

PCR/DNA/ Physical Gender determination methods - Total statistics

Generation→ Families ↓	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**Totals**
1	5:2:3	6:3:3	8:4:4	12:3:2:7	10:5:5	8:5:3	6:1:1:4	6:3:3	61:29:32
2	8:5:3	6:4:2	12:5:1:6	10:6:4	9:3:6	9:4:5	8:2:6	7:3:4	69:33:36
3	6:2:3:1	10:1:2:7	8:5:3	8:3:5	8:4:3:1	6:2:1:2:1	8:3:5	8:2:6	62:25:37
4	8:5:3	8:5:2:1	7:3:4	7:4:2:1	8:3:5	8:7:1	7:1:1:5	8:3:5	61:32:29
5	6:1:1:4	7:4:3	6:2:4	6:2:4	8:6:2	7:3:4	9:7:2	9:2:7	58:28:30
6	8:6:2	9:3:6	10:6:4	8:4:1:3	10:5:5	8:4:4	8:5:3	8:4:3:1	69:38:31
7	10:6:4	8:4:4	8:1:3:4	6:4:2	9:3:1:5	8:2:6	9:6:3	6:2:4	64:32:32
8	6:3:3	7:3:1:3	8:5:3	10:5:3:2	8:3:5	7:3:4	8:2:5:1	10:6:4	64:31:33
9	10:3:7	8:2:6	8:3:1:4	6:2:4	9:6:2:1	8:6:2	9:4:5	7:3:4	65:30:35
10	8:5:3	10:6:4	9:4:5	8:3:5	10:4:6	6:2:2:2	9:3:6	8:2:6	68:31:37
11	10:4:6	10:5:5	6:3:3	4:3:1	7:3:4	8:2:6	8:1:1:6	6:2:4	59:24:35
12	10:3:7	6:2:4	8:4:4	8:2:5:1	9:4:5	9:2:7	8:4:4	8:4:4	66:25:41
13	8:4:4	9:6:3	10:6:4	8:5:3	6:3:3	10:2:2:6	10:4:6	7:5:2	68:37:31
14	9:2:3:4	8:4:4	10:2:8	10:3:7	10:2:8	9:4:5	10:3:7	8:5:3	74:28:46
15	10:2:8	10:6:4	8:4:4	9:4:5	10:4:6	10:6:4	8:1:3:4	9:5:4	74:35:39
16	9:6:3	12:8:4	6:1:1:4	8:4:4	9:5:3:1	10:5:5	10:5:5	8:1:1:6	72:37:35
TOTAL	131:59:72	134:69:65	132:64:68	128:60 :68	140:64:76	131:62 :69	135:52 :83	123: 52 :71	1054:495:559
	8.2 :3.7:4.5	8.4:4.3:4.1	8.3:4:4.3	8: 4: 4	9 :4 : 5	8 :4 :4	8: 3 :5	7.5 :3 :4.5	

…/Table 4.73

Generation→ Familie ↓	9	10	11	12	Sub totals	Totals	No of deliveries	Average pups per delivery
1	7:6:1				7:6:1	68:35:33	9	7 : 4 : 3
2	8:4:3:1	5:2:1:2	3:2:1		16:8:8	85:41:44	9	8 : 4 : 4
3						62:25:37	8	7 : 3 : 4
4	9:5:1:2	6:2:1:1:2			15:9:6	76:41:33	9	7 : 4 : 4
5	6:3:2:1				6:3:3	64:31:33	9	7 : 3 : 4
6	7:2:4:1	4:2:2	4:2:2	5:1:1:1:2	20:8:12	89:46:43	9	8 : 4 : 4
7						64:32:32	8	8 : 4 : 4
8	6:2:1:3				6:2:4	70:35:35	9	8 : 4 : 4
9	7:5:1:1	6:3:1:2			13.8:5	78:38:40	9	8 : 4 : 4
10	8:4:4				8:4:4	76:35:41	9	8 : 4 : 4
11						59:24:35	8	7 : 3 : 4
12						66:25:41	8	8 : 3 : 5
13	6:1:1:2:2	7:4:1:2	3: 1:2	2:1:1	18:8:10	86:45:41	9	8 : 4 : 4
14	8:5:3				8:5:3	82:33:49	9	9 : 3 :6
15						74 :35:39	8	8 : 4 : 4
16		5:2:1:2			5:2:3	77:39:38	8	8 : 4 : 4
Totals	72:36:36	33:16:17	10:5:5	7:3:4	122:63:59	1176:560:616	138	
	4 : 2 : 2	2 : 1 1	0.6:0.3:0.3	0.4:0.2:0.2				

Table 4.74 Dietary study

PCR/DNA/ Physical Gender determination methods - Summary statistics

%	Group		Total	Females	Males	Demised	Females	Males
2.99	Group	1	1436	724	712	43	18	25
2.49	Group	2	1446	625	821	32	15	17
2.65	Group	3	1471	538	933	39	18	31
3.66	Group	4	1500	581	919	62	28	34
2.83	Group	5	1473	885	588	41	19	22
2.94	Group	6	1496	906	590	44	26	18
3.30	Group	7	1516	508	1008	50	21	29
2.78	Group	8	1526	1021	505	32	21	11
4.98	Group	9	1176	560	616	72	38	34
3.10	Total		13.040			415		

Conclusions

A total of 415 pups out of 13,040 perished before gender could be physically ascertained and their DNA materials subjected to PCR analysis. This constituted approximately 3.1% of the total pups delivered with means of 1,449 pups delivered per group and 46 demised pups per group respectively.

No significant statistical gender difference in sex ratios was found among the demised pups in the water, p>0.66.; glucose, p>0.166, sodium p>0.49, potassium p>0.339, calcium p>0.612, combined sodium + potassium p>0.104, and all-inclusive group p>0.7995, signifying absence of gender influence among the demised pups. There was significant sex ratio difference towards female progeny among the demised pups in the magnesium (p<0.005) and combined calcium + magnesium (p<0.044) groups towards the female progeny.

The study did not show evidence of gender bias among the demised pups. The overall perinatal mortality rate was 32/1000 which is comparable to other countries with high human perinatal mortality rates (Global health for all databases, Geneva: WHO).

Table 4.75 Comparison between physical and PCR gender determination - 50 mice paps sampled and sexed

Age	Method	Percentage	Number of paps
Day one / two	PCR	98%	49 out 0f 50
	Physical	45%	23 out of 50
Day Three	Physical	65%	33 out of 50
Day Seven	Physical	85%	43 out of 50
Day Fourteen	Physical	95%	48 out of 50

50 mice pups were sampled and sexed using physical methods of gender determination verses PCR method. The first 1 – 2 days only 45% were positively identified because only anal tail distance was used. On the third day 65% were positively identified as the breast spots appeared. After one week 85% could be identified. After two weeks the physical identification accuracy was close to the PCR method rendering the latter method unnecessary for use after two weeks.

4.27 Dietary study

To determine the effects of dietary chemical compositions on biochemical serum concentrations of the respective chemicals

A study aimed at examining the relationship between high dietary chemical supplementation and the relevant serum chemical concentrations was carried out. The rationale was to determine whether the respective high chemical element concentrations in the various feeding dietary solutions were reflected systemically. This was based on the fact that high dietary chemical components in our diets have been advocated to influence the sex ratio outcome through their systemic effects. These chemicals are absorbed from the intestines via the liver to the blood stream and subsequently to the extra cellular space where cells including gametes are bathed in this fluid. With the presence of increased amounts of chemicals in the extra cellular fluid, various cellular reactive responses take place through osmosis, passive diffusion and active ion channel movements. These adjustments attempt to maintain the extracellular and intracellular equilibrium state of the relevant chemicals and as these chemicals are charged, in the process of the cellular ionic dynamics create cell surface charges or in their immediate environs such as the attached proteins that influence their cellular activities including fertilization.

Methods

Among the nine study groups, each had 16 families for sex ratio experimentation and 2 extra families provided for other aspects of the study such as chemical analysis. The latter familes were too treated with the same chemicals as the experimental groups. Determination of the serum biochemical levels of the respective elements under investigation was done using families 17 & 18 ($F_{17\ \&\ 18}$) in all the nine groups N_{1-9}.

Blood samples of 0.5 ml of blood was drawn from each animal model by nipping off the tail and intra-orbitally, every week, centrifuged to separate plasma samples for subsequent analysis of serum chemistries to assess systemic concentrations. The biochemical analyses were performed using Humalyzer 2000 (Human GmbH, D65205, Wiesbaden, Germany**).** Samples of greater amounts are drawn through cardiac puncture, but were avoided in this study. Routine data collection and applicable statistical analytical methods were used.

Results

Table 4.76 Dietary study - Group one - Control water solution

Serum biochemical concentrations of the respective chemicals

	Sodium	**Sodium**	**Potassium**	**Potassium**	**Calcium**	**Calcium**	**Magnesium.**	**Magnesium**
	Females	**Males**	**Females.**	**Males**	**Females**	**Males**	**Females**	**Males**
1	140.00	142.00	7.60	7.62	2.16	2.35	1.72	1.74
2	138.00	140.00	7.12	7.14	2.14	2.16	1.62	1.63
3	145.00	144.00	8.00	8.00	2.24	2.28	1.66	1.67
4	140.00	141.00	7.76	7.80	2.30	2.50	1.70	1.70
5	140.00	142.00	7.65	7.70	2.20	2.59	1.68	1.70
6	135.00	136.00	6.77	7.80	2.32	2.60	1.71	1.70
7	136.00	137.00	6.12	6.32	2.88	2.92	1.70	1.75
8	140.00	139.00	7.20	7.31	2.55	2.60	1.73	1.82
9	138.00	140.00	6.63	7.68	2.77	2.80	1.80	1.69
10	141.00	141.00	6.69	6.80	2.80	2.90	1.69	1.66
11	138.00	142.00	6.22	6.20	2.14	2.30	1.62	1.66
12	144.00	143.00	8.00	7.8	2.36	2.40	1.74	1.78
13	140.00	142.00	6.72	6.20	2.34	2.20	1.79	1.70
14	139.00	140.00	7.12	7.20	2.38	2.42	1.66	1.66
15	143.00	142.00	6.66	6.70	2.18	2.40	1.68	1.70
16	141.00	143.00	6.12	6.30	2.91	2.95	1.60	1.80

Normal laboratory animal values were observed among the four chemicals of sodium (140 – 160 mg/ml) potassium (5 – 8 meq/l) calcium (7 – 10 meq/l) and magnesium (1.5 - 2.5 meq/l).

Table 4.77 Group two - Control glucose solution

Serum biochemical concentrations of the respective chemicals

	Sodium	**Sodium**	**Potassium**	**Potassium**	**Calcium**	**Calcium**	**Magnesium**	**Magnesium**
	Females	**Males**	**Females.**	**Males**	**Females**	**Males**	**Females**	**Males**
1	139.00	141.00	7.62	7.16	2.18	2.36	1.76	1.73
2	140.00	141.00	7.13	7.14	2.17	2.14	1.64	1.67
3	144.00	146.00	7.10	7.20	2.26	2.24	1.69	1.68
4	142.00	143.00	7.73	7.76	2.50	2.50	1.70	1.74
5	140.00	141.00	7.75	7.95	2.30	2.62	1.71	1.73
6	135.00	137.00	7.77	7.89	2.32	2.62	1.73	1.72
7	137.00	139.00	6.22	6.44	2.89	2.94	1.71	1.73
8	142.00	143.00	6.20	7.31	2.45	2.58	1.83	1.84
9	140.00	141.00	7.34	7.73	2.82	2.87	1.79	1.70
10	143.00	144.00	6.89	6.99	2.82	2.93	1.71	1.67
11	137.00	137.00	6.12	6.32	2.12	2.34	1.69	1.67
12	143.00	142.00	6.40	8.30	2.37	2.42	1.68	1.79
13	140.00	143.00	6.42	6.52	2.35	2.28	1.80	1.73
14	138.00	139.00	7.12	7.53	2.39	2.43	1.67	1.67
15	142.00	146.00	6.76	6.64	2.20	2.43	1.69	1.70
16	140.00	142.00	6.32	6.53	2.92	2.94	1.59	1.74

Normal laboratory animal values were observed among the four chemicals of sodium (140 – 160 mg/l) potassium (5- 8 meq/l) calcium (7 – 10 mg/l) and magnesium (1.5 - 2.5 meq/l).

Control groups

Presented below are summaries of the paired t-test results between the following:-

Serum sodium levels between the control groups

Serum potassium levels between the control groups

Serum calcium levels between the control groups

Serum magnesium levels between the control groups

Table 4.78 Comparing sodium serum levels in the control groups (1 & 2)

	Group 1	**Group 2**
Number	32.00	32.00
Mean	140.3750	140.8438
Standard deviation	2.4330	2.6532
SEM	0.4301	0.4690
standard error of difference = 0.330		
The two-tailed p-value - 0.1653		
Conclusion. The difference is not statistically significant.		

Paired serum sodium t-test results between the control groups 1 and 2. Showing absence of significant difference between the serum sodium levels of the water and glucose control groups (p>0.165).

Table - 4.79 Comparing potassium serum levels in the control groups (1 & 2)

	Group 1	**Group 2**
Number	32.00	32.00
Mean	7.0922	7.0719
Standard deviation	0.6367	0.6125
SEM	0.1126	0.1083
standard error of difference = 0.087		
The two-tailed p-value - 0.8178		
Conclusion. The difference is not statistically significant.		

Paired serum potassium t-test results between the control groups 1 and 2. Showing absence of significant difference between the serum potassium levels of the water and glucose control groups (p>0.8178).

Table 4.80 Comparing calcium serum levels in the control groups (1 & 2)

	Group 1	Group 2
Number	32.00	32.00
Mean	2.4700	2.4906
Standard deviation	0.2674	0.2671
SEM	0.0473	0.0472
standard error of difference = 0.087		
The two-tailed p-value - 0.8178		
Conclusion. The difference is not statistically significant.		

Paired serum calcium t-test results between the control groups 1 and 2. Showing absence of significant difference between the serum calcium levels of the water and glucose control groups ($p>0.8178$).

Table 4.81 Comparing magnesium serum levels in the control groups(1 & 2)

	Group 1	Group 2
Number	32.00	32.00
Mean	1.68194	1.71563
Standard deviation	0.10428	0.05334
SEM	0.01843	0.00943
standard error of difference = 0.021		
The two-tailed p-value - 0.1160		
Conclusion. The difference is not statistically significant.		

Paired serum magnesium t-test results between the control groups 1 and 2. Showing absence of significant difference between the serum magnesium levels of the water and glucose control groups ($p>0.1160$).

Experimental groups

Table 4.82 Group three - Sodium serum levels

Group three - Sodium										
	Na-Females	Na-Males								
1	148.00	147.00								
2	143.00	144.00								
3	139.00	138.00								
4	146.00	147.00								
5	145.00	146.00								
6	148.00	147.00								
7	146.00	148.00								
8	143.00	144.00								
9	148.00	148.00								
10	147.00	148.00								
11	149.00	149.00								
12	147.00	146.00								
13	146.00	147.00								
14	145.00	146.00								
15	146.00	147.00								
16	145.00	146.00								

Table 4.83 Comparing sodium serum levels in water control group 1 and group 3

	Group 1	**Group 3**
Number	32.00	32.00
Mean	140.38	146.03
Standard deviation	2.48	2.43
SEM	0.4388	0.4301
standard error of difference 0.715		
The two-tailed p-value 0.0001		
Conclusion. The difference is statistically significant.		

Paired serum sodium t-test results between control group 1 and group 3. Showing significant difference between the serum sodium levels of group water control group and sodium group (p>0.0001).

Table 4.84 Group four - Potassium serum levels

Group four - Potassium										
	K Females	K Males								
1	8.86	8.89								
2	9.68	9.70								
3	8.10	8.20								
4	9.10	9.00								
5	9.00	9.10								
6	8.89	9.00								
7	8.86	8.89								
8	8.10	8.12								
9	9.00	9.10								
10	8.80	9.00								
11	8.16	8.00								
12	8.21	8.25								
13	8.36	8.40								
14	9.12	9.20								
15	8.36	8.50								
16	8.15	8.20								

Table 4.85 Comparing potassium serum levels between water control group 1 and group 4

	Group 1	**Group 4**
Number	32.00	32.00
Mean	7.1234	8.6969
Standard deviation	0.6696	0.4728
SEM	0.1184	0.0836
standard error of difference - 0.125		
The two-tailed p-value - 0.0001		
Conclusion. The difference is not statistically significant.		

Paired serum potassium t-test results between control group 1 and group 4 showing significant difference between the serum potassium levels of group water control group and potassium group ($p>0.0001$).

Table 4.86 Group five - Calcium serum levels

Calcium serum levels										
1	2.90	2.80								
2	2.89	2.90								
3	3.00	3.00								
4	2.78	2.80								
5	2.69	2.70								
6	2.90	2.92								
7	2.80	2.85								
8	2.76	2.80								
9	2.66	2.70								
10	2.90	2.95								
11	2.71	2.80								
12	2.69	2.70								
13	2.77	2.78								
14	2.68	2.72								
15	2.80	2.82								
16	2.86	2.90								

Table 4.87 Comparing calcium serum levels between water control group 1 and group 5

	Group 1	**Group 5**
Number	32.00	32.00
Mean	2.44	2.81
Standard deviation	0.27	0.11
SEM	0.047	0.17
standard error of difference - 0.050		
The two-tailed p-value - 0.0001		
Conclusion. The difference is not statistically significant.		

Paired serum Calcium t-test results between control group 1 and group 5 showing significant difference between the serum calcium levels of water control group and the calcium group (p<0.0001).

Table 4.88 Group 6 - Magnesium serum levels

Group six - magnesium.										
	Mg Females	Mg Males								
1	1.88	1.90								
2	1.92	1.88								
3	1.76	1.80								
4	1.80	1.82								
5	1.90	1.91								
6	1.89	1.90								
7	1.76	1.80								
8	1.80	1.81								
9	1.76	1.80								
10	1.81	1.82								
11	1.90	1.91								
12	1.65	1.70								
13	1.82	1.84								
14	1.83	1.85								
15	1.79	1.80								
16	1.69	1.70								

Table 4.89 Comparing magnesium serum levels between water control group 1 and group 6

	Group 1	**Group 6**
Number	32.00	32.00
Mean	1.89	1.82
Standard deviation	0.0760	0.0705
SEM	0.0134	0.0125
standard error of difference - 0.021		
The two-tailed p-value - 0.0001		
Conclusion. The difference is statistically significant		

Paired serum magnesium t-test results between control group 1 and group 6 showing significant difference between the serum magnesium levels of group water control group and magnesium group (p>0.0001).

Table 4.90 Group seven - Sodium and Potassium serum levels

Sodium + Potassium group

		Group seven - Sodium + Potassium										
	Females	Males	Females	Males								
1	148.00	149.00	9.80	9.85								
2	147.00	148.00	8.10	8.25								
3	145.00	146.00	7.79	7.80								
4	143.00	145.00	8.00	8.10								
5	145.00	147.00	8.12	8.30								
6	149.00	148.00	9.10	9.20								
7	146.00	148.00	8.86	9.00								
8	147.00	149.00	8.10	8.20								
9	149.00	148.00	8.36	8.40								
10	148.00	149.00	8.80	9.00								
11	146.00	147.00	8.15	8.20								
12	145.00	146.00	8.00	8.10								
13	147.00	148.00	8.12	8.30								
14	146.00	147.00	8.01	8.20								
15	148.00	149.00	8.52	8.70								
16	147.00	148.00	8.60	9.85								

Table 4.91 Comparing sodium serum levels in water control group 1 and group 7

	Group 1	**Group 7**
Number	32.00	32.00
Mean	140.38	147.16
Standard deviation	2.43	1.5
SEM	0.43	0.26
Standard error of difference - 0.554		
The two-tailed p-value - 0.0001		
Conclusion. The difference is statistically significant		

Paired serum Sodium t-test results between control group 1 and group 7 showing significant difference between the serum sodium levels of the water control group and sodium group ($p > 0.0001$).

Table 4.92 Comparing potassium serum levels in water control group 1 and group 7

	Group 1	**Group 7**
Number	32.00	32.00
Mean	7.12	8.50
Standard deviation	0.67	0.57
SEM	0.12	0.10
Standard error of difference - 0.168		
The two-tailed p-value - 0.0001		
Conclusion. The difference is statistically significant.		

Paired serum potassium t-test results between control group 1 and group 7 showing significant difference between the serum potassium levels of the water control group and potassium group ($p>0.0001$).

Table 4.93 Group eight - Calcium + Magnesium group serum levels

		Group eight Calcium + Magnesium.										
	Calcium Females	Calcium Males	Magnesium Females	Magnesium Males								
1	2.49	2.50	1.77	1.82								
2	2.77	2.80	1.73	1.75								
3	2.96	2.40	1.77	1.80								
4	2.59	2.70	1.70	1.72								
5	2.69	2.85	1.68	1.72								
6	2.80	2.75	1.78	1.82								
7	2.55	2.60	1.72	1.76								
8	2.70	2.70	1.71	1.74								
9	2.88	2.90	1.63	1.58								
10	2.60	2.65	1.68	1.72								
11	2.78	2.80	1.73	1.75								
12	2.65	2.68	1.78	1.83								
13	2.67	2.72	1.78	1.80								
14	2.64	2.66	1.69	1.72								
15	2.79	2.78	1.73	1.75								
16	2.45	2.60	1.64	1.63								

Table 4.94 Comparing calcium serum levels between water control group 1 and group 8

	Group 1	Group 5
Number	32.00	32.00
Mean	2.44	2.67
Standard deviation	0.28	0.13
SEM	0.05	0.02
Standard error of difference - 0.056		
The two-tailed p-value - 0.0003		
Conclusion. The difference is statistically significant		

Paired serum calcium t-test results between control group 1 and group 8. Showing significant difference between the serum calcium levels of the water control group and calcium + magnesium combined group (p>0.0003).

Table 4.95 Comparing magnesium serum levels between water control group 1 and group 8

	Group 1	Group 5
Number	32.00	32.00
Mean	1.69	1.73
Standard deviation	0.0760	0.0590
SEM	0.0134	0.0104
Standard error of difference - 0.018		
The two-tailed p-value - 0.036		
Conclusion. The difference is statistically significant		

Paired serum magnesium t-test results between control group 1 and group 8. Showing significant difference between the serum magnesium levels of the water control group and calcium + magnesium combined group (p>0.036).

Table 4.96 - Group nine – Cocktail with all-inclusive of the four chemicals in solution

	Sodium Females	Sodium Males	Potassium Females	Potassium Males	Calcium Females	Calcium Males	Magnesium Females	Magnesium Males
1	140.00	140.00	8.10	8.30	2.29	2.30	1.82	1.90
2	141.00	142.00	7.80	8.00	2.39	2.40	1.70	1.80
3	143.00	144.00	7.66	7.70	2.40	2.45	1.88	1.87
4	142.00	145.00	8.00	8.10	2.33	2.40	1.76	1.80
5	147.00	146.00	8.12	8.00	2.59	2.70	1.80	1.81
6	145.00	144.00	8.70	8.80	2.60	2.68	1.77	1.79
7	140.00	141.00	7.16	7.5	2.32	2.40	1.56	1.72
8	143.00	142.00	7.22	8.88	2.46	2.50	1.66	1.70
9	145.00	146.00	7.16	8.00	2.39	2.40	1.55	1.60
10	146.00	147.00	7.02	7.50	2.16	2.30	1.67	1.70
11	143.00	140.00	7.14	7.80	2.44	2.40	1.77	1.80
12	144.00	145.00	8.00	7.50	2.49	2.50	1.78	1.81
13	142.00	141.00	7.25	7.50	2.56	2.60	1.80	1.78
14	141.00	145.00	7.76	8.00	2.38	2.40	1.66	1.70
15	145.00	144.00	7.39	8.10	2.76	2.81	1.72	1.71
16	142.00	146.00	7.71	7.90	2.68	2.65	1.65	1.68

Table 4.97 Comparing sodium serum levels in water control group 1 and group 9

	Group 1	**Group 2**
Number	32.00	32.00
Mean	140.3750	143.3438
Standard deviation	2.4330	2.1940
SEM	0.4301	0.3878
standard error of difference - 0.521		
The two-tailed p-value - 0.0001		
Conclusion. The difference is statistically significant		

Paired serum sodium t-test results between the control group 1 and the all-inclusive group 9. Showing significant difference between the serum sodium levels of the water control group and sodium serum levels in the all-inclusive group both with p>0.0001.

Table - 4.98 Comparing potassium serum levels in water control group 1 and group 9

	Group 1	**Group 2**
Number	32.00	32.00
Mean	7.0922	7.8053
Standard deviation	0.6367	0.4724
SEM	0.1126	0.0835
standard error of difference - = 0.106		
The two-tailed p-value - 0.0001		
Conclusion. The difference is statistically significant		

Paired serum potassium t-test results between the control group 1 and the all-inclusive group 9. Showing significant difference between the serum potassium levels of the water control group and sodium + potassium serum levels in the all-inclusive group both with $p>0.0001$

Table - 4.99 Comparing calcium serum levels in water control group 1 and group 9

	Group 1	**Group 2**
Number	32.00	32.00
Mean	2.4700	2.4728
Standard deviation	0.2674	0.1520
SEM	0.0473	0.0269
standard error of difference - 0.056		
The two-tailed p-value - 0.9603		
Conclusion. The difference is not statistically significant		

Paired serum calcium t-test results between the control group 1 and the all-inclusive group 9 showing absence of significant difference between the serum calcium levels of the water control group and calcium & magnesium serum levels in the all-inclusive group ($p>0.9603$).

Table 4.100 Comparing magnesium serum levels in water control group 1 and group 9.

	Group 1	**Group 2**
Number	32.00	32.00
Mean	1.9831	1.7413
Standard deviation	1.7918	0.0861
SEM	0.3168	0.0152
standard error of difference - 0.320		
The two-tailed p-value - 0.4552		
Conclusion. The difference is not statistically significant		

Paired serum magnesium t-test results between the control group 1 and the all-inclusive group 9. Showing absence of significant difference between the serum magnesium levels of the water control group and calcium + magnesium serum levels in the all-inclusive group ($p>0.4552$).

Summary

Normal levels of sodium, potassium, calcium and magnesium were found among the control groups with means of 140 mg/ml, 7 mg/ml, 2.4 meq/l and 1.7 mg/ml respectively. No statistical significant differences were found between the concentrations of these four chemicals between the two control groups with sodium ($p>0.165$), potassium ($p>0.818$), calcium ($p>0.878$) and magnesium ($p>0.116$). Significant statistical differences were found between the corresponding elements in the water control group and the experimental groups of sodium, potassium, calcium, magnesium, sodium + potassium, calcium + magnesium group and all-inclusive ($p>0.0001$) except between calcium and magnesium in group one and that of the all-inclusive group with ($p>0.9603$) and ($p>0.455$) respectively.

Conclusions

Normal serum levels were observed in the control groups while elevated serum levels were observed among the experimental groups except calcium levels and magnesium element in the all-inclusive group.

4.28 The weight study

The aim was to study the effects of various chemical formulations on the weights of the animal models as a measure of the wellbeing.

Background and rationale

Since the inception of the study, the various chemicals dissolved in tap water were fed to the animals and weight trends taken as a measure of the wellbeing of the animal model and success of the study.

In the initial stages, the animal models in groups four, five, six and eight that were constituted with potassium, calcium and magnesium formulations had poor feeding habits. Some models were refusing to feed on these formulations and were continually losing weight and some even starved to death. Following a series of trials we were failing to get them feed on these despite low dilutions prompting us to suspect that probably the taste could have been the contributing factor and not the concentrations. This was the first biggest challenge that we faced in this study and almost put a stop to it. As a desperate measure glucose was added to the feeding water and this worked well as solution was struck and this jam-started the study and since then, we have not had a major challenge neither looked back.

Methods

To study the effects of the various chemical formulations on the weights of the animal models, six pairs of animal models were selected from each of the nine groups and their weights taken on a weekly basis for a period of six months. The models were introduced to the study at the age of six weeks in line with the rest of the study's other aspects. The chemical formulations, constitutions and applications are as stipulated earlier.

Results

Routine data collection, summarization and analysis using the applicable statistical tools were done.

Presented below is the summarized data of the six families of each group. The data of six families is divided into two groups to save on space on presentation. F1 represents the means of the females in the first three families and F2 represents the means of the females of the rest of the six families. M1 represents the means of the males of the first three families and M2 represents the means of the males of the rest of the six families of each group. The weights were recorded in decigrams (dg) of which ten decigrams constitutes one gram (g).

Table 4.101 Group one: General weight trends of the animal models (1 g = 10d g)

Females	Females 1	Wt diff	Females 2	Wt diff	Males I	Wt diff	Males 2	Wt diff
1	145	0	150	0	165	0	170	0
2	146	1	152	2	166	1	172	2
3	160	14	155	3	169	3	173	1
4	165	5	161	6	173	4	179	6
5	170	5	173	12	186	13	190	11
6	178	8	184	11	198	12	202	12
7	198	20	200	16	212	14	215	13
8	216	18	220	20	234	22	237	22
9	240	24	242	22	236	2	238	1
10	245	5	247	5	238	2	240	2
11	250	5	258	11	240	2	242	2
12	269	19	268	10	245	5	246	4
13	280	11	276	8	250	5	252	8
14	260	-20	256	-20	255	5	260	8
15	260	0	260	4	272	17	273	13
16	260	0	262	2	275	3	276	3
17	260	0	263	1	276	1	276	0
18	270	10	263	0	275	-1	276	0
19	269	9	262	-1	275	0	276	0
20	259	-20	261	-1	275	0	275	-1
Total weight gain		114dg	111dg		110dg		105dg	

Group one - The general weight trends with F1 & M1, F2 & M2 representing the means of the first three families and the rest of the six families respectively.

Table 4.102 General weight trends - Summary statistics

	Group	Number	Females 1	Females 2	Males 1	Males 2
Mean	1.0000	10.5000	225.0000	225.1500	230.7500	233.4000
Std. deviation	0.00000	5.91608	47.63789	45.74791	41.04539	39.42134
Variance	0.00000	35.00000	2269.36842	2092.87105	1684.72368	1554.04211
Sum	20.00	210.00	4500.00	4503.00	4615.00	4668.00

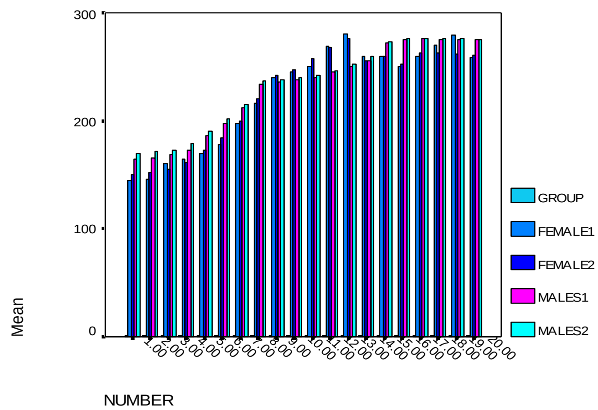


Figure 4.65 General weight trends **(**the spikes signify pregnancy states**)**

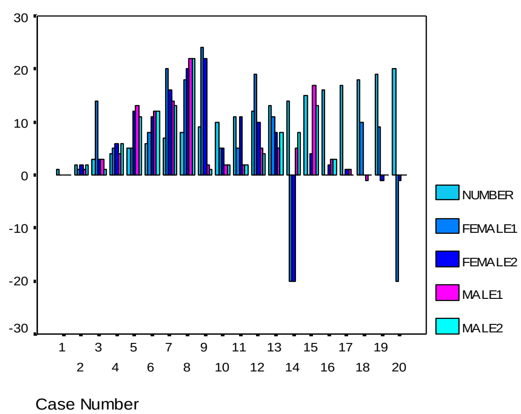


Figure 4.66 Detailed weight gain and loss trends

(The dips represent weight losses after delivery or minor weight changes)

Conclusion

In group one, the total mean weight gain among the female animal models was 11.25 g and 107.5 dg among the males translating into mean monthly weight gain of 18.75 dg and 1.8 g respectively. The maximum weight attained was 28.0 g among the females and 276 dg among the males which are within the average adult weight range.

Table 4.103 Group 2: General weight trends of the animal models (1 g = 10 dg)

	Females 1	**Females 2**	**Males 1**	**Males 2**
1	148	146	159	169
2	149	148	161	171
3	152	151	164	174
4	158	160	169	177
5	164	167	172	184
6	176	179	190	200
7	190	200	212	210
8	202	220	238	228
9	230	228	240	230
10	248	230	242	239
11	252	235	244	240
12	266	240	248	245
13	275	250	255	250
14	255	250	265	255
15	258	259	270	275
16	259	260	275	278
17	260	260	275	276
18	261	275	275	276
19	262	278	275	276
20	260	258	275	275
Weight gain	112dg	112dg	116dg	106dg

Group two - The general weight trends with F1 & M1, F2 & M2 representing the means of the first three families and the rest of the six families respectively.

Table 4.104 General weight trends - Summary statistics

	Group	Number	Females 1	Females 2	Males 1	Males 2
Mean	2.0000	10.5000	221.3000	219.7000	230.2000	231.4000
Std. deviation	0.00000	5.91608	47.52739	45.33629	44.46714	40.17121
Variance	0.00000	35.00000	2258.85263	2055.37895	1977.32632	1613.72632
Sum	40.00	210.00	4426.00	4394.00	4604.00	4628.00

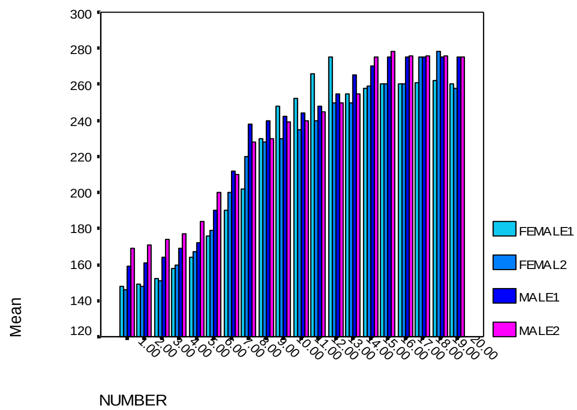


Figure 4.67 General weight trends (the spikes signify pregnancy states)

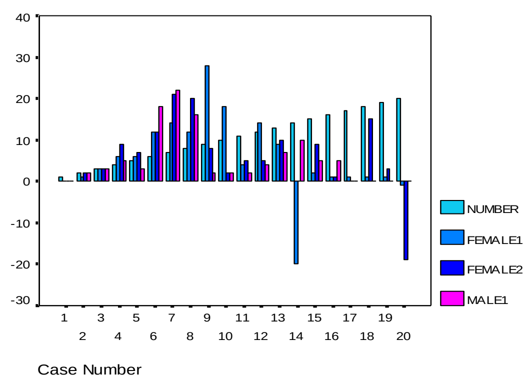


Figure 4.68 Detailed weight gain and loss trends

Detailed weight trends showing the gains and losses, the major dips represent weight losses following delivery and the minor dips represent the minor weight changes.

Conclusion

In group two, the total mean weight gain among the females was 11.2 g and 111 dg among the males translating into 1.87 g and 18.5 dg monthly weight gains. The maximum weight attained by both the females and males was 278 dg which was within the adult mice weight range.

Table 4.105 Group 3: General weight trends of the animal models (1g = 10dg)

Group three								
	Females 1	**Differences**	**Females 2**	**Differences**	**Males 1**	**Differences**	**Males 2**	**Differences**
1	144	0	150	0	169	0	170	0
2	147	3	152	2	170	1	171	1
3	150	3	154	2	173	3	175	4
4	156	6	160	6	176	3	180	5
5	162	6	170	10	186	10	191	11
6	178	16	192	22	206	20	210	19
7	192	14	208	16	220	14	225	15
8	202	10	228	20	240	20	242	17
9	228	26	243	15	242	2	246	2
10	240	12	248	5	244	2	248	2
11	258	18	260	12	246	2	250	2
12	268	10	267	7	250	4	254	4
13	276	8	279	12	255	5	260	6
14	256	20	260	-19	260	5	265	5
15	255	-1	260	0	270	10	272	7
16	260	5	262	2	276	6	278	6
17	263	3	262	0	276	0	276	-2
18	265	2	269	7	275	-1	275	-1
19	265	0	279	10	275	0	274	-1
20	261	-4	256	-23	273	-2	272	-2
Weight gains	117dg		106dg		104dg		102dg	

Group three - The general weight trends with F1 & M1, F2 & M2 representing the means of the first three families and the rest of the six families respectively.

Table 4.106 General weight trends - Summary statistics

	Group	Number	Females 1	Females 2	Males 1	Males 2
Mean	3.0000	10.5000	221.3000	227.9500	234.1000	236.7000
Std. deviation	0.00000	5.91608	48.85650	47.13751	39.81127	39.32871
Variance	0.00000	35.00000	2386.9578	2221.9447	1584.9368	1546.74737
Sum		210.00	4426.00	4559.00	4682.00	4734.00

Detailed statistical weight trends.

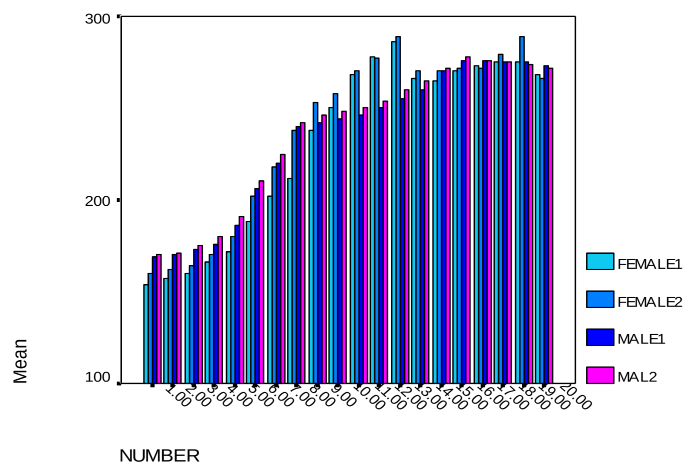


Figure 4.69 General weight trends

General weight trends among group three, the major spikes signify pregnancy states.

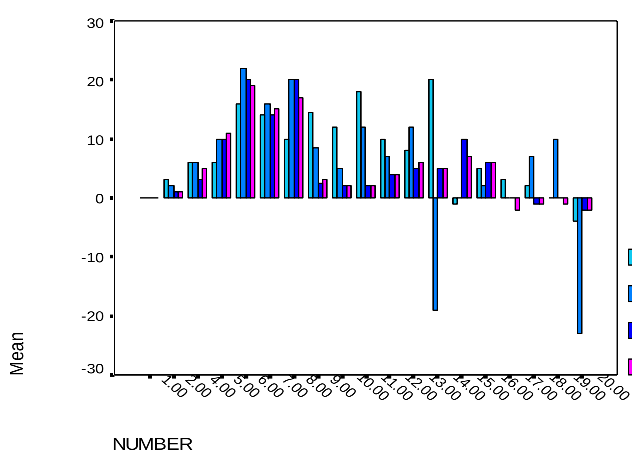


Figure 4.70 Detailed weight gain and loss trends

Trends of weight gains and losses. The major dips represent after delivery weight losses.

Conclusion

In group three, the total mean weight gain among the female and male animal models were 111.5 dg and 10.3 g representing mean weight gains of 18.6 dg and 1.72 g per month. The maximum weight attained among the females was 27.9 g and 278 dg among the males which are within the average adult weight range.

Table 4.107 Group 4 General weight trends of the animal models (1Ig = 10 dg)

Group four								
	Females 1	**Differences**	**Females 2**	**Differences**	**Males 1**		**Males 2**	**Difference**
1	164	0	150	0	179	0	168	0
2	166	2	151	1	180	1	172	4
3	167	1	153	2	181	1	174	2
4	170	3	160	7	185	4	178	4
5	178	8	170	10	193	8	189	11
6	191	13	186	16	204	11	202	13
7	202	11	190	4	215	11	218	16
8	226	24	216	26	238	23	240	22
9	230	4	232	16	239	1	242	2
10	232	2	248	16	240	1	243	1
11	235	3	256	8	245	5	250	7
12	240	5	264	8	250	5	252	2
13	250	10	279	15	262	12	265	13
14	255	5	250	-29	265	3	270	5
15	258	3	255	5	275	10	272	2
16	259	1	256	1	275	0	275	3
17	259	0	258	2	275	0	276	1
18	260	1	261	3	275	0	276	0
19	261	1	261	0	275	0	276	0
20	260	-1	270	9	274	-1	275	-1
Total weight gain	96		120		95		107	

Group four - The general weight trends with F1 & M1, F2 & M2 representing the means of the first three families and the rest of the six families respectively.

Table 4.108 Group 4 General weight trends - Summary statistics

	Number	Females 1	Females 2	Males 1	Males 2
Mean	10.5000	223.1500	223.3000	236.2500	235.6500
Std. deviation	5.91608	37.33811	46.15318	37.13117	40.58944
Variance	35.00000	1394.13421	2130.11579	1378.72368	1647.50263
Sum	210.00	4463.00	4466.00	4725.00	4713.00

Detailed statistical weight trends.

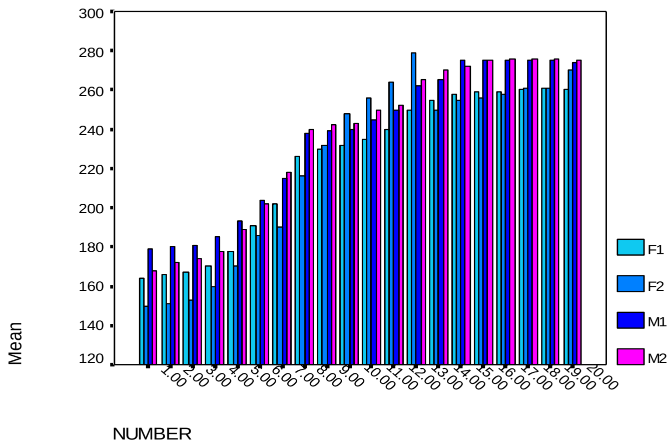


Figure 4.71 General weight trends

Group four - general weight trends.

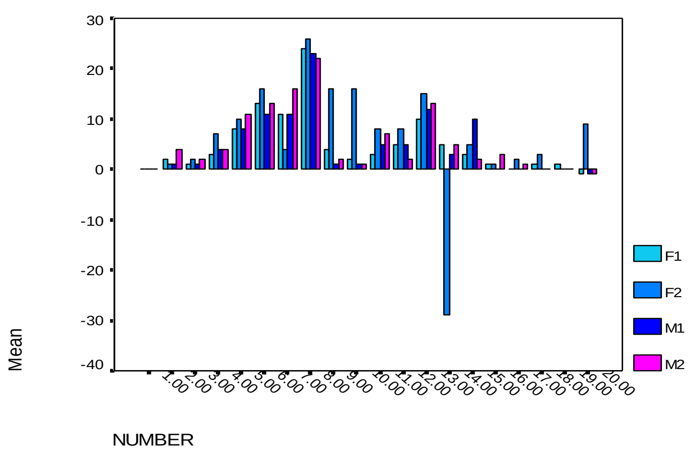


Figure 4.72 Detailed weight gain and loss trends

The trends of weight gains and losses, the major dips representing weight losses following delivery.

Conclusion

In group four, the mean total weight gain among the female and male animal models were 10.8 g and 101 dg respectively representing mean weight gains of 1.75 g and 1.68 g per month. The maximum weight attained among the females was 279 dg and 276 dg among the males which are within the average adult mice weight range.

Table 4.109 Group 5: General weight trends of the animal models (1 g = 10 dg)

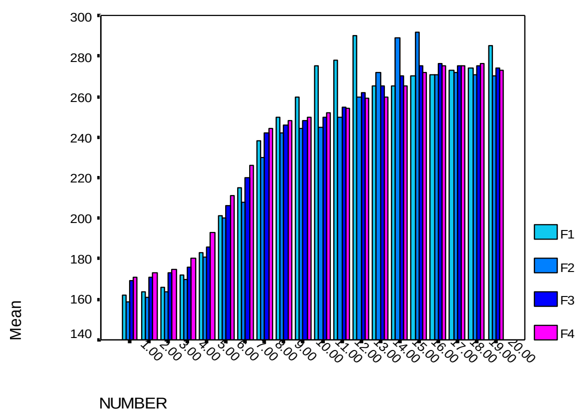
Group Five								
	Females 1	**Differences**	**Females 2**	**Differences**	**Males 1**	**Differences**	**Males 2**	**Differences**
1	152	0	149	0	169	0	171	0
2	154	2	151	2	171	2	173	2
3	156	2	154	3	173	2	175	2
4	162	6	160	6	176	3	180	5
5	173	11	171	11	186	10	193	13
6	191	18	190	19	206	20	211	18
7	205	14	198	8	220	14	226	15
8	228	23	220	22	242	22	244	18
9	240	12	232	12	246	4	248	4
10	250	10	234	2	248	2	250	2
11	265	15	235	1	250	2	252	2
12	268	3	240	5	255	5	254	2
13	280	12	250	10	262	7	259	5
14	255	-25	262	12	265	3	260	1
15	255	0	279	17	270	5	265	5
16	260	5	282	3	275	5	272	7
17	261	1	261	-21	276	1	275	3
18	263	2	262	1	275	-1	275	0
19	264	1	261	-1	275	0	276	1
20	275	11	260	-1	274	-1	273	-2
Total weight differences		123dg		111dg		105dg		102dg

Group five - The general weight trends with F1 & M1, F2 & M2 representing the means of the first three families and the rest of the six families respectively.

