Time-Lapse Analysis and Morphokinetic Evaluation of Fresh vs. Vitrified/Warmed Oocytes, Including Donor and Explorative Sibling Oocyte Cycles

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Study leaders

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DECLARATION

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SUMMARY

BACKGROUND: Infertility is defined as a disorder of the reproductive system whereby there is failure to achieve a clinical pregnancy after 12 months or more of regular unprotected sexual intercourse. The primary objective of Assisted Reproductive Technologies (ART) is to implement fertilization in instances where corrective therapy for male or female patients cannot yield fertilization. During the past three decades infertility has become more prevalent. In addition to this, the commercialized world has experienced a trend of women conceiving their first-born within their later reproductive years. This trend of delaying motherhood has thus led to the common use of oocyte vitrification protocols, which have become increasingly robust over the years. The validation of the oocyte vitrification protocol essentially came from the comparison of fresh versus vitrified/warmed oocytes and how they succeeded in *in vitro* fertilization (IVF)/intracytoplasmic sperm injection (ICSI) outcomes. It was reported that there were no differences in fertilization rates, implantation rate and pregnancy rates when comparing fresh vs. vitrified/warmed oocytes. Furthermore, there is a trend towards implementing morphokinetic analyses to examine the comparisons between fresh and vitrified/frozen oocytes. With the rapid progression in technology within the ART field of medicine, time lapse systems (TLS) presents an extremely unique and promising tool for improving embryo selection. Improvement of embryo selection will only advocate for the production of clinic-specific embryo kinetic models for prediction of success. The more models of embryo selection we create, the more we may understand whether an optimal morphokinetic profile exists.

AIMS: Primary aim: To investigate the comparison with fresh and vitrified/warmed oocytes, using TLS imaging, as well as creating a normative range to reference the classification of future embryo developments.
Secondary aim: To investigate the embryo development time lapse (TL) time points of sibling oocytes of patients having both fresh and vitrified oocytes used for treatment in the same insemination cycle.

MATERIALS AND METHODS: Retrospective study conducted from 2013 to 2017 at Wijnland Fertility Clinic on de-identified, aggregated TL patient oocyte and embryo development data. Data was filtered according to exclusion and inclusion criteria. Statistical analysis rendered descriptive statistics, quantile (median) regression tests, TOST tests, and matched design linear regression model tests.

RESULTS: Results indicated an overall delay in time points and durations between time-points for the vitrified/warmed oocyte population, when compared to their fresh counterparts. Using the quantile (median) regression model, it was found that almost all vitrified/warmed timings were slower than their fresh counterparts ($p<0.05$), whereby t5 ($p=0.068$; 95% CI) and t9 ($p=0.106$; 95% CI) were not. Using the TOST method, it was found that at the 5% level of equivalence, no time points showed equivalence ($p<0.05$; 90% CI; 5%). It was found at the 10% level that there was significant non-equivalence for time points tPB2, tPNa, t2, t4, t6, t8, tSC, tSB, tB and tHB ($p<0.05$; 90%CI; 10%). This indicated that for the times stated for non-equivalence there was a delay in timings within the vitrified/warmed oocyte population. Conversely, also at the 10% level, it was found that there was significant equivalence for time points tPNf, t3, t5, t7, t9+ and tEB ($p<0.05$; 90%CI; 10%), This indicated that for the time points stated there was no statistically significant difference in timings with regards to the fresh

and vitrified/warmed oocyte population. Lastly, for the sibling oocyte study, there were no consistent patterns found. This was due to the small population size (n=57).

CONCLUSION: In conclusion, this study showed that there was a statistically significant overall delay within the timings for vitrified/warmed oocytes when compared to their fresh counterparts. The most statistically significant findings within this study include the delayed vitrified/warmed oocyte time points for tPNa, t2, t4, t8, tSC, tSB and tHB ($p<0.05$). The most significant clinical finding of this study was the assumption that vitrified/warmed oocytes undergo mitochondrial stress post warming and requires 2-3 hours of culture in order to reboot the cellular machinery to full operating potential. As a result of this assumption it was suggested that vitrified/warmed oocytes exhibit a 1-hour insemination delay in order to give opportunity for mitochondrial recovery post warming. Another crucial finding was that there was a total delay in the vitrified/warmed oocyte population of 8,53 hours, which could lead to the assumption that even though there was a statistically significant lag exhibited within the vitrified/warmed oocyte population, this is most probably not of clinical significance.

OPSOMMING

AGTERGROND: Infertiliteit word gedefinieer as ‘n afwyking van die voortplantingsstelsel, waar daar versuim word om ‘n kliniese swangerskap te behaal na ‘n periode van 12 maande of langer met gereelde onbeskermde seksuele omgang. Die primêre doelwit van Geassisteerde Reproduktiewe Tegnologie (ART) is om bevrugting te bewerkstellig in gevalle waar natuurlike bevrugting onsuksesvol is. In die afgelope drie dekades het die voorkoms van infertiliteit wêreldwyd betekenisvol toegeneem. Studies, in eerste-wêreld lande, toon dat al hoe meer vrouens uitstel om ‘n familie te begin tot later in hul voorplantingsjare. Hierdie tendens, in terme van vertraging van moederskap, het dus gelei tot die algemene gebruik van oösiet preserveringstegnieke. Die sukses en waarde van oösiet preserveringstegnieke en -metodes is bevestig deur die uitkoms van in vitro bevrugting/intrasitoplasmatiese sperm inspuiting [IVB/ICSI] sukses tussen vars oösiet en gevriesde/ontdooide oösiet siklusse te vergelyk. Hierdie studies toon dat daar geen verskille in die bevrugtings-, implanterings- en swangerskapsyfer is, wanneer vars met gevriese/ontdooide oösiete vergelyk word nie. Daar is huidiglik ook ‘n neiging om die implementering van morfokinetieseanalise te gebruik om die vergelyking van vars en gevriese/ontdooide oösiete te ondersoek. Die toename in tegnologiese verwikkelinge binne die mediese ART veld, dui “time lapse systems” (TLS) aan as ‘n unieke en belowende hulpmiddel vir die verbetering van embrioseleksie. Die beskikbare TLS morfokinetiese data kan lei tot beter embrioseleksie. Kliniek spesifieke TLS morfokinetiese modelle kan moontlik gebruik word vir beter voorspelling van ART sukses. Die ontwikkeling van verskeie verskillende TLS modelle van embrio seleksie, sal toenemend beter insig gee in terme van ‘n optimale morfokinetiese profiel.

DOELWITTE: Primêre doelwit: Om die verskil tussen vars en gevriesde/ontdooide oösiet ontwikkeling te ondersoek deur gebruik te maak van TLS morfokinetiese beelde; en ook om verwysingsdata wat normale waardes identifiseer as verwysing en klasifikasie vir toeomstige embriostudies uit te wys.
Sekondêre doelwit: Om TL morfokinetiese tydpunte van geneties verwante oösiete van pasiënte wat beide vars en gevriesde/ontdooide oösiete gebruik het vir behandeling in dieselfde inseminasie siklus, te ondersoek .

MATERIALE EN METODES: Retrospektiewe studie op anonieme, saamgevoegde TL pasiënt oösiete en embrio ontwikkelingsdata vanaf 2013 tot 2017 by Wijnland Fertiliteitskliniek. Die data is gefiltreer volgens die uitsluitings- en insluitingskriteria voor statistiese analise. Statistiese analise het beskrywende statistiek, kwantielverhouding (mediaan) toetse, TOST toetse, asook ooreenstemmende ontwerp lineêre regressie model toetse ingesluit.

RESULTATE: Die resultate van die studie het ‘n algemene vertraging in tydpunte en tydsverloop tussen verskeie tydsperiodes vir die gevriesde/ontdooide oösiet populasie in vergelyking met die vars oösiet populasie aangedui.

Die statistiese kwantielverhouding (mediaan) regressie model het bevind dat amper alle gevriesde/ontdooide oösiet tydpunte stadiger was as die vars oösiete tydpunte ($p<0.05$), uitsluitend t5 ($p=0.068$; 95% CI) en t9 ($p=0.106$; 95% CI). Die TOST metode het bevind dat by ‘n 5% vlak van ekwivalensie, geen tydpunt ekwivalent ($p<0.05$; 90% CI; 5%) was nie. Daar was egter bevind dat by die 10% vlak ekwivalensie, daar beduidende nie-ekwivalensie was vir die volgende tydpunte: tPB2, tPNa, t2, t4, t6, t8, tSC, tSB, tB en tHB ($p<0.05$; 90% CI;

10%). In gevalle van nie-ekwivalensie was daar dus ‘n vertraging in die tydpunte van die gevriesde/ontdooide oösiet populasie. Daar was egter ook beduidende ekwivalensie by die 10% vlak vir sekere tydpunte: tPNf, t3, t5, t7, t9+ en tEB ($p<0.05$; 90% CI; 10%) wat aandui dat vir hierdie tydpunte daar geen beduidende verskil was tussen vars en gevriesde/ontdooide oösiet populasies nie. Ten slotte, vir die geneties verwante oösiet pasïent groep was daar geen betroubare uitkomste nie omdat die groep te klein was vir betroubare statistiese ontleding (n=57).