Table 4.110 General weight trends - Summary statistics

	Group	Number	Females 1	Females 2	Males 1	Males 2
Mean	5.0000	10.5000	227.8500	222.4500	235.7000	236.6000
Std. deviation	0.00000	5.91608	46.06434	45.33556	40.42810	38.28893
Variance	0.00000	35.00000	2121.92368	2055.31316	1634.43158	1466.04211
Sum	100.00	210.00	4557.00	4449.00	4714.00	4732.00

Detailed statistical weight trends.



Group four - general weight trends.

Figure 4.73 General weight trends

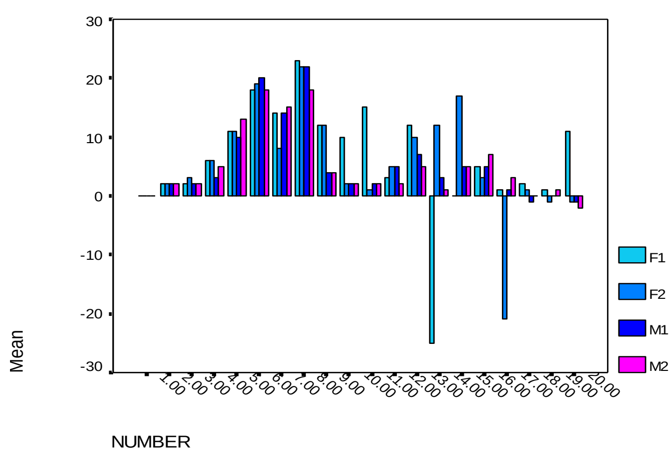


Figure 4.74 Detailed weight gain and loss trends

Weight gains and losses trends, the dips represent weight losses after delivery or minor weight changes.

In group five, the total mean weight gain among the female and male animal models were 11.7 g and 103.5 dg respectively representing mean weight gains of 19.5 g and 17.25 g per month. The maximum weight attained among the females was 28.2 g and 276 dg among the males which are within the average adult weight range.

Table 4.111 Group 6: General weight trends of the animal models (1 g = 10d g)

Group Six								
	Females 1	**Differences**	**Females 2**	**Differences**	**Males 1**	**Differences**	**Males 2**	**Differences**
1	153	0	148	0	169	0	171	0
2	155	2	151	3	170	1	173	2
3	158	3	154	3	172	2	175	2
4	163	5	160	6	175	3	181	6
5	171	8	170	10	187	12	194	13
6	193	22	195	25	210	23	211	17
7	208	15	212	17	228	18	231	20
8	225	17	233	21	248	20	246	15
9	242	17	246	13	249	1	250	4
10	252	10	250	4	250	1	252	2
11	266	14	265	15	253	3	254	2
12	270	4	268	3	255	2	256	2
13	285	15	276	8	256	1	260	2
14	260	-25	258	-18	265	9	270	10
15	260	0	260	2	275	10	276	6
16	260	0	261	1	276	1	277	1
17	262	2	262	1	275	-1	278	1
18	270	8	264	2	274	-1	277	-1
19	277	7	272	8	275	1	276	-1
20	256	-21	280	8	274	-1	275	-1
Total weight gains	103dg	103dg	132dg	132dg	105dg	105dg	104dg	104dg

Group five - The general weight trends with F1 & M1, F2 & M2 representing the means of the first three families and the rest of the six families respectively.

Table 4.112 General weight trends - Summary statistics

	Group	Number	Females 1	Females 2	Males 1	Males 2
Mean	6.0000	10.5000	229.3000	229.2500	236.8000	238.4500
Std. deviation	.00000	5.91608	46.64209	47.74811	40.54964	39.99010
Variance	.00000	35.00000	2175.48421	2279.88158	1644.27368	1599.20789
Sum	120.00	210.00	4586.00	4585.00	4736.00	4769.00

Detailed statistical weight trends.

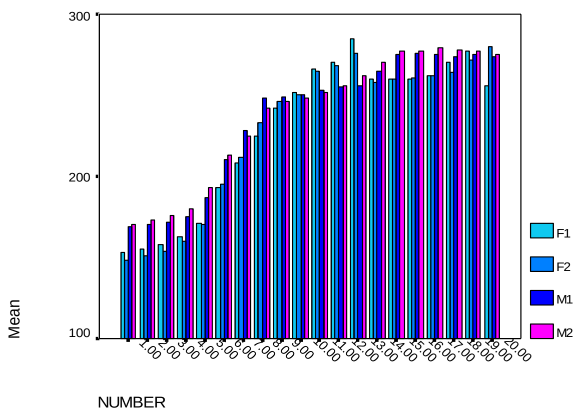


Figure 4.75 General weight trends

Group four - general weight trends.

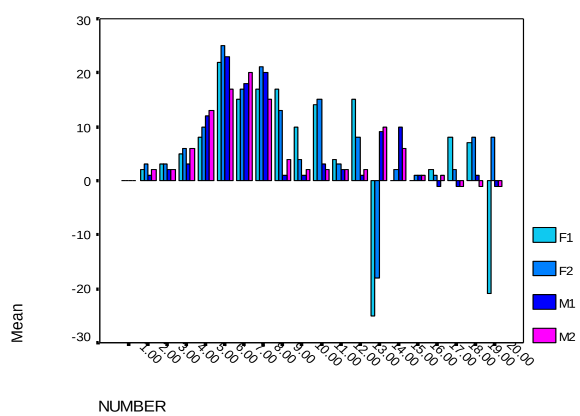


Figure 4.76 Detailed weight gain and loss trends

Weight gains and losses trends, the dips represent weight losses after delivery and other minor weight changes.

In group six, the total mean weight gain among the female and male animal models were 11.75 g and 104.5 dg respectively representing mean weight gains of 19.5 g and 174.2 dg per month. The maximum weight attained among the females was 285 g and 279 dg among the males which are within the average adult weight range.

Table 4.113 Group 7: General weight trends of the animal models (1g = 10dg)

Group seven								
	Females 1	**Differences**	**Females 2**	**Differences**	**Males 1**	**Differences**	**Males 2**	**Differences**
1	163	0	158	0	170	0	172	0
2	164	1	160	2	172	2	173	1
3	165	1	163	3	176	4	175	2
4	172	7	169	9	180	4	183	8
5	184	12	176	7	192	12	198	15
6	202	18	196	20	204	12	212	14
7	220	18	206	10	218	14	229	17
8	231	11	229	23	240	22	246	17
9	246	15	248	19	244	4	248	2
10	252	6	255	7	246	2	250	2
11	268	16	266	11	250	4	254	4
12	271	3	270	4	254	4	256	2
13	282	11	278	8	260	6	260	4
14	258	-24	255	-23	270	10	272	12
15	259	1	258	3	275	5	278	6
16	260	1	260	2	275	0	278	0
17	260	0	261	1	276	1	278	0
18	268	8	265	4	272	-4	277	-1
19	272	4	265	0	273	1	276	-1
20	256	-16	264	-1	275	2	274	-2
Total weight gains	93dg	93dg	106dg	106dg	105dg	105dg	102dg	102dg

Group seven - The general weight trends with F1 & M1, F2 & M2 representing the means of the first three families and the rest of the six families respectively.

Table 4.114 General weight trends - Summary statistics

	Group	Number	Females 1	Females 2	Males 1	Males 2
Mean	7.0000	10.5000	232.2000	230.1000	236.1000	239.4500
Std. deviation	0.00000	5.91608	42.22696	43.50910	39.55795	39.38939
Variance	0.00000	35.00000	1783.11579	1893.04211	1564.83158	1551.52368
Sum	140.00	210.00	4644.00	4602.00	4722.00	4789.00

Detailed statistical weight trends.

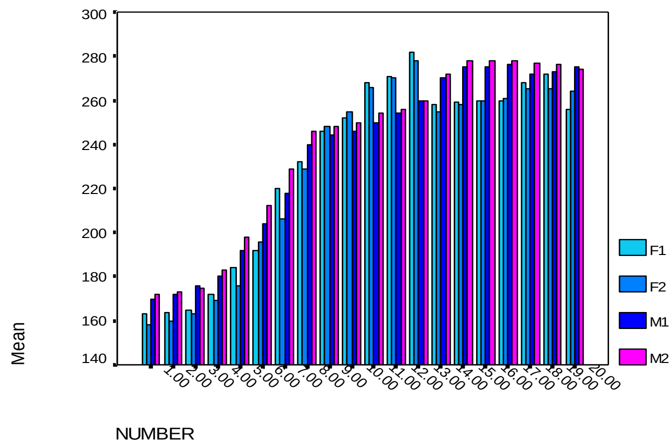


Figure 4.77 General weight trends

Group four - general weight trends.

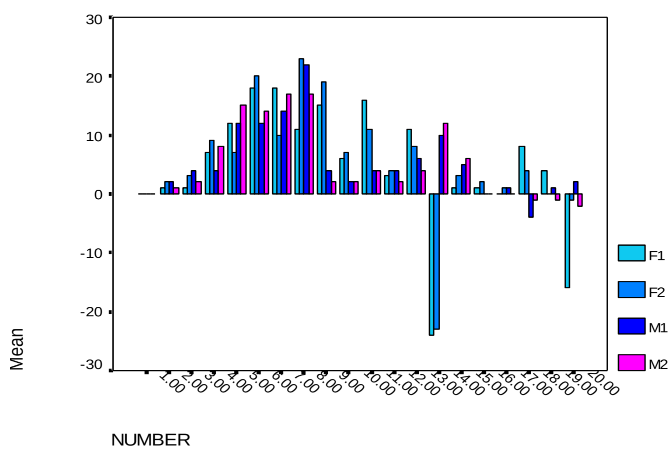


Figure 4.78 Detailed weight gain and loss trends

Weight gains and losses trends with the dips representing after delivery weight losses and other minor weight changes.

In group seven, the total mean weight gain among the female and male animal models were 9.95 g and 103.5 dg respectively representing mean weight gains of 1.658 g and 17.25 dg per month. The maximum weight attained among the females was 28.2 g and 27.8 dg among the males which are within the average adult weight range.

Table 4.115 Group 8: General weight trends of the animal models (1 g = 10 dg)

Group eight								
	Females 1	**Differences**	**Females 2**	**Difference s**	**Males 1**	**Differences**	**Males 2**	**Differences**
1	149	0	155	0	164	0	170	0
2	153	4	158	3	166	2	173	3
3	155	2	160	2	169	3	176	3
4	160	5	163	3	172	3	180	4
5	171	11	174	11	186	14	193	13
6	188	17	190	16	204	18	213	20
7	200	12	205	15	208	4	225	12
8	226	26	230	25	238	30	242	17
9	249	23	233	3	240	2	246	4
10	253	4	234	1	245	5	248	2
11	267	14	235	1	250	5	252	4
12	270	3	240	5	255	5	256	4
13	280	10	245	5	260	5	262	6
14	256	-24	255	10	270	10	270	8
15	260	4	278	23	275	5	277	7
16	261	1	281	3	275	0	277	0
17	261	0	262	-21	275	0	279	2
18	265	4	266	4	275	0	278	-1
19	265	0	268	2	274	-1	277	-1
20	263	-2	270	2	274	0	275	-2
Weight gains		114dg		115dg		111dg	109dg	109dg

Group eight - The general weight trends with F1 & M1, F2 & M2 representing the means of the first three families and the rest of the six families respectively.

Table 4.116 General weight trends - Summary statistics

	Group	**Number**	**Females 1**	**Females 2**	**Males 1**	**Males 2**
Mean	8.0000	11.0000	227.6000	225.1000	233.2500	238.4500
Std. error of mean	0.00000	1.56441	10.57017	9.78019	9.43227	8.94206
Variance	0.00000	48.94737	2234.56842	1913.04211	1779.35526	1599.20789
Sum	160.00	220.00	4552.00	4502.00	4665.00	4769.00

Detailed statistical weight trends.

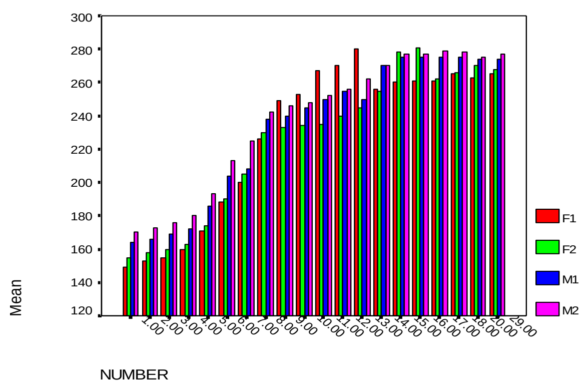


Figure 4.79 General weight trends among group

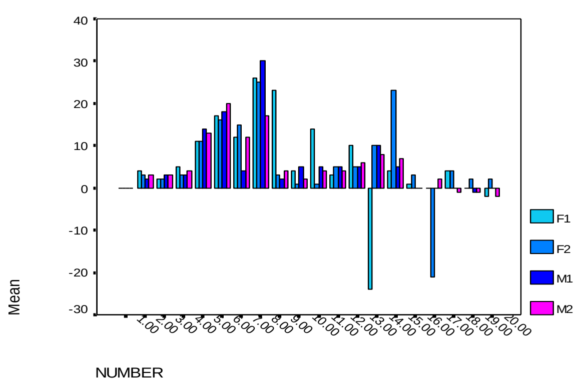


Figure 4.80 Detailed weight gain and loss trends

Trends of weight gains and losses. The major and minor dips represent after delivery weight losses and other minor weight changes.

In group eight, the total mean weight gain among the female and male animal models were 114.5 dg and 110.0 dg respectively representing mean weight gains of 19.08 g and 18.33 g respectively per month. The maximum weight attained among the females was 281dg and 279 dg among the males which is within the average adult weight range.

Table 4.117 Group 9: General weight trends

General weight trends of the animal models (1g = 10 dg)

Group nine								
	Females 1	**Differences**	**Females 2**	**Differences**	**Males 1**	**Differences**	**Males 2**	**Differences**
1	146	0	156	0	168	0	172	0
2	149	3	158	2	170	2	174	2
3	150	1	160	2	172	2	176	2
4	158	8	166	6	178	6	181	5
5	168	10	171	5	186	8	190	9
6	178	10	190	19	203	17	214	24
7	192	14	202	12	218	15	227	13
8	220	28	223	11	235	17	238	11
9	248	28	226	3	238	3	240	2
10	255	7	230	4	242	6	244	4
11	269	14	235	5	248	6	245	1
12	273	4	240	5	250	2	249	4
13	285	12	246	6	260	10	250	1
14	260	-25	255	9	270	10	266	16
15	262	2	272	17	275	5	273	7
16	265	3	285	13	275	0	274	1
17	268	3	260	-25	276	1	276	2
18	270	2	261	1	275	-1	276	0
19	279	9	262	1	275	0	275	-1
20	259 dmg	-20	260	-2	273	-2	275	0
Weight gains		113 dg		104 dg	108	104 dg	104 dg	104 dg

Group nine - The general weight trends with F1 & M1, F2 & M2 representing the means of the first three families and the rest of the six families respectively.

Table 4.118 General weight trends - Summary statistics

	Group 9	**Number**	**Females 1**	**Females 2**	**Males 1**	**Males 2**
Mean	9.0000	10.5000	227.7000	222.9000	234.3500	235.7500
Std. deviation	0.00000	5.91608	51.28158	42.40022	40.69434	38.20289
Variance	0.00000	35.00000	2629.80000	1797.77895	1656.02895	1459.46053
Sum	180.00	210.00	4554.00	4458.00	4687.00	4715.00

Detailed statistical weight trends.

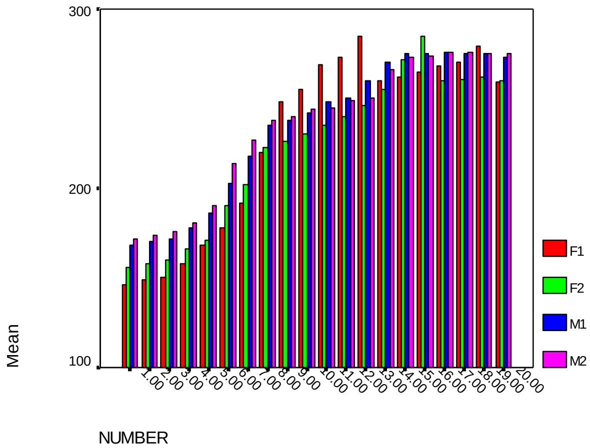


Figure 4.81 General weight trends

Group nine - general weight trends.

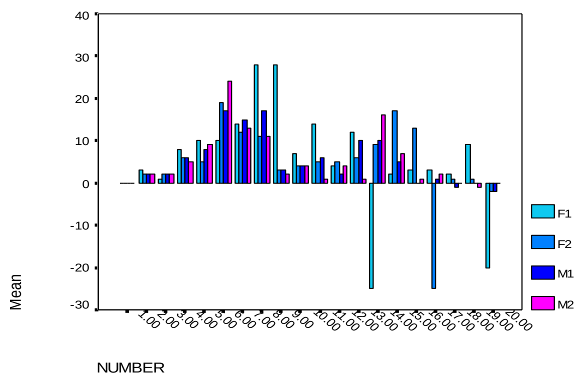


Figure 4.82 Detailed weight gain and loss trends

Trends of weight gains and losses. The major and small dips represent after delivery weight losses and other minor weight changes respectively.

In group nine, the total mean weight gain among the female and male animal models were 108 dg and 106.dg respectively representing mean weight gains of 1.81 g and 176.7 dg respectively per month. The maximum weight attained among the females was 285 dg and 27dg among the males which is within the average adult weight range.

Table 4.119 Total general weight trends - Summary statistics

	Minimum	Maximum	Sum	Mean	Standard deviation	Variance
Total weight gain females	9.95	11.75	100.10	11.12	0.55	0.301
Total weight gain males	10.10	11.10	95.00	10.55	0.34	0.113
Monthly weight gains males	1.68	1.85	15.85	1.76	0.056	0.003
Total weight gain females	27.80	28.60	253.70	28.19	0.29	0.086
Total weight gain males	27.60	27.90	249.60	27.73	0.13	0.017

The total mean weight gain among the female and male animal models were 11.12 g and 10.55 g respectively representing mean monthly weight gains of 1.85 g and 1.7 6 g respective. The maximum weight attained among the females was 28.19 dg and 27.73 dg among the males which are within the average adult weight range. There was general weight gain and absence of any weight loss.

Conclusion

This aspect of the study was included due to the initial challenges of the animal models refusing to take the chemicals in water until glucose was added and therefore it was prudent that a way of monitoring the well-being of animal models be sought so as to ensure their wellbeing as well as continued intake of chemicals under study. The general weight gain reaffirmed good general wellbeing of the animal models, intake of the study chemicals and validity of our results.

4.29 Dietary study. Effects of generations on sex ratios

Effects of birth order and sex ratios

A study aimed at examining the relationship between birth order and sex ratios was carried out using the data accrued. This study sought to find out whether birth order had significant influence on sex ratios of the progeny. The rational was based on the many unconfirmed allegations in society that sex ratios may be influenced by birth order.

Methods

Data on all the deliveries of nine groups each with sixteen families were collected over their entire life spans of the animal models of between two and three years. This ran between June

2008 and October 2010. Data on all deliveries were recorded and analysed. The delivery order was designated as generation one in the first delivery and was referred to as generation two the second delivery and similarly for the latter generations and deliveries.

Results

Among the 9 groups with sixteen families each, there were 1528 deliveries with a total of 13,040 pups comprising of 6,348 female pups and 6,692 male pups. The mean litter size per family was 8.5 per delivery. The mean female pups per family per delivery was 4.15 and the mean male pups delivered per family was found to be 4.38

The average pups delivered among the 1 – 9 generations (deliveries) were between 8 – 9 and dropped from the 10th delivery (generation) to 6 pups per delivery and down up to eventual 2 in the last 12th delivery. The sex ratio influence towards the female gender among groups 5, 6 and 8 was maintained and so was male gender inclination in groups 2, 3, 4 and 7. Absence of gender influence was maintained in groups 1 & 9.

Conclusions

The mean litter sizes were not found to be influenced by birth order and were universally similar in all the groups and families. In particular among the first ten deliveries there was an average litter size of 8.5 pups per delivery. Birth order was found to decline in numbers as from the 10, 11th & 12 deliveries with declining range of 6 - 12 pups per delivery. In all the nine groups, birth order was not found to affect sex ratios.

Table 4.120 Control group one - Water solution

Effects of families and generations on sex ratios

Generatios→ **Families ↓**	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**Totals**
1	8:3:5	8:5:3	8:2:6	10:6:4	8:4:4	7:3:4	8:4:4	8:3:1:3:1	65:31:34
2	8:4:4	8:4:3:1	5:3:2	9:4:5	8:4:4	6:3:3	7:2:5	9:5:4	60:29:31
3	11:6:5	6:3:3	5:1:4	8:3:5	10:6:4	8:2:1:3:2	9:3:6	8:6:2	65:31:34
4	13:3:10	8:6:2	6:3:3	10:5:5	8:2:6	9:2:1:6	8:4:4	6:3:3	68:29:39
5	12:5:1:6	10:4:6	10:5:5	12:7:5	10:3:7	8:3:4:1	9:4:5	8:4:4	79:36:43
6	8:3:5	10:6:4	8:5:3	8:2:1:5	9:4:5	10:5:5	8:5:3	10:6:4	71:37:34
7	10:6:4	12:8:4	6:2:3:1	8:4:4	9:6:3	8:2:1:5	8:4:4	8:4:4	69:37:32
8	10:2:8	8:4:4	8:4:1:3	9:5:4	12:5:7	7:4:3	9:3:6	7:3:4	70:31:39
9	10:5:5	10:6:4	7:4:3	8:4:3:1	8:6:2	8:4:4	6:3:3	10:6:4	67:38:29
10	10:6:4	8:1:1:6	8:3:5	9:6:3	10:7:3	9:4:5	9:4:5	8:4:4	71:36:35
11	8:2:1:5	8:4:4	10:5:5	10:5:5	8:5:2:1	10:6:4	10:5:5	9:5:4	73:38:35
12	8:5:3	9:3:6	6:3:3	10:4:6	10:5:5	8:4:4	10:6:4	8:6:2	69:36:33
13	10:6:4	8:6:2	10:6:1:4	8:2:6	10:6:4	10:6:4	9:3:6	8:4:4	73:39:34
14	12:6:6	8:3:5	8:5:1:2	10:6:4	8:4:4	9:3:6	10:4:6	7:3:3:1	72:35: 37
15	10:4:6	8:4:4	10:7:3	8:3:1:4	10:5:5	10:6:4	8:4:4	9:3:6	73:37:36
16	12:5:7	10:6:3:1	8:6:2	11:7:4	10:3:7	11:5:6	10:5:5	8:4:4	80:41:39
Totals	160:72:78	139:74:65	123:64:59	148:75:73	148:75:73	138:65:73	138:63:75	131:70:61	
Pups/Del	9 : 5 : 4	9 :5 : 4	8 : 4 : 4	9 : 5 : 4	9 : 5 : 4	9 : 4 :5	9 : 4 : 5	8 : 4 : 4	

…/Table 4.120

	9	**10**	**11**	**12**	**Sub total**	**Grand totals**	**No delivery**	**Average Pups**
1	9:4:5	8:4:4	5:2:2:1		22:10:12	87:41:46	11	8 : 4 : 4
2	8:4:4	4:2:2	6:4:2	4:3:1	22:13:9	82:43:39	12	7 : 4 : 3
3	6:2:4	3:2:1			9:4:5	74:36:38	10	7 : 3 :4
4	8:4:4	6:2:1:2:1			14:7:7	82:36:46	10	8 : 4 : 4
5	9:6:3	6:3:3			15:9:6	94:46:48	10	9 : 4 : 5
6	8:5:1:2	7:5:2	6:1:4:1	6:3:3	27:15:12	98:51:47	12	8 : 4 : 4
7	6:2:1:3	4:2:1:1			10:5:5	79:42:37	10	8 : 4 : 4
8	8:5:3	6:5:1	5:4:1	4:2:2	23:16:7	93:47:46	12	7 : 4 : 3
9	8:4:4	5:2:3			13:6:7	80:44:36	10	8 : 4 : 4
10	7:4:3	6:3:3	4:1:2:1		17:8:9	88:47:41	11	8 : 4 : 4
11	9:2:1:6	5:4:1	6:3:3	6:4:1:1	26:14:12	99:52:57	12	8 : 4 : 4
12	7:2:5	8:5:1:2	6:2:4		21:10:11	90:45:45	11	8 : 4 : 4
13	6:3:3	8:4:4	5:2:3	6:1:3:2	25:10:15	98:49:49	12	8 : 4 : 4
14	8:2:6	6:0:6	6:3:3	3:1:2	23:6:17	95:39:56	12	8 : 4 : 4
15	9:5:4	7:6:1	8:4:1:3		24:15::9	97:52:45	11	9 : 5 : 4
16	8:4:4	8:5:1:2	4:3:1		20:12:8	100:54:46	11	9 : 5 : 4
Totals	124:61:63	97:57:40	61:29:32	29 : 14:15		1436: 724 :712	176	8.00
	8 : 4 : 4	6 :3.5:2.5	4 : 2 : 2	2 : 1 : 1		8.2 : 4.1 : 4.1		

Total families and generations versus their litter sizes and sex ratios.

Table 4.121 Control group – water solution

Effects of generations on sex ratios

Generations		T : F : M	Families	T : F : M	Families	T : F : M
1	160:72:78	9 : 5 : 4	1	8 : 4 : 4	13	8 : 4 : 4
2	139:74:65	9 : 5 : 4	2	7 : 4 : 3	14	8 : 4 : 4
3	123:64:59	8 : 4 : 4	3	7 : 3 :4	15	9 : 5 : 4
4	148:75:73	9 : 5 : 4	4	8 : 4 : 4	16	9 : 5 : 4
5	148:75:73	9 : 5 : 4	5	9 : 4 : 5		
6	138:65:73	9 : 4 : 5	6	8 : 4 : 4		
7	138:63:75	9 : 4 : 5	7	8 : 4 : 4		
8	131:70:61	8 : 4 : 4	8	7 : 4 : 3		
9	124:61:63	8 : 4 : 4	9	8 : 4 : 4		
10	97:57:40	6 : 3.5:2.5	10	8 : 4 : 4		
11	61:29:32	4 : 2 : 2	11	8 : 4 : 4		
12	29 : 14:15	2 : 1 : 1	12	8 : 4 : 4		
Average		7.5 : 4 : 3.5				

Generations / families versus mean litter size and sex ratios.

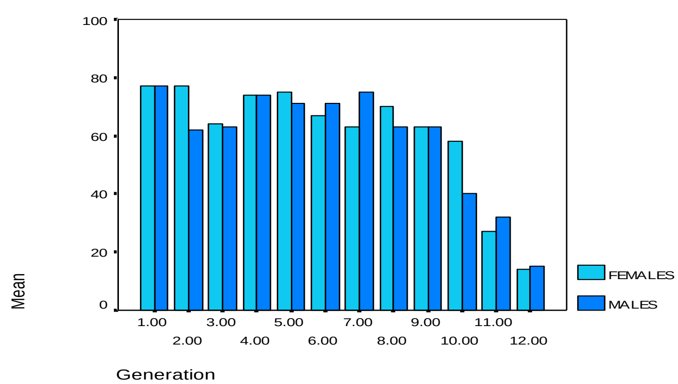


Figure 4.83 Generations versus litter size and sex ratios

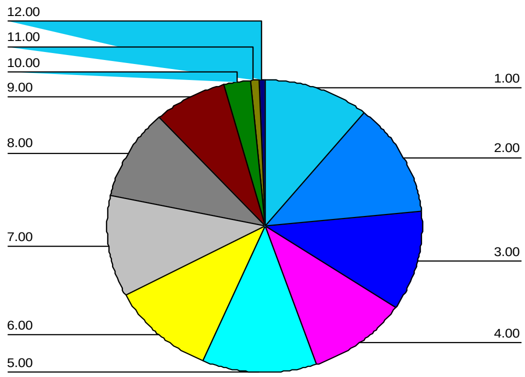


Figure 4.84 Generational representation

Conclusion

In group one, there were 176 deliveries with 1436 pups delivered comprising of 724 females and 712 males. The mean litter size was 8.2 pups per delivery and mean female to male litter sizes were 4.1 respectively. There was no evidence of sex ratio influence.

Table 4.122 Dietary study control group two - Glucose solution

Generations→ Families ↓	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**Totals**
1	8:3:5	8:6:2	4:1:3	12:4:8	10:5:5	8:2:6	9:2:7	6:2:4	65:25:40
2	8:4:3:1	6:2:4	8:4:4	12:6:6	12:7:5	9:4:5	6:2:4	8:2:1:5	69:31:37
3	6:3:3	9:6:3	6:2:3:1	14:8:6	10:5:5	10:3:7	8:3:5	6:1:5	69:31:38
4	7:5:2	11:4:7	12:6:6	8:2:6	8:2:6	9:3:5:1	6:3:3	8:2:6	69:27:42
5	10:5:5	8:3:5	10:7:3	6:1:1:4	8:3:5	8:2:5:1	8:2:6	9:4:5	67:28:39
6	8:2:6	10:6:4	12:4:8	10:5:5	10:4:6	7:3:1:3	9:3:6	10:5:5	76:33:43
7	8:5:1:2	10:2:8	10:5:5	8:4:4	9:2:7	8:5:3	10:4:6	8:2:1:5	71:31:40
8	10:6:4	11:6:5	10:4:6	7:3:3:1	8:3:5	10:6:4	9:3:1:5	8:4:4	73:36:37
9	12:7:5	8:3:1:4	9:3:6	10:5:5	8:2:6	8:3:3:2	8:3:5	10:5:5	73:32:41
10	12:6:6	10:6:4	6:3:2:1	8:3:5	10:4:6	10:5:5	9:3:5:1	10:4:6	75:34:41
11	9:6:1:2	9:2:7	11:5:6	10:6:4	8:5:2:1	10:3:7	10:4:6	8:4:4	75:36:39
12	10:7:3	11:4:7	6:2:4	10:2:8	8:4:4	9:4:5	8:4:3:1	9:3:6	71:30:41
13	12:5:7	10:4:6	8:2:1:5	10:4:6	10:6:4	8:2:4:2	10:4:6	10:6:4	78:34:44
14	10:4:6	8:3:5	10:5:5	8:4:4	9:5:4	10:3:7	8:4:4	8:4:4	71:32:39
15	9:4:4:1	10:4:6	8:3:5	10:4:6	9:3:6	10:4:6	10:3:7	9:2:1:1	75:27:48
16	11:4:7	10:4:6	10:7:3	12:4:8	11:5:6	10:3:7	10:4:6	7:5:2	81:36:45
TOTALS	150:78:72	149:66:83	140:64:76	155:66:89	148:65:83	144:56:88	138:52:86	134:57:77	1158:504:652
	9 :4.5:4:5	9 : 4 : 5	9 :4 : 5	10 :4 : 6	9 : 4 : 5	9 : 3.5 : 5.5	9 : 3 : 6	8 : 3.5 : 4.5	

.../Table 4.122

Effects of families and generations on sex ratios

Generationss→ Families ↓	**9**	**10**	**11**	**12**	**Sub-totals**	**Totals**	**Number of deliveries**	**Average Pups/delivery**
1	8:6:2	6:1:5	4:2:2		18:9:9	83:34:49	11	7 : 3 : 4
2	10:4:4:2				10:4:6	79:35:43	9	9 : 4 : 5
3	9:4:5	8:4:4	7:3:4	5:2:3	29:13:16	98:44:54	12	8 : 4 : 4
4	10:6:4	9:3:6	6:3:3	4:2:2	29:14:15	98:41:57	12	8 : 3 : 5
5	8:4:3:1	6:3:3	8:2:6	6:2:4	28:11:17	95:39:56	12	8 : 3 : 5
6	6:2:4	7:3:4	9:4:5		22:9:13	98:42:56	11	9 : 4 : 5
7	8:3:5	5:0:3:2	6:4:2	5:1:4	24:8:16	95:39:56	12	8 : 3 : 5
8	5:2:3	6:3:3	7:3:4		18:8:10	91:44:47	11	8 : 4 : 4
9	8:4:4	8:2:6			16:6:10	89:38:51	10	9 : 4 : 5
10	6:2:4	7:4:3	5:2:3	6:3:3	24:11:13	99:45:54	12	8 : 4 : 4
11	8:2:5:1				8:2:6	83:38:45	9	9 : 5 : 5
12	9:3:6	7:2:5			16:5:11	87:35:51	10	9 : 4 : 5
13	9:4:5				9:4:5	87:38:49	9	10 : 4 : 6
14	8:3:5	6:2:4	4:1:1:2	4:1:3	22:7:15	93:40:53	12	8 : 3 : 5
15	7:2:5				7:2:5	82:29:53	9	9 : 3 : 6
16	8:4:4				8:4:4	89:40:49	9	10 :4 : 6
TOTAL	127:57:70	75:27:48	56:25:31	30:12:18		1446: 622: 824	170	8.00
	8: 4 :4	5 : 2 : 3	4 : 2 :2	2. : 1 : 1		8.5 : 3.7 : 4.8		

Table 4.123 Dietary study control group two - Glucose solution

Effects of generations on sex ratios

Generations		**T : F : M**	**Families**	**T : F : M**	**Families**	**T : F : M**
1	150:78:72	9 : 4.5 : 4:5	1	7 : 3 : 4	13	10 : 4 : 6
2	149:66:83	9 : 4 : 5	2	9 : 4 : 5	14	8 : 3 : 5
3	140:64:76	9 : 4 : 5	3	8 : 4 : 4	15	9 : 3 : 6
4	155:66:89	10 :4 : 6	4	8 : 3 : 5	16	10 :4 : 6
5	148:75:73	9 : 4 : 5	5	8 : 3 : 5		
6	144:56:88	9 : 3.5 : 5.5	6	9 : 4 : 5		
7	138:52:86	9 : 3 : 6	7	8 : 3 : 5		
8	134:57:77	8 : 3.5 : 4.5	8	8 : 4 : 4		
9	127:57:70	8 : 4 : 4	9	9 : 4 : 5		
10	75:27:48	5 : 2 : 3	10	8 : 4 : 4		
11	56:25:31	4 : 2 : 2	11	9 : 5 : 5		
12	30:12:18	2 : 1 : 1	12	9 : 4 : 5		
Means	1446 : 622: 824	7.5 : 3.25 : 4.25				

Generations / families versus mean litter sizes and sex ratios

Total families and generations versus their litter sizes and sex ratios.

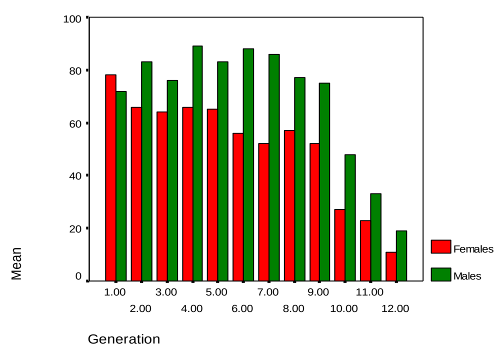


Figure 4.85 Generations versus litter size and sex ratios

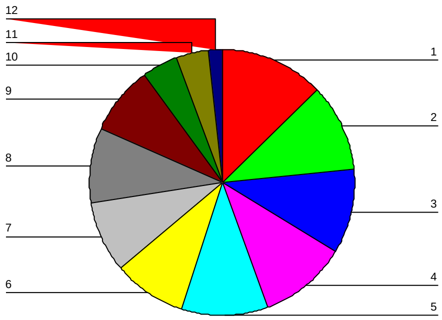


Figure 4.86 Generational representation

Conclusion

In group two, there were 170 deliveries with 1446 pups delivered comprising of 622 females and 824 males. The mean litter size was 8.5 pups per delivery and mean female to male litter numbers were 3.7 to 4.8 respectively. The dietary sex ratio influence towards the male gender was demonstrated but no generational influence or association was demonstrated or established. The average pups delivered among the 1 – 9 generations or deliveries were between 8 – 9 and dropped from the 10^{th} delivery from 6 pups per delivery to 2 in the last 12^{th} delivery.

Table 4.124 Dietary study group three - The all-inclusive cocktail solution

Gender distribution among families / generations and their effects on sex ratios

Generations → **Families ↓**	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**Totals**
1	9:3:6	6:4:2	6:2:4	10:3:7	8:1:1:6	6:2:4	7:3:3:1	8:4:4	60:23:37
2	8:2:6	8:3:5	8:2:6	12:4:8	7:1:6	8:2:5:1	6:3:3	8:6:2	65:23:42
3	11:5:6	8:4:4	10:3:7	8:2:5:1	8:1:7	10:4:6	8:2:6	9:4:5	72:25:47
4	11:3:1:6:1	6:2:4	10:3:7	12:2:10	6:2:4	8:3:5	9:4:5	6:2:4	68:22:46
5	8:3:5	5:3:2	10:4:6	10:2:1:7	10:4:6	9:5:4	8:4:3:1	8:3:5	68:29:39
6	8:4:4	10:3:7	10:4:6	8:2:6	8:1:1:5	10:4:6	9:3:6	9:3:6	72:26:46
7	5:2:3	10:4:6	12:5:7	10:3:6:1	10:2:8	8:3:5	10:4:6	8:4:4	73:27:46
8	8:2:6	12:4:8	8:3:5	9:3:6	11:3:8	10:7:3	9:5:1:3	8:4:4	75:32:43
9	7:2:5	10:4:6	8:2:6	11:3:8	10:4:5:1	8:5:3	10:4:6	9:6:3	73:30:43
10	12:4:8	10:4:6	8:1:7	10:2:8	8:3:5	9:3:1:5	10:5:5	9:3:5:1	76:26:50
11	10:2:8	9:2:6:1	10:3:7	12:3:1:8	10:3:7	8:2:6	9:3:6	6:2:1:3	74:22:52
12	12:4:6	10:2:8	9:1:8	8:3:4:1	10:5:5	9:2:7	10:5:5	8:4:4	74:26:48
13	10:1:1:8	8:2:6	10:4:6	10:5:5	10:4:6	9:3:5:1	8:4:4	8:3:1:4	73:28:45
14	11:2:9	10:1:1:8	8:2:6	10:6:4	10:3:7	10:3:7	10:4:5:1	7:4:3	76:26:50
15	12:4:8	10:2:1:7	8:3:5	10:3:7	12:4:8	10:4:6	8:3:5	9:3:5:1	79:27:52
16	11:2:9	10:4:6	10:4:1:5	9:6:3	12:6:5:1	11:5:6	10:3:7	9:2:7	82:33:49
Totals	151:47:104	142:54: 88	145:48:97	159:54:105	150:48:102	143:57:86	141:60 :81	129:59 :70	1150:425:725
	9.5 : 3 : 6.5	9 : 3 : 6	9 : 3 : 6	9 : 3 : 6	9 : 3 : 6	8.5 :3.5: 5	8:5.3.5:5	8: 3.5:4.5	

…/Table 4.124

Generations → Families ↓	**9**	**10**	**11**	**12**	**Sub totals**	**Totals**	**No delivery**	**Average Pups/delivery**
1	7:2:5	6:3:3	8:2:6		21:7:14	81:30:51	11	6 : 2 : 4
2	8:1:7	6:2:4			14:3:11	79:26:53	10	8 : 3 : 5
3	8:1:1:6	7:2:5	6:2:4	5:2:3	26:8:18	98:33:65	12	7 : 3 : 5
4	9:4:5	6:1:5	7:4:3	6:4:2	28:13:15	96:35:61	12	8 : 3 : 5
5	8:4:4	6:1:5	6:3:3		20:8:12	88:37:51	11	8 : 3 : 5
6	7:1:1:5	8:2:6			15:4:11	87:30:57	10	9 : 3 : 6
7	9:2:7	6:2:4	7:5:2	5:1:4	27:10:17	100:37:63	12	8 : 3 : 5
8	6:3:2:1	8:2:6			14:5:9	89:37:52	10	9 : 4 : 5
9	8:3:5	6:3:3	4:3:1		18:9:9	91:39:52	11	8 : 3 : 5
10	8:4:4	7:2:5	6:2:4	5:2:2:1	26:10:16	102:36:66	12	9 : 3 : 6
11	9:2:6:1	8:4:4			17:6:11	91:28:63	10	9 : 3 : 6
12	9:4:5	6:2:4	7:1:6		22:7:15	96:33:63	12	8 : 3 : 5
13	7:2:5				7:2:5	80:30:50	9	9 : 3 : 6
14	10:2:8				10:2:8	86:28:58	9	9 : 3 : 6
15	8:3:5	9:4:5	8:5:3	4:2:2	29:14:15	108:41:67	12	9 : 3 : 6
16	9:2:7	8:3:4:1			17:5:12	99:38:61	10	10 : 4 : 6
TOTAL	130:42 :88	97:33:64	59:27:32	25:11:14		1471:538:933	173	
	8 : 3.5 : 5.5	6 : 2 : 4	4 : 2 : 2	2 : 1 : 1				

Total generations and families versus mean litter sizes and sex ratios.

Table 4.125 Study group three - Sodium solution

Effects of generations on sex ratios

Generations	**T : F : M**	**Families**	**T : F : M**	**Families**	
1	9.5 : 3 : 6.5	1	6 : 2 : 4	13	9 : 3 : 6
2	9 : 3 : 6	2	8 : 3 : 5	14	9 : 3 : 6
3	9 : 3 : 6	3	7 : 3 : 5	15	9 : 3 : 6
4	9 : 3 : 6	4	8 : 3 : 5	16	10 : 4 : 6
5	9 : 3 : 6	5	8 : 3 : 5		
6	8.5 :3.5: 5	6	9 : 3 : 6		
7	8:5.3.5:5	7	8 : 3 : 5		
8	8: 3.5:4.5	8	9 : 4 : 5		
9	8 : 3.5 : 5.5	9	8 : 3 : 5		
10	6 : 2 : 4	10	9 : 3 : 6		
11	4 : 2 : 2	11	9 : 3 : 6		
12	2 : 1 : 1	12	8 : 3 : 5		
Mean	7.5:2.8:4.7				

Litter size / Sex ratios versus generations and families.

The average pups delivered were constantly between 8 – 9 among the first nine generations or deliveries and dropped from the 10th delivery from 6 pups per delivery to 2 in the last 12th delivery. The sex ratio influence towards the male gender was maintained. The generations had no effect on sex ratio outcomes.

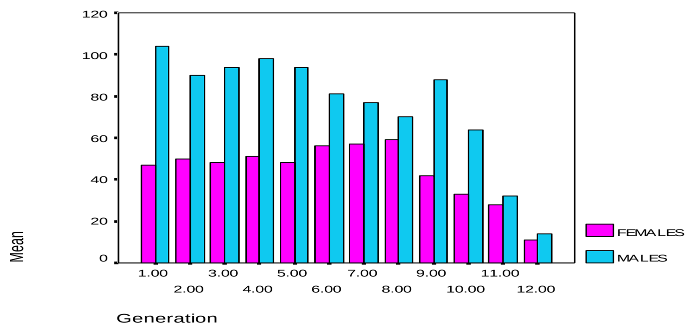


Figure 4.87 Generations versus litter size and sex ratios

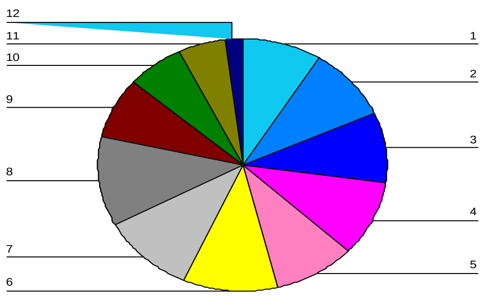


Figure 4.88 Generational representation

Conclusion

In group three, there were 173 deliveries with 1471 pups delivered comprising of 538 females and 933 males. The mean litter size was 8.4 pups per delivery and mean sex ratio of 3.1 females to 5.3 males. The gender male inclination demonstrated earlier was maintained but no generational association was established.