GEVOLGTREKKING: Die navorsing dui daarop daar ‘n algemene, statistiese beduidende vertraging van die morfokinetiese TL tydpunte vir gevriesde/ontdooide oösiete is wanneer dit vergelyk word met vars oösiet tydpunte. Veral beduidend was die vertraging van gevriesde/ontdooide tydpunte; tPNa, t2, t4, t8, tSC, tSB en tHB ($p<0.05$). Van kliniese waarde is die moontlikheid dat die vertraging in tydpunte van gevriesde/ontdooide oösiete daarop dui dat hierdie oösiete mitokondriale spanning na ontdooing ondervind en dus 2-3 uur langer in kultuur gehou moet word om sellulêre meganismes tot hul volle potensiaal te aktiveer en te laat herstel. As gevolg van dié aanname, word daar voorgestel dat gevriesde/ontdooide oösiete ‘n 1-uur inseminasie vertraging vergun moet word; om die geleentheid te bied vir mitochondriale herstelling na ontdooing.

Die bevinding dat daar ‘n algehele vertraging van 8,53 uur in embrioontwikkeling was in die gevriesde/ontdooide oösiet populasies was statisties beduidend , maar heel moontlik nie van kliniese belang nie.

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LIST OF ABBREVIATIONS

Abbreviation	Meaning
1PN	Presence of one pronucleus
2PN	Presence of two pronuclei
3PN	Presence of three pronuclei
AI	Artificial insemination
AMA	Advanced maternal age
ART	Assisted reproductive technologies
CI	Confidence interval
CLBR	Cumulative live birth rate
CO_2	Carbon dioxide
COC	Cumulus oocyte complex
CPA	Cryoprotective agents
CPR	Clinical pregnancy rate
DC	Direct cleavage
DCM	Distorted cytoplasmic movements
DMSO	Dimethyl sulfide oxide
DNA	Deoxyribonucleic acid
DUC	Direct uneven cleavage
ECC1	Duration of first cell cycle
ECC2	Duration of second embryo cell cycle
ECC3	Duration of third embryo cell cycle
ET	Embryo transfer
FET	Frozen embryo transfer
HA	Hyaluronic acid
HREC	Health Research Ethics Council
ICSI	Intracytoplasmic sperm injection
IMSI	Intracytoplasmic morphologically selected sperm injection
IR	Implantation rates
IUI	Intra-uterine sperm injection
IVF	*In vitro* fertilization
KID-	Known non-implantation data
KID+	Known implantation data
LBR	Live birth rate
LN_2	Liquid nitrogen
MINC	Brand-named microprocessor controlled, gassed, and humidified incubator
N_2	Nitrogen
NaCl	Sodium Chloride
NS	Not significant
O_2	Oxygen
OHSS	Ovarian hyperstimulation syndrome
PGT-a	Preimplantation genetic testing for aneuploidy
PGT-m	Preimplantation genetic testing
PGT-sr	Preimplantation genetic testing
pH_e	External pH
pH_i	Internal pH
PICSI	Physiological intracytoplasmic sperm injection

PN	Pronuclei
PROH	Propanediol
RC	Reverse cleavage
RCT	Randomized control trial
RICSI	Rescue intracytoplasmic sperm injection
s2	Synchronization of cell divisions
s3	Synchronization of cleavage pattern
SF	Slow-freezing
SOP	Standard operating procedure
SR	Survival rate
t0 – 9+	Time points from one cell to nine plus cells
tB	Time to blastulation
tHB	Time to blastocyst hatching
TL	Time-lapse
TLS	Time-lapse system
tM	Time to morula
tPB2	Time of second polar body appearance/extrusion
tPNa	Time to polar nuclei appearance
tPNf	Time to polar nuclei fading
tSB	Time to start of blastulation
tSC	Time to start of compaction
VP	PN Duration
WHO	World Health Organization
ZP	Zona pellucida

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CHAPTER 1

BACKGROUND

1.1 Overview of Assisted Reproductive Technologies (ART)

Infertility is defined as a disorder of the reproductive system whereby there is failure to achieve a clinical pregnancy after 12 months or more of regular unprotected sexual intercourse (WHO, 2010). Furthermore, infertility is categorized into primary and secondary infertility; primary being when a woman is unable to conceive and has never been able to ever bear a child, either due to the failure to become pregnant or the failure to carry a pregnancy to a live birth. Secondary being when a woman is unable to conceive, either due to the failure to become pregnant or the failure to carry a pregnancy to a live birth following either a previous pregnancy or a previous ability to carry a pregnancy to a live birth (WHO, 2010).

The primary objective of Assisted Reproductive Technologies (ART) is to implement fertilization in instances where corrective therapy for male or female patients cannot yield fertilization; this occurs by bringing the spermatozoa closer to the ova using advance technology and equipment via treatment options such as artificial insemination (AI), *in vitro* fertilization (IVF), intracytoplasmic sperm injection (ICSI) and physiological intracytoplasmic sperm injection (PICSI), to name the most commonly used treatments (Jones & Lopez, 2006).

With regards to the different treatments available in ART, the vital process that differentiates each treatment option is the method of insemination. AI involves the injection of processed spermatozoa (<0.5ml) via a catheter into the uterus of the female (Do Amaral et al., 2001). This process aims to bypass the cervical mucus, which may pose as a major stressor to spermatozoa during natural conception.

IVF is commonly suggested for patients who exhibit a good/normal male diagnosis. IVF involves insemination of oocytes via the addition of processed spermatozoa to oocyte cumulus complexes. This method of insemination allows spermatozoa to penetrate and fertilize the oocyte in a more natural selecting *in vitro* fashion, which resembles *in vivo* circumstances as close as possible.

ICSI involves the process of injecting a singular immobilized spermatozoon into the cytoplasm of a single ovum (*in vitro*) via micromanipulation. This treatment is usually indicated for patients with poorer spermatozoa samples, poor fertilization via IVF and repeated IVF failure. Several studies have shown that ICSI yields a more superior fertilization rate, while not negatively affecting the development of the subsequent embryo(s) (Yoeli et al., 2008; Johnson et al., 2013). Due to ICSI presenting with less total or near-total fertilization failure than IVF, it has led to the popular use over its counterparts.

Since the establishment of ICSI, there have subsequently been two sub-methods with the main goal being to enhance and improve outcomes of the original ICSI method. These methods include PICSI (as mentioned above) and intra cytoplasmic morphologically selected sperm injection (IMSI). The chief concepts for the basis of these alternative ICSI methods are based on specialized sperm selection. ICSI primarily uses sperm morphology, while enhanced morphology viewing and sperm maturity are the main selection tools used for IMSI and PICSI, respectively. The ICSI alternatives are also used for patients with poor ICSI outcomes, such as fertilization failure, chromosomal irregularities and failed or poor blastocyst formation (Mokánszki et al., 2014; Luna et al., 2015; Erberelli et al., 2017).

PICSI is based on indirectly selecting mature sperm. This concept is done via the use of hyaluronic acid (HA), which sperm with lower DNA fragmentation is more likely to bind to. Spermatozoa with less DNA fragmentation are said to be more likely mature, compared to immature spermatozoa which exhibit higher levels of DNA fragmentation (Beck-Fruchter et al., 2016). IMSI is based on enhancing the view of spermatozoa via high magnification (>6000 times) in order to observe morphological defects that would not have been observed on the ICSI magnification level. However, there is controversial literature around the effectiveness of PICSI (Parmegiani et al., 2012; Majumdar and Majumdar, 2013; Beck-Fruchter et al., 2016) and IMSI (Tanaka et al., 2012; Delaroche et al., 2013; Boitrelle et al., 2014; Gatimel et al., 2016).

Infertility may be the result of one or many factors, both from the male, female, both male and female, and unknown reasons (Jones and Lopez, 2006). On the female's behalf, the cause ranges from failure to ovulate, tubal blockage, advanced maternal age, gonadotropin deficiency, endometriosis, and excessive exercising or excessive malnutrition in the case of anorexic patients (Sherwood and Ward, 2013).

Furthermore, male factors that contribute to infertility may range from previous trauma to the testes, low sperm count, poor sperm transport, spinal cord injury (Kafetsoulis et al., 2006) to environmental factors such as smoking or carcinogenic factors such as radiation (Jones and Lopez, 2006; Sherwood and Ward, 2013). The combination of male and female infertility factors may range from idiopathic to multifactorial; often not clearly defined or known.