Table 4.126 Dietary study group four - Potassium solution.

Gender distribution across the families / generations and their effects on sex ratios

Generations → Families ↓	1	2	3	4	5	6	7	8	Sub totals
1	5:2:3	8:1:1:6	9:2:7	10:2:7:1	8:2:6	10:4:6	6:2:1:3	8:4:4	64:21:43
2	6:1:1:4	8:4:4	10:4:6	8:3:4:1	6:2:4	8:5:3	5:3:2	9:3:5:1	60:26:34
3	8:3:5	9:2:7	8:1:1: 6	9:2:7	10:3:7	10:6:4	8:4:3:1	6:3:3	68:25:43
4	10:5:5	8:3:5	12:4:8	8:1:7	10:2:8	12:9:2:1	9:3:6	9:4:5	78:31:47
5	8:3:5	10:2:8	10:3:7	8:3:5	12:2:1:9	10:5:4:1	8:4:4	9:5:1:3	75:29:46
6	8:4:3:1	12:4:8	8:2:6	10:4:6	10:4:6	8:3:5	10:2:1:7	8:4:4	74:28:46
7	6:2:4	9:2:6:1	10:4:6	8:3:5	12:7:1:4	8:2:6	9:6:3	9:5:3:1	70:32:39
8	7:1:1:5	12:3:9	10:5:4:1	12:3:9	10:6:4	7:3:1:3	10:5:5	7:4:2:1	75:32:43
9	8:2:6	10:2:1:7	9:2:7	10:4:6	9:4:5	10:4:6	8:3:4:1	8:4:4	72:26:46
10	12:3:9	8:1:1:5:1	8:2:6	12:4:8	10:6:4	8:4:4	10:5:5	6:1:1:4	74:28:46
11	12:3:1:8	9:3:5:1	10:4:1:5	10:4:6	8:4:3:1	9:4:1:4	10:4:6	8:2:6	76:33:45
12	12:4:7:1	10:4:6	9:2:7	12:7:4:1	10:4:6	10:4:6	10:5:1:4	9:4:5	82:35:47
13	10:2:8	8:3:4:1	10:5:5	10:4:1:5	9:5:4	10:5:5	9:4:5	8:3:4:1	74:33:41
14	11:3:8	10:4:6	12:4:8	8:2::15	8:6:2	11:4:7	10:5:5	6:3:3	76:32:44
15	10:3:7	10:4:6	9:2:1:5:1	10:3:7	10:4:6	10:6:4	9:6:3	8:6:2	76:35:41
16	12:4:8	8:3:5	10:6:3:1	12:5:7	9:2:1:6	9:4:5	10:6:4	8:5:3	78:36:42
TOTAL	145:48:97	149:46:103	154:55:99	157:56:101	151:66:85	150:74:76	141:70:71	126:62:64	1173:479:694
	9 : 3 : 6	9.6 :3.3:6.1	9.5:3.4:6.	10:3.6: 6.4	9.4:4.1:5.3	9.4:4.6:4.8	8.8:4.4:4.4	7.9:3.9:4	

Generations → Families ↓	9	10	11	12	Sub totals	Grand totals	No. deliveries	Average Pups/delivery
1	8:2:6	8:1:7	6:2:4		22:5:17	86: 26:60	11	8 : 2 : 6
2	9:4:5	8:3:5	7:1:6	5:2:3	29:10:19	89:36:53	12	7 : 3 : 4
3	8:1:1:6	6:1:5			14:3:11	82:28:54	10	8 :2 : 4
4	10:4:6	9:3:6	8:2:6	6:1:4:1	33:10:23	111:41:70	12	9 :3 : 6
5	9:2:7	8:3:4:1	8:4:4	7:2:5	32:11:21	107:40:67	12	9 : 3 : 6
6	6:1:5	7:3:4	5:2:3		18:6:12	92:34:58	11	8 : 3 : 5
7	7:3:4	6:3:3	8:1:6:1		21:7:14	91:38:53	11	8 :3 : 5
8	8:2:6	8:2:1:5			16:4:12	90:35:55	10	9 : 4 : 5
9	8:4:4	9:4:5	6:1:1:4		23:9:14	95:36:59	11	9 : 3 : 6
10	9:3:5:1	8:2:6	8:4:1:3	6:1:1:4	31:10:21	105:38:67	12	9 :3 : 6
11	8:2:6	6:3:3			14:5:9	90:38:52	10	9 : 4 :5
12	6:1:5	5:2:3			11:3:8	93:38:55	10	9 : 4 : 5
13	8:3:5	6:2:4	6:3:3		20:8:12	96:40:56	11	9 : 4 : 5
14	10:3:7	8:1:1:6			18:5:13	94:37:57	10	9: 4 : 5
15	8:3:4:1				8:3:5	84:38:46	9	9 : 4 :5
16	9:1:8	8:2:5:1			17:3:14	95:39:56	10	9 : 4 : 5
TOTAL	131:40:91	110:37:73	62:21:41	24:6:18		1500 : 582 : 918	172	
	8 : 2.5 :5.5	7 : 2.3:3.7	4 :1.3:2.7	1.5 : 0.4:1.1		7.8 : 3.1 : 4.7		

Total generations and families versus mean litter sizes and sex ratios.

Table 4.127 Study group three - Sodium solution

Effects of generations on sex ratios

Generations	**T : F : M**	**Families**	**T : F : M**	**Families**	**T : F : M**
1	9 : 3 .0 : 6.0	1	8 : 2 : 6	11	9 : 4 : 5
2	9.6 :3.3 : 6.1	2	7 : 3 : 4	12	9: 4 : 5
3	9.5 : 3.4 : 6.1	3	8 :2 : 4	13	9 : 4 :5
4	10 : 3.6 : 6.4	4	9 :3 : 6	14	9 : 4 : 5
5	9.4 : 4.1 : 5.3	5	9 : 3 : 6		
6	9.4 : 4.6 : 4.8	6	8 : 3 : 5		
7	8.8 : 4.4 : 4.4	7	8 :3 : 5		
8	7.9 : 3.9 : 4	8	9 : 4 : 5		
9	8 : 2.5 : 5.5	9	9 : 3 : 6		
10	7 : 3.3 : 3.7	10	9 :3 : 6		
11	4.0 : 1.3:2.7	11	9 : 4 :5		
12	1.5: 0.4:1.1	12	9 : 4 : 5		

Generations versus litter size and sex ratios.

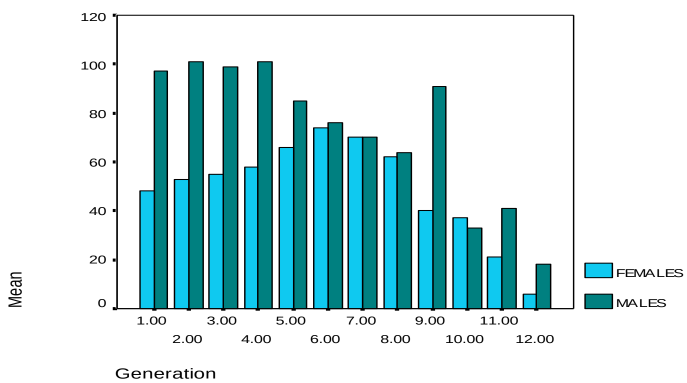


Figure 4.89 Generations versus litter size and sex ratios

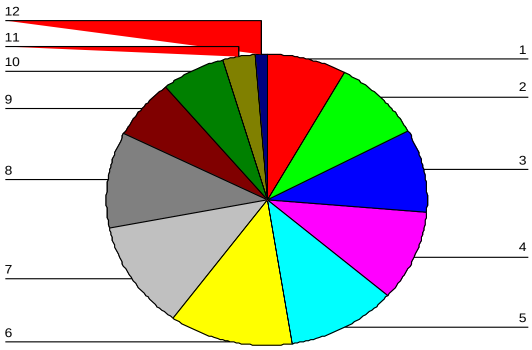


Figure 4.90 Generational representation

Conclusion

In group four, there were 172 deliveries with 1500 pups delivered comprising of 581 females and 919 males. The mean litter size was 7.8 per family and mean female to male litter sizes was 3.1 and 4.7 respectively. The dietary male gender inclination established earlier was maintained but no generational influenced was demonstrated.

Table 4.128 Study group five - Calcium solution

Gender distribution among the families / generations and their effects on sex ratios

Generations→ Families↓	1	2	3	4	5	6	7	8	Totals
1	5:4:1	7:3:1:3	8:6:2	10:6:4	6:3:3	10:4:6	6:3:2:1	8:4:4	60:34:26
2	7:5:2	6:3:3	10:6:4	12:9:3	9:7:1:1	8:4:4	9:5:4	7:5:2	68:44:24
3	7:3:4	8:5:3	8:5:1:2	6:4:1:1	8:6:2	9:6:3	6:2:4	9:5:4	61:37:24
4	6:3:3	10:6:3:1	10:8:2	10:5:1:4	8:5:3	8:4:4	6:2:1:3	8:3:5	66:38:28
5	8:5:3	12:8:4	8:4:4	8:6:2	9:6:3	10:3:1:6	8:3:5	9:3:5:1	72:39:33
6	6:4:2	10:6:3:1	9:7:2	10:6:4	10:5:1:4	8:3:5	8:4:4	6:4:2	67:40:27
7	5:3:1:1	10:5:5	10:7:3	8:4:3:1	8:6:2	9:5:3:1	7:3:1:3	8:6:2	65:41:24
8	8:4:4	12:8:4	9:4:5	10:6:4	9:6:2:1	8:4:4	10:6:4	8:4:4	74:42:32
9	12:6:6	10:5:5	8:6:2	9:7:2	12:4:8	10:6:4	8:4:4	10:4:5:1	79:42:37
10	8:5:2:1	8:5:3	12:8:4	10:7:3	10:4:6	9:4:5	10:3:7	9:3:1:5	76:40:36
11	12:10:2	7:5:2	8:5:2:1	12:7:1:4	8:5:3	8:4:4	9:4:5	10:5:5	74:46:28
12	10:6:4	9:6:3	10:7:3	9:5:4	8:3:5	6:4:2	10:6:4	9:5:4	71:42:29
13	11:7:1:3	10:7:3	10:6:1:1	9:4:5	8:3:5	10:4:6	9:6:2:1	8:6:2	75:44:31
14	12:9:3	11:7:1:3	10:5:5	8:3:5	10:7:2:1	11:6:5	9:6:3	8:4:4	71:44:27
15	10:6:3:1	10:5:5	9:4:5	10:7:3	12:4:8	8:6:2	10:4:1:5	6:4:2	69:37:32
16	12:8:4	10:7:3	10:5:5	9:7:2	10:8:2	12:6:6	10:6:4	9:6:3	73:41:22
TOTAL	130:81:49	149:92:57	149:94:55	153:98:55	145:83:62	144:74:70	135:70:65	132:72:60	1144:681:473
	8 : 5.: 3	9: 6 : 3	9: 6 :3	9: 6 : 3	9:5 : 4	9 : 5 : 4	8 : 4 :4	8 :4 : 4	

Generations Families	9	10	11	12	Sub total	Grand totals	Number of deliveries	Average pups per delivery
1	10:6:4	8:7:1	6:3:2:1		24:16:8	84:50:34	11	8 : 5 : 3
2	9:7:2	8:5:3	8:6:2	6:4:2	31:22:9	99:66:33	12	8 : 5 : 3
3	7:3:4	8:4:4	6:3:3	5:3:2	26:13:13	87:50:37	12	7: 4 : 3
4	8:5:1:2	7:5:2	5:3:1:1		20:13:7	86:52:34	11	8 : 5 : 3
5	9:7:2	8:5:3			17:12:5	89:51:38	10	9 : 5 : 4
6	10:7:3	9:6:3	8:4:4	6:4:1:1	33:21:12	100:61:39	12	8 : 5 : 3
7	6:2:1:3	6:2:4			12:5:7	77:46:31	10	8 : 5 : 3
8	8:6:2	8:5:3	6:3:3		22:4:8	96:56:40	11	9 : 5 : 4
9	6:3:3	5:2:3			11:5:6	90:47:43	10	9 : 5 : 4
10	9:5:1:3	8:6:2	6:5:1	6:3:2:1	29:20:9	105:60:45	12	9 : 5 : 4
11	8:5:3	7:3:3:1			15:8:7	89:54:35	10	9 : 5 : 4
12	8:5:2:1				8:5:3	79:49:30	9	9 : 5 : 4
13	9:8:1	8:5:3	6:4:2	7:4:3	30:21:9	105:65:40	12	9 : 5 : 4
14	7:5:2	6:3:1:2			13:9:4	84:53:31	10	8 : 5 : 3
15	8:5:3	7:3:4	6:5:1		21:13:8	90:49:41	11	7 : 5 : 4
16	10:8:2	7:6:1			17:14:3	90:55:35	10	9 : 5 : 4
TOTAL	128:92:36	110:68:42	57:36:21	30:18:12		1450:864:586	174	
	8 :6 : 2	7 : 4 : 3	3.5 : 2 .2:1.3	2 : 1 : I		8.3 :5 :3.3		

Table 4.129 Dietary Study group five - Calcium solution

Effects of generations on sex ratios

Generations	T : F : M	Families	T : F : M	Females	T : F : M
1	8 : 5.: 3	1	8 : 5 : 3	13	9 : 5 : 4
2	9: 6 : 3	2	8 : 5 : 3	14	8 : 5 : 3
3	9: 6 : 3	3	7: 4 : 3	15	7 : 5 : 4
4	9: 6 :3	4	8 : 5 : 3	16	9 : 5 : 4
5	9:5 : 4	5	9 : 5 : 4		
6	9:5 : 4	6	8 : 5 : 3		
7	8 : 4 :4	7	8 : 5 : 3		
8	8 : 4 :4	8	9 : 5 : 4		
9	8 :6 : 2	9	9 : 5 : 4		
10	7 : 4 : 3	10	9 : 5 : 4		
11	3.5 : 2 .2:1.3	11	9 : 5 : 4		
12	2 : 1 : I	12	9 : 5 : 4		

Generations and families versus mean litter sizes and sex ratios

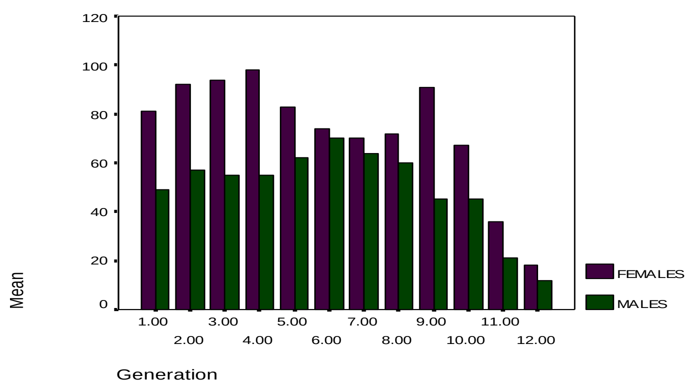


Figure 4.91 Generations versus litter size and sex ratios

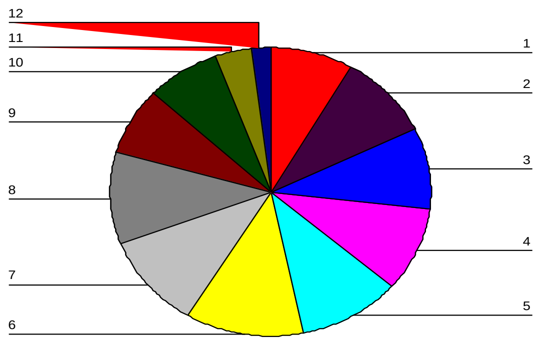


Figure 4.92 Generational representation

Conclusion

In group five, there were 174 deliveries with 1473 pups delivered comprising of 885 females and 588 males. The mean litter size was 8.3 per delivery and mean female to male litter sizes was 5 to 3.3 respectively. The dietary female gender inclination established before was maintained but no generational influenced was demonstrated.

Table 4.130 Dietary Study group six - Magnesium solution

Gender distribution among the families / generations and their effects on sex ratios

Generations → **Families ↓**	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**Totals**
1	5:3:1:1	2:0:2	12:4:8	10:6:4	8:5:3	8:4:4	6:4:2	8:6:2	59:33:26
2	6:5:1	8:4:1:3	8:4:4	8:2:6	10:6:3:1	9:4:5	8:6:2	9:5:1:3	66:38:28
3	5:2:2:1	4:2:2	6:4:2	11:6:5	9:6:3	10:4:6	8:5:2:1	9:7:2	62:36:26
4	8:4:4	8:6:2	10:7:1:2	10:6:4	8:6:1:1	10:5:5	6:3:1:2	7:5:2	67:44:23
5	6:3:3	8:4:3:1	8:7:1	11:5:1:5	10:6:4	8:2:1:5	7:4:3	8:4:4	66:37:29
6	7:3:1:3	12:8:4	12:8:4	8:6:2	6:4:1:1	7:4:3	8:6:2	9:3:1:5	69:44:25
7	8:6:2	12:9:3	10:5:5	9:7:2	10:6:4	8:3:5	9:5:1:3	8:5:3	74:47:27
8	9:6:3	10.7:3	12:8:4	8:6:2	8:4:1:3	9:5:4	10:6:4	9:6:3	75:49:26
9	10:3:1:6	8:6:2	8:6:1:1	7:4:3	12:4:8	10:5:1:4	10:8:2	8:6:1:1	73:44:29
10	12:8:4	9:5:4	10:8:1:1	10:8:2	11:5:6	9:6:2:1	12:7:5	8:3:1:4	81:51:30
11	12:7:5	10:6:4	12:8:4	9:5:1:3	10:4:6	8:6:2	8:5:2:1	9:6:3	78:48:30
12	8:5:2:1	10:4:6	10:4:1:5	12:8:4	9:2:1:6	10:7:3	8:6:2	8:6:1:1	75:44:31
13	10:4:6	10:7:3	10:6:4	8:5:3	10:5:5	10:7:3	8:4:4	9:7:2	75:45:30
14	12:8:4	8:3:1:4	10:5:5	8:3:5	13:7:1:5	10:6:4	10:6:4	8:5:3	79:45:34
15	10:7:3	12:7:5	9:2:7	11:4:7	9:7:2	12:8:3:1	10:7:3	9:7:2	82:49:33
16	14:8:6	10:6:4	10:3:1:6	12:8:4	10:5:5	12:7:5	8:4:4	6:3:3	82:45:37
TOTAL	142:85:57	141:86:55	157:92:65	152:91:61	153:85:68	150:85:65	136:88:48	132:87:45	1163:699:464
	9 : 5 : 4	9 : 5 : 4	10 : 6 : 4	9 :5 : 4	10 : 5 : 5	9 : 5 : 4	8 : 5 : 3	8 : 5 : 3	

.../Table 4.130

Generations → Families ↓	9	10	11	12	Totals	No delivery	Average pups per delivery
1	10:7:3	8 :6:2	6:3:3		83:49:34	11	8 : 5 : 3
2	8:4:4	9:5:4	7:5:2	6:4:2	96:56:40	12	8 : 5 : 3
3	8:6:2	6:4:1:1			76:47:29	10	7 : 5 : 3
4	8:7:1	9:3:6	6:3:3		90:57:33	11	8 : 5 : 3
5	6:4:2	6:4:2	6:5:1		84:50:34	11	8 : 5 : 3
6	4:1:1:2	8:5:2:1			81:51:30	10	8 : 5 : 3
7	8:5:3	8:4:4	7:4:3	5:4:1	102:64:38	12	8 : 5 : 3
8	6:4:2	7:2:4:1	6:3:3		94:58:36	11	8: 5 : 3
9	8:5:3	9:2:1:6	8:4:4	5:3:2	103:59:44	12	8 : 5 : 3
10	7:2:1:4	5:3:2			93:57:36	10	9 : 6 : 3
11	8:4:4	6:4:2	4:2:2		96:58:38	11	8 : 5 : 3
12	6:5:1	7:3:4	6:4:2	6:3:3	100:59:41	12	8 : 5 : 3
13	9:7:2	9:6:3			93:58:35	10	9 : 5 : 4
14	8:6:2	8:4:4	5:3:1:1		100:58:42	11	9 : 5 : 4
15	9:8:1	8:6:2	8:6:1:1	6:3:3	113:73:40	12	10 : 6 : 4
16	6:4:2	4:3:1			92:52:40	10	9 : 5 : 4
TOTAL	119:81:38	117:66:51	69:43:26	28:17:11	1496:906:590	176	
	7 : 5 : 2	7 : 4 : 3	4 : 3 : 1	2 : 1 : 1			

Total families and generations versus mean litter sizes and sex ratios

Table 4.131 Dietary study group six - Magnesium solution

Effects of generations on sex ratios

Generations	T : F : M	Females	T : F : M	Females	T : F : M
1	9 : 5 : 4	1	8 : 5 : 3	13	9 : 5 : 4
2	9 : 5 : 4	2	8 : 5 : 3	14	9 : 5 : 4
3	10 : 6 : 4	3	7 : 5 : 3	15	10 : 6 : 4
4	9 :5 : 4	4	8 : 5 : 3	16	9 : 5 : 4
5	10 : 5 : 5	5	8 : 5 : 3		
6	9 : 5 : 4	6	8 : 5 : 3		
7	8 : 5 : 3	7	8 : 5 : 3		
8	8 : 5 : 3	8	8: 5 : 3		
9	7 : 5 : 2	9	8 : 5 : 3		
10	7 : 4 : 3	10	9 : 6 : 3		
11	4 : 3 : 1	11	8 : 5 : 3		
12	2 : 1 : 1	12	8 : 5 : 3		

Generations and families versus mean litter sizes and sex ratios.

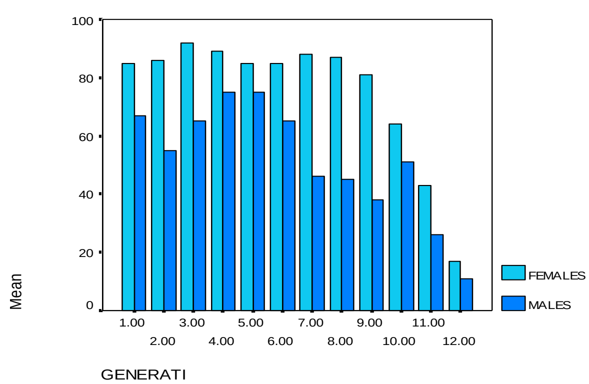


Figure 4.93 Generations versus litter size and sex ratios

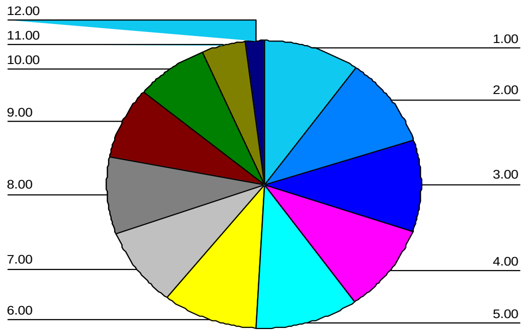


Figure 4.94 Generational representation

Conclusion

Group six had 176 deliveries with 1496 pups delivered comprising of 906 females and 590 males. The mean litter size was 8.5 per family and mean female to male litter sizes was 5.1 to 3.4 respectively. The dietary female gender inclination established before was maintained but no generational influence was demonstrated.

Table 4.132 Dietary Study group seven - Sodium/Potassium solution

Gender distribution among the families / generations and their effects on sex ratio

Generations → Families↓	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**Totals**
1	6:2:4	4:1:3	12:3:9	8:1:7	8:2:5:1	10:3:7	8:2:1:5	8:3:5	64:18:46
2	6:3:3	5:2:3	8:2:6	10:3:6:1	7:2:4:1	9:6:3	6:3:3	9:3:6	60:24:36
3	6:2:4	5:1:3:1	10:1:1:8	6:2:4	10:2:8	12:8:4	8:2:6	9:4:5	66:23:43
4	8:3:4:1	6:3:3	12:4:8	12:4:8	12:4:8	9:6:2:1	10:4:6	8:4:4	77:32:45
5	10:3:7	5:0:5	6:2:3:1	8:3:5	9:1:1:7	10:4:6	6:3:2:1	7:3:4	61:20:41
6	8:2:1:5	10:3:7	10:2:8	9:2:7	8:1:7	10:2:1:7	8:2:6	9:5:4	72:21:51
7	8:2:6	10:1:1:8	12:3:9	10:3:6:1	10:2:8	8:4:4	8:3:4:1	8:3:1:4	74:23:51
8	8:2:5:1	12:4:8	10:3:7	8:2:6	9:4:5	10:7:3	9:4:1:4	9:3:6	75:30:45
9	6:2:4	8:2:6	8:2:6	9:1:1:7	10:3:7	8:4:31:	10:5:5	8:2:6	67:22:45
10	7:3:4	10:3:6:1	11:4:7	10:3:7	8:1:1:6	8:2:6	8:3:5	9:2:7	71:22:49
11	12:4:8	8:1:1:6	9:3:6	10:2:7:1	9:2:7	9:4:1:4	10:6:4	8:4:4	75:28:47
12	12:3:9	8:3:4:1	12:4:8	9:1:8	10:5:5	8:4:3:1	8:5:3	9:3:6	76:28:48
13	10:2:7:1	8:1:1:6	10:1:9	10:6:4	8:3:5	10:7:3	8:3:1:4	8:2:6	72:27:45
14	12:2:10	9:3:6	8:2:6	12:6:5:1	10:4:6	9:3:6	10:4:6	8:3:5	78:27:51
15	11:2:9	10:3:7	12:5:7	10:4:6	10:5:5	12:6:6	10:2:1:7	9:1:8	84:29:55
16	10:2:8	12:3:9	9:5:4	12:6:6	10:5:1:4	10:3:7	11:6:5	7:2:4:1	81:33:48
TOTAL	140:40:100	130:37:93	159:47:112	153:50:103	148:49:99	152:76:76	138:61:77	133:48:85	1153:407:746
	9: 3 : 6	8 : 3 : 5	10 : 3 : 7	9 : 3 : 6	9 : 3 : 6	10 : 5 : 5	9 : 4 : 5	8 : 3 : 5	

.../Table 4.132

Generations → **Families ↓**	**9**	**10**	**11**	**12**	**Totals**	**No delivery**	**Average pups per delivery**
1	8:3:5	7:1:6	6:2:4		85:24:61	11	8 : 2 : 6
2	7:3:3:1	6:1:5			73:28:45	10	7: 3 : 4
3	10:3:7	8:2:6	7:3:4	5:1:3:1	96:32:64	12	8 : 3 : 5
4	8:1:7	6:2:4			91:35:56	10	9 : 4 : 5
5	10:2:7:1	8:1:7			79:23:56	10	8 : 2 : 6
6	9:3:6	8:3:5	8:1:7	4:2:2	101:30:71	12	9: 3 : 6
7	8:3:5	6:3:3	8:1:6:1	6:1:5	102:31:71	12	9:3 : 6
8	9:3:1:5	10:3:6:1	8:2:6	5:1:4	107:40:67	12	9 :3 : 6
9	8:2:6	8:3:4:1			83:27:56	10	8 :3 : 5
10	9:1:8	7:2:5	8:2:5:1		95:27:68	11	9 : 3 : 6
11	8:3:5	9:2:7	8:1:7		100:34:66	11	9 : 3 : 6
12	7:1:6	6:3:3	6:1:1:4		95:34:61	11	9 : 3 : 6
13	8:4:4	7:1:6	8:3:5	6:1:5	101:36:65	12	8 : 3 : 6
14	9:2:7	8:2:6	7:3:4		102:34:68	11	9 : 3 : 6
15	8:2:1:5	8:4:3:1			100:36:64	10	10 : 3 : 6
16	8:1:6:1	9:2:7	8:1:7		106:37:69	11	10 : 3 : 6
TOTAL	134:39:95	121:35:86	82:20:62	26 : 6 :20	1516:508:1008	177	
	8 : 2 : 6	7 : 2 : 5	5 : 1 : 4	2 : 0.5: 1.5:	8.5 :2.8 : 5.7		

Total families and generations versus mean litter sizes and sex ratios

Table 4.133 Dietary study group seven - Sodium / Potassium solution

Effects of generations on sex ratios

Generations	T : F : M	Families	T : F : M	Families	T : F : M
1	9: 3 : 6	1	8 : 2 : 6	13	8 : 3 : 6
2	8 : 3 : 5	2	7: 3 : 4	14	9 : 3 : 6
3	10 : 3 : 7	3	8 : 3 : 5	15	10 : 3 : 6
4	9 : 3 : 6	4	9 : 4 : 5	16	10 : 3 : 6
5	9 : 3 : 6	5	8 : 2 : 6		
6	8 :4 :4	6	9: 3 : 6		
7	9 : 4 : 5	7	9:3 : 6		
8	8 : 3 : 5	8	9 :3 : 6		
9	8 : 2 : 6	9	8 :3 : 5		
10	7 : 2 : 5	10	9 : 3 : 6		
11	5 : 1 : 4	11	9 : 3 : 6		
12	2 : 0.5: 1.5:	12	9 : 3 : 6		

Generations and families versus litter size and sex ratios.

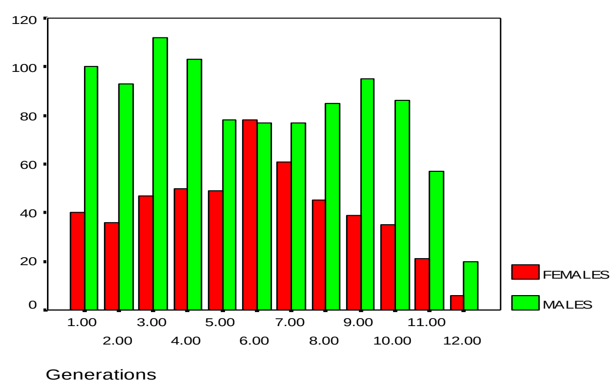


Figure 4.95 Generations versus litter size and sex ratios

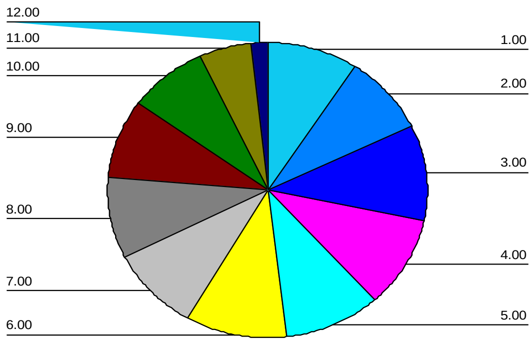


Figure 4.96 Generational representation

Conclusion

In group seven, there were I77 deliveries with 1516 pups delivered comprising of 508 females and 1008 males. Mean litter size was 8.5 pups per family and mean female to male litter sizes were 2.8 and 5.7 respectively. The dietary male gender inclination established before was maintained but no generational influence was demonstrated.

Dietary study

Table 4.134 Dietary Study group eight - Calcium / Magnesium solution

Gender distribution among families / generations and their effects on sex ratios

Generations→ Families	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**Totals**
1	7:5:2	6:3:3	10:8:2	12:9:3	8:6:2	8:4:4	6:3:1:2	8:6:2	65:45:20
2	9:5:1:3	8:5:3	10:7:3	10:8:2	10:8:2	10:6:4	8:4:4	6:2:1:3	71:47:24
3	6:4:2	6:4:2	12:8:4	12:8:4	9:5:1:3	7:3:4	9:3:5:1	8:5:3	69:41:28
4	8:4:4	10:6:3:1	8:6:2	8:4:1:3	8:5:3	10:7:3	8:6:2	9:6:3	69:45:24
5	7:3:4	5:3:1:1	8:7:1	6:4:2	10:7:1:2	10:6:4	9:8:1	9:4:5	64:44:20
6	8:5:2:1	12:8:4	9:7:2	8:6:1:1	8:6:2	11:56	10:73	8:6:2	74:51:23
7	6:4:1:1	8:6:2	8:4:4	10:73	8:6:2	10:64	8:3:5	9:7:2	67:44:23
8	12:10:2	10:7:3	10:7:3	9:7:2	10:82	8:3:1:4	7:4:3	8:3:1:4	74:51:23
9	13:9:4	12:9:4	12:102	10:8:1:1	12:93	6:4:2	9:3:6	9:6:3	83:57:26
10	12:9:3	9:6:3	6:4:2	9:7:2	8:5:3	10:73	8:4:4	9:4:5	72:47:25
11	10:6:4	12:9:2:1	9:6:3	11:83	10:46	8:4:1:3	8:6:2	8:6:2	76:50:26
12	8:6:2	10:8:1:1	10:7:3	9:2:7	11:4:1:6	10:64	8:5:3	9:8:1	75:48:27
13	10:7:3	12:10:2	12:9:3	8:3:5	9:6:3	10:5:5	10:7:3	9:7: 2	80:54:26
14	12:8:4	9:6:3	10:7:3	10:3:7	7:3:4	12:6:6	9:4:1:4	8:7:1	77:45:32
15	10:7:3	12:8:4	8:5:3	10:4:6	10:6:4	10:6:4	12:8:4	7:6:1	79:50:29
16	12:9:3	10:6:1:3	11:6:5	8:4:4	12:8:4	12:8:4	10:6:4	9:5:1:3	84:54:30
TOTAL	152:103:49	151:106:45	153:108:45	150:94:56	150:99:51	152:88:64	139:83:56	133:91:42	1178:772:406
	9.5:6 : 3	9.5:6 :3	9.5:6:3	9.5:6:3	9.5:6:3	9.5:5.5:4	8.6:5.1 :3.5	8.3:5.7:2.6	

Generations → Families	**9**	**10**	**11**	**12**	**Sub totals**	**Grand totals**		**Average pups per delivery**
1	10:7:3	8:7: 1	6:4:2		24 : 18 : 6	89 :63:26	11	7 : 5 : 2
2	8:5:1:2	9:7:2	8:5:3	6:5:1	31 : 23 : 8	102:70: 32	12	7 : 5 : 2
3	7:4:3	8:6:2	4:2:2		19 : 12 : 7	88:53:35	11	7 : 4 : 3
4	9:8:1	8:5:3			17: 13 : 4	86:58:28	10	8 : 5 : 3
5	10:8:2	9:7:1:1			19 : 16 : 3	83:60:23	10	8 : 6 : 2

6	10:7:3	9:6:3	8:6:2	5:4:1	32 : 23 : 9	106:74:32	12	7 : 5 : 2
7	8:4:1:3	6:3:3			14 :8 : 6	80:51:29	10	8 : 5 : 3
8	9:7:2	8:7: 1	8:5:3		25: 19 : 6	99:70:29	11	8 : 6 : 2
9	8:6:2	6:3:1:2			14 : 9 : 5	97:67:30	10	9 : 6 : 3
10	9:6:3	8:6:2	9:6:3	6:4:2	32 : 22 : 10	104:69:35	12	7 : 4 : 3
11	9:4:5	7:5:1:1			16 : 9 : 7	93:59:34	10	8 : 5 : 3
12	8:5:2:1	9:7:2	6:3:3		23 : 15 : 8	98:63:35	11	8 : 5 : 3
13	9:8:1	10:8:2	8:5:3	7:5:2	34 : 26 : 8	114:80;34	12	7 : 5 : 2
14	8:6:2	9:5:4			17 : 11 : 6	94:56:38	10	8 : 5 : 3
15	6:5:1	8:7:1			14 : 12: 2	93:62:31	10	9: 6 : 3
16	9:6:3	7:6:1			16 : 12 : 4	100:66:34 1526	10	10 : 6 : 4
TOTAL	137:98:39	132:99:33	56:36:20	24: 18:6		1526 : 1021 : 505	172	
	8.5 : 6 : 2.5	8 : 6 : 2	3.5 :2.25:1.25	1.5:1 : 0.5		8 : 5 :3		

Table 4.135 Dietary Study group eight - Calcium / magnesium solution

Effects of generations on sex ratios

Generations	T : F : M	Family	T : F : M	Family	T : F : M
1	9.5:6 : 3	1	7 : 5 : 2	13	7 : 5 : 2
2	9.5:6 : 3	2	7 : 5 : 2	14	8 : 5 : 3
3	9.5:6 : 3	3	7 : 4 : 3	15	9: 6 : 3
4	9.5:6 : 3	4	8 : 5 : 3	16	10 : 6 : 4
5	9.5:6 : 3	5	8 : 6 : 2		
6	9.5:5.5:4	6	7 : 5 : 2		
7	8.6:5.1 :3.5	7	8 : 5 : 3		
8	8.3:5.7:2.6	8	8 : 6 : 2		
9	8.5 : 6 : 2.5	9	9 : 6 : 3		
10	8 : 6 : 2	10	7 : 4 : 3		
11	3.5 :2.25:1.25	11	8 : 5 : 3		
12	1.5:1 : 0.5	12	8 : 5 : 3		

Total families and generations versus mean litter sizes and sex ratios

Generations and families versus mean litter sizes and sex ratios.

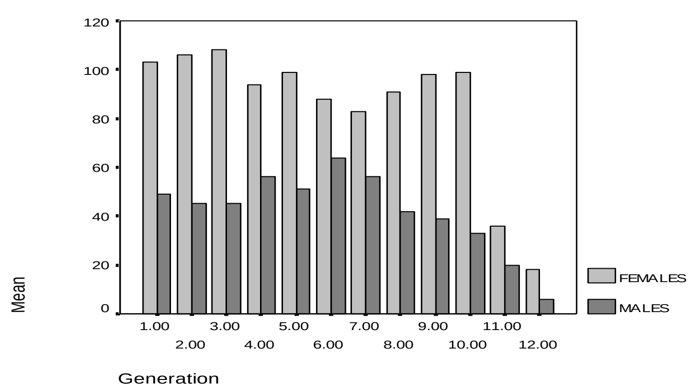


Figure 4.97 Generations versus litter size and sex ratios

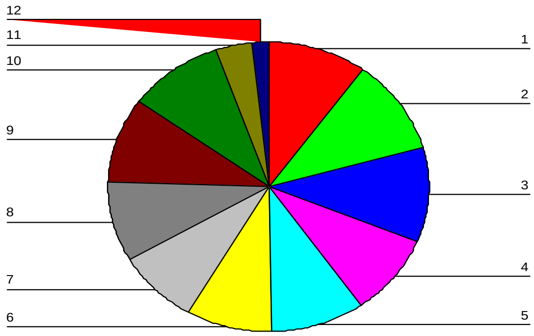


Figure 4.98 Generational representation

Conclusion

Group eight, had I72 deliveries with 1526 pups delivered comprising of 1021 females and 505 males. Mean litter size was 8 per family and mean female to male litter sizes were 5 and 3 respectively. The dietary female gender inclination established before was maintained but no generational influenced was demonstrated.

Table 4.136 Dietary study group nine – All-inclusive cocktail solution

Gender distribution among the families / generations and their effects on sex ratios

Generations→ Families ↓	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**Totals**
1	7:3:4	6:3:3	8:4:4	12:3:2:7	10:5:5	8:5:3	6:1:1:4	6:3:3	63:30:33
2	8:5:3	6:4:2	12:5:1:6	10:6:4	9:3:6	9:4:5	8:2:6	7:3:4	69:33:36
3	6:2:3:1	10:1:2:7	8:5:3	8:3:5	8:4:3:1	6:2:1:2:1	8:3:5	8:2:6	62:25:37
4	8:5:3	8:5:2:1	7:3:4	7:4:2:1	8:3:5	8:7:1	7:1:1:5	8:3:5	61:32:29
5	6:1:1:4	7:4:3	6:2:4	6:2:4	8:6:2	7:3:4	9:7:2	9:2:7	58:28:30
6	8:6:2	9:3:6	10:6:4	8:4:1:3	10:5:5	8:4:4	8:5:3	8:4:3:1	69:38:31
7	10:6:4	8:4:4	8:1:3:4	6:4:2	9:3:1:5	8:2:6	9:6:3	6:2:4	64:32:32
8	6:3:3	7:3:1:3	8:5:3	10:5:3:2	8:3:5	7:3:4	8:2:5:1	10:6:4	64:31:33
9	10:3:7	8:2:6	8:3:1:4	6:2:4	9:6:2:1	8:6:2	9:4:5	7:3:4	65:30:35
10	8:5:3	10:6:4	9:4:5	8:3:5	10:4:6	6:2:2:2	9:3:6	8:2:6	68:31:37
11	10:4:6	10:5:5	6:3:3	4:3:1	7:3:4	8:2:6	8:1:1:6	6:2:4	59:24:35
12	10:3:7	6:2:4	8:4:4	8:2:5:1	9:4:5	9:2:7	8:4:4	8:4:4	66:25:41
13	8:4:4	9:6:3	10:6:4	8:5:3	6:3:3	10:2:2:6	10:4:6	7:5:2	68:37:31
14	9:2:3:4	8:4:4	10:2:8	10:3:7	10:2:8	9:4:5	10:3:7	8:5:3	74:28:46
15	10:2:8	10:6:4	8:4:4	9:4:5	10:4:6	10:6:4	8:1:3:4	9:5:4	74:35:39
16	9:6:3	12:8:4	6:1:1:4	8:4:4	9:5:3:1	10:5:5	10:5:5	8:1:1:6	72:37:35
TOTAL	133:60:73	134:69:65	132:64:68	128:60 :68	140:64:76	131:62 :69	135:52 :83	121: 50 :71	1056:496:560
	8.2 :3.7:4.5	8.4:4.3:4.1	8.3:4:4.3	8: 4: 4	9 :4 : 5	8 :4 :4	8: 3 :5	7.5 :3 :4.5	

.../Table 4.136

Generations→ Families ↓	9	10	11	12	Sub totals.	Totals		No. of deliveries	Average pups per delivery
1	7:6:1				7:6:1	70:36:34		9	7 : 4 : 3
2	8:4:3:1	5:2:1:2	3:2:1		16:8:7	87:41:44	61:369:39	9	8 : 4 : 4
3						62:25:37	69:33:36	8	7 : 3 : 4
4	8:5:1:2	6:2:1:1:2			14: 9:5	76:34:42	62:25:37	9	7 : 4 : 4
5	6:3:2:1				6:3:3	67:35:32	61:32:29	9	7 : 3 : 4
6	7:2:4:1	4:2:2	4:2:2	5:1:1:1:2	20:8:12	78:36:42	58:28:30	9	8 : 4 : 4
7						69:38:31	69:38:31	8	8 : 4 : 4
8	6:2:1:3				6:2:4	70:34:36	64:32:32	9	8 : 4 : 4
9	7:5:1:1	6:3:1:2			13.8:5	77:39:38	64:31:33	9	8 : 4 : 4
10	8:4:4				8:4:4	76:35:41	68:31:37	9	8 : 4 : 4
11						59:24:35	59:24:35	8	7 : 3 : 4
12						66:25:41	66:25:41	8	8 : 3 : 5
13	6:1:1:2:2	7:4:1:2	3: 1:2	2:1:1	18:8:10	84:33:51	66:25:41	9	8 : 4 : 4
14	8:5:3				8:5:3	82:33:49	68:37:31	9	9 : 3 :6
15						74 :35:39	74:28:46	8	8 : 4 : 4
16		5:2:1:2			5:2:3	79:37:42	74:35:39	8	8 : 4 : 4
Totals	72:36:36	33:16:17	10:5:5	7:3:4	122:63:59	1176:560:616	72:37:35	138	

Total families and generations versus mean litter sizes and sex ratios.

Table 4.137 Dietary Study group nine – All-inclusive cocktail solution

Effects of generations on sex ratios

Generations	**T : F : M**	**Family**	**T : F : M**	**Family**	**T : F : M**
1	8.2 :3.7:4.5	1	7 : 4 : 3	12	8 : 4 : 4
2	8.4:4.3:4.1	2	8 : 4 : 4	13	9 : 3 :6
3	8.3:4:4.3	3	7 : 3 : 4	14	8 : 4 : 4
4	8: 4: 4	4	7 : 4 : 4	15	8 : 4 : 4
5	9 :4 : 5	5	7 : 3 : 4	16	8 : 4 : 4
6	8 : 4 : 4	6	8 : 4 : 4		
7	8 : 3 : 5	7	8 : 4 : 4		
8	7.5 :3 :4.5	8	8 : 4 : 4		
9	4 : 2 : 2	9	8 : 4 : 4		
10	2 : 1 1	10	8 : 4 : 4		
11	0.6:0.3:0.3	11	7 : 3 : 4		
12	0.4:0.2:0.2	12	8 : 3 : 5		

Generations and families versus litter size and sex ratios.