In the last decade, infertility has become increasingly prevalent. In relation to this increase in prevalence, parenthood is unquestionably one of the most globally anticipated ambitions in adulthood (Boivin et al., 2007). However, not all couples who desire a pregnancy will achieve one spontaneously. A failure to conceive, then, is often taken on by individuals or couples as a major life stressor, which can inflict havoc on otherwise well-adjusted couples and/or individuals. Based on a study conducted in 2007 based on the world population of 6,6 billion (Prb.org, 2019), 72.4 million people were identified as infertile and of those, 40.5 million were seeking infertility medical care (Boivin et al., 2007). In addition to this, a subsequent study matched with a similar infertility prevalence of up to 20% of all couples that are in their reproductive years (Kruger et al., 2016), with this number increasing per decade.

1.1.1 Embryo environment

With regards to ART, it is commonly known that human gametes are highly sensitive to the culture environment and its variations, thus it is very important to have reliable culture media; and even more vital to have a reliable incubator (Swain, 2010; Swain, 2011). As with many notions in the field of ART, the goal is to imitate the physiological or *in vivo* conditions in order to achieve optimum embryo development. Optimization and selection of the most efficient incubator for the laboratory is essential to the development of embryos *in vitro* as well as for clinical outcomes of the ART clinic.

It is well accepted that the improvement of the quality of gametes and developing embryos is directed by the management of stress inflicted within the IVF laboratory (Swain, 2010). It has been established that these potential stressors may include an assortment of environmental parameters that can be controlled in the laboratory. Such stress may be attributed to unsuitable media energy substrate composition, gas composition, temperature, osmolality and/or pH fluctuations.

Reference values for each environmental component exist and are conscientiously monitored along with the preferred medium and incubator in order to achieve a superior culture environment (Swain, 2011). The optimal values for temperature, oxygen and pH are 37.0°C, 5% and 7.20 to 7.35 respectively (Quinn, 2014; WHO, 2010). Notably, additional literature to the World Health Organization (WHO) manual have suggested embryo temperatures should remain safely below 37.0°C at 36.7°C (Higdon et al., 2008). The internal pH (pH_i) of embryo is predominantly responsible for the maintenance of intracellular homeostasis (Will and Swain, 2012). pH_i is responsible for the regulation of several cellular processes including enzymatic activity, cell division, differentiation, membrane transport, protein synthesis, cell communication, cytoskeleton elements and microtubule dynamics (Swain, 2011; Quinn, 2014). The optimization of carbon dioxide (CO_2) within the embryo's microenvironment is also essential. The gas phase of CO_2 is used to control the pH; this is achieved by controlling the pressure of CO_2. CO_2 is affected by the atmospheric pressure (i.e. the level above sea level) and thus a definite value for CO_2 are not recommended because different laboratories at different above sea levels will need varying CO_2 concentrations to obtain their desired pH. Carbon dioxide dissolves in the culture media which results in concentrations of carbonic acid. This compound is what is responsible for the changes in pH. Therefore, if the pressure of CO_2 is decreased, an increase in pH is observed, and this is the manner in which the pH is achieved via CO_2 pressure manipulation (WHO, 2010).

1.1.2 Culture media

Notably, with regards to embryo culture media, there are two competing notions that have been widely implemented by commercial media brands. These include sequential- and one-step mediums. Both mediums aim to culture embryos to blastocyst stage (5 to 7 days of culture) (Salvaing et al., 2016). Sequential media aims to culture embryos until day 3 of development in one medium. The rationale behind this theory is that the cleaving embryo (day 1 to 3) requires different concentrations of components when compared to what that same embryos

need during the blastulation phase of development (day 3 to day5/6/7). Thus, the 'cleavage' medium is changed on day 3 of embryo development and replaced with the 'blastocyst' culture medium for culture until full blastocyst stage (Morbeck et al., 2014).

One-step media, or otherwise known as 'monoculture media', was designed with the concept of 'letting the embryo choose'. This concept operates by culturing the embryo in the same media for its full development from cleavage- to blastocyst stage. The implementation of this media is based on the rationale that all the possible 'nutrients' that an embryo needs for successful *in vitro* development is present; the embryo then chooses what it needs at what time it needs it (Morbeck et al., 2017). One-step media is also considered the most convenient method for embryo culture when using a time-lapse incubator, which necessitated the development of monoculture systems (Basile et al., 2013).

It can be said that human embryos can develop *in vitro* in rather different types of media from basic systems to sequential complex culture media. There are various commercially available culture media today, making this market highly competitive placing the responsibility in choosing the 'best' culture media in the hands of the embryologist. It is furthermore important to remember that commercial culture media is almost always constant, therefore special care must be administered by embryologist to maintain the external confounding factors that exist in the laboratory, in order to keep the environment beneficial for embryos to develop healthily and ultimately result in healthy pregnancies.

1.2 Cryopreservation

1.2.1 History

Reproductive biology has made use of the freezing of human gametes for several decades. The first successful freezing method was in fact discovered by accident, by C. Polge, A.U. Smith, and A.S. Parkes in 1948 (Pegg, 2002; Clarke, 2004). The discovery that glycerol can protect cells from freezing damage initiated a period of rapid development in the techniques we now know as 'cryopreservation'. Compounds that aid in preventing the damaging effects of freezing, such as glycerol, have since been defined as 'cryoprotectants' or cyroprotective agents (CPA) (Gook, 2011).

Trailing that early (accidental) discovery, almost all the subsequent developments of the classical freezing methods have relied upon the addition of a cryoprotective compound until shown experimentally to affect survival. During the development of these freezing methods, various observations were found to be essential to survival. These include the nature and concentration of the CPA and the temperature at which it is added, the rates of cooling and warming, the storage temperature, and the temperature and rate at which the CPA is removed (Pegg, 2002; Gook 2011). Optimizing these factors subsequently resulted in the success of freezing spermatozoa, and other relatively basic cell structures such as various endocrine cells and strains of tissue culture cells (Pegg, 2002). The practical successes stimulated an even further drive to improve the then novel freezing protocol. Fundamental research that was done in the 1960s disclosed a number of the key concepts that are involved: the central importance of the total quantity of ice that is formed, the position of the ice crystals relative to the cells,

the toxicity of CPAs and the temperature dependence of that toxicity, and the magnitude of osmotically induced changes in volume.

In summary, the primary concepts of cryobiology which yielded the most superior survival rates included: CPA to toxicity ratio, rates of freezing and warming, ice crystal formation, rate of CPA addition and removal. Slow freezing was the initial established freezing protocol, which was then enhanced to the superior method of vitrification, which is commonly used today. Both well-established protocols were developed on the premise of the key principles of cryopreservation, as mentioned.

Glycerol has been the most common CPA used to freeze spermatozoa within the early freezing protocols along with propylene glycol and ethylene glycol, which were primarily used for variant species slow-freezing (SF). Ethylene glycol and dimethyl sulfoxide (DMSO), along with sucrose are more commonly used during vitrification protocols today, however DMSO along with propanediol (PROH) was also commonly used during the initial SF protocols (Gook, 2011). Notably, recent vitrification protocols consist of varied equilibration times for oocytes and blastocysts to allow for different CPA infiltration rates for the varied cell structures, instead of experimenting with various concentrations of different CPAs, as done in the past.

1.2.2 Damage of ice crystal formation

During the freezing of cellular structures, it was found that the formation of ice crystals was detrimental to the survival of the cell as observed in the poor success rates upon rapid warming post SF. This concept was subsequently researched, and it was found that the ice formed from freezing has a very low ability to dissolve solutes. The undissolved solutes thus concentrate in the diminishing volume of unfrozen liquid (Pegg, 2002). This concept clarified why freezing of cells caused an increase in concentration salt/sodium chloride (NaCl). During the early developments of cryopreservation, it was not yet clear whether ice crystal formation or the concentration of salt as a result thereof, was the main stressor to the cell damage during freezing. It was then established that ice crystal formation was the primary obstacle to overcome, however the ‘salt-damage’ was not disregarded as being troublesome to the cell survival (Pegg, 2002). Thus, the introduction of CPAs (permeable and non-permeable) were developed to aid in decreasing the temperature at which ice crystal formation occurred, as well as decreasing the salt concentration within the dehydrated cell (Figure 1) (permeable CPAs specifically) (Pegg, 2002; Gook, 2011; Gosden, 2011).

1.2.3 The toxicity of cryoprotectants

CPAs, as most compounds, are toxic when used in excess. However, when compared to compounds such as NaCl which is abundant within a cell being frozen without a CPA, the NaCl is more toxic than the CPAs in the same concentration (Pegg, 2002). It is known that CPAs are toxic for cells, however they have the advantages of reducing the concentration of salt as well as decreasing the temperature at which ice crystal formation occurs. Therefore, a delicate relationship exists between the correct concentration of CPA needed to aid successful cryopreservation and the concentration at which the CPA itself becomes toxic to the cell. Different types of CPAs

also have different ways in which it permeates the cell (Figure 1); DMSO being one of the most effective (Medicine, 2012). The size of the cell also influences the rate at which the CPA permeates and dehydrates the cell, as well as the method of diffusion (Figure1A,C) (Medicine, 2012).