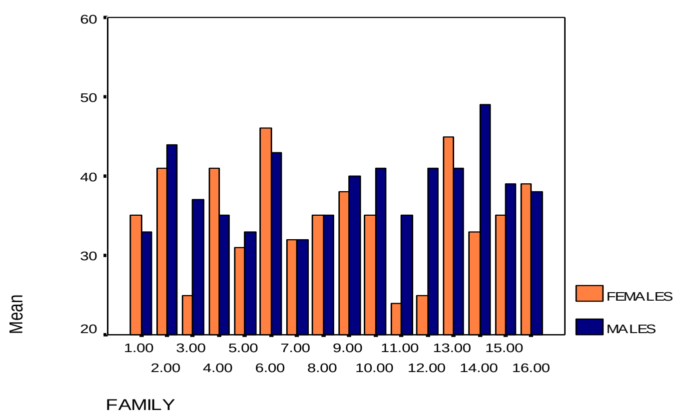


Figure 4.99 Generations versus litter size and sex ratios

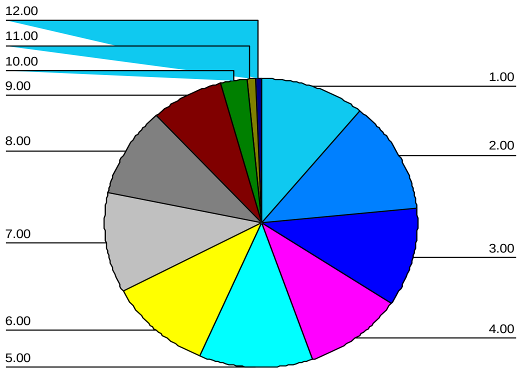


Figure 4.100 Generational representation

Conclusion

Group nine had 138 deliveries with 1176 pups delivered comprising of 560 females and 616 males. Mean litter size was 8.5 per family and mean female to male litter sizes was 4.1 to 4.4 respectively. Absence of dietary gender influence established before was maintained. Absence of generational influence was demonstrated.

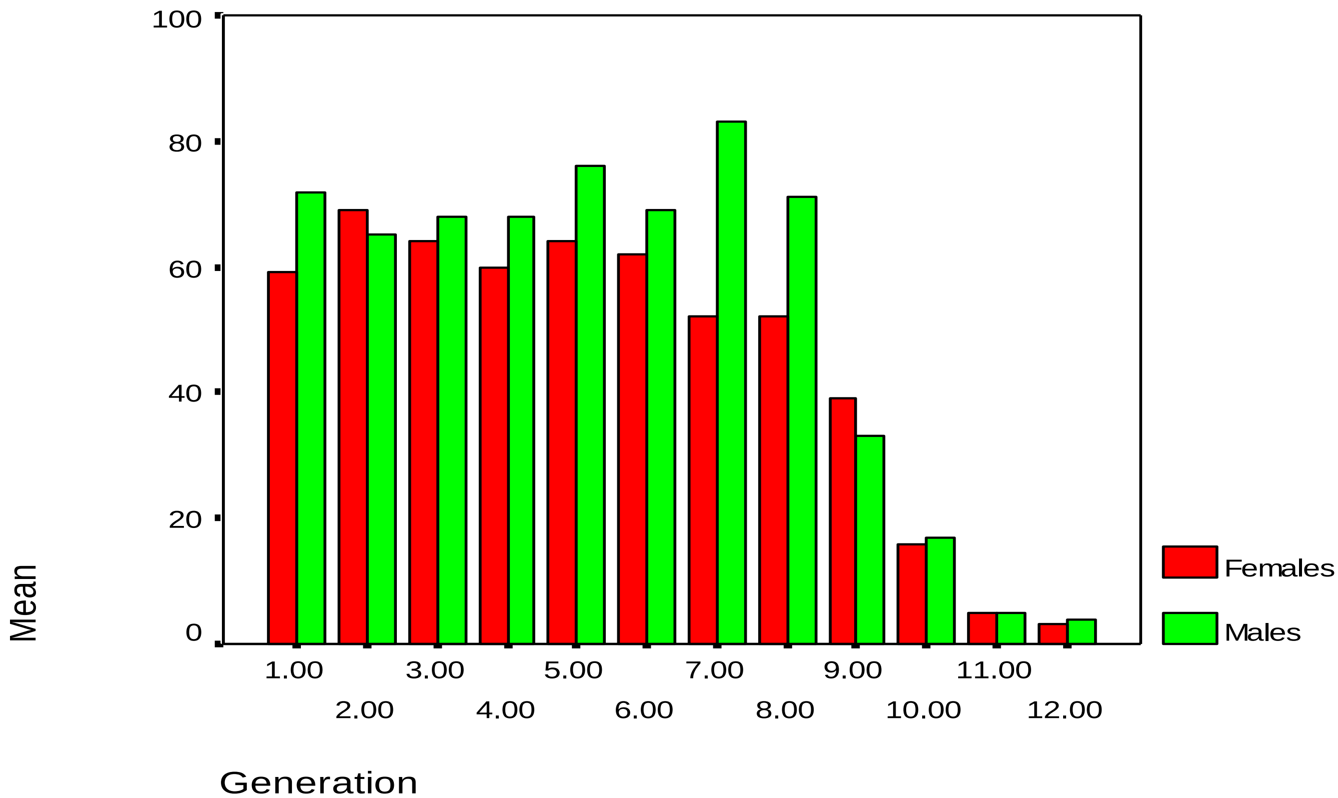


Figure 4.101 Sex ratio trends among the 12 generations / Deliveries

Conclusion

The mean litter size was 8.5litters per delivery in all the groups and generations. The litter size was not found to be influenced by birth order and was universally similar in all the groups and families. This was found in particular among the first ten deliveries with average litter size of 8.5 pups per delivery. Birth order was found to decline in numbers as from the 10, 11th & 12 deliveries with in the declining range of 6 - 2pups per delivery. In all the nine groups, birth order was not found to influence sex ratios except declining litter sizes as from the 10th delivery onwards.

The sex ratio influence was maintained as influenced by the study modulators (sodium, potassium, calcium and magnesium). No clear evidence of generational sex ratio influence was demonstrated.

4.30 Dietary Study

Effect of seasonal variations on sex ratios

A study aimed at examining the relationship between seasonal variations and sex ratios was carried out using the accumulated data. The study sough to find out if food, water and the environment were held relatively constant, whether there could be any sex ratio influence in their progeny. The rational was based on the fact that studies in the animal kingdom in their natural habitats have shown sex ratio influence related to the availability of food as well as seasonal variations.

In East Africa, there are three major climatic seasons that include the dry, rainy and cold seasons. The dry seasons last five months in a year, between the months of January to March as well as August and September. The rainy season last a period of five months too and run between the months of April and May and between October, November and December. The cold seasons last for a period of two months per year during the months of June and July.

Methods

Data on all the deliveries of the nine groups each with sixteen families was collected over their entire life spans of between two and half years. The number of deliveries were analysed against the seasons during which the study was carried out. The seasons considered were those prevailing in our local region. These include the hot, cold and the rainy seasons. The local hot seasons run in the months of January, February, March, August and September. The rainy seasons are represented by the months of April, May, October, November and December and the cold seasons prevail in the months of June and July. The study ran between August 2008 and October 2010 through a period of 26 months comprising of 12 months of dry season, 10 months of rainy season and 4 months of cold season.

Results

There were 1528 deliveries that took place, with 13,040 pups delivered. -- were delivered during the dry season at the rate of 415 pups per month, and during the rainy seasons at the rate of 730 pups per month and -- during the cold season at the rate of -- per month. More pups were therefore delivered during the rainy season followed by the dry season and the least occurred during the cold season.

Presented below is data pertaining to the study topic, analysis and conclusions.

Table 4.138 Dietary study control group one - Water solution

Effect of seasonal variations on sex ratios.

Dry

DATE	FEMALE		MALES		TOTAL	F %	M %	No. Delivery
August 2008	33.00	33.00	31.00	31.00	64.00	52%	48%	8
September 2008	71.00	38.00	74.00	43.00	145.00	48%	52%	10
TOTAL		**71.00**		**74.00**	**145**	**48%**	**52%**	**18**
Rainy								
October 2008	165	94.00	158	84.00	323	51%	49%	22
November 2008	262	97.00	251	93.00	513	51%	49%	24
December 2008	339	67.00	323	72.00	662	51%	49%	17
TOTAL		**268.00**		**249.00**	**517**	**51.8%**	**48.2%**	**63**
Dry								
January 2009	420	81.00	408	85.00	828	50%	50%	20
February 2009	445	25.00	437	29.00	882	50%	50%	6
March 2009	475	30.00	470	33.00	945	50%	50%	8
TOTAL		**136.00**		**147.00**	**283**	**48%**	**52%**	**34**
Rainy								
April 2009	502	27.00	493	23.00	995	50%	50%	6
May 2009	525	23.00	516	23.00	1041	50%	50%	6
TOTAL		**50.00**		**46.00**	**96**	**52%**	**48%**	**12**
Cold								
June 2009	560	22.00	546	18.00	1106	50%	50%	5
July 2009	577	17.00	569	23.00	1146	50%	50%	5
TOTAL		**52.00**		**53.00**	**105**	**49.5%**	**50.5%**	**10**
Dry								
August 2009	597	20.00	580	11.00	1177	51%	49%	4
September 2009	613	16.00	601	21.00	1214	50%	50%	5
TOTAL		**36.00**		**32.00**	**68**	**53%**	**47%**	**9**
Rainy								
October 2009	615	2.00	606	5.00	1221	50%	50%	1
November 2009	624	9.00	615	9.00	1239	50%	50%	2
December 2009	630	6.00	619	4.00	1249	50%	50%	1
TOTAL		**17.00**		**18.00**	**35**	**48.5%**	**51.5%**	**4**

.../Table 4.138

Dry

January 2010	670	40.00	645	26.00	1315	51%	49%	8
February 2010	678	8.00	652	7.00	1330	51%	49%	2
March 2010	701	23.00	683	31.00	1384	51%	49%	7
TOTAL		**71.00**		**64.00**	**135**	**54.4%**	**45.6%**	**17**

Rainy

April 2010	713	12.00	701	18.00	1414	51%	49%	4
May 2010	716	3.00	706	5.00	1422	51%	49%	1
TOTAL		**15.00**		**23.00**	**38**	**45.9%**	**54.1%**	**5**

Cold

June 2010	722	6.00	708	2.00	1430	51%	49%	1
July 2010	723	1.00	709	1.00	1432	51%	49%	1
TOTAL		**7.00**		**3.00**	**10**	**70%**	**30%**	**2**

Dry

August 2010	723	0.00	711	2.00	1434	51%	49%	1
September 2010	724	1.00	712	1.00	1436	51%	49%	1
TOTAL		**1.00**		**3.00**	**4**	**25%**	**75%**	**2**

Summary

Season	**Duration**	**Female**	**%**	**Male**	**%**	**Totals**	**%**	**No. delivery**	**p/del**
Dry season	12	318	49.84	320	50.16	638	44	80	7.97
Rainy season	10	347	50.81	336	49.19	683	47	84	8.13
Cold season	4	59	51.30	56	48.70	115	9	12	9.58
Totals	**26**	**724**		**712**		**1436**		**176**	**8.15**

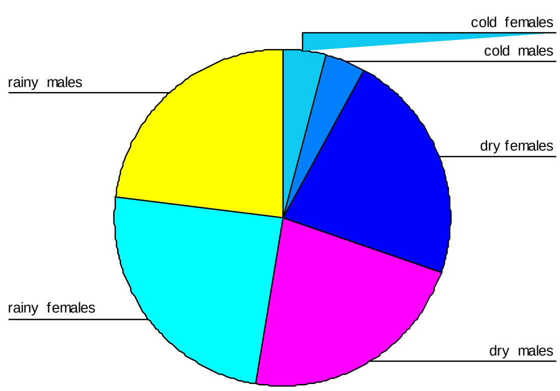


Figure 4.102 Seasonal gender representation

Summary

In group one, the dry season ran a span of 12months during the entire study period and was represented by the months of January, February, March, August and September. During this dry period, a total of 638 (53.2 per month) pups were delivered comprising of 318 (49.84%) female pups against 320 (50.16%) male pups. There was no statistical significant difference between the female and male progeny p>0.7037.

The rainy season ran a span of 10 months during the entire study and was referred to as between the months of April and March and between October, November and December. During this period, 683(68.3 per month) pups were delivered comprising of 347 (50.81) female against 336 (49.19) male pups. There was no statistical significant gender difference between the female and male progeny (p>4099).

The cold season constituted four months of the entire study period and prevails during June and July during the year. During this period, a total of 115 (28.8 per month) pups were delivered comprising of 59 (51.30%) female and 56 (48.70%) male pups delivered.

Conclusion

More pups were delivered per month during the rainy season 68.3 compared to the dry season of 53.2 pup per month and cold season of 28.8 per month. There were no statistical significant differences between the female and male progenies in all these seasons. From this data seasons appear to affect the number of pups delivered per season but not the progeny.

Table 4.139 Dietary study control group two - Glucose solution

Effect of seasonal variations on sex ratios

Dates	Females		Males		Total	%	%	No. delivery
Dry season								
August 2008	21	21.00	17	17.00	38	55%	45%	4
September 2008	69	48.00	69	52.00	138	50%	50%	12
TOTAL		**69.00**		**69.00**	**138**	**50%**	**50%**	**16**
Rainy season								
October 2008	192	123.00	208	139.00	400	48%	52%	32
November 2008	271	79.00	322	119.00	593	45%	55%	25
December 2008	339	68.00	413	91.00	742	45%	55%	19
TOTAL		**270.00**		**344.00**	**614**	**44%**	**56%**	**76**
Dry season								
January 2009	393	54.00	491	78.00	884	44%	56%	17
February 2009	415	22.00	526	25.00	941	44%	56%	5
March 2009	432	17.00	554	28.00	986	43%	57%	5
TOTAL		**93.00**		**131.00**	**224.00**	**40%**	**60%**	**27**
Rainy season								
April 2009	452	20.00	589	35.00	1041	43%	57%	6
May 2009	467	15.00	612	23.00	1079	43%	57%	4
TOTAL		**35.00**		**58.00**	**93**	**38%**	**62%**	**10**
Cold season								
June 2009	480	13.00	625	13.00	1107	43%	57%	3
July 2009	509	29.00	650	25.00	1161	43%	57%	6
TOTAL		**42.00**		**38.00**	**80.00**	**52%**	**48%**	**9**
Dry season								
August 2009	529	20.00	676	26.00	1207	44%	56%	6
September 2009	540	11.00	694	18.00	1236	43%	57%	3
TOTAL		**31.00**		**44.00**	**75.00**	**41%**	**59%**	**8**
Rainy season								
October 2009	544	4.00	701	7.00	1245	43%	57%	1
November 2009	548	4.00	710	9.00	1260	43%	57%	1
December 2009	550	2.00	722	12.00	1272	43%	57%	1
TOTAL		**10.00**		**28.00**	**38.00**	**26%**	**74%**	**3**
Dry season								
January 2010	560	10.00	735	13.00	1295	43%	57%	2
February 2010	574	14.00	753	18.00	1329	43%	57%	4
March 2010	599	25.00	784	31.00	1385	43%	57%	7
TOTAL		**49.00**		**62.00**	**111.00**	**44%**	**56%**	**13**
Rainy season								
April 2010	610	11.00	803	9.00	1415	43%	57%	2
May 2010	612	2.00	806	3.00	1418	43%	57%	1
TOTAL		**13.00**		**12.00**	**25.00**	**52%**	**48%**	**3**
Cold season								
June 2010	614	2.00	809	3.00	1425	43%	57%	1
July 2010	616	2.00	812	3.00	1428	43%	57%	1
TOTAL		**4.00**		**6.00**	**10.00**	**40%**	**60%**	**2**
Dry season								
August 2010	620	4.00	816	4.00	1436	43%	57%	1
September 2010	625	5.00	821	5.00	1446	43%	57%	1
TOTAL		**9.00**		**9.00**	**18.00**	**50%**	**50%**	**2**

…/Table 4.139

Summaries - Gp 2

Season	Duration	Female	%	Males	%	Totals	%	No. delivery	p/del
Rainy season	10	330	41.88	458	58.12	788	54	92	8.5
Dry season	12	249	43.84	319	56.16	568	39	67	8.4
Cold season	4	46	51.11	44	48,89	90	7	11	8.18
Totals	**26**	**625**		**821**		**1446**		**170**	**8.50**

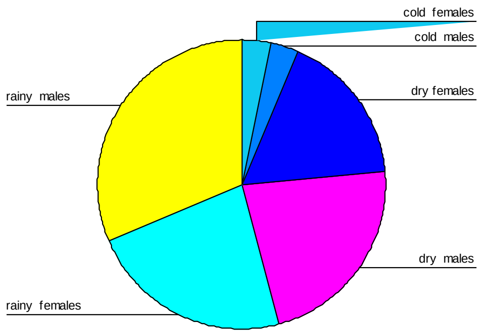


Figure 4.103 Seasonal gender representation

In the second group, the dry season ran a similar span of 12months during the entire study and was referred to as between the months of January to March and August and September. During the dry season a total of 568 pups (47.33 pups per month) comprising of 249 (43.84%) female pups against 319 (56.16%) male were delivered. There was statistical significant difference between the female and male progeny with a two-tailed p-value of 0.0240.

The rainy season ran a span of 10months during the entire study and was represented by the periods between the months of April and March and between October and December. During the rainy season, there was a total of 785 (65.4 per month) pups delivered comprising of 330 (41.88%) females and 458 (58.12%) representing a significant statistical gender difference ($p<0.0099$).

The cold season constituted four months of the entire study period between June and July. During this period, there were a total of 90 (22.5 per month) pus comprising of 46 females and 44 males delivered. There was no statistical significant difference between the female and male progeny with a two-tailed p-value of 0.7713.

Conclusion

There were more pups that were delivered during the rainy season (67.1 per month) than the dry (47.6 per month) and the cold season (22.5 per month). Statistical gender difference was observed during the rainy and the dry seasons but not during the cold seasons.

Table 4.140 Dietary study group three - Sodium solution

Effect of seasonal variations on sex ratios

Dry

Date	Female		Male		Total	%	%	No. delivery
August 2008	21	21.00	32	32.00	53	39%	61%	6
September 2008	53	32.00	89	57.00	142	37%	63%	11
TOTAL		**53.00**		**89.00**	**142**	**37%**	**63%**	**17**
Rainy								
October 2008	124	71.00	246	157.00	370	33%	67%	27
November 2008	185	61.00	412	166.00	597	30%	70%	27
December 2008	251	66.00	499	87.00	750	33%	67%	19
TOTAL		**198.00**		**410.00**	**608**	**33%**	**67%**	**73**
Dry								
January 2009	308	57.00	587	88.00	895	34%	66%	18
February 2009	335	27.00	610	23.00	955	35%	65%	6
March 2009	356	1.00	637	27.00	993	35%	65%	3
TOTAL		**105.00**		**138.00**	**243**	**43%**	**57%**	**27**
Rainy								
April 2009	382	26.00	667	30.00	1049	36%	64%	7
May 2009	402	20.00	692	25.00	1094	36%	64%	5
TOTAL		**46.00**		**55.00**	**101**	**46%**	**54%**	**12**
Cold								
June 2009	416	14.00	709	17.00	1125	36%	64%	3
July 2009	435	19.00	738	29.00	1173	37%	63%	6
TOTAL		**33.00**		**46.00**	**79**	**42%**	**58%**	**9**
Dry								
August 2009	445	10.00	760	22.00	1205	36%	64%	4
September 2009	457	12.00	781	21.00	1238	36%	64%	4
TOTAL		**22.00**		**43.00**	**65**	**34%**	**66%**	**8**

.../Table 4.140

Rainy								
October 2009	465	8.00	796	15.00	1261	36%	64%	2
November 2009	470	5.00	810	14.00	1280	37%	63%	2
December 2009	478	8.00	820	10.00	1298	37%	63%	2
TOTAL		**21.00**		**39.00**	**60**	**35%**	**65%**	**6**
Dry								
January 2010	485	7.00	836	16.00	1321	36%	64%	3
February 2010	491	6.00	852	16.00	1343	36%	64%	2
March 2010	518	27.00	885	33.00	1402	37%	63%	7
TOTAL		**40.00**		**65.00**	**105**	**40%**	**60%**	**12**
Rainy								
April 2010	529	11.00	899	14.00	1427	37%	63%	3
May 2010	533	4.00	910	12.00	1443	37%	63%	1
TOTAL		**15.00**		**26.00**	**41**	**42%**	**58%**	**4**
Cold								
June 2010	536	3.00	925	15.00	1461	37%	63%	2
July 2010	537	1.00	928	3.00	1465	37%	63%	1
TOTAL		**4.00**		**18.00**	**22**	**18%**	**82%**	**3**
Dry								
August 2010	537	0.00	930	2.00	1467	37%	63%	1
September 2010	538	1.00	933	3.00	1471	37%	63%	1
TOTAL		**1.00**		**5.00**	**6**	**16%**	**84%**	**2**

Summary

Season	**Duration**	**Female**	**%**	**Males**	**%**	**Totals**	**%**	**No. delivery**	**p/del**
Rainy season	10	280	34.56	530	65.44	810	55	95	8.52
Dry season	12	221	39.46	339	60.54	560	38	66	8.48
Cold season	4	37	36.63	64	63.37	101	7	12	8.41
Totals	**26**	**538**		**933**		**1471**		**173**	**8.50**

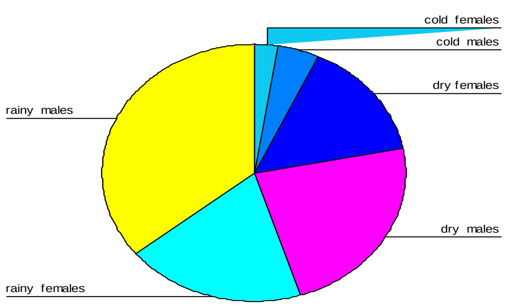


Figure 4.104 Seasonal gender representation

Summary

The dry season that span between the months of January to March and August to September constituted a total of 12 months during the entire study. During this period, there were 560 (46.67) pups per month comprising of 221 (39.46%) female pups against 339 (60.54%) male pups. No statistical significant gender difference was observed $p>0.0774$.

The rainy season constituted 10months during the entire study and ran between the months of April and March and between October November and December. During this period, there were 810 (81.0) pups delivered per month) comprising of 280 (34.56%) female pups delivered against 530 (65.44%) male pups delivered. Significant gender difference was observed between the female and males $p<0.0011$.

The cold season comprised of four months of the entire study between June and July. During this period, there were 101 (25.25 pups delivered per month) comprising of 37 (36.63%) female and 64 (63.37%) male pups delivered. No statistical significant gender difference was observed between the female and male progeny ($p>0.0926$).

Conclusion

There were more pups delivered during the rainy season of 81 pups per month than the dry and the wet seasons of 46 and 26 pups per month respectively. Statistical gender difference was observed skewed towards the male progeny during the rainy season but not during the dry and cold seasons. This gender difference was also observed in the dietary study and is most likely attributed to the dietary chemical ionic influence.

Table 4.141 Dietary study group four - Potassium solution

Effect of seasonal variations on sex ratios.

Dry season

DATE	FEMALE		MALES		TOTAL	%	%	No. delivery
August 2008	4	4.00	7	7.00	11	36%	64%	1
September 2008	39	35.00	72	65.00	111	35%	65%	12
T0TAL		**39.00**		**72.00**	**111**	**35%**	**65%**	**13**
Rainy season								
October 2008	112	73.00	240	168.00	352	32%	68%	30
November 2008	190	78.00	392	152.00	582	32%	68%	28
December 2008	283	93.00	474	82.00	757	37%	63%	20
TOTAL		**244.00**		**402.00**	**646**	**38%**	**62%**	**78**
Dry								
January 2009	346	63.00	557	83.00	903	38%	62%	18
February 2009	367	21.00	582	25.00	949	38%	62%	5
March 2009	390	23.00	609	27.00	999	39%	61%	6
TOTAL		**107.00**		**135.00**	**242**	**44%**	**56%**	**29**
Rainy								
April 2009	420	30.00	639	30.00	1059	39%	61%	7
May 2009	450	30.00	662	23.00	1112	40%	60%	6
TOTAL		**60.00**		**53.00**	**113**	**53%**	**47%**	**13**
Cold								
June 2009	462	12.00	681	19.00	1143	40%	60%	3
July 2009	475	13.00	703	22.00	1178	40%	60%	4
TOTAL		**25.00**		**41.00**	**66**	**38%**	**62%**	**7**
Dry								
August 2009	492	17.00	723	20.00	1215	41%	59%	4
September 2009	504	12.00	743	20.00	1247	40%	60%	4
TOTAL		**29.00**		**40.00**	**69**	**42%**	**58%**	**8**
Rainy								
October 2009	508	4.00	750	7.00	1258	40%	60%	1
November 2009	510	2.00	759	9.00	1269	40%	60%	1
December 2009	520	10.00	769	10.00	1289	40%	60%	2
TOTAL		**16.00**		**26.00**	**42**	**38%**	**62%**	**4**
Dry								
January 2010	528	8.00	777	8.00	1305	40%	60%	2
February 2010	543	15.00	814	7.00	1357	40%	60%	2
March 2010	564	21.00	855	41.00	1419	39%	61%	7
TOTAL		**44.00**		**86.00**	**130**	**34%**	**66%**	**11**
Rainy								
April 2010	570	6.00	876	21.00	1443	39%	61%	3
May 2010	573	3.00	886	10.00	1460	39%	61%	1
TOTAL		**9.00**		**30.00**	**41**	**24%**	**76%**	**4**
Cold								
June 2010	577	4.00	900	4.00	1475	39%	61%	1
July 2010	579	2.00	908	8.00	1488	39%	61%	2
TOTAL		**6.00**		**22.00**	**28**	**21%**	**79%**	**3**

.../Table 4.141

Dry

August 2010	580	1.00	913	5.00	1494	39%	61%	1
September 2010	581	1.00	919	6.00	1500	39%	61%	1
TOTAL		**2.00**		**11.00**	**13**	**27%**	**73%**	**2**

Summary

Season	**Duration**	**Female**	**%**	**Males**	**%**	**Totals**	**%**	**Deliveries**	**p/del**
Rainy season	10	329	39.07	513	60.93	842	56	99	8.50
Dry season	12	221	39.18	343	60.82	564	38	63	8.95
Cold season	4	31	32.9	63	67.02	94	6	10	9.40
Totals	**26**	**581**		**919**		**1500**		**172**	**8.72**

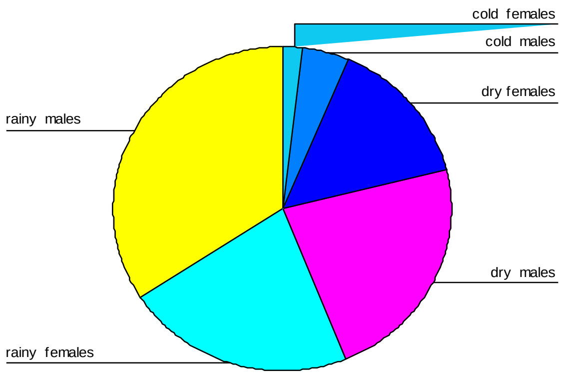


Figure 4.105 Seasonal gender representation

Summary

In the fourth group, 12 months represented the dry season during the entire study period that ran between the months of January to March and August and September. During this period, there was a total of 564 (46.7 pups per month) comprising of 221 (39.18%) female pups and 343 (60.82%) male pups that were delivered. Significant statistical difference was observed between the female and male progeny with a two-tailed $p<0.0012$.

The rainy season had a span of 10months during the entire study and was represented by the periods between the months of April and March and between October and December. During this period, 842 (84.2 per month) comprising of 329 (39.07%) female and 513 (60.93%) male pups were delivered. Statistical significant gender difference was observed with a two-tailed $p<0.0034$.

The cold season comprised of four months of the entire study period represented between June and July. During this period, there was a total of 94 (23.5 per month) comprising of 31 (32.98%) female and 63 (67.02%). No statistical significant difference was observed between the female and male progeny with a two-tailed $p>0.1898$.

Conclusion

There were more pups delivered during the rainy season of 84 pups per month than the dry and wet seasons of 47 and 24 pups per month respectively. Statistical significant gender differences skewed towards the male gender were observed during the rainy season and the dry seasons but not during the cold seasons. This difference was also observed in the dietary study and was most probably attributed to the dietary chemical ionic influence.

Table 4.142 Dietary study group five - Calcium solution

Effect of seasonal variations on sex ratios

Dry

DATE	FEMALE		MALES		TOTAL	%	%	No delivery
August 2008	16	16.00	10	10.00	26	62%	38%	3
September 2008	70	54.00	43	33.00	113	61%	39%	10
TOTAL		**70.00**		**43.00**	**113**	**61%**	**39%**	**13**
Rainy								
October 2008	236	166.00	134	93.00	370	63%	37%	30
November 2008	385	149.00	222	88.00	607	63%	37%	27
December 2008	449	64.00	300	78.00	749	59%	41%	17
TOTAL		**373.00**		**257.00**	**636**	**59%**	**41%**	**74**
Dry								
January 2009	525	76.00	349	49.00	874	60%	40%	15
February 2009	552	27.00	381	32.00	933	59%	41%	7
March 2009	582	30.00	401	20.00	983	59%	41%	6
TOTAL		**133.00**		**101.00**	**234**	**56%**	**44%**	**28**
Rainy								
April 2009	612	30.00	426	25.00	1038	58%	42%	6
May 2009	641	29.00	453	27.00	1094	58%	42%	7
TOTAL		**59.00**		**52.00**	**111**	**53%**	**47%**	**13**
Cold								
June 2009	667	26.00	464	11.00	1121	59%	41%	4
July 2009	689	22.00	476	12.00	1155	60%	40%	4
TOTAL		**48.00**		**23.00**	**71**	**67%**	**33%**	**8**
Dry								
August 2009	713	24.00	491	15.00	1194	59%	41%	4
September 2009	733	22.00	502	11.00	1225	60%	40%	4
TOTAL		**44.00**		**26.00**	**70**	**63%**	**37%**	**8**
Rainy								
October 2009	743	10.00	506	4.00	1249	60%	40%	1
November 2009	751	8.00	508	2.00	1259	60%	40%	1
December 2009	761	10.00	518	10.00	1279	60%	40%	2
TOTAL		**28.00**		**16.00**	**44**	**63%**	**37%**	**4**
Dry								
January 2010	781	20.00	536	18.00	1317	60%	40%	4
February 2010	808	27.00	546	10.00	1354	60%	40%	4
March 2010	839	31.00	567	21.00	1406	60%	40%	7
TOTAL		**78.00**	**49**	**49.00**	**127**	**63%**	**37%**	**15**
Rainy								
April 2010	852	23:00	579	12.00	1431	61%	39%	5
May 2010	862	10.00	580	11.00	1442	61%	39%	2
TOTAL		**33:00**		**23.00**	**56**	**60%**	**40%**	**7**
Cold								
June 2010	871	9.00	582	2.00	1453	61%	39%	1
July 2010	877	6.00	584	2.00	1461	61%	39%	1
TOTAL		**15.00**		**4.00**	**19**	**79%**	**21%**	**2**

…/Table 4.142

Dry

August 2010	880	3.00	587	3.00	1467	50%	50%	1
September 2010	885	5.00	588	1.00	1473	61%	39%	1
TOTAL		**8.00**		**4.00**	**12**	**89%**	**11%**	**2**

Summary – Calcium

Season	**Duration**	**Female**	**%**	**Males**	**%**	**Totals**	**%**	**Deliveries**	**p/del**
Rainy season	10	489	55	338	57	827	56	98	8.40
Dry season	12	333	37	223	38	556	38	66	8.42
Cold season	4	63	8	27	5	90	6	10	9.00
Totals	**26**	**895**		**588**		**1473**		**174**	**8.46**

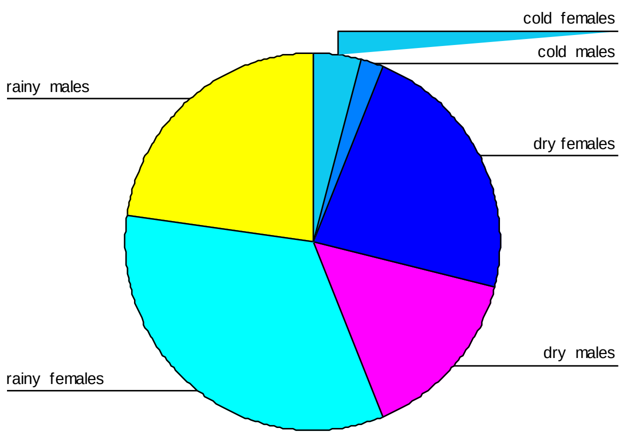


Figure 4.106 Seasonal gender representation

Summary

In group five, during the dry seasons of 12 months of the study period, there were 556 (46.33 pups per month) comprising of 333 (59.90%) female and 223 (40.10%) male pups. Statistical significant difference was found between the female and the male progeny with a p-value of <0.0004.

During the 10 month rainy seasons, 827 (82.7 pups per month) were delivered comprising of 489 (59.13%) female and 338(40.87 %) male pups. Statistical significant gender difference was found between the female and the male progeny (p<0.0025).

During the cold season, 90 (22.5 pups per month) pups were delivered comprising of 63 (70.00%) female and 27 (30%) male pups. There was statistical significant gender difference found between the female and the male progenies ($p<0.0447$).

Conclusion

There were more pups delivered during the rainy season (82.7 pups per month) than the dry and the wet seasons with 40.87 and 22.5 pups delivered per month respectively. Significant statistical gender difference was observed during all the seasons. Similar female gender inclination was observed in the dietary study in this group and is most probably attributed to the dietary chemical ionic influence although it was of no statistical significant.

Table 4.143 Dietary study group six - Magnesium solution

Effect of seasonal variations on sex ratios

Dates	Females		Males.		Totals.	%	%	No delivery
Dry season								
August 2008	9	9.00	4	4.00	13	69%	31%	2
September 2008	59	50.00	37	33.00	96	61%	39%	10
Total		**59.00**		**37.00**	**96**	**61%**	**39%**	**12**
Rainy season								
October 2008	227	68.00	150	113.00	377	60%	40%	24
November 2008	370	143.00	231	81.00	601	61%	39%	28
December 2008	441	71.00	327	96.00	768	57%	43%	22
Total		**382.00**		**290.00**	**672**	**56%**	**44%**	**74**
Dry season								
January 2009	539	98.00	374	47.00	913	59%	41%	20
February 2009	570	31.00	393	19.00	963	59%	41%	7
March 2009	601	31.00	412	19.00	1013	59%	41%	7
Total		**160.00**		**85.00**	**245**	**65%**	**35%**	**34**
Rainy season								
April 2009	633	32.00	430	18.00	1063	59%	41%	6
May 2009	655	22.00	443	13.00	1098	59%	41%	5
Total		**54.00**		**31.00**	**85**	**63%**	**37%**	**11**
Cold season								
June 2009	671	16.00	452	9.00	1123	59%	41%	3
July 2009	706	35.00	468	16.00	1174	60%	40%	6
Total		**51.00**		**25.00**	**76.00**	**67%**	**33%**	**9**
Dry season								
August 2009	734	8.00	481	13.00	1215	61%	39%	2
September 2009	755	21.00	495	14.00	1250	60%	40%	4
Total		**49.00**		**27.00**	**76**	**64%**	**36%**	**6**
Rainy season								
October 2009	765	10.00	498	3.00	1263	60%	40%	1
November 2009	773	8.00	500	2.00	1273	61%	39%	1
December 2009	783	10.00	520	20.00	1303	61%	39%	4
Total		**28.00**		**20.00**	**53**	**53%**	**47%**	**6**
Dry season								
January 2010	806	13.00	535	15.00	1341	61%	39%	3
February 2010	828	22.00	545	10.00	1373	60%	40%	4
March 2010	871	43.00	571	26.00	1442	60%	40%	8
Total		**88.00**		**51.00**	**139**	**63%**	**37%**	**15**
Rainy								
April 2010	888	17.00	582	11.00	1470	60%	40%	3
May 2010	891	3.00	584	2.00	1475	60%	40%	1
Total		**20.00**		**13.00**	**33**	**61%**	**39%**	**4**
Cold season								
June 2010	901	10.00	587	3.00	1488	60%	40%	2
July 2010	903	2.00	589	2.00	1492	60%	40%	1
Total		**12.00**		**5.00**	**17**	**70%**	**30%**	**3**
Dry season								
August 2010	904	1.00	589	0.00	1493	60%	40%	1
September 2010	906	2.00	590	1.00	1496	60%	40%	1
Total		**3.00**		**1.00**	**4**	**75%**	**25%**	**2**

Summary - Magnesium

Season	Duration	Female	%	Males	%	Total	Pups/Month	%
Dry season	12	359	64.11%	201	35.89%	560	46.67	37.43%
Rainy season	10	484	57.41%	359	42.59%	843	84.3	56.35%
Cold season	4	63	67.74%	30	32.23%	93	23.25	6.22%
Totals	**26**	**906**		**590**		**1496**		

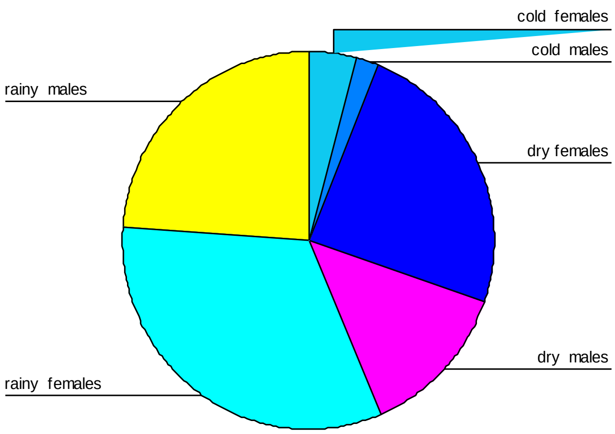


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Figure 4.107 Seasonal gender representation

Summary

During the dry season, in group six, a total of 560 pups (47.7 pups delivered per month) were delivered comprising of 359 (64.11%) female and 201 (35.89%) male pups. Statistical significant difference was observed between the female and the male progeny in this group (p<0.0024).

During the rainy seasons, 843 pups (84.3 pups were delivered per month) comprising of 484 (5741%) female and 359 (42.59%) male pups. Statistical significant difference was found between the female and the male progeny in this group (p<0.0180).

During the cold seasons, a total of 93 pups (23.25 pups delivered per month) comprising of 63 (67.74%) female and 30 (32.23%) male pups were delivered. No statistical significant difference was found between the female and the male pups (p>0.1107)

Conclusion

There were more pups delivered during the rainy season (84 pups per month) than the dry and the wet seasons with 48 and 23 pups delivered per month respectively. Significant statistical gender difference was observed during the rainy and the dry seasons but not during the cold seasons. Similar female gender inclination was observed in the dietary study in this group and is most probably attributed to the dietary chemical ionic influence.

Table 4.144 Dietary study group seven - Sodium / Potassium solution

Effect of seasonal variations on sex ratios

DATE	**FEMALE**		**MALES**		**TOTAL**		**F %**	**M %**	**No delivery**
Dry									
August 2008	8	8.00	14	14.00	22		36%	64%	2
September 2008	39	31.00	87	73.00	126		30%	70%	13
Total		**39.00**		**87.00**	**126**		**30%**	**70%**	**15**
Rainy									
October 2008	121	82.00	305	218.00	426	69.00	28%	72%	34
November 2008	171	50.00	456	151.00	627	31.00	27%	73%	23
December 2008	258	87.00	541	85.00	799	35.00	32%	68%	21
Total		**219**		**454**		**673**	**33%**	**67%**	**77**
Dry									
January 2009	301	43.00	601	60.00	902	27.00	33%	67%	10
February 2009	333	32.00	626	25.00	959	57.00	34%	66%	7
March 2009	350	17.00	649	23.00	999	40.00	35%	65%	4
Total		**92**		**108**		**200**	**46%**	**54%**	**21**
Rainy									
April 2009	372	22.00	677	28.00	1049	50.00	35%	65%	6
May 2009	390	18.00	705	28.00	1095	46.00	35%	65%	5
Total		**40**		**56**		**96**	**42%**	**58%**	**11**
Cold									
June 2009	396	6.00	716	11.00	1112	17.00	35%	65%	2
July 2009	414	18.00	756	40.00	1170	58.00	35%	65%	7
Total		**24**		**51**		**75**	**32%**	**68%**	**9**
Dry									
August 2009	427	13.00	785	29.00	1212	42.00	34%	66%	6
September 2009	438	11.00	814	29.00	1252	40.00	34%	66%	5
Total		**14**		**58**		**82**	**19%**	**81%**	**11**
Rainy									
October 2009	440	2.00	824	10.00	1264	12.00	34%	66%	1
November 2009	444	4.00	833	9.00	1277	13.00	34%	66%	1
December 2009	456	12.00	853	20.00	1309	32.00	34%	66%	4
Total		**18**		**39**		**57**	**32%**	**68%**	**6**

…/Table 4.144

Dry									
January 2010	462	6.00	906	53.00	1368	59.00	34%	66%	7
February 2010	478	16.00	912	6.00	1380	22.00	34%	66%	2
March 2010	498	20.00	966	54.00	1464	74.00	33%	67%	9
Total		**42**		**113**		**155**	**27%**	**73%**	**18**
Rainy									
April 2010	504	6.00	986	20.00	1490	26.00	33%	67%	3
May 2010	505	1.00	996	10.00	1501	11.00	33%	67%	1
Total		**7**		**30**		**37**	**16%**	**84%**	**4**
Cold									
June 2010	507	2.00	1001	5.00	1508	7.00	33%	67%	1
July 2010	507	0.00	1004	3.00	1511	3.00	33%	67%	1
Total		**2**	**8**	**8**		**10**	**20%**	**80%**	**2**
Dry									
August 2010	507	0.00	1006	2.00	1513	2.00	33%	67%	1
September 2010	508	1.00	1008	2.00	1516	3.00	33%	67%	1
Total		**1**		**4**		**5**	**20%**	**80%**	**2**

Summary

Season	**Duration**	**Female**	**%**	**Males**	**%**	**Totals**	**%**	**Delivery**	**p/del**
Rainy season	10	289	56	581	58	870	57	98	8.8
Dry season	12	193	38	368	37	561	37	67	8.37
Cold season	4	26	6	59	5	85	6	11	7.72
Totals	**26**	**508**		**1008**		**1516**		**177**	**8.56**

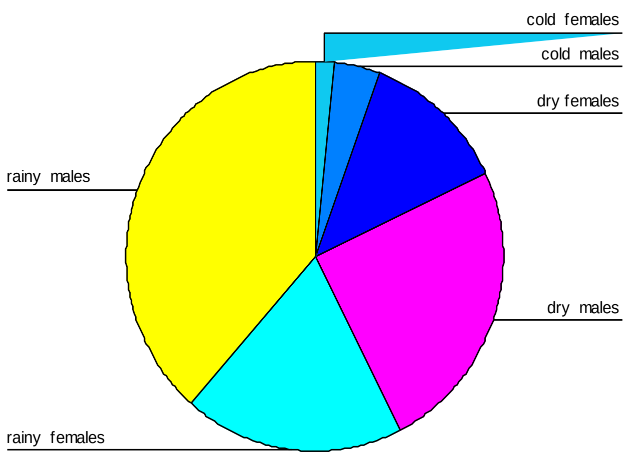


Figure 4.108 Seasonal gender representation

Summary

In group seven, during the 12 months of dry season, a total of 561 pups (46.75 delivered per month) were delivered comprising of 193 (34.40%) female and 368 (65.60%) males were delivered. Statistical significant gender difference between the female and male progeny was observed ($p<0.0079$).

In the 10months of the rainy season study period, a total of 870 pups (87.0 per month) comprising of 289 (33.22%) female and 581 (66.78%) male pups were delivered. Statistical significant gender difference between the females and males gender was observed ($p>0.0001$).

During the 4 months of the cold seasons, 85 pups (21.25 per month) comprising of 26 (30.60%) female pups and 59 (69.40%) male pups were delivered. No significant statistical difference was found between the female and male progeny ($p>0.2390$).

Conclusion

There were more pups delivered during the rainy season (87 pups per month) than the dry and the wet season with 48 and 21 pups delivered per month respectively. Significant statistical gender difference was observed during the rainy and the dry seasons but not during the cold seasons. Similar gender difference was also observed in the dietary study and is most probably attributed to the dietary chemical ionic influence.