Essentially, two consequences of CPA toxicity exist: the highest concentration that the cell will tolerate prior to cryopreservation is restricted, and, during freezing, the concentration will rise as ice crystal formation takes place. In vitrification, as opposed to freezing, a much higher initial concentration is necessary, but no additional concentration occurs during cooling because the cell goes from a solid to a glass state, bypassing the freezing process. In both protocols (freezing and vitrification), one seeks the highest tolerable CPA concentration to lessen the salt concentration and in vitrification to achieve the vitreous state without freezing (Pegg, 2002).

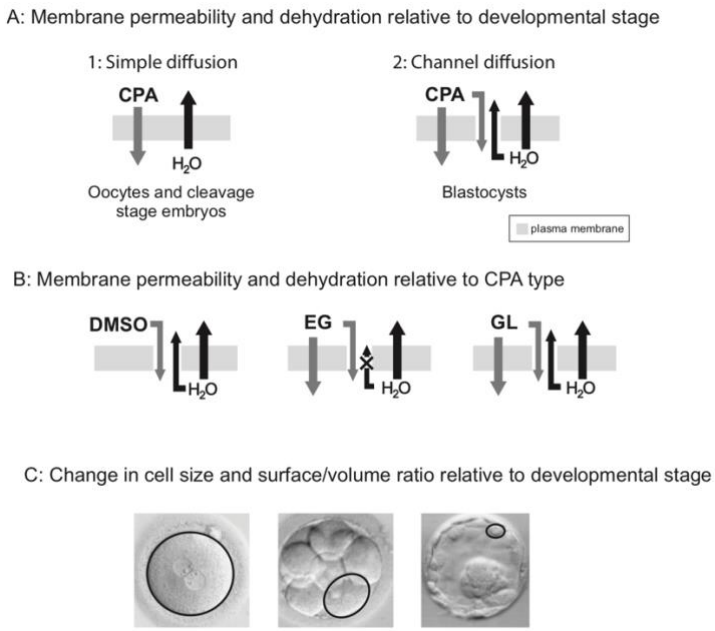


Figure 1: Movement of water and CPAs across the plasma membrane, movement of water relative to type of CPAs (B) and efficiency of dehydration and CPA uptake relative to cell size (C) (Medicine, 2012)

1.2.4 Osmotic shock and cryoprotectants

As mentioned before, effective CPAs infiltrate the cell membranes, but they do so at a slower pace than water. It is not surprising that due to this difference in pace of penetration of water and CPA into the cell, an osmotic imbalance is unavoidable throughout the addition or removal of these compounds. Major osmotic shock results in cell damage and therefore cell lysis (in most cases) (Pegg, 2002). In order to avoid this effect, it is essential to observe and control the alterations in cell volume, so that satisfactory limits are maintained. This maintenance will subsequently ensure the avoidance of structural and functional damage.

1.2.5 Slow-freezing

The first pregnancy from SF and rapid thawing oocytes using DMSO was reported in 1986 (Gook, 2002). This success within the ART community proved that human gametes and embryos be can successfully preserved and stored by specially developed cryopreservation methods. The process of vitrification, more recently developed, is one of these methods and has been well accepted and adapted in IVF laborites today and show robust results regarding survival rates of embryos, oocytes and spermatozoa (Cobo et al., 2017).

The SF method was a predecessor of vitrification, which consisted of numerous steps of controlled rates of cooling through different temperature phases using liquid nitrogen (LN) (Cobo & Diaz, 2011). SF is a lengthy process that requires specific equipment, which increases costs unnecessarily. SF has also been shown to cause osmotic shock due to solution effects and intracellular ice crystallization leading to cell damage.

Many variants of SF were developed and experimented with when the protocol was relatively new to the ART field. However, none successfully enhanced the protocol with regards to increasing the pregnancy rates and clinical efficacy. These alterations of the protocol included: changing the concentration of sucrose from 0.1 mol/L to 0.2- to 0.3- and then back to 0.1-mol/L. The increases from 0.1 to 0.2 mol/L and from 0.2 to 0.3 mol/L were both recorded as detrimental to the cell. The change to 0.2 mol/L resulted in an increase in spindle damage and at 0.3 mol/L decreased implantation rates and underdeveloped cleavage development were observed (Gook, 2001).