Table 4.145 Dietary study group eight - Calcium / Magnesium solution

Effect of seasonal variations on sex ratios

DATE	**FEMALE**		**MALES**		**TOTAL**		**F %**	**M %**	**No delivery**
Dry									
August 2008	19	19.00	11	11.00	30		63%	37%	3
September 2008	80	61.00	40	29.00	120		66%	34%	11
Total		**80.00**		**40.00**	**120**		**66%**	**34%**	**14**
Rainy									
October 2008	301	221.00	124	84.00	425	77	70%	30%	35
November 2008	453	152.00	178	54.00	631	30	71%	29%	23
December 2008	532	79.00	255	77.00	787	44	67%	33%	16
Total		**452.00**		**215.00**		**667**	**67%**	**33%**	**74**
Dry									
January 2009	601	69.00	313	58.00	914	34	65%	35%	12
February 2009	624	23.00	330	17.00	954	40	65%	35%	5
March 2009	655	31.00	349	19.00	985	50	66%	34%	5
Total		**123.00**		**94.00**		**217**	**56%**	**44%**	**22**
Rainy									
April 2009	692	37.00	369	20.00	1042	57	66%	34%	5
May 2009	718	26.00	384	15.00	1083	41	66%	34%	5
Total		**63.00**		**35.00**		**98**	**64%**	**36%**	**10**
Cold									
June 2009	738	20.00	398	14.00	1117	34	66%	34%	4
July 2009	785	47.00	411	13.00	1196	60	66%	34%	7
Total		**67.00**		**27.00**		**94**	**71%**	**29%**	**11**
Dry									
August 2009	809	24.00	421	10.00	1230	34	66%	34%	4
September 2009	837	28.00	436	15.00	1273	43	66%	34%	5
Total		**52.00**		**25.00**		**77**	**67%**	**33%**	**9**
Rainy									
October 2009	848	11.00	438	2.00	1286	13	66%	34%	1
November 2009	856	8.00	440	2.00	1296	10	67%	33%	1
December 2009	870	14.00	454	14.00	1324	28	67%	33%	3
Total		**33.00**		**18.00**		**51**	**64%**	**36%**	**5**
Dry									
January 2010	916	46.00	462	8.00	1378	54	67%	33%	6
February 2010	939	23.00	471	9.00	1410	32	67%	33%	4
March 2010	975	36.00	472	21.00	1467	57	67%	33%	7
Total		**105.00**		**38.00**		**143**	**73%**	**27%**	**17**
Rainy									
April 2010	993	18.00	478	6.00	1491	24	67%	33%	3
May 2010	1002	9.00	493	8.00	1508	17	67%	33%	2
Total		**27.00**		**14.00**		**41**	**66%**	**34%**	**5**
Cold									
June 2010	1009	7.00	501	15.00	1510	22	67%	33%	2
July 2010	1016	7.00	504	3.00	1520	10	67%	33%	1
Total		**14.00**		**18.00**		**32**	**43%**	**67%**	**3**
Dry									
August 2010	1019	3.00	504	0.00	1523	3	67%	33%	1
September 2010	1021	2.00	505	1.00	1526	3	67%	33%	1
Total		**5.00**		**1.00**		**6**	**83%**	**17%**	**2**

…/Table 4.145

Summary

Season	Duration	Female	%	Males	%	Totals	%	No delivery	p/del
Rainy season	10	575	56	272	54	847	56	94	9.01
Dry season	12	365	36	188	37	553	36	64	8.64
Cold season	4	81	8	45	9	126	8	14	9.00
Totals	**26**	**1021**		**505**		**1526**		**172**	**8.87**

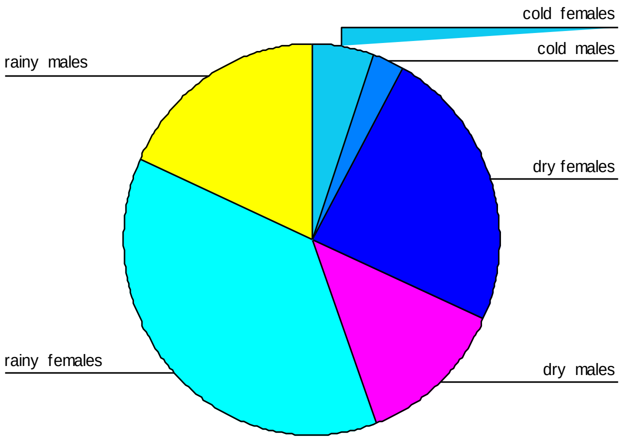


Figure 4.109 Seasonal gender representation

Summary

In group eight, during the 12 months dry seasons of study period, a total of 553 pups (46.08 delivered per month) comprising of 365 (66.00%) females and 188 (34.00%) males were delivered. Significant difference between the female and male progeny was observed ($p<0.0079$).

In the 10months rainy season study period, a total of 847 pups (84.70 per month) comprising of 575 (67.89%) female and 272 (32.11%) male pups were delivered. A statistical significant difference between the female and male gender was observed ($p<0.0001$).

During the 4 months cold seasons, 126 pups (31.50 per month) comprising of 81 (64.29%) female pups and 45 (35.71%) male pups were delivered. No significant statistical difference was found between the female and male progeny ($p>0.2390$).

Conclusion

There were more pups were delivered during the rainy season (85 pups per month) than the dry and the wet seasons with 46 and 31 pups delivered per month respectively. Significant statistical gender difference was observed during the rainy and the dry seasons but not during the cold seasons. Similar difference was observed in the dietary study and is most probably attributed to the dietary chemical ionic influence.

Table 4.146 Dietary study group nine – All-inclusive cocktail solution

Effect of seasonal variations on sex ratios.

Dry									
DATE	**FEMALE**		**MALES**		**TOTAL**		**F %**	**M %**	**No delivery**
August 2008	9	9.00	10	10.00	19		51%	49%	2
September 2008	38	29.00	35	25.00	73		52%	48%	6
Totals		**38.00**		**35.00**	**73**		**52%**	**48%**	**8**
Rainy									
October 2008	151	113.00	147	112.00	298	45.00	51%	49%	24
November 2008	228	77.00	229	82.00	457	29.00	50%	50%	20
December 2008	308	80.00	314	85.00	622	36.00	49%	51%	18
Totals		**270.00**		**279.00**	**549**		**49%**	**51%**	**62**
Dry									
January 2009	347	39.00	374	60.00	721	45.00	48%	52%	15
February 2009	365	18.00	401	27.00	766	45.00	47%	53%	5
March 2009	386	21.00	425	24.00	811	45.00	47%	53%	5
Totals		**78.00**		**111.00**	**189**		**42%**	**58%**	**25**
Rainy									
April 2009	400	14.00	450	25.00	850	52.00	47%	53%	4
May 2009	415	15.00	470	20.00	885	48.00	47%	53%	5
Totals		**29.00**		**45.00**	**74**		**34%**	**66%**	**9**
Cold									
June 2009	426	11.00	488	18.00	914	29.00	42%	58%	3
July 2009	458	32.00	504	16.00	962	48.00	43%	57%	6
Totals		**43.00**		**34.00**	**77**		**55%**	**45%**	**9**
Dry									
August 2009	465	7.00	518	14.00	983	21.00	43%	57%	2
September 2009	477	12.00	527	9.00	1004	21.00	43%	57%	2
Totals		**19.00**		**23.00**	**42**		**45%**	**55%**	**4**
Rainy									
October 2009	480	3.00	531	4.00	1011	7.00	43%	57%	1
November 2009	484	4.00	534	3.00	1018	7.00	43%	57%	1
December 2009	504	20.00	554	20.00	1058	40.00	43%	57%	5
Totals		**27.00**		**27.00**	**54**		**61%**	**39%**	**7**
Dry									
January 2010	509	5.00	559	5.00	1068	10.00	48%	52%	2
February 2010	515	6.00	566	7.00	1081	13.00	47%	53%	3
March 2010	522	7.00	572	6.00	1094	13.00	47%	53%	2
Totals		**18.00**		**18.00**	**36**		**50%**	**50%**	**7**

.../Table 4.146

Rainy									
April 2010	532	10.00	583	11.00	1115	21.00	47%	53%	2
May 2010	535	3.00	594	11.00	1129	14.00	47%	53%	1
Totals		**13.00**		**22.00**	**35**		**37%**	**63%**	**3**
Cold									
June 2010	539	4.00	601	7.00	1140	11.00	47%	53%	1
July 2010	549	10.00	603	2.00	1152	12.00	47%	53%	1
Totals		**14.00**		**9.00**	**23**		**60%**	**40%**	**2**
Dry									
August 2010	556	37.00	612	9.00	1168	16.00	47%	53%	1
September 2010	560	4.00	616	4.00	1176	8.00	50%	50%	1
Totals		**11.00**		**13.00**	**24**		**47%**	**53%**	**2**

Summary

Season	**Duration**	**Female**	**%**	**Males**	**%**	**Totals**	**%**	**No delivery**	**p/del**
Rainy season	10	338	60	378	61	716	61	81	8.83
Dry season	12	165	29	195	32	360	31	46	7.82
Cold season	4	57	11	43	7	100	8	11	9.09
Totals	**26**	**560**		**616**		**1176**		**138**	**8.52**

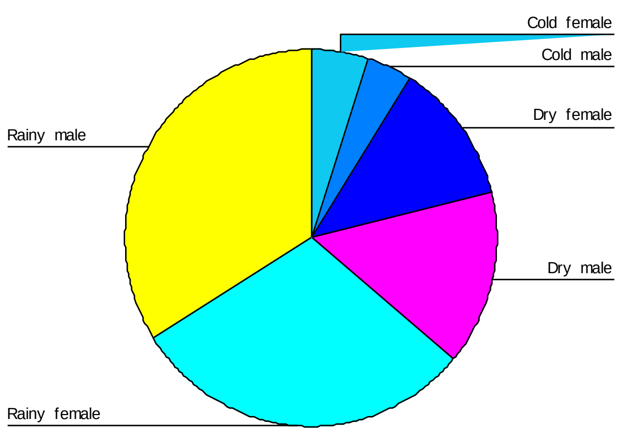


Figure 4.110 Seasonal gender representation

Summary

In group nine, during the 12months dry seasons of study period, a total of 360 pups were delivered translating into 30.00 pups per month comprising of 165 (45.83%) female and 195

(54.17%) males. No significant statistical difference was observed between the female and male progeny (p>0.0609).

During the rainy season, there were 716 (71.6 pups per month) comprising of 338(47.21 %) females and 378 (52.79 %) males delivered. No statistical significant difference was observed between the female and the male progeny in this group (p>0.4701).

During the cold season, there were 100 (25 pups/month) comprising of 57 (57.00%) females and 43 (43.00%) males. No statistical significant difference was observed between the female and the male progeny in this group (p>0.749).

Table 4.147 Dietary study summaries of the nine study groups

Effect of seasonal variations on sex ratios.

Group 1 - Water solution

Season	Duration	Female	%	Male	%	Totals	%	No delivery	p/del
Dry season	12	318	49.84	320	50.16	638	44	80	7.97
Rainy season	10	347	50.81	336	49.19	683	47	84	8.13
Cold season	4	59	51.30	56	48.70	115	9	12	9.58
Totals	**26**	**724**		**712**		**1436**		**176**	**8.15**

Group 2 - Glucose Solution

Season	Duration	Female	%	Males	%	Totals	%	Deliveries	p/del
Rainy season	10	330	41.88	458	58.12	788	54	92	8.5
Dry season	12	249	43.84	319	56.16	568	39	67	8.4
Cold season	4	46	51.11	44	48,89	90	7	11	8.18
Totals	**26**	**625**		**821**		**1446**		**170**	**8.50**

Group 3 - Sodium solution

Season	Duration	Female	%	Males	%	Totals	%	No delivery	p/del
Rainy season	10	280	34.56	530	65.44	810	55	95	8.52
Dry season	12	221	39.46	339	60.54	560	38	66	8.48
Cold season	4	37	36.63	64	63.37	101	7	12	8.41
Totals	**26**	**538**		**933**		**1471**		**173**	**8.50**

Group 4 - Potassium Solution

Season	Duration	Female	%	Males	%	Totals	%	No delivery	p/del
Rainy season	10	329	39.07	513	60.93	842	56	99	8.50
Dry season	12	221	39.18	343	60.82	564	38	63	8.95
Cold season	4	31	32.9	63	67.02	94	6	10	9.40
Totals	**26**	**581**		**919**		**1500**		**172**	**8.72**

.../Table 4.147

Group 5. Calcium solution

Season	Duration	Female	%	Males	%	Totals	%	No delivery	p/del
Rainy season	10	489	55	338	57	827	56	98	8.40
Dry season	12	333	37	223	38	556	38	66	8.42
Cold season	4	63	8	27	5	90	6	10	9.00
Totals	**26**	**895**		**588**		**1473**		**174**	**8.46**

Group 6. Magnesium Solution

Season	Duration	Female	%	Males	%	Total	Pups/Month	%
Dry season	12	359	64.11 %	201	35.89%	560	46.67	37.43%
Rainy season	10	484	57.41 %	359	42.59%	843	84.3	56.35%
Cold season	4	63	67.74 %	30	32.23%	93	23.25	6.22%
Totals	**26**	**906**		**590**		**1496**		

Group 7. Sodium / Potassium Solution

Season	Duration	Female	%	Males	%	Totals	%	Deliveries.	p/del
Rainy season	10	289	56	581	58	870	57	98	8.8
Dry season	12	193	38	368	37	561	37	67	8.37
Cold season	4	26	6	59	5	85	6	11	7.72
Totals	**26**	**508**		**1008**		**1516**		**177**	**8.56**

Group 8. Calcium /Magnesium Solution

Season	Duration	Female	%	Males	%	Totals	%	No delivery	p/del
Rainy season	10	575	56	272	54	847	56	94	9.01
Dry season	12	365	36	188	37	553	36	64	8.64
Cold season	4	81	8	45	9	126	8	14	9.00
Totals	**26**	**1021**		**505**		**1526**		**172**	**8.87**

Group 9. All-inclusive cocktail Solution

Season	Duration	Female	%	Males	%	Totals	%	No delivery	p/del
Rainy season	10	338	60	378	61	716	61	81	8.83
Dry season	12	165	29	195	32	360	31	46	7.82
Cold season	4	57	11	43	7	100	8	11	9.09
Totals	**26**	**560**		**616**		**1176**		**138**	**8.52**

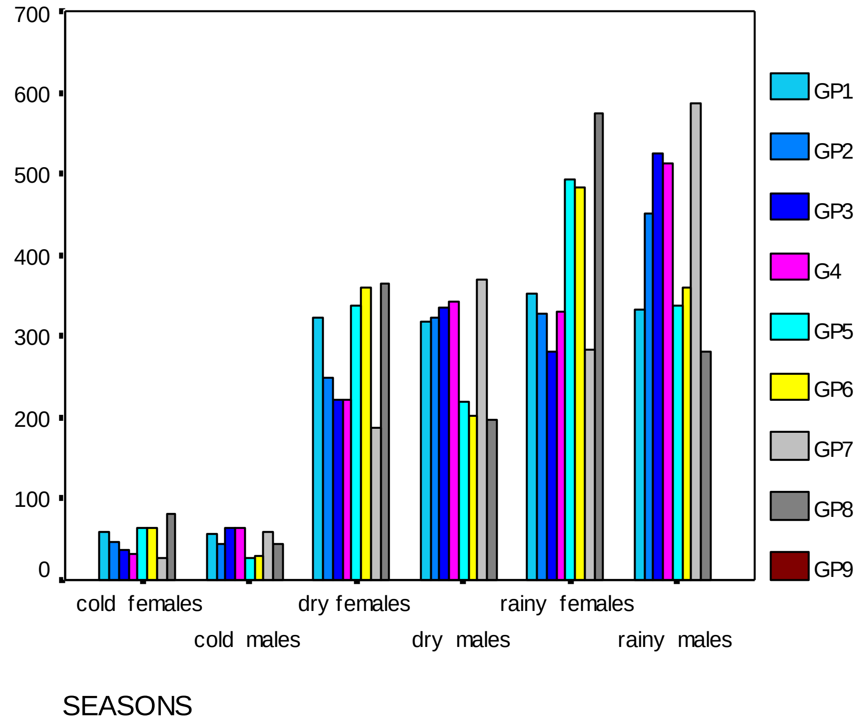


Figure 4.111 Gross seasonal gender representation among the nine groups

General comment

Summary

Seasonal variations had similar sex ratio results of absence of gender influence in groups one and nine towards neither gender with the control water group ($p>0.61$) and group nine with the cocktail of all the combined elements ($p>0.0609$) while, glucose, sodium, potassium and sodium + potassium supplementation groups influenced the sex ratios towards male progeny ($p<0.001$). Calcium, magnesium and calcium + magnesium ($p<0.001$) influenced sex ratios towards the female progeny. In all the nine groups showed significant influence on more pups delivered during the rainy season than the other seasons.

Conclusions

From the studies, there is enough evidence that suggest that seasons appear to mainly affect the litter size in particular the rainy seasons but not the gender ratios. The gender influences that were observed were mainly attributed to the ionic effects of the dietary chemical components and the seasons did not necessarily have overriding dietary ionic effects.

Chapter 5

Cat fish oocyte membrane potential studies

5.1 To evaluate effects of dietary chemical formulations on the oocyte membrane electrical potential.

Following the dietary study results discussed above, a study aimed at examining the relationships between dietary chemical ionic supplementation and oval membrane electrical charges was carried out. The rationale of the fish oocyte study was based on the fact that the oocyte membrane may have alternating membrane electrical charges that are influenced by dietary ionic effects. The influencing factors are postulated to act as modulators that exert allosteric modifications or electrotactism that allow selective gamete attraction and subsequently sex ratios. Some of these modulators have been postulated to be among our dietary components. There are possibly many modulators in our diets and out of the various alleged modulators four were selected namely sodium, potassium, calcium and magnesium and put under scrutiny in this study.

This study was done in three phases starting with search for a study design and an appropriate animal model that would accommodate varying swimming water chemical concentrations and lay eggs externally. The second phase was the pilot study and finally the main study.

Three phases were adopted starting with identification of an animal model appropriate for the study and a pilot study carried out towards preparation of the main study. These were done in an effort to identify an appropriate animal model befitting the study, evaluating the background logistics, assessing the study limitations, strengths and inherent challenges. The results of phase one and two were mainly qualitative and laid a firm background for the main study.

Phase one - Study design and animal model selection

Naturally when the thought of externally laid eggs accessible for experimentation comes alive, the frog comes in the minds of many as the first option and therefore it was not surprising that this was our starting step. The frog was initially identified and a pond set up. Female Xenopus laevis frogs were initially used and various trials attempted. The nine different ponds were set up and filled with water solutions containing the various chemical formulations as stated before. Female Xenopus laevis frogs were housed in these ponds for an acclimatisation period of 60 days prior to experimentation. The female frogs were subsequently injected with human chorionic gonadotropin (HCG) in order to induce ovulation and laying of eggs. This was however abandoned following the realization that it is difficult to domesticate them and that their breeding patterns are seasonal, difficult to manipulate and prohibitive pond management.

Following abandonment of the frog species, various fish species both fresh and sea water species were sampled, and attempts to extract their oocytes made in the same ponds. Many were abandoned due to their scarcity, failure to domesticate, inability to retrieve oocytes from them externally and difficulties in manipulating their immediate environs. In particular, sea fish were faced with complexities of their swimming salty water compositions that interfered with the study. Following various trials and failures, the cat fish met the set criteria of availability, ability to retrieve eggs from the body, ability to survive in fresh water where chemical manipulations could be done with various concentrations of salts in their swimming water. A further vital advantage is its ability to survive outside water due to its capability to breathe in such a scenario. Following the choice of the cat fish animal model, the ponds were abandoned and replaced with aquariums for easier operations and a pilot study started.

5.2 Pilot study

In a double blind control study, eighteen female cat fish were randomly divided into 9 groups each receiving different ionic formulations in their swimming solutions. The chemical effects on the oocyte membrane electrical potential were tested. They were exposed to the same chemical compositions as the mice in their swimming water but with one third dosage formulations to dietary study the effect of:-

1) High dietary sodium, potassium, calcium and magnesium as single elements on oocyte electrical charges.
2) Combined high dietary
   a) Sodium and potassium
   b) Calcium and Magnesium elements on the oocyte electrical charges.
3) Combined four elements as a cocktail on the oocyte membrane electrical charges.

They were followed up for one week and their ova retrieved on giving them pituitary extract intramuscularly. The harvested eggs from the respective groups were subjected to electrical fields and their behavioural patterns studied. This procedure was repeatedly done six times in each group and the findings recorded.

The second phase was carried out between February - August 2011 to identify the challenges that would be encountered in the main fish oocyte membrane study and solutions looked for where feasible. The results of the pilot study were presented in Stellenbosch university on the 12th October 2011 and corrections suggested. The results of the challenges obtained are described in details below as well as the solutions that were suggested and applied.

5.3 Location and infrastructure

The fish oocyte studies were done in collaborations with the University of Nairobi, and the IPR that seconded the work to Sagana Fisheries Research Station, both of which are under the Ministry of Heritage and National Museums aided by Jambo Fisheries, a private fish firm in the outskirts of Nairobi. The latter two institutions have personnel with enough expertise on fish research and the necessary infrastructure for fish experimentation.

5.4 Methods

A pilot study similar to that of the mice study was carried out following identification of cat fish as an appropriate animal model that can lay eggs externally for testing the effects of the various chemicals on the oocyte membrane electrical potential and other incidental challenges. Nine different aquariums were randomly set up containing different chemical formulations. They were designed in four categories of experimentation. The first category was set up as controls with water and glucose respectively. The second category was solutions of single elements of sodium, potassium, calcium and magnesium. The third category was solutions constituted with double elements of sodium with potassium and calcium with magnesium. The fourth category was a solution constituted with a cocktail of all these chemicals.

5.5 Fishing and fish handling

Routine fishing is done but slightly deep fishing is recommended as cat fish is usually in the deep muddy areas. Due to the latter reason, where the water level can be lowered by draining some of it is a preferred option. In this regard, it is usually hard to catch cat fish in ponds during rainy season due the high water levels.

Photo 5.1 Fish pond Sagana Fisheries Research Station on a rainy day with vaporization.

Photo 5.2 Routine pond fishing

A fish pond on the outskirts of Nairobi. The cat fish stay in muddy and not necessarily clean ponds. Routine fishing is done manually as shown above. Each fish was retrieved from the pond and ascertained that it had oocytes through physical examination.

Photo 5.3 Different types of fish are usually caught in the fishing net

Different types of fish are usually caught in the fishing net female catfish isolated.

Major Cat fish identification feature

The whiskers make identification of catfish quite easy from other fish among other features.

Some differences between the female and male fish

The difference between the female and male fish is the external genitalia and fairly easy to identify. The external genitalia are situated in front of the anal opening and differ anatomically in that whereas the male has a clear penile protrusion the female has a vaginal orifice. Physically the difference between a male fish and female fish of the same age is that the female fish is generally smaller while a male fish is generally bigger and a lot more aggressive in character.

Photo 5.4: Female catfish isolated

Transportation

With the collaboration of Sagana Fisheries Research Station based 100km from Nairobi and Jambo Fisheries based at the outskirts of Nairobi, domesticated cat fish were obtained on order and transported to the animal house and given a week to settle and to acclimatize to the new environment.

Initially we would transport several in a bucket but on the way some would fight and hurt each other. Although they usually do not kill each other but they do sustain serious injuries that get infected that often times lead to their demise. This was mitigated by transporting each one or two in one bucket at a time.

Some notable observed behavioural patterns.

Observations and challenges - Cat fish is an aggressive species and tend to fight even among themselves especially the males in presence of females. Despite considerable time of domestication, the aggressive behaviour still does tend to persist. This aggression makes them vulnerable to hurting themselves as they often times jump off the aquarium to the floor.

One of the interesting incident worth mentioning was as we initially started some catfish used to Jump off the aquarium and the watchmen who came from the fish eating area used to pull our legs by taking them and claiming were dead yet were alive. Over the time we found that they could survive even outside water as they could still breathe.

This challenge was mitigated by observing the minimum fish to aquarium surface area ratio which can provide ample space and minimize fighting among themselves. This is referred to as one inch of fish per twenty inches of the surface area of the aquarium as discussed earlier. It is always important to note that the eventual fish size needs to be known beforehand because though most aquariums can accommodate many small fish, they may accommodate only one grown cat fish like in our scenario.

Feeding patterns

Observation - The fish pellets feeds were put in the swimming water after every three days.

Comment

The fact that the experimental chemicals are in the swimming water was good for the study so that the immediate environs chemical level is constantly kept high. Fluids are best changed after three days to avoid contamination and infection in the aquariums.

Temperature

The ideal temperature range was found to be between 26 - 30^{o} C with an average of 28^{o} C.

Adaptability

Following the pilot study, the cat fish was found to be handy to use in our study because it could stand different conditions especially if introduced.

Manual confirmation of presence of eggs and their retrieval

Identification of female fish with oocytes is easy as the female fish develop bulging bellies when having substantial amounts of eggs in their ovaries. Gentle palpation towards the genital area is enough to expel some eggs which are clear proof of the presence of eggs in its ovaries. The female fish whose belies are empty without eggs are thin physically. When the oocytes are premature, they are usually also not ready to come out and usually require more time or ovulation induction. Often times, despite ovulation induction the eggs may not be able to be retrieved after 24 hours and at times, giving them more time is the solution where if given like six extra hours they will come out without difficulty.

Photo 5.5 Manual oocyte retrieval

Fish oocyctes retrieved in a petri dish by gently squeezing the belly of the fish.

Surgical retrieval of the fish eggs

For commercial breeding, the oocytes may be retrieved under anaesthesia whereby the fish is dissected on the anterior abdominal wall and the oocytes aspirated from the ovaries. The abdomen is stitched back and the fish kept in salt solution for three days. It usually heals within a week and continues with its life as usual. This process may be repeated for purposes of egg retrieval especially for purposes of commercial breeding.

Photo 5.6 Dissection showing the ovaries full of oocytes.

Dissection showing the ovaries full of oocytes.

5.6 Ovulation induction products - Pituitary extract / Ovaprim (Jambo Fisheries, Nairobi, Kenya)

Oocyte retrieval was done following ovulation induction. This was done either by laboratory prepared pituitary extract or factory prepared product named Ovaprim given at a dosage of 1 ml/kg intramuscularly. If the oocytes are required the following day the pituitary extract is prepared the day before. To avoid sacrificing fish, the pituitary extracts were obtained from the fish that died on the way from the fish pond usually from injuries as cat fish are aggressive and are fond of fighting in particular the males. On some occasions some were outsourced from the fish market.

The female fish does not generally lay eggs without the presence of the male fish but to circumvent this, either pituitary extract can be given or the factory prepared Ovaprim. The pituitary extract can be retrieved from either female or male but requires an experienced fish technician to do it. It requires dissecting the underside of the scull where a slit is made by the use of a chisel blade. The pituitary comes into view as shown below. It is then extracted and placed in a petri dish.

Photo 5.7 Showcasing the pituitary gland in vivo

Photo 5.8 The pituitary extract

Handling of the Pituitary extract

It is then pounded into a paste that is diluted with 2 cc normal saline which is given as an intramuscular injection in a dosage of 1 ml per fish. One pituitary fish extract is adequate for two fish and this is injected intramuscularly into the fish and left overnight.

The pituitary gland is extracted and placed in a petri dish as shown in picture below. It is then pounded into a paste made. The paste is made into a solution by mixing it with 1 ml of normal saline. This pituitary extract is immediately injected intramuscularly into the fish at the lateral distal 1/3 and left overnight.

5.7 Oocyte handling after retrieval

The oocytes are removed into a petri dish and placed on a temperature regulated plate set approximately 30^0C so as to maintain the ovarian temperature. A 4cc syringe is prepared ready for the oocyte transfer to the plastic Perspex tissue bath. The electrical assembly is set up as well as the heating system ready for experimentation before oocyte retrieval.

For the determination of membrane potential a small plastic Perspex tissue bath was set up measuring 5 cm x 6 cm x 10 cm. Saline phosphate buffer was used so as to maintain the cell morphology. To the opposite sides of the bath an adjustable anode and cathode electrode was attached. These electrodes were provided with a direct currents ranging between 1.5 - 6.0 volts. The bath was filled with a phosphate buffer of pH -7.0. The ova were subsequently placed in the centre of the container. The poles were moved towards the ova till they came into contact and given 3 - 4 minutes then removed and the number of attached ova onto the pole counted, as well as recorded for all the 9 different chemical formulation groups.

Timing of exposure and counting of the oocytes

After a series of observations it was found that three minutes to be optimal electrical field exposure to the oocytes in the Perspex tissue bath as less than this is too early and some oocytes are still attaching while more than five minutes, others start detaching. A timer is therefore used preferably a stopwatch for this purpose so as to standardize the experiments. After removing the electrode with the oocytes they are spread on a white paper with the help of a spatula. The counting was done manually with the help of a lighted magnifying glass to enhance the process by highlighting the oocytes aided by the ordinary microscope in case of any difficulties in identification of the oocytes.

For each study group twelve fish were included and the behavioural patterns of their oocytes evaluated in each fish.

5.8 The following is a summary of the laboratory set up assembly followed by detailed discussions on the operations of the various items and the results of observational findings and the challenges encountered The solutions to the challenges where applicable are also discussed among the pilot study results:-

1) Timer.
2) Batteries.
3) Voltmeter.
4) Aquariums
5) pH meter
6) Microscope
7) Thermometer
8) Perspex tank
9) Cooling system
10) Phosphate buffer.
11) Electrodes and connecting electrical wires.
12) Ovulation induction products - Pituitary extract / Ovipram.

Dosages used among 3 - 8 study groups in the oocyte fish studies.

Sodium chloride - 0.8 g/L

Potassium chloride - 1.9 g/L

Calcium chloride - 3.1 g/L

Magnesium chloride - 0.6 g/L

Dosages used in study group nine in the oocyte fish study.

Sodium chloride - 0.40 g/L

Potassium chloride - 0.95 g/L

Calcium chloride - 1.55 g/L

Magnesium chloride - 0.30 g/L

5.9 Pilot study results

Summary of observations on the operations of the different components, challenges met and their solutions where applicable

Various knowledge and experimental gaps were identified and noted as tabulated below and their solutions sought among the collaborating institutions and other relevant experts. Additional solutions to the various observations and challenges were found through experimentation. The results of the pilot study were mainly observational or qualitative and were presented in the Division of Medical Physiology on the 12th of October 2011 during which further suggestions on various applicable adjustments were given regarding the modalities of the main study. The results are presented below.

General operational parameters, challenges observed and suggested solutions

1a Timer

A stop watch was used for setting the required time of exposure of the oocytes to the electrical fields among the various chemical formulations.

1b Time of exposure

The time of exposure of the cells to the electric current was standardized as optimal at between 3 – 6minutes and not beyond these time limits. Before 3minutes, the oocytes are yet attaching to the electrode. Beyond six minutes clouding of the buffer and heating of the buffer occurs and dislodging of the attached oocytes begins.

1c Batteries

Routine dry batteries of 1.5 volts of ever ready type were used singly or in series for experimentation.

2 The voltmeter and voltage optimization

A voltmeter was used and it was noted that the voltage need to be optimized between 3.0 volts and 4.5 volts. It was observed that voltages below 3 volts were associated with minimal cellular electrode attraction while voltages above 4.5 were associated with increased heat production and clouding of the solution. The considerable heat generated and clouding can adversely affect the cellular function and the study results.

3a Aquariums - were acquired to replace the fish ponds that had been prepared to accommodate the toads.

3b Different aquarium materials and sizes were tried and the ideal types for the study found to be plastic aquariums measuring approximately 56 cm x 22 cm x 36 cm. This size had enough surface area that conformed with the general standard surface areas recommended of one inch of fish per twenty inches of the surface area of the aquarium. This offers enough swimming space and enough surface area for provision of oxygen for the aquaria fish. This size of tank could accommodate 6 fish based on the formulae length x width /40/5 = 56 cm x 22 cm /40 = 1232 cm^2/40 - 30.8/5 = 6.

4 The microscope

The microscope was used to study and determine the different characteristics of the oocytes in reference to their different developmental stages. Premature, over mature and the dead oocytes were excluded to ensure that only mature ones are under study.

5 Thermometer

Clinical thermometer with an alert system was used to monitor the temperature dynamics of the solutions.

6a Perspex tank

The Perspex tank needs to be carefully designed with various considerations in mind because the tank size has a direct bearing on the experimentation, study costs and results as well. After a series of experimentation, the following was found.

6b A big tank does require more current, more buffer, more oocytes and all these will increase the cost and may even interfere with results if the necessary proportions are not correctly met. A very small tank interferes with results in particular by causing overheating of the buffer that interferes with cellular function. A wide tank with the electrodes very wide apart is associated with less current effect whereas a small tank with close electrodes is likely to have the positive and negative electrical fields interfering with each other. In this study following a series of trials the optimal tank size was set at 5cm x 6cm x 10cm as per the needs of our work.

7 Cooling system

Ordinary fan was used for purposes of keeping Perspex tank temperatures within optimal temperatures assisted by a clinical thermometer for fine monitoring.

8 Heating system

To avoid chilling the ova after retrieval, a heating system similar to that keeps coffee cups warm with thermoregulation was used.

9a Buffer solution

A saline phosphate buffer was used so as to maintain the cell morphology. Its factory made with a pH of 7.0 which is of neutral electric charge so as to avoid buffer polarization as the study concerns electrical charges. One tablet of 0.01 mg of Rankem type is dissolved in 100 ml of distilled water at 20 degrees centigrade These are outsourced from the from RFCL limited representatives in Nairobi.

9b The buffer visibility

The visibility of the buffer solution and the cells were affected by bubbles emitted from the electrodes and the effect of the solution by the current on the solution. This challenge was addressed by optimizing the currents and the time periods of exposure. Currents generated from voltages above 6volts were faced with this challenge and therefore it was found that optimal voltage was around 4.5volts. Time of exposure was also found to affect this phenomenon and a series of trials, it was found that 3 minute to be optimal time period to read the results and beyond this the quality of buffer and hence the visibility is affected.

10a Electrodes and the connecting wires

Copper, iron and carbon electrodes were tested for suitability in our study. Carbon was found to be the best as it produced the least heat in the buffer solution. Zinc electrodes were not tested due to difficulties in their availability in the market.

10b The electrodes remnant charge

The electrodes were noted to have a remnant charge from previous exposure and this persistent charge effect was noted to have the potential of affecting the subsequent results of the study. The solution to this effect was found in reversing the electrodes such that the negative is briefly attached to the positive and vice versa. The voltage in the respective electrodes is then measured to confirm neutral charge status.

11 Temperature control

A routine clinical thermometer was used to determine the heat that is generated. This was an effort to avoid or control overheating as there is always some heat generated. Generally overheating is avoided by keeping the voltage within the range of 3.0 to 4.5 volts and an optimal tank size. Higher voltage potential is associated with generating more heat. Cooling system in case the cells have to be exposed for longer periods was provided by the use of the ordinary heaters with temperature control. Warming system was provided particularly on to the retrieved oocytes as they await the relevant exposure. This was done by the routine coffee warming electrical gadget with proper and relevant temperature control.

12 Oocyte count

Once the oocytes are put in the tank, two counts were done in each session. The first was done with the electrodes having no charge and the second count was done with the current on. The first count acted as the control while the second as experimental. These were recorded as either exposed those with current and non-exposed those without current.

13 Perspex tank for ova experimentation

The Perspex tank needs to be carefully designed with various considerations in mind because the tank size has a direct bearing on the experimentation, study costs and results as well. A big tank does require more current, more buffer, more oocytes and all these will increase the cost and may even interfere with results if the necessary proportions are not correctly met. A very small tank interferes with results in particular by causing overheating of the buffer that interferes with cellular function. A wide tank with the electrodes wide apart is associated with less current effect whereas a small tank with close electrodes is likely to have the positive and negative electrical fields interfering with each other. In this study following a series of trials the optimal tank size was set at 5cm x 6cm x 10cm as per the needs of our work.

14 Continuous pH Monitoring

The pH was ascertained and the neutral status confirmed before start of experimentation. Follow up measuring was done to monitor the pH particularly after passing the current. This is done because with the passage of the current and with time changes the pH status of the solution under study.

15 pH Monitoring

The pH meter was used to ascertain and confirm the neutral status before start of experimentation. Follow up measuring of the same was done to monitor the pH particularly after passing the current as this may change the pH of the solution.

16 Oocyte count

Once the oocytes are put in the tank, two counts are done in each session. The first is done with the electrodes having no charge and the second count is done with the current on. The first count acts as the control while the second as experimental. These are recorded as either exposed those with current and non-exposed those without current.

17 Physical characteristics of different oocytes

The physical characteristics of the oocytes were examined to find out whether there are notable physical differences among premature, mature, live and dead oocytes and are tabulated below.

Table 5.1 Physical characteristics of different oocytes

	Premature	**Mature**	**Dead**
Shape	Round in shape	Round shape	Oval / round / irregular.
Margins	Regular margins	Regular margins	Irregular margins
Size	Less than I.00mm	0.5mm - 1.00 mm	Less than 1.00mm
Colour	Dark green	Clear	Off white.
Nucleus	Nucleus not seen	Nucleus seen	Nucleus prominent

18 Statistics

Routine data collection and reporting was done. Oocyte counts were done before the currents were connected and after connection and recorded as counts between non exposed (no current) versus the exposed (with current) in the various groups of the chemical formulations. Using SPSS programme applicable statistics were used for analysis.

Summary of the oocyte electrophysiological results

Ova retrieved from the two control groups had little or no oval polar attraction to either the negative or positive electrodes.

There were significant ova polar attraction towards the positive electrode among the ova retrieved from the sodium, potassium and the combined sodium and potassium solutions and little or no attraction to the negative pole.

Ova retrieved from Calcium, magnesium and combined calcium magnesium solutions had significant affinity towards the .negative electrode and little or no affinity towards the positive pole.

Ova harvested from the solutions constituted with all the salts demonstrate dual attraction with some ova being attracted towards the positive pole and others towards the negative pole. More attraction was however noted on the negative pole than the positive.

Conclusion

From these observations from the pilot study, there was ample evidence that suggest that: electrical orientation of the ova does have direct contribution in gamete attraction, fertilization and consequently sex ratios.

Following the pilot study

After the pilot study with the above observations, we were fairly well versed with the work as most of the practical issues had been addressed and therefore were looking forward to the next main phase of the study. Before proceeding to the the finall phase, the study results were presented in the division of medical physiology in the university of Stellenbosch, on the 12 10 2011 where various adjustments and additions were suggested towards the main study.

5.10 Phase three (main) study of the Oocyte membrane study

Following presentation of the pilot study results in the division of medical physiology in the university of Stellenbosch, on the 12 10 2011 various changes and additions were suggested towards the main study and was carried out during the 2012/13 time period in the same infrastructure.

The major differences between the pilot study and the main study were that :-

- that whereas the pilot study was done for six months the main study was undertaken within a period of two years.
- Eighteen cat fish animal models were used in the former study while 108 animal models were used in the main study.
- Pituitary extracts were used for ovulation induction in the former study while ovuprim was used in the main study.
- The results of the pilot study were mainly qualitative while those of the main study results were quantitative.

Main oocyte membrane studies

Methods

Armed with the framework of the first phase and the pilot phase of the study the third phase was started as a double blind control study with 108 female cat fish that were randomly divided into 9 groups each comprising of twelve female fishes. These were randomly exposed to the same chemical formulations as the mice study was earlier done in four categories. The first category comprised of the controls with the first group in fresh water, second in glucose and water. The second category was constituted with single elements that comprised of the third, fourth, fifth and sixth groups of sodium, potassium, calcium and magnesium solutions. The third category was constituted with double chemical compositions that comprised of groups seven and eight of sodium with potassium and calcium with magnesium formulations. The last category was constituted with the last group that was set up with the cocktail of all the chemicals.

Of the 9 groups were two control groups and seven study groups each receiving different ionic formulations in their aquarial water. Nine different aquariums were randomly set up containing the following chemical formulations. Two aquariums with water and glucose solutions, four aquariums containing solutions of single elements of sodium, potassium, calcium and magnesium, two aquariums with combination groups of sodium with potassium, calcium with magnesium and one aquarium of a cocktail of all these elements.

Routine fishing was done, and the female fish selected in particular those with ova. Each fish was retrieved from the pond and ascertained that it had eggs through physical examination. Physically the difference between a male fish and female fish is easy as a male fish has an easily identifiable penis and the female has an easily noticeable vaginal orifice both next to the anal opening. The female fish is generally smaller and less aggressive while the male fish is generally bigger and a lot more aggressive in character.

A female fish with eggs is easy to identify as its belly is usually full unlike those without eggs whose belies are empty and thin. Due to the complexity of the study set up and the distance of the fish origin of more the 100km, each time 2 fish representing each group were transported and also studied at any time so that in case oocytes were not yielded from one fish for any reason the other fish would be a ready substitute. This gave rise to a two fish for one approach that gave a better control of the work.

Each fish was transported in its own container to avoid fights and injuries that often resulted to their demise. On arrival the fish were allowed to acclimatize to the new environment for 24 hours and put in their respective chemical formulation environment that was only known the principle researcher. These were followed up for one week and the eggs retrieved on giving them Ovaprim extract.

The eggs were retrieved by gently squeezing the belly of the fish and they usually come out with ease. If they are not yet ready to come out as long as they are present time factor is the solution as if given more time such as six extra hours they do come out without difficulty.

The harvested eggs from the respective group were subjected to electrical fields and their behavioural patterns studied. The same process was done repeatedly and the findings recorded.

For the determination of membrane potential a small plastic Perspex tissue bath was used. To the opposite sides of the bath an adjustable anode and cathode electrode was attached. These electrodes were provided with various direct currents of between 1.5 - 6 volts. The bath was be filled with a physiological phosphate buffer of pH=7.2. These were placed in the opposite sides of the Perspex bath. After the assembly is set up, the oocytes are placed in the middle and their direction of movement observed and recorded for all the 9 different chemical formulation groups.

Observations/Comments

Oocytes retrieved from the first and second control groups had no significant oval polar attraction to either the negative or positive electrodes. There was however significant oocyte polar attraction towards the positive electrode among the ova retrieved from the sodium, potassium and the combined sodium and potassium group solutions.

Oocytes retrieved from calcium, magnesium and combined calcium with magnesium solutions had significant affinity towards the negative electrode and with no significant affinity towards the positive pole.

Oocytes harvested from the solutions constituted with all the salts demonstrated dual attraction with some ova being attracted towards the positive pole and others towards the negative pole. There was however more attraction noted on the positive pole than the negative pole although this observation was not found to be of statistical significance.

In all the groups, only a limited number of ova out of the total ova that were retrieved were attracted to the respective electrical poles leaving the majority in the background of the experimental tray**.**

Conclusion

Solutions constituted with sodium and potassium elements appear to have skewed ionic influence over the oocyte membrane electrical charge towards the negative charge hence their attraction towards the positive pole. Their common electrical factor is their valency one (+). Practically if the oval electrical charge is influenced towards the negative pole, then during fertilization it will selectively favour the positively charged gamete which is the Y bearing sperm resulting to a male conceptus.

Similarly, Solutions constituted with calcium and magnesium appears to have skewed ionic influence over the oocyte membrane electrical charge towards the positive charge influencing hence their attraction towards the negative pole. Their common electrical factor is their valency two (++). Practically if the oval electrical charge is influenced towards the positive charge then during fertilization it will selectively favour the negatively charged gamete which is the X bearing sperm resulting to a female conceptus.

5.11 Main study Results

Below are the tabulated and analysed results of the main study.

Table 5.2 Control study group one - Electrolyte solution

Quantitative analysis of oval electrode attraction

	Electrolyte in water solution.					
	No. of eggs attached to anode			No. of eggs attached to cathode		
	Current Off	Current On	Difference 1	Current Off	Current On	Difference 2
Fish 1	4.00	9.00	5.00	3.00	5.00	2.00
Fish 2	3.00	8.00	5.00	2.00	2.00	0.00
Fish 3	4.00	9.00	5.00	4.00	3.00	1.00
Fish 4	3.00	16.00	13.00	2.00	8.00	5.00
Fish 5	6.00	25.00	19.00	7.00	16.00	9.00
Fish 6	1.00	22.00	22.00	5.00	0.00	3.00
Fish 7	3.00	17.00	13.00	6.00	5.00	3.00
Fish 8	2.00	11.00	9.00	2.00	17.00	15.00
Fish 9	1.00	13.00	12.00	5.00	3.00	2.00
Fish 10	5.00	9.00	4.00	3.00	7.00	4.00
Fish 11	2.00	6.00	4.00	4.00	2.00	2.00
Fish 12	3.00	22.00	19.00	4.00	2.00	2.00
Mean	3.08	13.92	10.83	3.92	5.83	4.18
SD	1.51	6.37	6.52	1.62	5.37	4.30

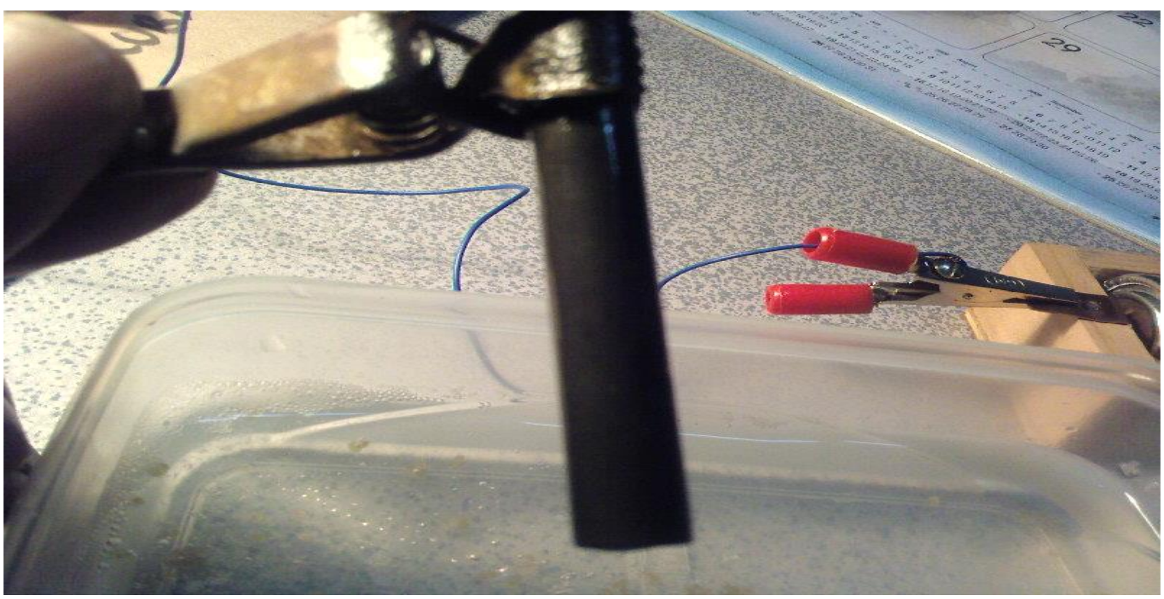


Photo 5.9 Control Group with electrolyte water solution - -ve Electrode

The above picture shows group one negative electrode in electrolyte water solution with power on. It shows absence of attraction of the ova towards the negative electrode.

Photo 5.10 Control Group with electrolyte water solution +ve Electrode

The above picture shows group one positive electrode in electrolyte water solution with power on. It shows minimal attraction of the ova towards the positive electrode.

-ve +ve

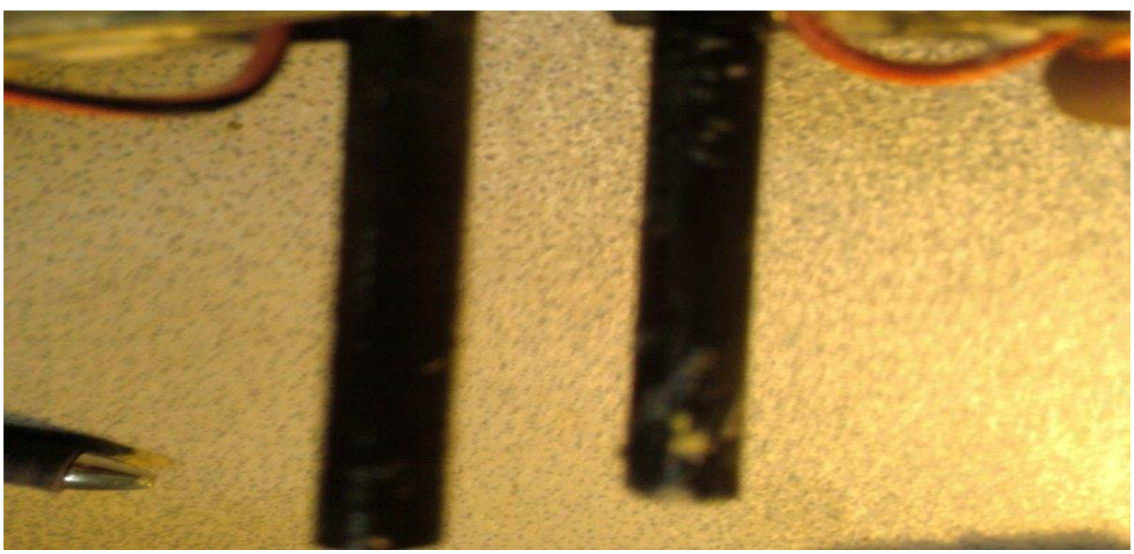


Photo 5.11 Control Group with electrolyte water solution with –ve & +ve electrodes

The above picture shows group one positive and negative electrode assembly in electrolyte water solution with power on. It shows minimal affinity towards both electrodes but more on the the positive electrode powered by the red coloured wiring.

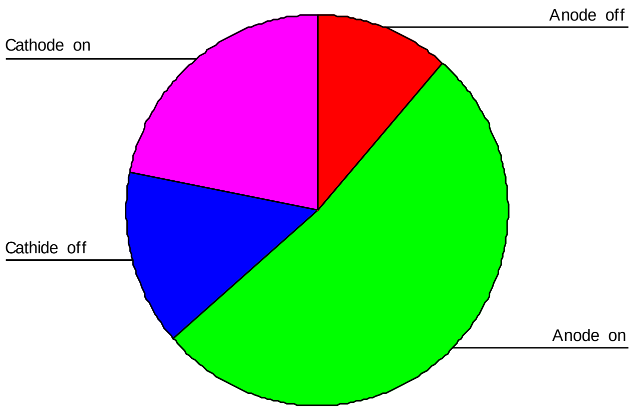


Figure 5.1 Oocyte polar distribution in the electrolyte solution

Summary

In group one of electrolyte solution, more ova were attracted to the positive electrode than the negative electrode with a statistical significant difference found between them with a two-tailed p-value of 0.0003.

There was statistical significant difference in oval attraction to the positive electrode with power on and without power with a two-tailed p-value of 0.0001.

There was no statistical significant difference in oval attraction to the negative electrode with power on and without power with a two-tailed p-value of 0.2684.

There was no statistical significant difference between ova attracted to the positive electrode and the negative electrode without power in the electrolyte solution with a two-tailed p-value of 0.1842.

Conclusion

There was minimal baseline oval attraction to both the electrodes, more on the positive electrode of significance (p-value of 0.0003).

Table 5.3 Control study group two - Glucose solution

Quantitative analysis of oval electrode attraction

	Glucose experiment					
	No of eggs attached to Anode			**No of eggs attached to Cathode**		
	Current Off	**Current On**	**Difference 1**	**Current Off**	**Current On**	**Difference 2**
Fish 1	3.00	19.00	16.00	6.00	9.00	2.00
Fish 2	0.00	15.00	15.00	1.00	7.00	6.00
Fish 3	7.00	18.00	11.00	6.00	5.00	1.00
Fish 4	5.00	20.00	15.00	6.00	6.00	0.00
Fish 5	7.00	15.00	8.00	4.00	9.00	5.00
Fish 6	3.00	18.00	15.00	7.00	8.00	1.00
Fish 7	3.00	26.00	23.00	1.00	9.00	14.00
Fish 8	5.00	20.00	15.00	6.00	.00	2.00
Fish 9	4.00	16.00	12.00	7.00	8.00	8.00
Fish 10	5.00	16.00	11.00	5.00	4.00	1.00
Fish 11	3.00	10.00	7.00	7.00	12.00	8.00
Fish 12	4.00	20.00	16.00	5.00	11.00	12.00
Mean	4.08	17.75	13.67	5.08	10.17	5.00
SD	1.83	3.89	4.25	6.072.11	6.07	4.67

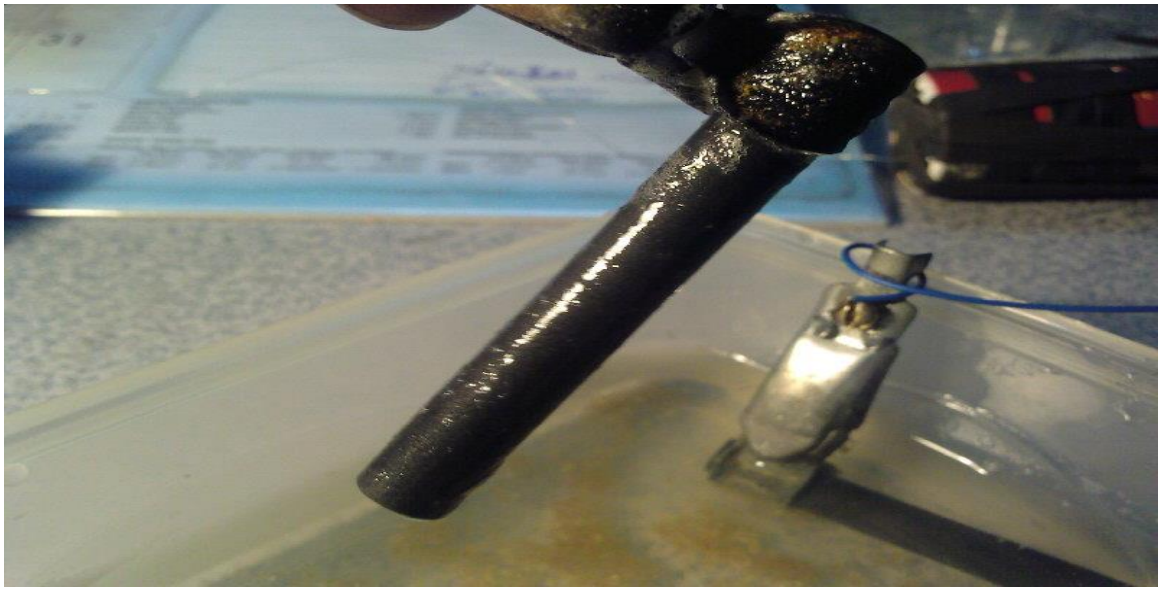


Photo 5.12 Control Group with glucose -ve Electrode

The above picture shows group two negative electrode in glucose water solution with power on. It shows absence of attraction of the ova towards the cathode.

Photo 5.13 Control Group with glucose +ve Electrode

The above two pictures are representative of all others taken from the glucose solution control group. It shows among others with varying no or very little appreciable affinity of the ova to either the positive or the negative electrode.

-ve +ve

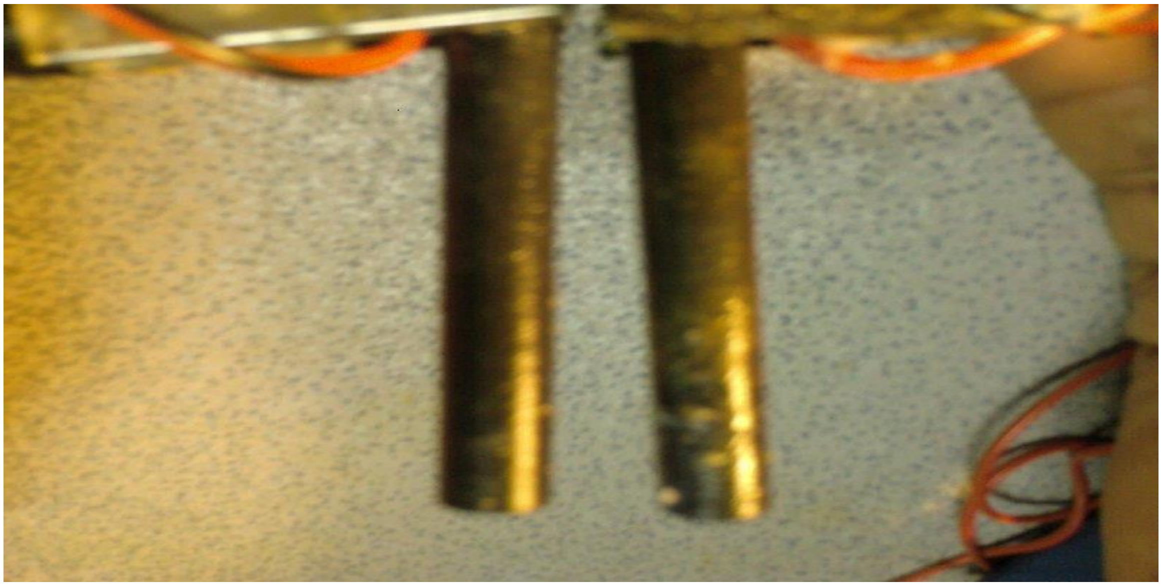


Photo 5.14 Shows group two negative and positive electrode assembly

The above picture shows group two negative and positive electrode assembly in electrolyte solution with power on. It shows minimal affinity towards both electrodes but more on the the positive electrode powered by the red coloured wiring.

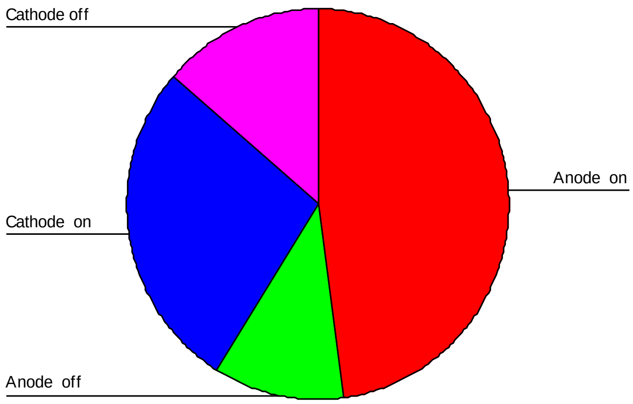


Figure 5.2 Oocyte polar distribution in the glucose solution

Summary

In group two glucose solution, more ova were attracted to the positive electrode than the negative electrode with a statistical significant difference found between them with a two-tailed p-value of 0.0001.

There was no statistical significant difference in oval attraction to the negative electrode with or without power with a two-tailed p-value of 0.0634.

There was no statistical significant difference in oval attraction to the positive electrode with power on between those in the electrolyte and glucose control solutions with a two-tailed p-value of 0.0561.

There was no statistical significant difference in oval attraction to the negative electrode with power on between those in the electrolyte and glucose control solutions with a two-tailed p-value of 0.155.

There was no statistical significant difference between ova attracted to the positive electrode and the negative electrode without power in the electrolyte solution with a two-tailed p-value of 0.1530.

Conclusion

There was minimal baseline oval attraction to both the electrodes, more on the positive electrode of significance (p-value of 0.0001).

Table 5.4 Study group three - Sodium solution

Quantitative analysis of oval electrode attraction

	Sodium experiment					
	No. of eggs attached to Anode			**No. of eggs attached to Cathode**		
	Current off	**Current on**	**Difference 1**	**Current off**	**Current on**	**Difference 2**
Fish 1	5.00	32.00	27.00	2.00	3.00	1.00
Fish 2	2.00	46.00	44.00	1.00	4.00	3.00
Fish 3	5.00	34.00	29.00	3.00	3.00	0.00
Fish 4	3.00	31.00	28.00	2.00	6.00	4.00
Fish 5	1.00	34.00	33.00	1.00	4.00	3.00
Fish 6	2.00	15.00	13.00	1.00	3.00	2.00
Fish 7	4.00	24.00	20.00	2.00	2.00	0.00
Fish 8	1.00	75.00	74.00	1.00	0.00	1.00
Fish 9	6.00	30.00	24.00	2.00	6.00	4.00
Fish 10	1.00	27.00	26.00	1.00	3.00	2.00
Fish 11	4.00	17.00	13.00	3.00	4.00	1.00
Fish 12	5.00	35.00	30.00	2.00	4.00	2.00
Total	39.00	400	361	21.00	43.00	23.00
Mean	3.25	33.33	30.08	1.75	3.58	1.92.00
STD	1.82	15.52	16.19	0.75	1.44	1.38

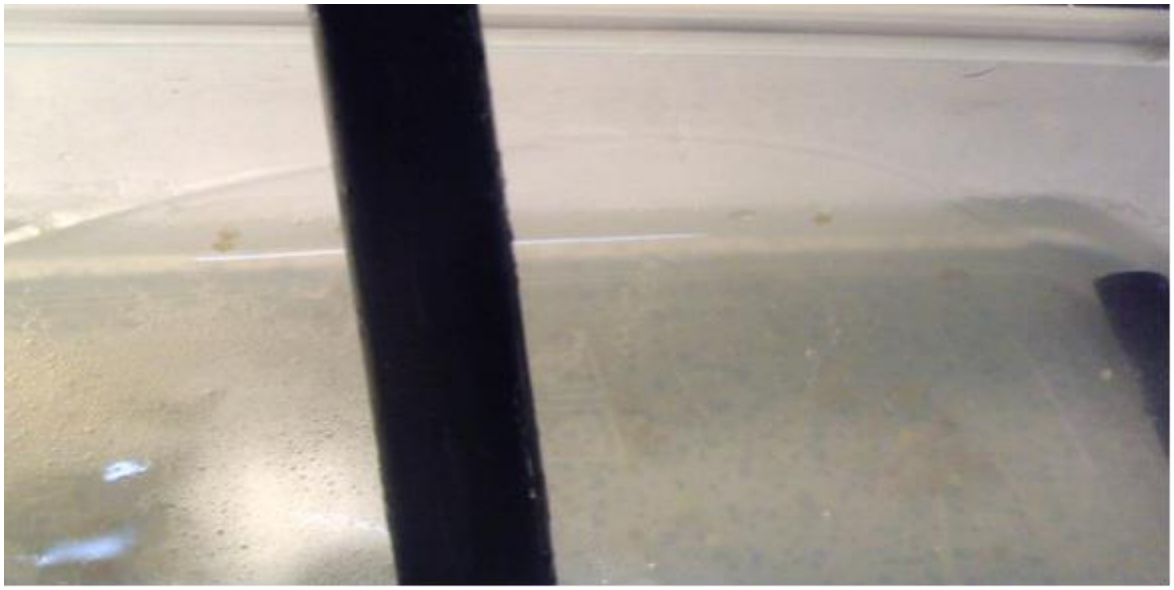


Photo 5.15 Group 3 -b- Sodium solution -ve Electrode

The above picture represents the negative electrode in group four sodium solution with power on. It shows absence of attraction of the ova towards the cathode.

Photo 5.16 Group 3 -a- Sodium solution - +ve Electrode

The above picture shows group three positive electrode in sodium solution with power on. It shows appreciable affinity of the ova towards the anode.

-ve *+ve*

Photo 5.17 Group 3 Sodium solution - ve & +ve electrodes assembly.

The above picture shows group three negative and positive electrodes assembly in sodium solution with power on. It shows similar findings as above of appreciable affinity of some ova to the anode pole and absence of attraction of the ova towards the cathode.

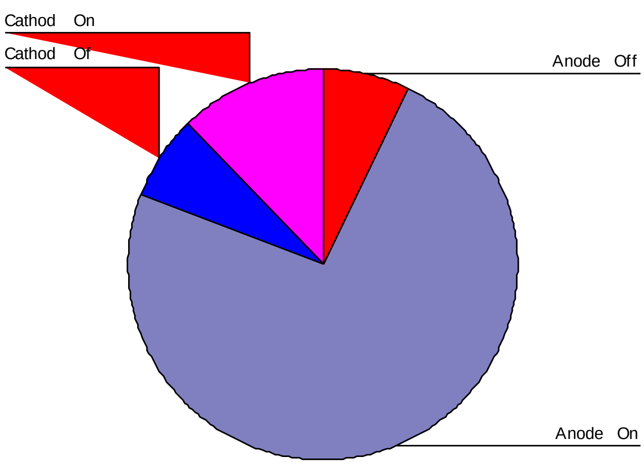


Figure 5.3 Oocyte polar distribution in the sodium solution

Summary

In group three sodium solution, more ova were attracted to the positive electrode than the negative electrode. A high statistical significant difference was found between them with a two-tailed p-value of less than 0.0001

There was statistical significant difference in oval attraction to the positive electrode with power on and with power off with a two-tailed p-value of 0.0001.

There was no statistical significant difference in oval attraction to the negative electrode with power on and off in the sodium solution with a two-tailed p-value of 0.0579.

There was no statistical significant difference between oval attracted to the positive electrode and the negative electrode without power in the sodium solution with a two-tailed p-value of 0.845.

Conclusion

There was modest baseline oval attraction to both the electrodes, more on the positive electrode than the negative electrode of statistical significance ($p<0.0001$).

Table 5.5 Study group four - Potassium solution

Quantitative analysis of oval electrode attraction

	Experiment					
	No. of eggs attached to Anode			No. of eggs attached to Cathode		
	Current off	Current on	Difference	Current off	Current on	Difference
Fish 1	4.00	20.00	18.00	0.00	3.00	3.00
Fish 2	3.00	16.00	13.00	1.00	5.00	4.00
Fish 3	5.00	17.00	12.00	1.00	5.00	4.00
Fish 4	7.00	20.00	13.00	0.00	3.00	3.00
Fish 5	4.00	30.00	26.00	0.00	5.00	5.00
Fish 6	6.00	32.00	26.00	4.00	1.00	3.00
Fish 7	2.00	42.00	40.00	0.00	3.00	3.00
Fish 8	3.00	20.00	17.00	2.00	5.00	3.00
Fish 9	1.00	26.00	25.00	8.00	2.00	6.00
Fish 10	2.00	11.00	9.00	1.00	3.00	2.00
Fish 11	1.00	26.00	25.00	3.00	5.00	2.00
Fish 12	4.00	32.00	28.00	1.00	6.00	5.00
Total	42.00	292.00	250.00	21.00	46.00	25.00
AVG	3.50	24.33	21.25	1.75	4.33	4.08
SD	1.88	8.66	8.94	2.34	2.10	1.98

Photo 5.18 Group 4 -b- Potassium -ve pole

The above picture represents the negative electrode in group four potassium solution with power on. It shows absence of attraction of the ova towards the cathode.

Photo 5.19 Group 4 -b- Potassium +-ve pole

The above two pictures represent group four of potassium solution with the current on. It shows appreciable affinity of the ova towards the positive electrode and none towards the negative electrode.

Photo 5.20 Group 4 potassium solution - ve & +ve electrodes assembly

The above picture shows group four negative and positive electrodes assembly in sodium solution with power on. It shows similar findings as above of appreciable affinity of some ova to the anode pole and minimal attraction of the ova towards the cathode.

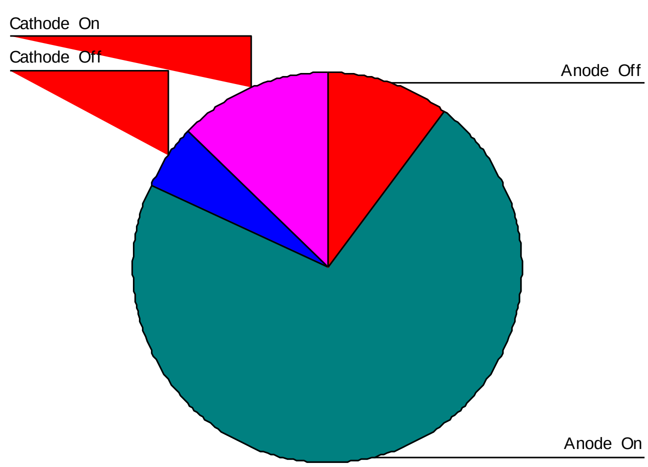


Figure 5.4 Oocyte polar distribution in the potassium solution

Summary

In the group four potassium solution, more ova were attracted to the positive electrode than the negative electrode. A high statistical significant difference was found between them with a two-tailed p-value of less than 0.0001.

There was statistical significant difference in oval attraction to the positive electrode with power on and with power off with a two-tailed p-value of 0.0001.

There was no statistical significant difference between oval attracted to the negative electrode with or without power in the potassium solution with a two-tailed p-value of 0505.

There was no statistical significant difference between oval attracted to the positive electrode and the negative electrode without power in the potassium solution with a two-tailed p-value of 0.1129.

Conclusion

There was preferential and substantial more oval attraction to the positive electrode and minimal to the negative electrode of statistical significance ($p<0.0001$).

Table 5.6 Study group five - Calcium solution

Quantitative analysis of oval electrode attraction

	Calcium solution					
	No. of eggs attached to Anode			**No. of eggs attached to Cathode**		
	Current off	**Current on**	**Difference**	**Current off**	**Current on**	**Difference**
Fish 1	3.00	1.00	2.00	5.00	40.00	35.00
Fish 2	0.00	9.00	9.00	0.00	80.00	80.00
Fish 3	6.00	15.00	7.00	7.00	60.00	53.00
Fish 4	7.00	10.00	3.00	2.00	85.00	83.00
Fish 5	4.00	8.00	4.00	2.00	67.00	65.00
Fish 6	3.00	2.00	1.00	6.00	78.00	72.00
Fish 7	1.00	10.00	9.00	0.00	66.00	66.00
Fish 8	3.00	11.00	9.00	4.00	75.00	71.00
Fish 9	0.00	2.00	2.00	6.00	78.00	72.00
Fish 10	3.00	8.00	5.00	2.00	67.00	65.00
Fish 11	2.00	3.00	1.00	3.00	65.00	62.00
Fish 12	2.00	15.00	13.00	1.00	79.00	78.00
Total	34.00	94.00	65.00	38.00	840.00	802.00
Mean	2.80	7.80	5.42	3.17	70.00	66.83
SD	2.12	4.88	3.92	2.41	12.13	13.02

Photo 5.21 Group 5 -a- Calcium solution +ve Pole

Photo 5.22 Group 5 -b- calcium solution - ve Pole

-ve ***+ve***

Photo 5.23 Group 5 calcium solution - ve & +ve electrodes assembly

The above picture shows group five negative and positive electrodes assembly in calcium solution with power on. It shows similar findings as above of appreciable affinity of some ova to the cathode pole and minimal attraction of the ova towards the anode.

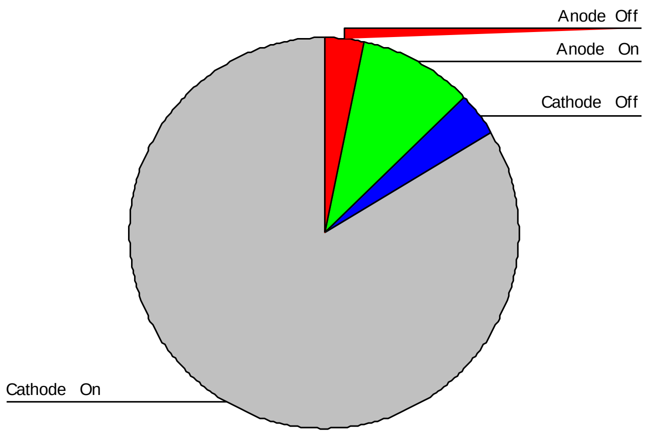


Figure 5.5 Oocyte polar distribution in the calcium solution

Summary

In the group five calcium solution, more ova were attracted to the negative electrode than the positive electrode. A statistical significant difference was found between them with a two-tailed p-value of less than 0.0001

More ova were attracted to the negative electrode with power on than without with a high statistical significant difference found between these two with a two-tailed p-value of less than 0.0025

There was statistical significant difference in oval attraction to the positive electrode with power on and off in the calcium solution with a two-tailed p-value of 0.0033.

There was no statistical significant difference between oval attracted to the positive electrode and the negative electrode without power in the calcium solution with a two-tailed p-value of 0.6817.

Conclusion

There was substantial and preferential more oval attraction to the negative electrode and less oval attraction to the positive electrode of statistical significant difference (p>0.0001).

Table 5.7 Study group six - Magnesium solution

Quantitative analysis of oval electrode attraction

	Magnesium solution					
	No. of eggs attached to Anode			**No. of eggs attached to Cathode**		
	Current off	**Current on**	**Difference**	**Current off**	**Current on**	**Difference**
Fish 1	2.00	22.00	20.00	6.00	88.00	82.00
Fish 2	11.00	20.00	9.00	10.00	122.00	112.00
Fish 3	3.00	3.00	00.00	3.00	57.00	54.00
Fish 4	6.00	4.00	2.00	7.00	52.00	45.00
Fish 5	2.00	8.00	6.00	1.00	84.00	83.00
Fish 6	7.00	21.00	14.00	8.00	138.00	130.00
Fish 7	4.00	10.00	6.00	6.00	141.00	135.00
Fish 8	0.00	4.00	4.00	4.00	52.00	48.00
Fish 9	2.00	2.00	0.00	5.00	22.00	17.00
Fish 10	0.00	1.00	1.00	2.00	18.00	16.00
Fish 11	2.00	0.00	2.00	0.00	77.00	77.00
Fish 12	3.00	10.00	7.00	4.00	41.00	37.00
Total	42.00	105.00	63.00	56.00	892.00	836.00
Average	3.5	12.08	6.31	4.67	74.33	8.62
SD	3.15	15.08	10.56	2.93	41.97	14.51

Photo 5.24 Group 6 -b- Magnesium solution -ve Pole**.**

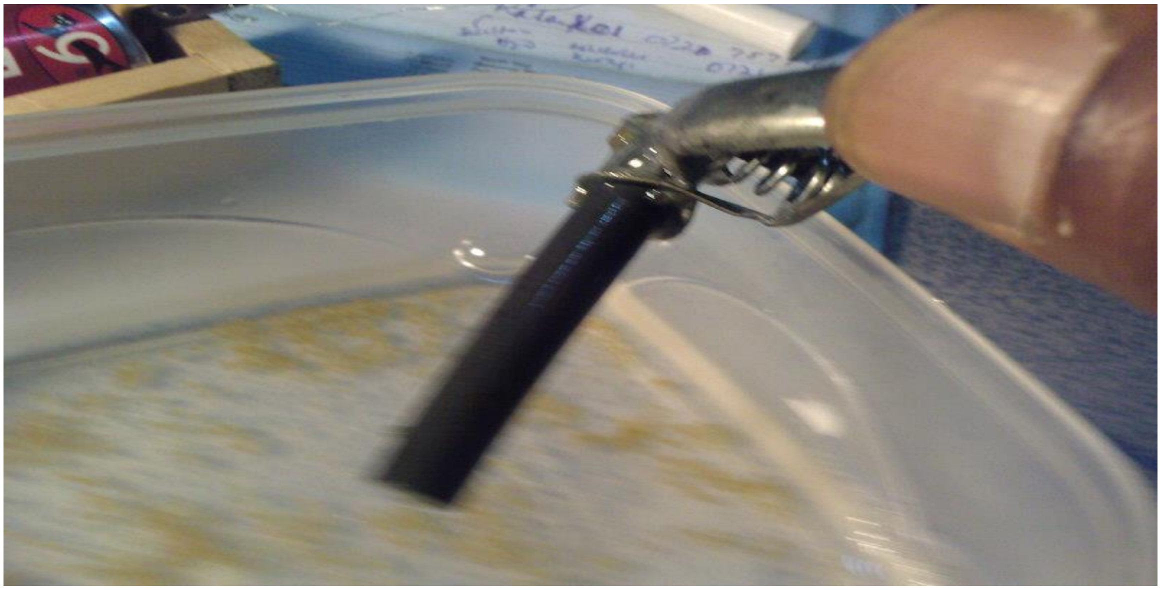


Photo 5.25 Group 6 - a - Magnesium solution +ve Pole

The above pictures represent electrodes in group six magnesium solution with the current on. They show significant affinity of the ova towards the negative electrode and minimal attraction towards the negative electrode or its assembly.

-ve ***+ve***

Photo 5.26 Group 6 Magnesium solution - ve & +ve electrodes assembly

The above picture shows group six negative and positive electrodes assembly in magnesium solution with power on. It shows similar findings as above of significant affinity of the ova to the cathode and minimal attraction towards the anode.

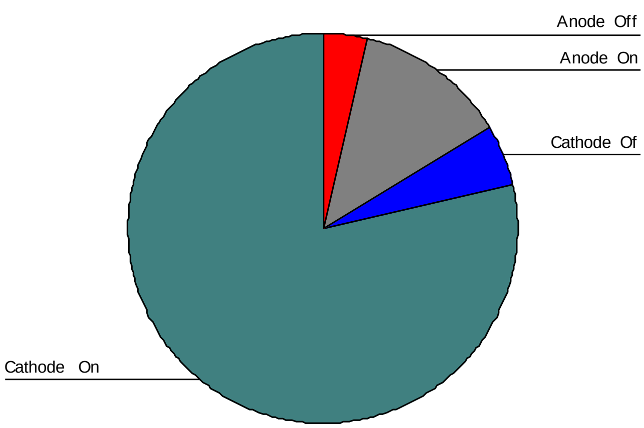


Figure 5.6 Oocyte polar distribution in the magnesium solution

Summary

In the magnesium solution, more ova were attracted to the negative electrode than the positive electrode. A high statistical significant difference was found between them with a two-tailed p-value of less than 0.0001

More ova were attracted to the negative electrode with power on that without with a high statistical significant difference found between these two with a two-tailed p-value of less than 0.0001

There was moderate statistical significant difference in oval attraction to the positive electrode with power on and off in the magnesium solution with a two-tailed p-value of 0.0200.

There was no statistical significant difference between oval attracted to the positive electrode and the negative electrode without power in the magnesium solution with a two-tailed p-value of 0.0621.

Conclusion

There was substantial and preferential oval attraction to the negative electrode and less oval attraction to the positive electrode of statistical significance ($p<0.0001$).

Table 5.8 Study group seven - Sodium / Potassium solution

Quantitative analysis of oval electrode attraction

	Experiment					
	No. of eggs attached to Anode			**No of eggs attached to Cathode**		
	Current off	**Current on**	**Difference**	**Current off**	**Current on**	**Differenc e**
Fish 1	2.00	105.00	103.00	4.00	6.00	2.00
Fish 2	1.00	39.00	38.00	4.00	8.00	4.00
Fish 3	3.00	62.00	59.00	2.00	2.00	0.00
Fish 4	8.00	78.00	54.00	3.00	3.00	0.00
Fish 5	.00	57.00	57.00	2.00	10.00	8.00
Fish 6	6.00	83.00	77.00	5.00	4.00	1.00
Fish 7	8.00	84.00	76.00	3.00	7.00	4.00
Fish 8	5.00	76.00	71.00	2.00	5.00	3.00
Fish 9	2.00	31.00	29.00	1.00	2.00	1.00
Fish 10	2.00	49.00	47.00	3.00	3.00	0.00
Fish 11	5.00	79.00	73.00	6.00	4.00	3.00
Fish 12	2.00	65.00	63.00	7.00	8.00	1.00
Total	44.00	808	747	42.00	62.00	27
Mean	3.67	67.30	3.67.	3.50	5.17	2.25
SD	2.67	21.03	17.83	1.78	2.62	2.34

Photo 5.27 Group 7 -a- Sodium + Potassium solution +-ve Pole

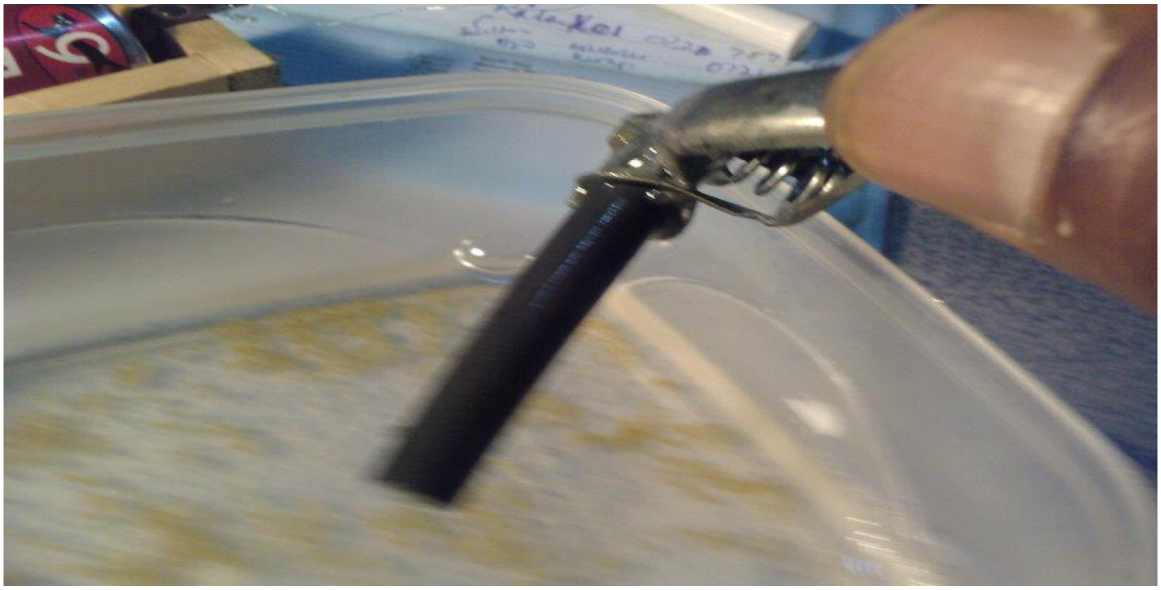


Photo 5.28 Group 7 -b- Sodium + Potassium solution -ve Pole

The above pictures represent electrodes in group seven of sodium and potassium solution with the current on. They show significant affinity of the ova towards the positive electrode and minimal attraction towards the negative electrode or its assembly.

-ve ***+ve***

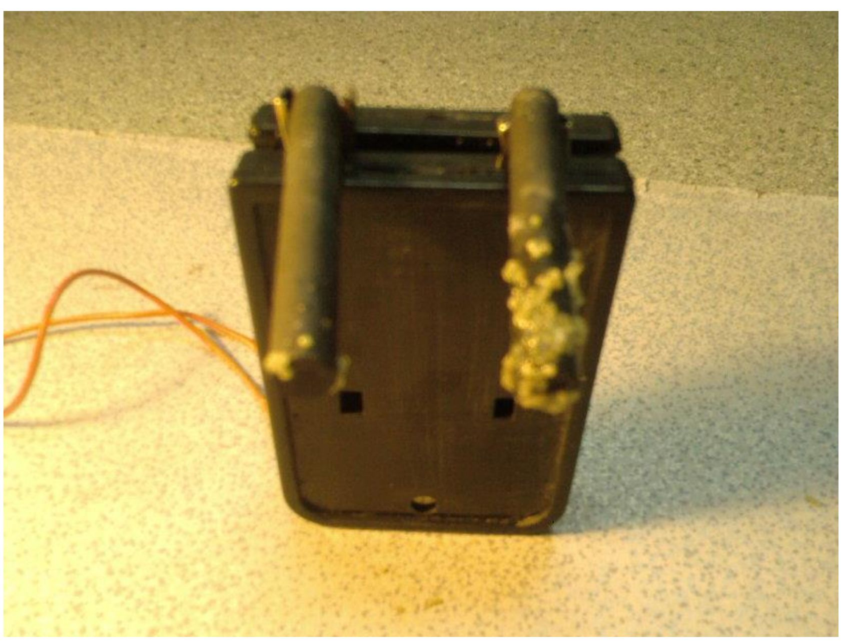


Photo 5.29 Group 7 -a- Sodium + Potassium solution +-ve Pole

The above picture shows group seven negative and positive electrodes assembly in sodium and potassium solution with power on. It shows similar findings as above of significant affinity of the ova to the anode pole and minimal attraction towards the cathode.

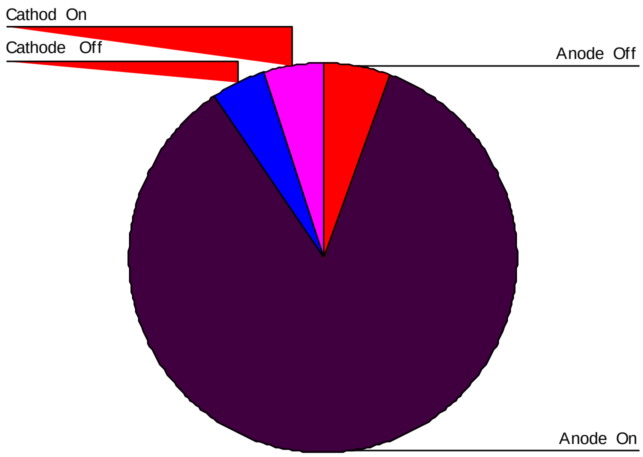


Figure 5.7 Oocyte polar distribution in the sodium + potassium solution

Summary

In the sodium and potassium combined solution, more ova were attracted to the positive electrode than the negative electrode. A high statistical significant difference was found between them with a two-tailed p-value of less than 0.0001

More ova were attracted to the positive electrode with power on than without with a high statistical significant difference found between these two with a two-tailed p-value of less than 0.0001

There was no statistical significant difference between ova attracted to the negative electrode with or without power in the sodium and magnesium solution with a two-tailed p-value of 0.0590.

There was no statistical significant difference between ova attracted to the positive electrode and the negative electrode without power in this group with a two-tailed p-value of 0.8556.

Conclusion

There was more substantial and preferential oval attraction to the positive electrode than the negative electrode of statistical significance (p<0.0001)

Table 5.9 Study group eight - Calcium + Magnesium solution

Quantitative analysis of oval electrode attraction

	Experiment					
	No. of eggs attached to Anode			**No. of eggs attached to Cathode**		
	Current off	**Current on**	**Difference one**	**Current off**	**Current on**	**Difference two**
Fish 1	2.00	3.00	1.00	5.00	19.00	14.00
Fish 2	5.00	1.00	4.00	2.00	35.00	33.00
Fish 3	4.00	9.00	5.00	3.00	43.00	40.00
Fish 4	3.00	8.00	5.00	2.00	110.00	78.00
Fish 5	6.00	5.00	1.00	4.00	38.00	34.00
Fish 6	1.00	9.00	8.00	5.00	88.00	83.00
Fish 7	2.00	1.00	1.00	1.00	43.00	42.00
Fish 8	1.00	3.00	2.00	2.00	35.00	33.00
Fish 9	1.00	5.00	4.00	6.00	19.00	30.00
Fish 10	5.00	8.00	3.00	7.00	38.00	31.00
Fish 11	1.00	1.00	0.00	4.00	110.00	86.00
Fish 12	4.00	9.00	5.00	1.00	48.00	47.00
Total	35.00	62.00	39.00	42.00	626.00	584.00
Mean STD	2.92 1.83	5.17 3.33	2.67 1.78	3.5 1.97	52.17 32.12	48.67 32.47

Photo 5.30 Group 8 -a- calcium + magnesium solution -ve Pole

Photo 5.31 Group 8 -b- Calcium + Magnesium solution +ve Pole

The above two pictures represent group eight with calcium and magnesium solution It shows significant affinity of the ova to the negative electrode and minimal towards the positive electrode.

-ve +ve

Photo 5.32 Group 7 -a- Sodium + Potassium solution +-ve Pole

The above picture shows group eight negative and positive electrodes assembly in calcium and magnesium solution with power on. It shows similar findings as above of significant affinity of the ova towards the cathode pole and minimal attraction towards the anode.

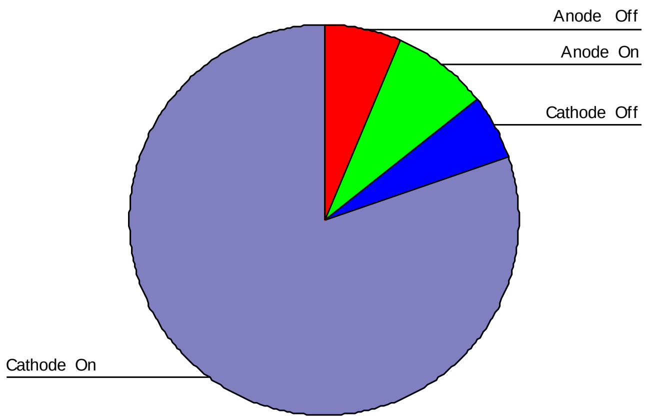


Figure 5.8 Oocyte polar distribution in the Calcium / Magnesium solution

Summary

In the group eight of calcium and magnesium solution, more ova were attracted to the negative electrode than the positive electrode. A high statistical significant difference was found between them with a two-tailed p-value of less than 0.0003.

More ova were attracted to the negative electrode with power on that without with a high statistical significant difference found between these two with a two-tailed p-value of less than 0.0003.

There was moderate statistical significant difference in oval attraction to the positive electrode with power on and off in this group with a two-tailed p-value of 0.0420.

There was no statistical significant difference between oval attracted to the positive electrode and the negative electrode without power in the magnesium solution with a two-tailed p-value of 0.4822

Conclusion

There was more substantial and preferential oval attraction to the negative electrode than the positive electrode of statistical significance ($p<0.0001$)

Table 5.10 Study group nine – All-inclusive cocktail solution

Quantitative analysis of oval electrode attraction

	Experiment					
	No. of eggs attached to Anode			**No. of eggs attached to Cathode**		
	Current off	Current on	Difference	Current off	Current on	Difference
Fish 1	0.00	20.00	20.00	2.00	0.00	2.00
Fish 2	3.00	32.00	29.00	7.00	7.00	0.00
Fish 3	12.00	80.0	68.00	21.00	90.00	69.00
Fish 4	0.00	48.00	48.00	6.00	23.00	17.00
Fish 5	16.00	90.00	74.00	12.00	40.00	28.00
Fish 6	4.00	34.00	30.00	15.00	40.00	25.00
Fish 7	5.00	4.00	1.00	4.00	25.00	21.00
Fish 8	16.00	60.00	44.00	9.00	20.00	11.00
Fish 9	1.00	80.00	79.00	2.00	28.00	26.00
Fish 10	2.00	22.00	20.00	1.00	5.00	4.00
Fish 11	4.00	00.00	4.00	5.00	21.00	16.00
Fish 12	20.00	80.0	60.00	16.00	60.00	44.00
Totals	83.00	550	477	100	359	263
Mean	6.92	45.83	39.75	8.33	29.92	21.92
STD	7.09	31.73	7.68	6.39	7.29	19.41

Photo 5.33 Group 9 -a- All elements inclusive -ve Pole

Photo 5.34 Group 9 -a- All elements inclusive +ve Pole.

-ve ***+ve***

Photo 5.35 Group 9 all-inclusive cocktail solution +-ve Pole

The above picture shows group nine negative and positive electrodes assembly in all-inclusive cocktail solution of the four elements with power on. It shows similar findings as above of appreciable affinity of some ova to the anode pole and similar attraction towards the cathode. More attraction is however demonstrated in the anode pole.

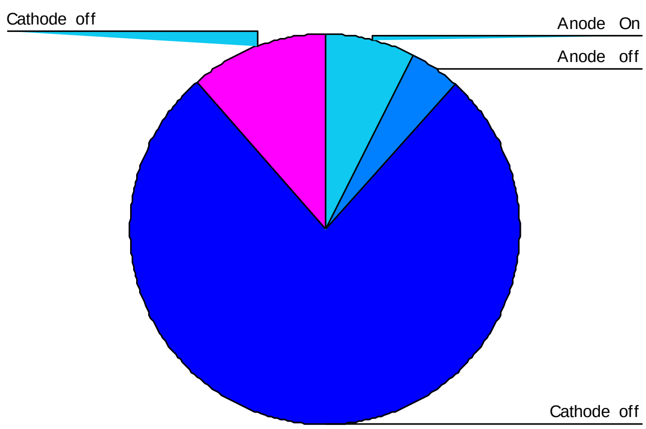


Figure 5.9 Oocyte polar distribution in the all-inclusive cocktail solution

Summary

In the group nine of all elements inclusive, more ova were attracted to the positive electrode than the negative electrode. However, there was no statistical significant difference found between them with a two-tailed p-value of 0.0530.

More ova were attracted to the negative electrode with power on that without with a high statistical significant difference found between these two with a two-tailed p-value of less than 0.0031.

There was significant statistical difference in oval attraction to the positive electrode with power on and off in this group with a two-tailed p-value of 0.0005.

There was no statistical significant difference between oval attracted to the positive electrode and the negative electrode without power in the magnesium solution with a two-tailed p-value of 0.4416.

Conclusions

There was more substantial and preferential oval attraction to the positive electrode than the negative electrode but of no statistical significance ($p>0.0530$).

5.12 Comparing the pilot and the main study:-

Control groups

- The first water control group had similar sex ratio outcomes without influence over the sex ratios.
- ***In contrast***
- The second glucose control group had influence over sex ratio outcomes towards the male progeny.

The single and combined chemical groups.

The following *status quo* was maintained as earlier noted in the pilot study.

- Sodium group three, potassium group four and the combined group seven of the latter two elements had significant sex ratios skewed towards the male progeny.
- Groups five calcium, six magnesium and eight of the combined group eight, of the latter two elements, had significant sex ratios skewed towards the female progeny.

All-inclusive - Cocktail.

- Group nine constituted of the whole cocktail of all the chemicals had no influence over sex ratio outcomes.

5.13 Comments / Conclusions

The study reaffirms the societal allegations that there are factors in our diets that do influence sex ratio outcomes and that these factors could be chemically mediated.

It further reaffirms that:
Sodium and potassium do influence sex ratios towards the male gender.
Calcium and magnesium do influence sex ratios towards the female gender.

The finding in the main study does go further to throw light into long standing allegations that sweet things do influence sex ratios towards the females but our study conveys the very opposite message that the influence with glucose is actually towards the male progeny.

The finding among the all-inclusive or cocktail does bring in an interesting natural aspect of sex ratio outcomes where the effects of various chemical elements may tend to cancel each other and modulate sex ratios towards 1:1 ratios.

Chapter 6

Discussion

6.1 Introduction

Sex ratio adjustment as a subject is emotive, controversial and complex because it does not only deal with humans but the very start of new life, the blueprint and the genes which make the stuff that humanity are made of (Scott et al., 1999). Unfortunately, the subject often times stirs people's emotions more than their minds and cause people often times to take up strong views on what is right and what is wrong (Benagiano et al., 1999). At an individual level, the concept is intriguing as it concerns the very basic question that each person always has in mind, the unspoken concern of the ability to procreate and the capacity to achieve the desired family size with the aspired gender balance (Shushan et al., 1993). Socially, it touches on the core of humanity with overtones affecting the social, legal, medical, ethical and religious aspects of our lives (Campbell, 2002; Loras College, 2002; Savulescu, 2002).

Most concerns are majorly focussed on the ethical standpoints rather than the actual work done in exploring further areas in an attempt to advance the subject (Liu, 1996). This often times has been observed to elicit knee jerk like reactions that challenge further rational debates while other times impressions may be created that scientists should refrain from this work because they are rather becoming too big for their boots and trying to play God by tinkering with the genes, the blueprint of humanity and that it may be far better if they abandoned this subject because it is far too beyond human understanding (Pembrey, 2002; Scott et al., 1999). Without fear of contradiction, research is simply search of knowledge and it is vital in all areas of human life but the use of the information acquired from it need to be legislated upon to ensure proper use (Benagiano et al., 1999; Robertsoet al., 2011; Robertson et al., 1995).

Despite the controversies of this subject, mankind has historically desired to find solutions to his challenges, find cure for his ailments in order to alleviate suffering and improve life (Barabasi et al., 2011). In the process, a lot has been achieved however, hereditary diseases such as –X–

linked disorders have remained a big challenge without eminent hope of finding complete cure except prevention which has remained as the only recourse to date (El-Hazmi, 1999).

Due to failure of getting solutions to various human challenges, therefore the importance of research cannot be underestimated because to date, the acquired diseases have significantly reduced over the recent past due to improved economic status, better nutrition, better medical care and expansion of screening services (Shaikha et al.,1999).This has ushered a paradyme shift to the overall disease prevalence thus making genetic diseases a major cause of morbidity and mortality (El – Hazmi, 1999). If this trend is not arrested by finding more preventive and curative measures, then genetic diseases are headed to be more prevalent in years to come (Arrayed, 1999; Owain et al., 2012).

Looking at the pathophysiology of -X- Linked diseases, these are conditions that are expressed mainly in situations where - X chromosome exist in single status as in the male chromosomal expression of -XY- as opposed to the double - XX - female chromosomal representation (Adam, 2004). The double - X- chromosome acts as a preventive tool against these conditions and are therefore more prevalent among the males than the females because females are mosaics with two cell lines of maternal X chromosomes and the paternal –X- chromosomes (Orstavik, 1999; Wutz, 2007). In normal circumstances one X chromosome is randomly inactivated in early embryonic life and stays permanently inactivated (Orstavik, 2006). In circumstances where there is X linked diseases, there is preferential or skewed X inactivation where the mutant X chromosome or the affected chromosome is inactivated (Orstavik, 2009).

Logically, it therefore follows that when a couple is affected by an - X- linked disease and the foetus is a female, there is a high probability that the child will not be affected but a carrier status may present. However, if the child is a male, the odds are such that the child will have a 1 : 2 chance of being affected (Barron et al.,1996). It is therefore natural that, influencing conception towards predominantly female offspring among the affected can circumvent some of these conditions and their subsequent social, financial and emotional costs that go with bearing the affected progeny (Baron et al., 1996; Seguy, 1975).

Considering that over 350 conditions have so far been reported (Adam, 2004; College, 2002; El–Hazmi, 1999), it is therefore comforting that sex ratio adjustment methods for medical reasons and in particular for genetic diseases have been made permissible towards alleviation of human suffering from genetic diseases (Vanden, 1995, Schroeder, 1994; Savulescu 2002, Burke, 2002). It is in this regard that this study has been undertaken in support of this noble cause.

The practice of sex ratio adjustment precedes the written history (Hewit, 1987). The first documented gender selection having been presumably done by God when after creating Adam, He went further to create Eve as a balancing act (Genesis 1: 29). The rest was left to mankind as he was commissioned to go forth and fill the earth (Genesis 1: 29; Psalms, 139:13-14, The Holy Quran chapter 23, verses 12 - 14). Man has since been preoccupied by the desire to influence the sex ratios of his off springs since creation (Hewit, 1987). Failure to achieve the aspired or desired family gender distribution has led to many sex ratio adjustment methods that span from the ancient cultural myths and practices to the modern day high technological methods (Folk wisdom, 2002; Schenker, 2002; College, 2002).

There are three main principles of sex ratio adjustment that have so far been advocated and practiced over the years. These include the traditional practices, natural or indirect influencing methods and the direct artificial sex ratio selection methods. Their differences are reflected in the times of discovery, advocacy and practice in human history (Folk wisdom, 2002; College., 2002; Vidal 1998).

The principles of traditional methods are based on assumptions, myths, long term observations and astrology (Folk wisdom, 2002; Savulescu, 1999). The principles of natural methods are based on physiological dynamics of the body and the gametes (Guerrero 1971; Billings, 1972 Whelan, 1974). These are aimed at creating favourable environs that create competitive advantage for the desired gender and are therefore defined as influencing and not selecting (Landrum et al., 1984; Seguy, 1975; Wilcox et al., 1995; Wilcox, 2001) The latest technology manipulates the gametes directly and selectively cause them to meet hence are defined as selection methods (Ericsson, 1994;. Barron-Vallejo, 1996; Hewit, 1996).

Every conceptional attempt has a natural wish of a normal pregnancy, delivery of either female or male child through exploiting the natural 50:50 chance of either gender based on individuals' predicament (France, 1981; Screenay, 1993). Willfully, subconsciously or out of curiosity, many couples do practice sex ratio adjustment depending on their cultural, religious and other needs and wishes before conception and the method used defines the principle in question (College, 2002; Celik et al., 2003). In general, for those who attempt these adjustment methods for curiosity may attempt the easy and less complex methods such as the traditional or the natural methods whereas those with other compelling needs and wishes are likely to attempt the more complex and expensive methods (Erickson, 1994; Fugger et al., 1998).

The initial traditional sex ratio adjustment methods were based on either long term observations or rudimentary scientific knowledge and many have already fallen out of use (Folk wisdom, 2002). Over the years, these methods translated into the indirect natural methods of sex ratio influencing that exploit the natural physical properties of the gametes or the body's physiological properties reflecting increasing understanding of the human anatomy, physiology and their clinical applications (Edwards, 2000; Carvalho et al., 1998; Screenay., 1993). With increasing knowledge and understanding of human reproduction, came the latest artificial sex ratio adjustment methods that are based on direct manipulation of the gametes or the conceptus defining the current direct sex selection methods (Erickson, 1994; Seguy 1975; Fugger et al., 1998; Vidal et al., 1998).

Most of the traditional gender ratio adjustment methods have fallen into disuse and out of favour and therefore remain as myths or tales that hold little value and use (Folk wisdom, 2002). To date, the majority of sex ratio adjustments methods that are practiced fall in the category of natural or indirect sex ratio influence. These include billings, smart stork and dietary methods (Screenay, 1993; Whelan, 1985; Billings, 1972; Shettles, 1970; Odeblad, 1997). Among the direct sex ratio selection methods include Erickson method that exploit the oocyte kinetic or density differential and the oocyte membrane electrical potential properties (Erickson, 1994; Vidal et al., 1998; Wang 2006).

The dietary method which is under scrutiny in this study is among the various indirect natural gender influencing methods that have been advocated (Stolkowski 1982). The rational is based on the fact that the oocyte membrane may have alternating membrane electric potential to negative, positive or neutral status (Papa et al., 1983). This electrical potential may be influenced by modulators in the diet that are under scrutiny in this study. It is postulated that the oocyte membrane in its' negative state, attracts the androsperm while at its positive state, attracts the gymnosperm while at its neutral status accepts either gamete at a 50:50 chance (Celik et al., 2003). This phenomenon has been described as galvanotropism (Collin et al., 2005, Hardy et al., 2002).

Galvanotropism is a concept whereby natural cells are attracted to one another due to their inherent membrane electrical charges (Collins et al., 2005; Gruler et al., 1990; Okamoto et al 1977; Richard et al., 2009). The attraction is proportional to the electrical membrane potential generated between them (Wong et al., 210; l). The dietary method is based on this concept (Celik et al., 2003). Through ionic influence, the chemical contents of the diets act on the receptor sites or some proteins on the oval membrane that the spermatozoa bind as it fertilizes the ova and exert some specific allosteric modification or electrotactism that allows selective attraction towards either gamete and subsequently influence the gender of the conceptus (Celik et al., 2003; Hardy, 2002; Rajan, 1999).

Gametes have cellular membrane charges and are therefore electrogenic and function through the various transmembrane ionic transport systems that allow various ionic currents to mediate various biologic and physiological events during cell growth, maturation, division and fertilization (Tosti, 2004).

Sex ratio adjustment is not only of interest in the humans but also in the animal kingdom where studies have shown that breeding patterns can be influenced by various factors such as the prevailing climatic conditions, dietary intake, age, and the adult sex ratio. Sex ratio manipulation is exploited for commercial purposes in particular in the dairy farming (Chenyi et al., 2003; Elissa et al., 1999). Among the crocodiles, sex differentiation can be pre -determined by the temperature at which the eggs are incubated (Warner, 2005).

The practice of gender selection captures a lot of interest and appears not destined to stop any time soon considering the fact that the process started from time immemorial and is still practised world over (College, 2002; Hewit, 1987). Unlike many other global practices that are largely influenced by race, education, culture, and status, these practises stand above and beyond these and are practiced in varying degrees by all societies in various ways (Schenker, 2002).

Looking at the principles of the methods used, it is clear that adjustment are either preconceptional or postconceptional done through simple prediction or luck among the traditional, through influencing certain physiological environments created or manipulated to give certain gametes some competitive advantage over the other and finally through direct gamete or foetal selection (Billings, 1972; Odeblad, 1997; Screenay, 1993; Shettles, 1970; Whelan, 1985). When serious interrogations on the methods are put into question, it becomes evident that whereas the ethical questions on direct sex selection methods may in no doubt considered in particular by the opponents of this subject to be an unwelcome debate, questions still linger as to where to place the grey aspects of the indirect natural gender influencing practices that are in practice today and difficult to enforce legislation on (Kalaca et al, 1985; Robertson, 2001; Simcock, 1985; Vidal et al., 1998 ; Wang, 2006).

As scientists, we certainly have a challenge with us pertaining to this subject because it is real and begging for answers and we cannot simply wish it away as we arm ourselves with excuses. This is because over all the years humanity has existed, a lot of practices have been speculated and practised but with little in-depth research on these methods. With this in mind therefore, it is prudent and timely to focus on various societal practices that are prevalent to enlighten us further on probable useful practices that should be encouraged as well as harmful ones that should be discouraged. It will be naive to assume that the controversies on this subject will fizzle out without justification. This study focused on one of the dietary gender adjusting practices among other methods of gender adjustments that are commonly alleged to work in many societies world over with an in-depth analysis, conclusions and recommendations.

Discussed below therefore is an in-depth look at all aspects pertaining to gender adjustment methods. Tracing the practices from their inceptional stages through various evolutionary landmarks, the religious and social aspects and inputs to the subject are also considered in brief. This is done cause they too form part and parcel of this subject as they provide the link between our study to the past, the evolutionary new findings that make the matrix upon which all new findings stand while the social fabric make the icing of the cake. Details of the various methods are discussed in the literature review and the scientific foundation chapters.

In this study, dietary allegations are put into in-depth study with a selection of four elements to test our hypothesis. Their dietary ionic effects on the progeny of the study models is presented and analysed coupled with other aspects of the same study pertaining to DNA testing, serum chemical dynamics, weight gains, effects of birth order, seasonal effects are discussed and conclusions made. The ionic effects of these four chemical elements are further subjected to yet another study that looks at their effects of the cellular or oval membrane and attempts to show case their uses done.

6.2 The historical perspective of sex ratio adjustment practices is fascinating and it would be rather incomplete to discuss this subject without a touch of the past so that we can appreciate where we have come from, the milestones covered, our current status and the future perspectives (Bull, 1981; Schenker, 2002) What is intriguing is the fact that irrespective of the method, however unscientific, attempts over the years were made to explain as accurately as possible depending on the times and places of practice(Savulescu, 2002). “Darwin 1862 described the process as difficult to understand why evolution is through human sexuality and not pathogenesis and appears like the subject is hidden in darkness” (Stearns, 1987).

The practices of sex ratio adjustment have evolved in line with the advancement of society from traditional myths of the distant past (Folk wisdom, 2002) to the natural influencing methods of yester years (Carvalho et al., 1998; Edwards., 2000; Screenay, 1993) and lately to current artificial selection methods (Vidal et al., 1998; Wang 2006).

The practice can be traced from the traditional myths (Folk wisdom, 2002) to the natural influencing methods (Carvalho et al., 1998; Edwards., 2000; France, 1981; Screenay., 1993) and finally to the current artificial methods (Hewit, 1987). In all these methods, sex ratio adjustment process is achieved either through natural or artificial methods applied before or after conception through influencing the natural characteristics of the body or direct manipulation of the gametes or the conceptus (Erickson et al., 1992; Silverman et al., 2002).

Both traditional and natural gender influencing methods and techniques are still in full practice in various parts of the world (Savulescu, 2002; Schenker, 2002). Their prevalence and impact are difficult to assess because they are practiced privately at the comfort of peoples' homes and therefore difficult to assess their prevalence nor apply legislation on them (Billings, 1972; Schenker, 2002; Shettles, 1970).

The dietary method under scrutiny in this study stems from the idea that changing a woman's diet for several weeks before she conceives can influence her conceptus is based on German studies done in the 1940s which looked at the environmental influence on the reproduction of worms (Stolkowski, 1980). In 1980, a research study was published aimed at testing this theory as to whether diet can influence the sex of babies in humans. It was thought that through diet, the mineral ionic balance in the woman's body could facilitate gender selection. The ionic balance was thought to affect the chemical make-up of her vaginal secretions and gametes (Celik et al., 2003; 1980 Rajan, 1999; Stolkowski).

The oocyte membrane potential study under study is based on the concept of bioelectricity that sprung into the scientific helms with the epic frog studies carried out by Galvani in the late 1700s (Hebbel, 1936; Miller, 1907). Using the frog animal model, he elicited muscular contractions through electrical stimulus. Since then, bioelectricity has provoked numerous research projects on the subject (Hebbel, 1936). Over the years this bio-electrical concept has been advanced to involve various ionic distributions between the outside and inside of the cells that create electrical gradients across the cell membranes known or electrical potential (Tosti et al, 2004).

6.3 Religious perspectives of sex ratio

The religious perspectives of sex ratio adjustment touches on ethics, culture and other diversities that do sometimes make us get so polarised on to our own opinions that we do not want to listen, hear or appreciate each other's story. Sex ratio adjustment is no exception and therefore it is in this regard and premises that all players need to accommodate each other.

For those that believe in God's creation, the area of creation can be said to unquestionably belong to God and the moment man ventures into this territory is like playing God or breaching the earlier covenants as stated in The Holy Bible which states that God created man in his own image, in the image of God he created him male and female he created them, blessed them and told them to be fruitful and increase in number and fill the earth (Genesis 1:27 - 8).

In the Muslim version, The Holy Quran describes the development of an embryo into full human person. "We Created (Khalaqna) man of an extraction of clay, then we set him a drop in a safe lodging, then we created of the drop of a clot, then we Created of the clot a tissue, then we created of the tissue bones, then we covered the bones in flesh, therefore we produced it as another creature so blessed be God, the best of creator (Quran chapter 23, verse 12-14).

So, God presumably did the first gender balance when after creating Adam , He went further and created Eve as a balancing act (Genesis 1: 27 - 28, The Holy Bible, Holy Quran chapter 23, verse 12-14) After this gender balancing, the rest was left to mankind when he was commissioned to go forth and fill the earth. Man set forth with a lot of zeal to accomplish his God given right of filling the earth and this resulted in creation of large families where gender balance was not an issue but globally led to over population that led to depletion of resources, poverty, diseases and other social ills (Butler, 1994). Man was therefore forced to reduce the population through small family sizes but as he achieved small family sizes he was faced with the problem of achieving the desired gender family balance (Campbell 2002; Savulescu, 1999).

Religion has played its rightful role of condemning the social direct sex ratio adjustment methods that are practised in various countries of the world (College, 2002; Schenker, 2002). However, though contentious and paradoxical, religion interestingly may have played some role in sex ratio influencing adjustment methods by default through the well cherished and intended yet noble doctrine of monogamous marriage system of which the Christian faiths are based (Schenker., 2002). Of particular interest is the Billings method of family planning advocated among the

Catholics (Billings 1972). One of the facets of this method involves discussions on how to avoid or achieve conception and further details on how to influence a particular gender in favour of ones wishes (James B Brown 1981). The begging question that bacon always is whether this information is given for knowledge purposes or for practice. To avoid harsh judgements, I usually find it comfortable to leave the verdict to the audience. However, the Muslims are more liberal in this matter as they have options of marrying up to four wives and this tends to take care of possible gender imbalances within the larger family (Schenker, 2002).

Religion has on occasions appeared to cherish the male progeny and there are areas in the bibble where there is enough evidence towards this in the church practices or hierarchy (Mathew 14: 21). Without going into details, there are various areas that in religion may need to be modified to reflect the current social status quo of gender equality cause nothing stays the same but moves along the evolutionary path or ladder (Mathew 15: 38).

This study involved the use of naturally oriented products and practices that most religions, cultures and ethical guidelines are not opposed to (Screenay, 1993; Whelan, 1985; Billings, 1972; Shettles, 1970; Odeblad, 1997). All the necessary steps needed to ensure the ethical, moral confines as well as the international scientific guidelines were carried out. The study sought to look for only ways of preventing sex linked diseases and only aspects of sex ratio influence pertaining to genetic disorders were considered. The study was limited to factors that had sex ratio influence in a laboratory set up with the use of animal models. No human subjects were used in the study.

Looking at creation, it appears like everything was created with a clear purpose but man was created with limited knowledge of these purposes. It is through bio - research that man can try to unravel the unknown purposes of all creations so as to expand his boundaries and limits of knowledge. The information accrued may at times be like weapons that must be with us for use if need be but need to be used with wisdom and correctly for the benefit of society. Research need to continue in all aspects of our lives so as to gain more knowledge but the gained information should be used for the betterment of society and not *vice versa*.

6.4 Dietary chemical ionic influenced sex ratio patterns

The social claims that diets do affect sex ratios was evaluated through a double blind study that was designed aimed at examining the chemical serum ionic influence of sodium potassium calcium and magnesium using mice animal models. The rationale of the study was based on the fact that dietary chemical influence on sex ratio outcomes has been advocated from time immemorial though not fully proven as yet.

Using 144 families of Swiss (Webster) breed mice, these were randomly divided into 9 groups receiving different ionic formulations in their drinking water. They were followed up throughout their life spans of between 2 – 3 years and data on the sex ratios of their progeny analysed using applicable statistics.

With 1528 deliveries a total of 13,040 pups were delivered comprising of 6,348 female pups and 6,692 male pups. The mean pups per delivery were 8.5/delivery. There was no significant statistical difference in sex ratio found in the water control group, $p=0.61$ while, glucose, Sodium, potassium and sodium + potassium supplementation influenced the sex ratios towards male progeny ($p<0.001$). Calcium, magnesium as single elements and their combinations influenced sex ratios towards the female progeny ($p<0.001$). The cocktail of all the combined elements together had no significant influence towards either gender ($p>0.0609$).

This study did confirm our earlier pilot study findings that dietary chemical contents do influence sex ratios through their serum ionic influence along either female or male gender lines and that glucose does seem to have similar influence on sex ratios towards the male gender.

The idea of pre conceptional gender influence through dietary inputs originated from German studies in the 1940s which looked at the environmental influence on the reproduction of worms where their gender was found to be influenced by the soil chemical constitution (Papa et al., 1983). In 1980, research findings were published following a study done aimed at testing this theory in humans. In this study, it was postulated that through the dietary mineral ionic balance in the woman’s body could facilitate sex ratio influence possibly through her ova and or her vaginal secretions. The study recruited 281 couples, allocating them into either the boy group, which was a daily diet high in salt Na^+ and potassium K^+ or the girl group which was a daily diet high in calcium Ca^{2+} and Mg^{2+}. The men were also asked to go on the diet primarily to support the women rather than for the purpose of sex selection. 80% conceived the child of their own choice (Stolkowski, 1980).

Following this study, many proponents, supporters and even users of this method came on board because it is the easiest to use as it is used in the privacy of one's home at low cost, in absence of the use of fertility charts and without requiring fertility clinic interventions. It has been found to offer better results if combined with other naturally oriented sex ratio influencing methods with reported success rates of 65% - 75% (Seguy, 1975, Rajan, 1999, Papa et al., 1983).

In this study, the control water group, 50.42% were female pups and 49.58% male pups showing absence of sex ratio influence $p>0.61$ that is within the natural gender ratios world wide of 48 - 9 females to 51- 2 males per 100 singleton births (Hammound, 1965). There was however sex ratio influence in the glucose, sodium, potassium as single elements and in combination of the latter two elements towards the male gender $p<0.0001$ in the following percentages towards the male gender, glucose group 57%, Sodium group 63% potassium group 61%, and 66% for the sodium and potassium combination group. In this study although there was similar gender influence but of less percentages compared to 65 - 75% (Rajan, 1999, Papa et al., 1983).

Equally, there was sex ratio influence in the calcium and magnesium groups as single elements and in combination of the latter two elements towards the female gender $p<0.0001$ of the following percentages towards the female gender: calcium group with 60%, magnesium group with 61% and 67% for the calcium and magnesium combination group. Of these in our study, although there was gender influence towards the female gender, the percentages were less compared to up to 80% as reported earlier (Stolkwiski et al., 1980).

Conclusion

The study reaffirms the societal allegations that there are factors in our diets that do influence sex ratio outcomes and that these factors could be chemically mediated.

It further re affirms that sodium and potassium do influence sex ratios towards the male gender. Calcium and magnesium do influence sex ratios towards the female gender.

The new finding in the main study does go further to throw light into long standing allegations that sweet things do influence sex ratios towards the females but in the findings in this study are the very opposite in that the influence with glucose is actually towards the male progeny.

The finding among the all-inclusive or cocktail does bring in an interesting natural aspect of sex ratio outcomes where the effects of various chemical elements tend to cancel each other thus modulating sex ratios towards 1 : 1 ratios.

Looking at the overall figures, there were 13,040 pups delivered comprising of 6,348 female pups and 6,692 male pups that constituted 49%, 51% respectively. This translates into 1 : 1 ratio which is a further re affirmation of Fishers principle of evolution described in 1930 stating that sexually in reproducing species have their sex ratios around 1 : 1 proportions or inclined towards equal sex proportions. This is due to natural sex selection through the accumulation of autosomal alleles that direct the parental reproduction efforts toward the rare sex (Bull, 1981; Cavalho et al., 1998).

6.5 Sex ratio influence and the Glucose link

Glucose has been claimed to be associated with sex ratio influence and in particular skewing it towards the male conceptus with some confirming and others refuting it (Naoko et al., 2013; Issa et al., 2008). The Trivers–Willard hypothesis (TWH) states that there are environmental factors that affect reproductive success (Caneroon, 2008). In this regard, mothers in good conditions with resources are advantaged and tend to produce sons, while mothers in disadvantaged environs with fewer resources would rather invest in daughters (Elissa et al, 1999; Bradbury et al., 1998).

New evidence has put focus on the time of exposure as the vital variable pointing to the time of conception as the crucial point in time when the influence occurs and not later. It states that addition of glucose to growth cultures tend to encourage growth and development towards male conceptus (Caneroon, 2008). Stress too has been shown to be associated with increased serum glucose levels and to skew sex ratios towards male conceptus an effect that has been shown to be reversed by the administration of dexamethasone that reduces blood sugar levels (Hahan et al., 1999). Further evidence has been deduced from diabetic candidates whom with increased serum blood sugar levels have been observed to have a tendency of having male skewed progenies (Issa et al., 2008).

It appears that glucose and LH are important in reproduction as changes in glucose levels may be associated with maternal hormonal status and in particular the serum LH levels which has been found to be the link between the two and therefore defining the link between glucose in reproduction. Glucose induces release of LH which has a glycolytic effect that avails more glucose to the oocyte during the ovulation time (Murahashi et al., 1996). This explains why it has traditionally been postulated or observed that more male conceptuses are achieved during the mid-cycle than other times (James, 2000).

6.6 Early gender determination by PCR/DNA and perinatal mortality

Perinatal mortality is a good measure of the health status in humans and by extension can equally be used in animal health. In order to determine perinatal mortality and sex ratios, gender determination was done using either physical makers or the PCR. To avoid losing data the counting of the pups and cutting off tail (DNA) material was done within 24 hours of delivery. Sex determination by physical means was done based on the anal - genital distance which is about 1 ml in the females and twice this distance in the males. The optimal time for this determination is about three weeks of age. The pups whose gender could not be determined physically because they either died before attaining this age or were eaten by the adults had their DNA extracts subjected to PCR.

A total of 415 pups out of 13,040 perished before gender could be physically ascertained and their DNA materials subjected to PCR analysis. This constituted approximately 3.1% of the total pups delivered with means of 1,449 pups delivered per group and 46 demised pups per group respectively.

No significant statistical gender difference in sex ratios was found among the demised pups in the water (p>0.66); glucose (p>0.166), sodium (p>0.49), potassium (p>0.339), calcium (p>0.612), combined sodium + potassium (p>0.104), and the all-inclusive group (p>0.7995), signifying absence of gender influence among the demised pups. There was significant sex ratio difference towards female progeny among the demised pups in the magnesium (p<0.005) and combined calcium + magnesium (p<0.044) groups towards the female progeny.

The study did not show evidence of gender bias among the demised pups. The overall perinatal mortality rate was 32/1000 which is comparable to the countries with high human perinatal mortality rates (1994 Global health for all databases, Geneva: WHO).

The mouse is the most preferred animal model in biomedical research, however little attention has been given to early causes of pup deaths (Weber et al., 2013). During the pilot study this phenomenon was one of the observations that was made as one of our study challenges and was therefore designed and factored as one of our study arms. This was considered because if early pup deaths were not factored then data would be lost and this would render the study inconclusive. To avoid this all pups delivered were counted as soon as possible and not later than 24 hours of delivery and had all their tail tips aseptically cut and preserved for DNA / PCR gender determination if need be.

In humans, sex ratios are generally within the 1:1 ratio worldwide with a few exceptions of some few countries that have strong gender preferences (Hammound., 1965, Samuli., et al., 2009, Dickens, 2002). This is the natural status without influence and is in conformity with the Fishers principle that sex ratios are always naturally influenced towards sex ratios of 1:1 as directed through accumulation of autosomal alleles that direct the parental reproduction effort toward the rare sex (Cavalho et al., 1998). Without any influence the prevailing natural sex ratio is 102:100 with more males being born than females to accommodate for the slightly higher male mortality rates along life’s journey (Dahl et al., 2003, McCarthy, 2001).

Despite the widespread use of the mouse in various research projects, its perinatal mortality is not quite well studied. The most common causes of mortality that have been reported include hypothermia, infections especially respiratory infections, genetic disorders, starvation and traumatic injuries or infanticide by the adults (Weber., et al., 2013, Labov et al., 1985).

The perinatal mortality rate was 32/1000 which is comparable to many countries with the highest human perinatal mortality rates (1994 Global health for all databases, Geneva: WHO). Globally birth sex ratios are similar (1:1) and is higher among males except the countries with strong gender preference who report otherwise (Booth et al., 1994; Pflafker et al., 1994). In this study due to dietary ionic sex ratio influence, there was no preferential male oriented perinatal mortality.

6.7 Serum chemical concentrations

Serum chemical concentrations examinations were done to establish whether there is relationship with sex ratio influence. The study was aimed at examining the relationship between high dietary chemical supplementation and the relevant serum chemical concentrations. It sought to examine whether the respective high chemical element concentrations in the various feeding dietary solutions were reflected systemically.

The rational was based on the fact that high dietary chemical components in our diets have been advocated to influence the sex ratio outcome through their systemic effects. These chemicals are absorbed from the intestines via the liver to the blood stream and subsequently to the extra cellular space where cells including gametes are bathed in this fluid. With the presence of increased amounts of chemicals in the extra cellular fluid, various cellular reactive responses take place through osmosis, passive diffusion and active ion channel movements. These adjustments attempt to maintain the extracellular and intracellular equilibrium state of the relevant chemicals and as these chemicals are charged, in the process of the cellular ionic dynamics create cell surface charges and by extension their immediate environs such as the attached proteins that influence their cellular activities including fertilization (Hardy, 2002; Stephanova et al., 2012; Wang et al., 2001).

The determination of the serum biochemical levels of the respective elements under investigation was done in all the nine groups where blood samples of 0.5 ml were drawn through nipping off the tail or intra orbital routes. Using the standard automated systems the biochemical analysis was done on weekly basis.

Normal levels of sodium, potassium, calcium and magnesium were found among the control groups with means of 140 mg/ml, 7 mg/ml, 2.5 mg/ml and 1.7 mg/ml respectively. No statistical significant differences were found between the concentrations of these four chemicals between the two control groups of sodium ($p>0.165$), potassium ($p>0.818$), calcium ($p>0.878$) and magnesium ($p>0.116$). Significant statistical differences were found between the corresponding elements in the water control group and the experimental groups of sodium, potassium, magnesium, sodium, potassium, calcium and calcium + magnesium group and the all-inclusive group ($p>0.0001$) except between magnesium in the all-inclusive group ($p>0.036$).

The physiological aspects of the chemicals under study still remains one of the areas that still need a lot more work to be done because the serum biochemical data still remains to be refined despite various work done on the laboratory animal models because only limited information is available on this subject from the previous studies (Seldom, 2007**).** Despite various efforts towards standardization of normal values, various disparities still exist due to various observed technical differences in the modes of collection of the specimens, transportation and other differences among the animal models and work related modalities (Schnell et al., 2002; Dzięciołowska et al., 2009).

The control of cellular internal and external environs is done through refined regulatory systems that use negative and positive feedbacks mechanisms so as to maintain a homeostatically and balanced internal milieu with some normal deviations and disparities. (Cogswell et al., 2012). Armed with these defined normal baselines upon which somatic and germ cells have direct dependency on, light can be shed on the chemical effects on their cellular functions by exposing them to different chemical concentrations (Tefferi, 2001).

Sodium is a monovalent ion whose serum levels range between 140 mg – 160 mg (Tefferi, 2001, Seldom, 2007). It is actively involved in body fluid and blood pressure regulation, muscular activities, neural transmission and bone integrity (Cogswell et al., 2012; MacCarron, 2000). Most natural foods are good sources of sodium such as milk, soy, onion vegetables and drinking water. The major source of sodium however is common salt and most processed consumables (Rolfes et al, 2008).

Excess sodium intake or consumption includes fluid overload, elevated blood pressure, and cardiac as well as kidney disease. Low serum levels are caused by excess water consumption, diarrhoea and vomiting and presents with weakness, muscular cramps, confusion, fainting, convulsions and respiratory arrest (MacCarron, 2000, Rolfes et al., 2008).

In this study the mean sodium levels were found to be within the normal serum mean level of 135 – 160 mg/l. Paired serum sodium t test results between the control groups 1 and 2 showed absence of significant difference between the serum sodium levels of the water and glucose control groups ($p>0.165$). Similar absence of gender bias was observed in the ionic dietary study control group one with water ($p>0.61$). Significantly elevated serum sodium levels were found among the experimental groups three, four and seven with corresponding significant male gender bias ($p<0.0001$).

Potassium is a monovalent ion with serum levels that range between the levels of 5 - 8.0 ± 0.85 meq/L or even greater in the laboratory mice though these levels are incompatible with human life situations (Ryan et al., 2010). Circumstances that contribute to the high levels include the site of collection, method of collection, time allowed for clot formation before serum separation and the time between collection and analysis of the samples (Schnell et al., 2002, Ryan et al., 2010)

95% of Potassium is intracellular and works together with sodium to maintain fluid balance in all cellular and fluid electrophysiological functions in the body (Cogswell et al., 2012). Other functions that potassium is involved in include nerve conduction activities, cell to cell interations, muscular contractions, nerve conduction activities and metabolic activities (Rolfes et al., 2008). Some clinical uses include prevention of blood pressure and by extension strokes, reducing the incidence of kidney stones by aiding excretion of citrate that usually combines with calcium to make kidney stones (Ryan et al., 2010).

Good amounts of potassium are found in bananas, almonds, spinach, potatoes, oranges, sunflower seeds, sweat potatoes, tomatoes and mushrooms. Its deficiency causes weakness, muscle cramps, dry skin, mood changes, slow reflexes, acne and irregular heartbeats (Rolfes et al., 2008). Excess amounts of potassium leads to mild to deadly arrhythmias whose treatment involves administration of calcium to compete with potassium. This explains why by giving excess calcium will render the external charges of the cell or its immediate environs to be positive (Walter et al., 2006). In this study the mean potassium serum level was 7.09 which are within the normal serum levels of between the control groups ranging between 5 - 8.0 ± 0.85 meq/L. There was no significant difference between the serum potassium levels of the water and glucose control groups $p>0.8178$. Corresponding absence of gender bias in the progeny was observed in the water control group as well $p>0.61$. Significantly high serum potassium levels were found in the experimental groups four and seven $p<0.001$. Corresponding similar significant male gender biased progeny $p<0.0001$ was observed in the same groups in the dietary ionic study.

Calcium is a divalent ion with serum levels ranging between 7 mg – 10 mg whose main functions in the body are for structural development and maintenance (Bonilla et al., 1968, Tefferi, 2001). Other functions include muscle contraction, heart beats, nervous system, immune system, enzyme function. It interacts with sodium, potassium and magnesium for control of blood pressure among others (Rolfes et al., 2008).

Calcium is mainly found in dairy food products such as milk, cheese and yogurt in 70% of cases. Other minor sources include vegetables, fruits, meat products and grains (Rolfes et al., 2008, Cogswell et al., 2012).

In humans, calcium deficiency leads to weakness, muscle cramps, irregular heartbeats, rickets, weak bones that easily lead to easy fractures. Its deficiency is caused by inadequate intake, high phosphate and citrate intake as these tend to enhance calcium excretion (Rolfes et al., 2008). Excess calcium levels range above 10.5 mg/dl and are caused by disease states such as hyperthyroidism, malignancies, kidney diseases, medications e.g. thiazides, antacids and excess calcium intake e.g. milk (Munro et al., 2010).

In this study the mean calcium serum level in the control group was 2.44 meq/L which is within the normal serum levels of 2.24 – 2.46 meq/L. There was no significant difference between the serum calcium levels in the water and glucose control groups ($p>0.8178$). Corresponding absence of gender bias in the progeny was observed in the water control group ($p>0.61$).

There was significantly high serum calcium levels recorded in the experimental groups five ($p<0.0001$) and group eight ($p>0.0003$) and collateral significant female gender biased progeny observed in both the experimental groups five and eight in the dietary ionic study ($p<0.001$).

Potassium is a monovalent ion with serum levels that range between the levels of 5 - 8.0 + - 0.85 meq/L or even greater in the laboratory mice though these levels are incompatible with life situations (Tefferi, 2001; Ryan et al., 2010). Conditions that have been sited to contribute to some high levels include the site of collection, method of collection, time allowed for clot formation before serum separation and the time between collection and analysis of the samples (Schnell et al., 2002; Ryan et al., 2010).

95% of Potassium is intracellular and works together with sodium to maintain fluid balance in all cellular and fluid electrophysiological functions in the body (Cogswell et al., 2012). Other functions potassium is involved in include nerve conduction activities, cell to cell interactions, muscular contractions, nerve conduction activities and metabolic activities. Some clinical uses include prevention of blood pressure and by extension strokes, reducing the incidence of kidney stones by aiding excretion of citrate that usually combines with calcium to make kidney stones (Ryan et al., 2010).

Good amounts of potassium are found in bananas, almonds, spinach, potatoes, oranges, sunflower seeds, sweat potatoes, tomatoes and mushrooms. Its deficiency causes weakness, muscle cramps, dry skin, mood changes, slow reflexes, acne and irregular heartbeats (Rolfes et al., 2008). Excess amounts of potassium leads to mild to deadly arrhythmias whose treatment involves administration of calcium to compete with potassium. This explains why by giving excess calcium will render the external charges of the cell or its immediate environs to be positive (Walter et al., 2006).

Magnesium is a divalent ion whose serum levels serum levels range between (1.5 meq/l – 2.5 meq/l) and is an important element in the body as it is involved in many clinical functions (Tefferi, 2001; Seldom, 2007). It is incorporated in the integrity of the bone and heart muscle where it is 18 times more concentrated and this helps it to pump more effectively and optimally. It is involved in neurotransmission, protein synthesis, activation of vitamin B complex, blood coagulation and acts as a co factor in many enzymatic reactions e.g. ATP reactions (Mason, 2011; Mauskop, 2009).

Magnesium relaxes smooth muscles and this has a beneficial effect of lowing blood pressure, relieving asthma and dysmenorrhoeal and therefore has been used in their management. It has also been used in the treatment of migraine (Mauskop, 2009). It is predominantly found in whole unprocessed foods with high amounts in nuts, soybeans, wheat and bran. It is also found in good amounts in corn, peas, carrots, barley, oats, wheat, green brown rice, potatoes, banana, sweat potato, blue berries, orange and milk (Fox 2003, Mason 2011). Its deficiency leads to weakness, fatigue, irritability, anxiety, weakness, dysmenorrhea, blood pressure. Diarrhoea is the main side effect of excessive magnesium intake and reduced calcium absorption because it competes with calcium (Fox et al., 2003).

In this study the mean magnesium serum level was 1.82 meq/l which is within the normal serum levels of between the control groups ranging between 1.5 meq/l – 2.5 meq/l. There was no significant difference between the serum magnesium levels in the water and glucose control groups (>0.1160). Corresponding absence of gender bias in the progeny was observed between these control groups as well ($p>0.61$). Significantly high serum magnesium levels were found in the experimental groups six and eight ($p<0.0001$) and with corresponding similar significant female gender biased progeny ($p<0.0001$) observed in the same groups in the dietary ionic study.

Normal levels of sodium, potassium, calcium and magnesium were found among the control groups with means of 140 mg/ml, 7 mg/ml, 2.4 meq/l and 1.7 mg/ml respectively. No statistical significant differences were found between the concentrations of these four chemicals between the two control groups with sodium ($p>0.165$), potassium ($p>0.818$), calcium ($p>0.878$) and magnesium ($p>0.116$). Significant statistical differences were found between the corresponding elements in the water control group and the experimental groups of sodium, potassium, calcium, magnesium, sodium + potassium, calcium + magnesium and all-inclusive group ($p>0.0001$) except between calcium and magnesium and the all-inclusive group with p-values being $p>0.9603$ and $p>0.455$ respectively.

There are variations in determining the actual serum levels of various blood parameters due to several technical challenges as cited by various authors and each laboratory is best placed to set its reference values (Schnell et al., 2002). Serum magnesium levels for example have been found to be poor indicators of systemic concentrations and often times the levels in the white blood cells have even been suggested to be more predictable (Mason, 2011; Tefferi, 2001).

6.8: Monitoring the study progress

To monitor the study progress, the weight trends were used as a monitoring tool of the animal model wellbeing and the study. This was done through examining the effects of various chemical formulations on the weight trends on the animal models (Nicholson et al., 2009). The rationale was based on the fact that weight trends have been used before as a good measure of the wellbeing of research animals and success of studies (Jirkof et al., 2013). Based on the background of the study since its inception, the various chemicals under study were dissolved in tap water, and then fed to the animals and their weight trends examined. In the initial stages, the animal models in groups four, five, six and eight that were constituted with potassium, calcium and magnesium had poor feeding habits as some were refusing to feed and were continually losing weight and this persisted despite various dilutions till glucose was added.

To study the effects of the various chemical formulations on the weights of the animal models, six pairs of animal models were selected from each of the nine groups and their weights taken on a weekly basis for a period of six months. The models were introduced to the study at the age of six weeks in line with the rest of the study’s other aspects. The chemical formulations, constitutions and applications were done as stipulated earlier.

There was general weight gain and absence of recorded weight loss. The total mean weight gains among the female and male animal models were 11.12 g and 10.55 g respectively, representing mean monthly weight gains of 1.85 g and 1.76 g respectively. The maximum weight attained among the females was 28.19 dg and 27.73 dg among the males which are within the average adult weight ranges (Bachmanov et al., 2002).

The average mice weight has a wide range of between 1.8 g - 3.5 g with the males weighing slightly more (Greehami et al., 1977). The adult maximum weight gained was within this range. Weight gains and losses have been used in the past as a measure of the animal model wellbeing and for studying various cause effects of medications, diseases, congenital disorders and cancers (Jirkof et al., 2013, Nicholson et al., 2009).

Weight trends parameters have been used in research in agriculture and veterinary sectors where wide range of characteristics have been studied including growth rates, body compositions, metabolite measurements, cage telemetry and micro environment (Jirkof et al., 2013). The accrued findings indicate that the housing densities has a lot of bearing on weight related parameters and numbers that by extension have a strong bearing on the bio-economics of commercial animals (Nicholson et al., 2009). In this study, there was general poor feeding and loss of weight among the potassium, calcium and magnesium groups as well as their combinations in the initial stages but this was followed by steady weight gain in all the animal models following addition of glucose.

The general universal and steady weight gain among all the animal models reaffirmed good general feeding of the animal models that translated into their general wellbeing, intake of the study chemicals and validity of the results. The maximum weight attained by the adult mice was within the findings of other authors confirming good cage densities, telemetries and microenvironments which go further to ratify the results of the study (Bachmanov et al., 2002; Greehami et al., 1977).

6.9 The effects of birth order on sex ratios

The effects of birth order on sex ratios were examined in an effort to study the relationship between birth order and sex ratios using the accruing data. This study sought to know whether birth order had significant influence on sex ratios of the progeny. The rational was based on the many unconfirmed allegations in society that sex ratios may be influenced by birth order (Bull, 1981).

Armed with the data on all the deliveries of the nine groups each with sixteen families that accumulated over their entire life spans of the animal models of between two and three years the data on all deliveries was analysed. The delivery order was designated as generation in that the first delivery was referred to as generation one and the second delivery as generation two and so on.

With 1528 deliveries, a total of 13,040 pups were delivered comprising of 6,348 female pups and 6,692. The mean litter size was 8.5 per delivery comprising of 4.15 female pup number per delivery and 4.38 males per delivery.

The average pups delivered among the 1 – 9 generations (deliveries) were between 8 – 9 and dropped from the 10th delivery (generation) to 6 pups per delivery and down to eventually 2 in the last 12th deliveries. The sex ratio influence towards the female gender among groups 5, 6 and 8 was maintained and so was the inclination towards the male gender maintained in groups 2, 3, 4 and 7. Absence of gender influence was maintained in groups 1 and 9 as earlier reported.

The litter size was not found to be influenced by birth order and was universally similar in all the groups and families. In particular, the first ten deliveries had an average litter size of 8.5 pups per delivery. Birth order was found to decline in numbers from the 10th, 11th & 12th deliveries with in the declining range of 6 - 2 pups per delivery. In all the nine groups, birth order was not found to influence sex ratios except declining litter sizes as from the 10th delivery onwards.

The sex ratios at birth and subsequently the population sex ratios remain fairly constant in human populations without manipulation (Hesketh et al., 2006). Studies carried out in relation to various other correlations such as age between birth order and the sex ratios without manipulation have shown no significant positive correlations (Caqnacci et al., 2003).

Naturally slight variations with some excess female and male births have been reported in various places but it was after the First World War that significant excess male births were reported, but was followed by cautionary remedial measures (Samuli et al., 2009) as represented in figure 6.1. Despite these measures there still exist various countries with either son or daughter preferences (Plafker, 2002) as discussed below.

The traditional gender preference in particular with son inclination has remained despite modernization and has influenced and fuelled many to seek methods that can assist in achieving their goals (Schenker., 2002; Savulescu, 2002). In this study, the control represented society in absence of gender influence whereby there was no significant gender influence. However, the study chemicals did influence gender outcomes and this mimicked society where there is gender influence with sex ratio inclination.

6.10: The social inputs

The sex ratio influence and the social connection are based on society's various allegations and speculations that are at times practiced due to lack of clear scientific guidance (College, 2002); Folk wisdom, 2002; Schenker, 2002; Schroeder, 1992). Although many allegations can easily be wished away as they are clearly unscientific and even laughable, some however appear to be based on some rudimentary scientific knowledge and require further scientific verification (Savulescu 2002; Scott et al., 1999). Over the years many modern methods have emerged triggering many fears of unnatural harmful outcomes and social disruptions including routine families (Benangiono et al., 1999; Campbell, 2002; McCathy, 2001; Miller, 2007).

This study attempts to address some of these questions, allegations and speculations that have been rife in many societies concerning interplay between diet, fertilization and sex ratios. Attempts have been made to verify the validity of these claims by taking social claims to the laboratory for experimental verification.

The link between allegations and facts is the premise for search for knowledge or research because there are many allegations (social practices) that are put forth but upon testing are found to be untrue while others are found to be amazingly true (Dickens 2002). A case in point is the much feared allegation that gender selection will lead to societies with significantly skewed populations but what is on the ground is much different because for this to happen, there are some requisites that will have to be in place (Dahl et al., 2003). These include the need for significant preference of a particular gender and secondly enough demand for gender selection (Plafker, 202). Some studies done towards these allegations have disapproved this for example in Canada, United Kingdom and German, the studies done in this connection showed that over 70% of the respondents did not mind the gender of their children and would not seek gender selection methods. Many would be happy with a gender balanced family but without making significant extra efforts (Dickens 2002, Dahl et al., 2003, McCarthy, 2001).

In most countries, sex ratio adjustments for medical reasons is permissible but not for social reasons as stipulated by the human rights and biomedicine guidelines that recommend sex ratio adjustment for purposes of preventing hereditary diseases or any other relevant medical challenge (Burke, 2002; Savulescu, 2002; Schroeder, 1994;Vanden, 1995). It is however only 36 countries worldwide with clear policy guidelines on this subject (Dickens 2002, Marcy D, 2009). In Israel for example, it is permissible for a couple with four children of the same gender to request for sex selection (Judy et al., 2005).

Interestingly, despite so much debate and talk and legislation on attempts to influence sex ratios, it is important to note that traditional and naturally oriented sex ratio influencing methods are practiced in the comforts of people’s homes world over by the minute. This is the reality on the ground and there is limited influence that culture, religion or legislation can do about (Schenker, 2002). However it is consoling and comforting to note that these naturally oriented sex ratio influencing methods have been shown not to skew social sex ratios to any significant levels. This is in conformity with the natural Fishers genetical principle of natural selection upheld in 1930 which states that, despite external influence nature directs sex ratios towards 1:1 ratios (Carvalho et al., 1998l, Edwards 2000, Schenker., 2002). Our challenges today and direction of our legislation should therefore be focused on the artificial sex ratio selection methods that are practiced mainly in institutions and have clearly been shown to skew social sex ratios significantly (Scott et al., 1999).

Of interest is the fact that unlike our common perception, sex selection for personal reasons may not have significant social gender skewing effects because they are sporadic and not necessarily of significant numbers to affect gender proportions in the society. Of particular interest is where there is no common influencing factor towards a particular gender like in Europe (Dickens 2002, Dahl et al., 2003, McCarthy, 2001). What have been found of significance in affecting gender proportions world over are the external factors that affect families in particular government policies, religious inclinations and cultural pressures. Other external factors include wars, temperature changes, ecological disasters, and economic crisis (Samuli et al., 2009).

Naturally it has been found that parental investment is geared towards the fitness pay offs depending on the varying abiotic and biotic conditions as per Trivers-Willard hypothysis that in favourable conditions mothers would invest in males and the opposite would hold true (Bradbury et al., 1998; Elissa et al.,1999).

Looking at the global sex ratio perspectives, it is clear that there are areas with certain gender preferences such as china and India leading with the male child preference while Japan leads in the female child preference (Plafker, 2002). Africa is fairly silent on sex ratios but has a balanced sex ratios due to polygamous practices that tend to sort out gender issues per family while the west is more open without any particular gender inclination except a well expressed wish for gender balance in a family which does not have to be fulfilled through the modern sex ratio adjustment methods (Dahl et al., 2003, McCarthy, 2001).

As illustrated in figure 6.1 below, sex ratios have been observed to mirror the existing global outlook. Post world war two (11), there were too many women and few men. This was followed up by readjustment and the advent of sex ratio adjustment methods that led to too many men in the seventies, nineties up to 2010 followed by stabilization of ratios to date following legislation, religious and social condemnation of social sex ratio adjustments (Samuli et al., 2009).

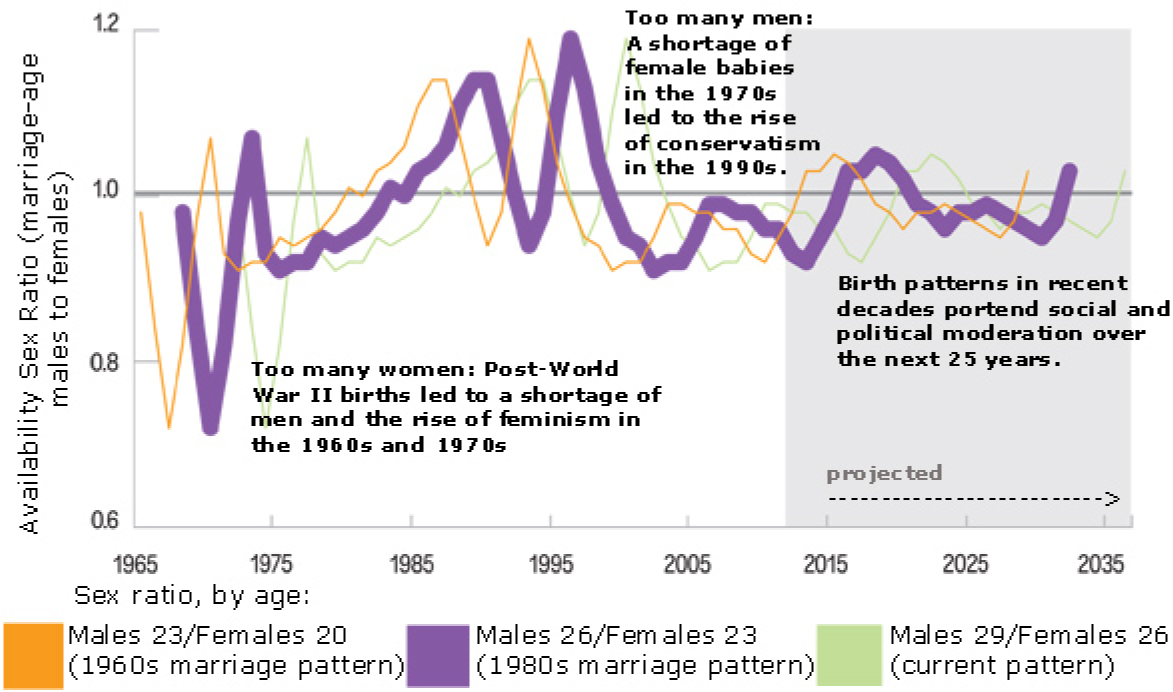


Figure 6.1 Sex ratios mirror the existing social global outlook or dynamics.

This study was not carried out for purposes of gender influence for social reasons and unlike the social sex ratio adjustment methods that are skewed towards the male gender (Booth et al., 1994). The work supports the female gender and does not support firms or persons who support sex ratio influence or selection for social reasons. The researchers do not support sex ratio influence for social reasons except for research purposes in an effort to gain more knowledge and understanding of the management of genetic disorders.

Darwin's theory as described in 1871 is a biological evolutionary explanation of how species develop given by Charles Darwin with others that explain how all species of organisms develop through the natural selection of various inherited qualities that increase individuals ability to compete, survive and reproduce (Ayala, 2009).This explains human sexual differences that evolved based on their sexual selection of certain physical, mental or psychological traits that evolved in order to aid competition among individuals so as to access preferred mates or traits that may enhance traits that help attract mates (Padian, 1999; Paul, 2002).

6.11 The effects of seasonal variations on sex ratios

The effects of seasonal variations on sex ratios were done through examining the relationship between seasonal variations and sex ratios. This was done using the accruing data along the study. The study sough to find out if food, water and the environment were held relatively constant in a laboratory environment, whether there could be any sex ratio influence in their progeny. The rational was based on the fact that studies in the animal kingdom in their natural habitats have shown sex ratio influence related to the availability of food as well as seasonal variations (Janzen et al., 2008, Chenyi et al., 2003, Raymond et al., 2012; Warner et al., 2005).

The seasons considered were those prevailing in our local region. These are the hot, cold and the rainy seasons. The local hot seasons run in the months of January, February, March, August and September, and the cold seasons prevails in the months of June and July. The rainy seasons are represented by the months of April, May, October November and December. The data on all the deliveries of the nine groups that was collected over their entire life spans of between two and half years showed absence of significant seasonal influence.

Seasonal variations was however found to have similar sex ratio results as was found in the earlier dietary ionic examination with absence of gender influence in groups one and nine showing neither gender with the control water group ($p>0.61$) and group nine of the cocktail of all the combined elements ($p>0.0609$). The glucose, sodium, potassium and sodium + potassium supplementation groups influenced the sex ratios towards male progeny ($p<0.001$). Calcium, magnesium and calcium + magnesium supplementation influenced sex ratios towards the female progeny ($p<0.001$). It was only during the rainy season that was found to have more pups delivered significantly over the other seasons among the nine groups.

To understand seasonal effects on sex ratios one probably needs to understand the seasonal and temperature dynamics that are at play at various stages (Warner et al., 2005). Thermoregulation is an expensive venture that cannot be wished away and in particular in early stages of life after conception and even after delivery so as to keep the small ones warm (Brandon et al., 2013).

There are four suggested types of gender determination. The first and second are based on genetic constitution modulated by hormonal input that was described in the ***Fishers principle of evolution based on the X and Y gametes*** in 1930 where sexually reproducing species have their sex ratios inclined towards equal sex proportions of 1 : 1 proportions. This is due to natural gender selection by the accumulation of autosomal alleles that direct the parental reproduction efforts toward the rare sex (Cavalho et al., 1998).

The third type of gender determination is that described by ***Charnov – Bull model*** referred to as temperature dependant sex determination (TSD) which is also referred to as environmental sex determination that is dependent on the temperatures prevailing during certain critical embryonic development most commonly observed among the reptiles. This occurs during certain critical embryonic periods defined as thermosensitive periods during which sex differentiation of the gonads is determined which is usually during the middle third of their embryonic development (Allsop, 2006; Cagnacci et al., 2003; Crews, 1996; Warner et al., 2005).

The fourth gender determination was observed to be a clear maternal influence over the eventual gender of the offspring depending on her eventual investment interest on the progeny sometimes and largely influenced by the prevailing environmental circumstances as defined by ***Trivers-Willard hypothesis*** In 1973, a biologist Robert Trivers and a mathematician Dan Willard in Harvard university who expounded an evolutionary theory that mothers in better living conditions produce relatively more sons while those in poorer living conditions tend to produce relatively more daughters (Bradbury et al., 1998; Cameron et al.,2004; Elissa et al., 1999; Marsteller et al., 1987; Nuno et al., 2012; Westber et al.; 2001).

It appears that sex ratios can therefore be manipulated through certain behavioural patterns and characteristics, such as placing nesting in certain locations that are affected by temperature that ultimately affect the resulting gender of the resultant progeny through a process called ***Plasticity in phenology.*** The latter mechanism has been used by some species to buffer themselves from the impacts of climate change (Raymond et al., 2012; Samuli et al., 2009; Schwanz et al., 2008; Telemeco et al., 2013). In humans seasonal variations have been reported to influence sex ratios in various places like Germany and United states (Lerch, 1998; Slatis, 1953).

Conclusions

In this study, there was no enough evidence to suggest that seasons influenced sex ratios but there was evidence that suggested that seasons affected the litter size in particular during the rainy seasons with clear improved reproductive performance during these seasons. The gender differences that were observed among the different groups were chiefly attributed to the ionic effects of the dietary components. The seasonal effects did not override the dietary ionic gender influence**.**

6.12 The electrophysiological studies

Chemical ionic effects on the oocyte membrane electrical potential

This study aimed at examining the relationship between dietary ionic supplementation, oocyte membrane electrical potential and sex ratios and was carried out among the cat fish following the earlier dietary studies in the mice reported above. The rational was based on the fact that the oocyte membrane may have alternating membrane electrical charge that is influenced by dietary ionic effects (Stolkowski et al., 1982). These ions are said to exert allosteric modification or electrotactism that allows selective gamete attraction and subsequent gender outcome following fertilization (Rajan, 1999; Celik et al., 2003).

Using a double blind control study, 108 female cat fish were randomly divided into 9 groups each receiving different ionic formulations in their swimming solutions. Each group comprised of twelve female catfish. These were exposed to different respective chemical formulation in their swimming water and were followed up for a period of one week. Their ova were retrieved following ovulation induction by Ovaprim 0.4 ml/kg intramuscularly. The harvested oocytes from the respective groups were subjected to electrical fields and their subsequent behavioural patterns examined. This procedure was repeatedly done twelve times in each group and the findings recorded.

Oocytes retrieved from the two control groups had no significant oval polar attraction to either the negative or positive electrodes. Ova retrieved from the sodium, potassium and the combined sodium and potassium solutions had significant oocyte polar attraction towards the positive electrode. Oocytes retrieved from Calcium, magnesium and combined calcium magnesium solutions had significant affinity towards the negative electrode and no significant affinity towards the positive pole. Oocytes harvested from the solutions constituted with all the salts demonstrated dual attraction with some ova being attracted towards the positive pole and others towards the negative pole. There was however more attraction noted on the positive pole than the negative pole although this was not statistically significant.

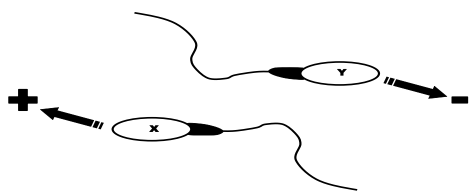
Solutions constituted with sodium and potassium elements appear to have skewed ionic influence over the oocyte membrane electrical charge towards the negative charge hence their attraction towards the positive pole. Their common electrical factor is their valency one (+). Practically if the oval electrical charge is influenced towards the negative then during fertilization it will selectively favour the positively charged gamete which is the Y-bearing sperm resulting to a male conceptus (Fugger et al., 1998).

Similarly, Solutions constituted with calcium and magnesium appears to have skewed ionic influence over the oocyte membrane electrical charge towards the positive charge influencing their attraction towards the negative pole. Their common electrical factor is their valency two (++). Practically if the oval electrical charge is influenced towards the positive charge then during fertilization it will selectively favour the negatively charged gamete which is the X-bearing sperm resulting to a female conceptus (Tosti et al., 2011).

The electrical control of cell physiology became apparent following the famous experiments of Galvani in the epic frog muscle studies in 1700 (Collin et al., 2005). Since then many more studies have demonstrated evidence that suggest that living cells both somatic and germ cells are electrogenic and that they do respond to electrical fields (Mikhai,l N. Z). Living cells are produced with specific set of functions that are executed through well-orchestrated set of events (Tosti et al., 2011). Some of these involve ionic movements inside, outside the cells and across the cell membranes that generate electrical potential that can be converted into mechanical energy for various cell functions including fertilization (Mcguiness et al., 1978; Valic et al., 2003).

Gametes too are electrogenic and function through the trans-membrane ionic transport and channel systems, that allow various ion currents to mediate various biologic and physiological events during cell growth, maturation, division and fertilization (Tosti, 2004).

From earlier studies, there is enough evidence that point to gametes having charges on their membranes which are useful presumably for fertilization and other functions as yet discovered (College, 2002; Tosti et al., 2011). With fixed charges on the Y or X bearing sperms then attraction of either sperm to the ova may be explained by alternating positive or negative charges of the oval membrane (Julian et al., 2010).



Studies have shown that there is clear communication between the sperms and the ova. This communication appears to be mediated by some factors that operate at different sites and distances. Some appear to operate at some distance, others at close range and the rest within the immediate environs or intra – gametal (Eisenbach,1999; Miller, 1985).

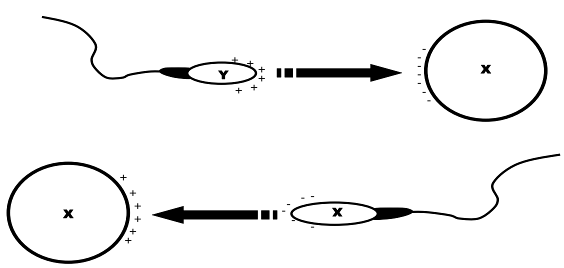
The distant communication factors are mediated through thermotaxis which control the sperms and directs them to a closer range of the ova where chemotaxis takes over and the guides them to the immediate environs of the ova (Anat et al., 2006; Dina et al., 1991; Fei Sun et al., 2005). Galvonotropism finally selectively influences which sperm finally fertilizes the ova (Hyman et al). This presumably appears to be the order of events along the journey of a sperm to its destination as natural things are in some kind of control and not left to chance (Hardy, 2002; Tosti et al., 2011; Wang et al., 1997).

There is clear evidence that the internal and external cell electrophysiological status are always in a dynamic status (Tosti et al., 2004). There is a difference in the distribution of the electrical

ions and subsequently charges inside and outside the cell which create electrical gradients across the membrane giving rise to transmembrane resting potential.
(Richard et al., 2009).

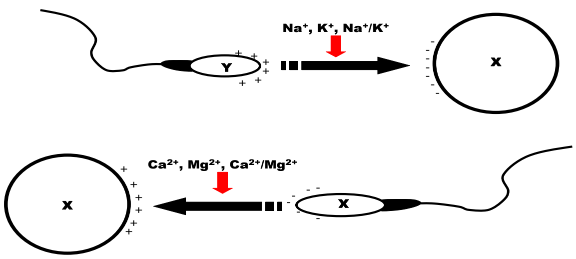
In this study, solutions constituted with sodium and potassium exhibited some skewed ionic influence over some of the oval membrane electrical charges towards the negative and was attracted to the positive pole. Their common electrical factor was valency one (+). Practically if the oval electrical charge is influenced towards the positive then the oval membrane or immediate environs must be bearing a negative charge and during fertilization it will selectively favour the positively charged gamete which is the Y bearing sperm resulting to a male conceptus.

Modulator sites of action



The strength or the affinity of the ova towards the electrode is relative and not total in that although all the ova were exposed to the respective chemical, not all the ova were attracted to the positive pole and this may explain the fact that in our dietary studies, whereas we fed the mice with the respective chemicals we only get partial and not total influence towards either gender. Likewise, it has been found that it is only a fraction sperms approximately 10% that undergo capacitation and ultimately become responsive to dynamic forces that propel them to their eventual fertilization sites (Anat Bahat et al., 2006).

Similarly, solutions constituted with calcium and magnesium showed some skewed ionic influence over the oval membrane electrical charge towards the positive charge and was attracted towards the negative pole. Their common electrical factor was their valency two (++). Practically if the oval electrical charge is influenced towards the negative charge then it must be bearing positive charge on its membrane or immediate environ and during fertilization it will selectively favour the negatively charged gamete which is the X bearing sperm resulting to a female conceptus (Edwards., 2000; Carvalho et al., 1998).



Conclusions

The electrical role in physiological functions shot to fame following the famous Galvani frog muscle studies in the 1700's (Collin et al., 2005). Many follow-up studies have confirmed that living cells are electrogenic and that they function through ionic movements across the cell membranes that create electrical potentials that can be useful in various functions including fertilization (Mikhail et al., 2001, Valic et al., 2003).

Studies have demonstrated that the X and Y sperms are produced in 50:50 proportions and there is no evidence that suggest this can be influenced. However nature appears to provide a soft valve in which proportions of its sex ratios can be influenced if need be without jeopardizing existence. The charges on the gametes offer a way out that nature can fine tune its sex ratios among other methods such as temperature or hormone dependant gender determination (Warner et al., 2005).

From these findings, it is clear that the social allegations that there is a relationship between dietary components and sex ratios are true (Seguy 1975, Rajan 1999, Papa et al., 1983). The components in this study were the chemicals under study that acted as the dietary modulators that ultimately influenced the electrical cellular gametal charges and subsequently the resulting progeny. The study further reaffirms that the dietary ionic effects on sex ratios can be used for prevention of -x- linked disorders (Seguy, 1975).

The mechanical attraction generated is proportional to the electrical membrane potential generated either intracellular or between the cells (Wong et al., 2010). The monovalents generate less electrical potential while the divalent cations generate more potential and this defines their different attraction capacities as explained previously. This explains the molecular basis upon which ions of single valency attracts the Y sperm leading to male conceptus and how cations of double valency attracts the X sperm leading to a female conceptus yet the modulators are both positively charged reaffirming the natural feminine supremacy by demonstrating that it is the ova and by extension the woman who determines the sex of the conceptus.

Although all the ova were exposed to the respective chemicals, not all were electrogenic to be attracted to the respective electrode but only a limited number. This co related well with the dietary studies that showed that despite feeding the mice with the respective chemicals, there was only partial gender influence towards the respective progeny (Anat Bahat et al., 2006).

With the above findings, it is evident that it is naturally not possible to achieve close to 100% results in sex ratio influence using natural means conforming to the Fishers principle which predicts evolution towards the 1:1 proportions of gender balance. This is a natural safeguard against extinction of a species by over production of a particular gender (Carvalho et al., 1998).

Chapter 7

7.1 Conclusions

General

The practice of gender selection captures a lot of interest and appears not destined to stop any time soon considering the fact that the process started from time immemorial and is still practised world over. Unlike many other global practices that are largely influenced by race, education culture and status, these practises stand above and beyond these and are practiced in varying degrees by all societies in various ways.

Dietary influence on sex ratio outcomes has been advocated through the ages but has not fully been proven. If proven this concept can be of great use in the prevention of X-linked diseases that manifest mainly in males while the females present as carriers. These diseases have remained a major medical challenge without definite cure despite the immense medical advances made over the years. To - date only preventive measures can offer hope to the affected couples.

***Through evolution*,** the methods have evolved in three phases from the presumptive traditional methods; to the natural predictive influencing practices to the modern day certain gender selection methods. The traditional and the natural practices of gender influencing are used on a daily basis by society out of reason or curiosity though the statistics are difficult to quantify. Studies have however provided us with comforting conclusions that these methods in particular the traditional and the naturally based methods do not have any significant sex ratio influence on society despite their widespread use.

Besides the bedroom practices on gender influencing matters, are cultural inputs that influence the latter. All regions of the world have their share of the story on gender influence be they traditional, natural or modern day practices. The traditional societies either rely on diets, practices or astrology. The societies that lack the latter rely on polygamy to complete the puzzle. What is universal and interesting is how the world shares the same platform in thought in justifying the need for gender balancing practices without going into details as this outsteps the confines and scope of our study.

Religion has had its share of contribution in practices of gender influence directly or directly. The birth of a son is often times given prominence in many religions thus influencing the thoughts, aspirations, wishes and practices of many. Some religions are explicit in their teaching such as the billing method that shares a sub topic of how to select the gender of the subsequent progeny.

The story of human creation has two proponents world over. Those who believe in God's creation and those who do not. Irrespective of one's religious standpoint, natural things were created with a clear purpose but unfortunately man was created with limited knowledge of these purposes. It is through bio - research that man tries to unravel the unknown purposes of these creations so as to expand his boundaries and limits of knowledge. Although information is useful, it can also be like a weapon that needs to be used with wisdom, caution and correctly for the benefit of society.

The religious stand points are fairly well defined. The western religions categorically condemn sex ratio influence while the eastern is fairly tolerant because of the polygamy factor that also makes Africa be silent about it.

Dietary chemical influence on gender

The dietary chemical influence on gender, which is under scrutiny in this study, falls among the natural influencing practices that are widely practiced but pose no danger to society. The overall results of this study attests to this global observation and confirms the Fishers principle of evolution that sexually reproducing species have their sex ratios around 1 : 1 proportions or are inclined towards equal sex proportions as long as the methods of gender influence are naturally oriented.

This observation is further confirmed by studies on natural gender influence that show low statistics on their abilities to influence gender outcome. What this study has contributed mainly is to distinquish between short term studies that may occasionally show reasonably high statistics of influence and the long run studies that tend to give low statistics in conformity to the fishers principal towards 1:1 ratios in the long run.

The purpose of nature in providing some ingredients in diets, various characteristics among living things, varying environmental dynamics that may influence gender ratios are presumably safety valves that are used to correct or fine tune the minor changes in the sex ratios that may lead to excess gender occurrences present in some areas. These methods are difficult to monitor, regulate or even have legislation on them. The world of comfort is that they pose no risk to human gender ratio dynamics worldwide because they have inherent self-modulating systems that cannot allow excesses of either gender.

The current modern day sex election methods belong to an entirely different platform and with an entirely different ball game all together as they are poised to different intents, purposes and destiny. They are majorly practiced for clear gender selection reasons, some for good medical reasons, but majorly for social reasons. They have no inherent self-regulation systems and tend to skew gender ratios as they have done it in some places where they have frequently been practised. There are therefore reasons to be concerned about them and legislation appears to be necessary.

The story of ***Glucose link to gender influence*** is not new starting with many kings' palaces where more sons were traditionally born due to plenteous sugar and delicious diets. This observation has been reported among the diabetics. Interestingly, although a lot has been discussed of the Trivers and Willard hypothesis that has been studied extensively and found true that mothers in better environs unlike those in wanting states have been found to produce relatively more sons than those in poorer living conditions who tend to produce relatively more daughters. It is possible that what is described as better living conditions in this hypothesis could as well be more glucose that defines the plenty and those in poorer conditions representing less glucose thus the latter simply defining the difference.

PCR /DNA test. The ***PCR /DNA*** test is increasingly becoming a common test tool as more and more people seek to reaffirm their parental status. In this study, the test was done to ascertain the gender status of the demised pups before physical gender determination could be done. Without sex ratio influence the prevailing natural sex ratio is 102:100 with more males being born than females to accommodate for the slightly higher male mortality rates. In humans, sex ratios are generally within the 1:1 ratio worldwide with a few exceptions of some few countries that have strong gender preferences

The causes of perinatal mortality both in humans and mice have well been studied and found higher among males except in humans in the countries with strong gender preference who report otherwise. Whereas in unfortunate circumstances infanticide has been reported in family balancing attempts, it remains unclear why it also happens among the mice.

Whereas the countries with low perinatal mortality report figures in the regions of 5 - 6/1000, the perinatal mortality rate of this study was 32/1000 which is comparable to the countries with high human perinatal mortality rates. Globally more male oriented perinatal deaths are reported except the countries with strong gender preference who report otherwise. In this study due to dietary ionic sex ratio influence, there was no preferential high male oriented perinatal mortality.

The animal model body weights trends

The animal model body weights trends were observed to be a challenge since the inception of the study due to the poor feeding habits following constitution of some of the solutions. These difficulties were by extension reflected in the weight trends among other effects. It was therefore found necessary to devise a method of monitoring the study and weight was singled out as an ideal objective tool for monitoring the progress of the study. During the pilot stages of the study, there was general poor feeding and loss of weight among the potassium, calcium and magnesium groups and their combinations but this was followed by steady weight gain in all the animal models following addition of glucose.

The weight parameter has been used to study characteristics in research in a wide range of characteristics including housing densities that has a lot of bearing on weight related parameters and numbers that by extension have a strong bearing on the bio-economics of animals.

The general universal steady weight gain among all the animal models reaffirmed general good feeding translating into good general wellbeing, intake of the study chemicals and validity of the results. The ultimate total weight gained correlated well with other research findings confirming good cage densities, telemetries and microenvironments.

Social inputs

Whereas gender adjustment for purposes of genetic diseases is no doubt a welcome debate, gender adjustments for social reasons is unwelcome and beyond the scope of this work. The early man had many children that led to population explosion and the need for contraception to curb the latter. As family sizes were reduced arose the need for gender balance in their families.

There are many cultures throughout the world that promote the son preference for various reasons. Culture is difficulty to beat in any part of the world as it is embedded within the whole matrix of the particular society in question.

The past global wars had their impact on sex ratios by decreasing the male numbers drastically. This was followed by compensatory increase of the male gender which has been maintained to date. Legislation in particular on the number of children has led many to the drawing board to design their families' gender wise. Political input has not been left as some communities have been urged to have many children in efforts to realizing political supremacy through the tyranny of numbers.

There are endless arguments for and against gender adjustment methods that fall in the realms of gender influence for social reasons that will not be considered as it is outside the scope of this work and will therefore not be discussed.

Birth order and sex ratio connection

The birth order and sex ratio connection has not fully been established as sex ratios at birth and subsequently the populations have been found to remain fairly constant in absence of manipulation. Related studies on other parameters such as age have also not shown any significant correlation without external influence.

Natural slight excesses of female and male births of non-significant proportions have been reported in various places but it was until the First World War that significant excess male births were observed. Despite remedial measures that were instituted to mitigate this, resulted into countries with either son or daughter preferences.

The traditional gender preference in particular the son inclination has remained despite modernization and has propelled many to seek methods to achieve this goal. In this study, absence of gender sex ratio influence in the control group confirmed the findings that without influence, sex ratios remain the same as seen in society. However, the study groups with chemicals did influence gender outcome and this mimicked society where there is gender influence.

The purpose of sex ratios influence is natural as there must be safety valves that fine tune the finer details of sex ratios in various places of the world. In this regard, where there may be some slight excess of certain gender then nature counters that as per Fishers principle by directing sex ratios towards 1:1 ratios.

Seasonal contributions

There are four modes of gender determination, first the genetical as was described by Fisher 1933 based on the x and y gametes and secondly the latter with hormonal modulation whereby testosterone input leads to male gender in the early stages of development. The third is temperature or environmental oriented as described by The Charnov – bull model. The fourth gender determination is based on maternal influence as expounded by Trivers-Willard hypothesis based on maternal investments in the future progeny. Majorly, gender determination follows the Fishers genetically based gender determination but the latter two come into play when fine tuning of minor gender excesses or maternal interests come into play.

Thermoregulation is vital in animals especially to the new-borns and no wonder that reproduction is low during the cold seasons both in the tropics and temperate climates as the cost of keeping warm is prohibitively high. As stated earlier, the temperature oriented gender influence has been reported but is probably for correcting minor gender imbalances that do not have major impact on the general or global gender balance.

In this study, there was no enough evidence to suggest that seasons significantly affect sex ratios but there was ample evidence that showed that seasons do appear to affect the litter size and in particular during the rainy seasons. In general more litter were delivered during the rainy season an observation that was recorded universally in all the nine groups. The gender variations among the nine groups mirrored that of the ionic effects of the dietary components and seasonal effects did not seem to override these.

Oocyte membrane electrical potential

The fish oocyte study was based on the rational that the oval membrane may have alternating membrane electrical charges that are influenced by dietary ionic effects. The influencing factors were postulated to act as modulators that exert allosteric modifications or electrotactism that allow selective gamete attraction. This concept was found true in this study as some oocytes were found to be electrogenic and responsive to electrical environs. Similar findings were found in other studies where only a limited number of sperms were found to be fertilizable.

There are various forces that ultimately get the gametes together starting with inherent contractions of the reproductive organs. Thermotaxis appear to be the distant influencing factor that direct the sperms to the gametes, while chemotaxis comes into play to guide them to the oval immediate environs and finally galvonotropism gets them together by playing the role of selectively accepting either the X or Y bearing sperm to fertilise the ova and presumably ultimately determine the gender. During this final fertilization process electrical forces may have a role to play even if in collaboration with other factors.

Since the initial studies of Galvani, many more studies have reaffirmed that living cells are electrogenic and that many cellular functions rely on the electrophysiological dynamics operating intracellular and extracellular. As these processes take place electrical potentials and charges are created that serve different functions within and without the cells. In this study electrical cell membrane surface charges are created and the difference between monovalent and divalent cations in their difference in polar attraction rests on the magnitude of influence on the cellular resting potential. The basic cellular surface charge is negative that is weak and not significant to upset intercellular interactions. With increased amounts of the monovalent ions in the solution, causes compensatory and similar increase of the same element intracellullar that leads to subsequent increase of the negative resting potential on the cellular membrane. This increase however is modest and its influence is limited to the cellular membrane and not beyond. This charge is therefore not significant enough to cause significant attraction to the surrounding proteins. The negative charge status leads to attraction to the positive pole. It is the same charge status that is attracted to the positively bearing male (Y) sperm leading to male conceptus.

Increased amounts of the divalent cations in the solution, causes compensatory and similar increase of the same element intracellular and leads to subsequent increase of the negative resting potential on the cellular membrane. This increase however is significant and its influence is not limited to the cell wall but goes beyond the cellular membrane. The charge generated is therefore significant enough to cause considerable attraction to the surrounding positively charged proteins. These positively charged proteins complexes on cellular membrane and the immediate environs create a positively charged status and leads to attraction to the negatively charged pole. In vivo, these positively charge status of the ovum attracts the negatively bearing female (X) sperm leading to female conceptus. Of interest is the fact that with good current among the calcium and magnesium combined solution, one can actually see some surrounding proteins moving towards the negative electrode.

In the above description, the strength of the electrical potential created by the chemicals under study on the cellular electrical potential correlates well with the valency of the chemical in question and defines the attraction that is created as described above.

In both circumstances the oocyte cellular surface membrane electrical potential is defined by the interplay between the extra cellular and intracellular ionic dynamics. With the presence of excess monovalents extracellular, this status makes the ions move into the cell making the cells more positive intracellular. In order to balance the cellular charges, a negative extracellular status is created. However with the divalent ions, as these cells move intracellular, they cause monovalent ions to move outwardly thus creating a positive extracellular status.

7.2 Summary of the new contributions of this study

Gender determination

Gender determination is majorly about interplay between genetics, hormones, environmental influence, physiological and maternal inputs. The paternal inputs appear to be modest and the ova able to be influenced in favour of the maternal wishes.

Natural sex ratio adjustment methods

The natural sex ratio adjustment methods that include dietary influence are practiced at the comfort of many peoples' homes and are difficulty to quantify or have legislation on. However this study shows that their long term effects conforms to the Fishers principle of evolution towards 1 : 1 sex ratios and may not have significant social gender skewing on a long term basis.

Electrical membrane charges

The placement of the electrical charges on the gametal cellular surface demonstrates that it is most probable that electrical charges are involved in fertilization and if the latter is true then the ova is likely to be responsible for determining the gender of the conceptus unlike earlier beliefs that man is the sole determinant of the gender of his progeny.

The study clearly explains the molecular basis upon which ions of single valency attracts the Y sperm leading to male conceptus and how cations of double valency attracts the X sperm leading to a female conceptus yet these are both positively charged. In this regard the study further demonstrates the natural feminine supremacy by demonstrating that it is the ova and by extension the woman who determines the sex of the conceptus.

Social cultural inputs

The social cultural inputs are many and diverse. However, the multiple cultural influences is in disarray with varied concepts on the way the world views women, men and gender balance. There is therefore urgent need of global teaching on how to harmonise the way we view, value and treat the different gender and gender balance so as to bring the world to the same level of thinking. It is unfortunate to blame society on matters that no attempts have been deliberated upon and direction of approach given.

The religious contribution

The religious contribution appears to be in clear disharmony on matters of gender ratios with some supporting and others in disagreement. A common religious standpoint needs to be sought so that an all-inclusive front can be set as a benchmark for the desired practices.

The economic influence

The economic influence appears to favour the stable economies with bankable promises of taking care of the aged as this assurance takes away the great fear among the aged of being left alone and hence the need for sons to take care of them. The strong son preference is reflected among societies that base their after retirement on their children to take care of them. In general the after retirement policies set in a particular country has a lot of bearing on the gender preference of the individual families. It is needless to say that the more affluent and better placed economically families or societies become do they also become less reliant on their children's support in their later years.

Politics

Politics affect every aspect of our lives including family gender compositions. Most political decisions, dispensation and goodwill are reflected on their citizenry. Wrong political decisions do upset the country in question and worse political status often times boil down to wars that greatly upset gender balances with more men dying in the war zones.

Legislation

Legislation mirrors the political will everywhere world over and drives a lot of policies that ultimately affect the lives of the citizens affected. The policy that limits the number of children per family by extension lays good grounds for methods of gender influencing to come into play.

It is apparent that there are many religious, social cultural, economic, political and legislative matters that do affect sex ratio adjustment practices. By addressing many of these challenges, it is possible to stem down the need for the social sex selection methods that appear to be detrimental to social gender balance.

7.3 Recommendations

In the past, we have watched by the side as various members of society engage in various public debates in both audio visual and print media on diverse medical and other issues touching our lives in various aspects that have unfortunately ended up shelved in archives due to lack of conclusive evidence or failure of the scientists to come to our rescue. We probably need to declare that time of silence as over and at hand now is a season to roll up sleeves and offer some answers to these social claims and allegations. Many attempts that have been made towards this direction have been reviews on the subject that have tended to revolve around the very same short studies that have been done in the past many of which are difficult to draw sound judgments. Other reviewers have dwelt mainly on the very same ethical issues that have been discussed and long exhausted. Unfortunately, all these appear to be like trying to reinvent the very same wheel instead of advancing it.

Time is now rife and prudent to mainstream in our studies some of the most important social allegations, myths and practices that truly affect all sectors of our lives so as to advance these subjects to better understanding and if necessary be put in good practical uses. This would save society from misguidance, mal–practice and even exploitation by some un scrupulous members of the same society. Unfortunately there are times when we blame the society of various practices yet it is our failure as scientists to guide them appropriately through scientific verifications of the alleged social claims. It is also probably failure on our part as all subjects need to be studied for in depth knowledge and value addition including inviting social, cultural, ethical, moral and legal inputs to the scientific findings for purposes of practical application of the results. This is an all involvement of the stake holders approach that brings everybody on the table and brings forth a well-blended scientific work that embraces all aspects of our lives be it moral, legal, cultural or ethical for better application or use.

Shelving, or shunning of some important subjects that are in the public domain or debate forums without going steps further by the professionals to verify them is surely a wanting approach that needs to be revised and different practical approaches developed and applied.

To lay the many social allegations to rest therefore, studies need to be carried out on various aspects of our lives so as to base allegations on facts, statistics and real time events. There are many times that scientists and members of society engage each other in unnecessary long and protracted arguments over allegations and issues rather than getting down to research upon which to base arguments on.

Research on sensitive matters such as this need to be done not necessarily in support of gender ratio influence, but for knowledge purposes so that the society is not misled by scrupulous people who may take advantage of the many grey areas of knowledge.

Usually changes in any practice or customs in the highly emotive areas have followed fairly established pattern of initial horrified negation then diminished horror of curiosity followed by interrogation, examination, in depth studies and finally steady acceptance with gradual practice as it has been observed by many including dickens and others.

7.4 Future research

Various arms of research need to be allowed to continue, be it on religious matters, scientific subjects, social debates, sensitive bio matters or even on weapons of mass destruction for knowledge purposes. The research findings need to be assessed and categorised among those that can be discarded, those that can be put in good use and those to await future further research or use.

Value addition in all aspects of our lives is a major pre-occupation in this day and era and days to come. We strive to achieve this in areas of business, industry, music and this should be extended to our academic premises. This concept need to be applied as well in our publications, reviews and research so as to avoid the very same old views, ideas and facts being presented only in different wordings and places.

Future lies in listening and managing expectations of the silent majority in any group of people as echoed in many labour wards following a delivery where reactions of jubilations or disappointments from relatives and friends are always overheard expressing that it is either a baby girl !!!!! or a baby boy!!!!!. These reactions are governed by factors such as welcoming the new baby, being happy that the baby is born safely, having achieved the desired or the aspired gender or having achieved many other issues that come with a new baby like being named after someone within the family.

Among the future challenges that will face us will be as we support those pursuing various human rights of different categories such as gays, we should not forget, ignore or silence those who may be seeking their natural justice from gender imbalance in their families. They too have natural and individual rights to gender balance.

It is always challenging to draw conclusions on matters that do not quite affect us and often times unfortunate that sometimes those who champion certain causes are not victims of the same. It is perhaps prudent and timely that the opinions of the affected be sought analysed and deliberated upon for justice to prevail. Democracy is the voice of the majority which may at times translate into simply tyranny of numbers but may not be necessarily the right things although that is why they have their way. However, the minority still must have their rights not only heard but addressed and not ignored.

I salute in advance those that will endeavour to venture in this line of study for its path and journey is not easy to navigate and wish them the best of luck.

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