Research has also shown that chill-sensitive oocytes may survive cryopreservation if the temperature is very rapidly lowered from a safe temperature (e.g. body temperature) to one which is so low that chemical and biological processes cease (Sansinena et al., 2011). This concept (along with the failed attempts to improve the protocol) led the movement from SF to the development of rapid cooling of oocytes via a process called vitrification.

Since the development of vitrification, a study done in 2010 reported that their results suggest that vitrification/warming is currently the most efficient means of oocyte cryopreservation in relation to subsequent success in establishing pregnancy (Smith et al., 2010). However, in terms of the fundamental principles of cryobiology the survival rates between SF and vitrification are similar (Medicine, 2012).

1.2.6 Vitrification

The principle of vitrification involves the solidification of a sample into an amorphous, glassy state while upholding the nonexistence of both intracellular and extracellular ice crystals. Essentially, the combination of high cooling rates and high CPA concentration is what is responsible for the successful outcome of avoiding ice crystal formation during vitrification (Sansinena et al., 2011).

Since the development of vitrification of oocytes, SF has become obsolete (Cobo & Diaz, 2011). One of the original concerns when vitrification of oocytes was introduced and implemented, was that of fears of high risks

of toxicity caused by the high concentration of CPAs. Since the development of more recent vitrification protocols such risks have been avoided. This is mainly due to the extreme high cooling rates, which eliminates the concerns of toxicity damage (Sansinena et al., 2011) and this was mainly achieved via "open system" vitrification methods whereby the oocyte comes into direct contact with the LN.

There have been concerns regarding cross-contamination via this open system, however, no cases of cross-contamination have been recorded to date (Cobo & Diaz, 2011). Albeit this fact, it has been suggested that methods should be adapted in order to, in all cases, consider safety and attempt to avoid contamination.

Upon warming of vitrified oocytes, cells rehydrate, and CPAs are removed. Whether all physicochemical changes cause any alteration in embryo morphokinetics is still not well known, however no differences in clinical outcomes and embryo morphology have been observed or reported in several previous studies comparing fresh and vitrified oocytes. Therefore, the time-lapse imaging of embryos from vitrified oocytes can help to clarify whether vitrification can cause subcellular effects that are able to alter cell division dynamics (Cobo et al., 2017).

1.2.7 Vitrification of oocytes

Cryopreservation of oocytes has been a controversial topic since its conception about a decade ago. During the early stages of developing the oocyte SF protocol, a low survival rate of 30% was obtained (Gosden, 2011). Development of the oocyte SF protocol was also put to a halt shortly after it was developed due to the discovery of the concept of zona pellucida hardening post warming. However, this issue was subsequently bypassed by the introduction of ICSI (Gosden, 2011). The freezing protocol was then modified by attempts to alter the CPA compositions and initial seeding temperature; however, the protocol was still not widely accepted. Studies speculated that the reason for the failure of proposed oocyte freezing protocols while the embryo protocols were succeeding, was mainly due to the fact that oocytes require more exposure to CPAs to allow more penetration due to the larger cell mass than blastocysts exhibit (Pegg, 2002).

Since the development of the oocyte vitrification protocol, studies suggest that vitrification for oocyte cryopreservation significantly improves oocyte survival and pregnancy rates. In humans, most studies suggest that post thaw survival rates of vitrified oocytes are superior to those that have undergone SF protocols (Oktay et al., 2006). Several randomized control trials (RCT) exist that compared pregnancy rates of slow freeze vs. vitrified oocytes (Cao et al., 2009; Smith et al., 2010; Boldt, 2011; Glujovsky et al., 2014). One such paper proved that vitrification resulted in better oocyte survival (81% vs. 67%; $P<0.001$), fertilization (77% vs. 67%, P1⁄4.03), and clinical pregnancy rate (CPR) per thawed oocyte (5.2% vs. 1.7%, P1⁄4.03) compared to slow freezing (Smith et al., 2010). Another study included the review of 2 RCTs which both supported the notion of oocyte vitrification yielding superior results to oocyte SF. Both RCTs did not evaluate LBR, however observations regarding CPR were found to be in favor of vitrification of oocytes (Glujovsky et al., 2014).

The validation of the oocyte vitrification protocol essentially came from the comparison of fresh versus vitrified oocytes and how they succeed in IVF/ICSI outcomes. There were 4 RTCs that were focused on by the *The Practice*

Committees of the American Society for Reproductive Medicine and the *Society for Assisted Reproductive Technology* in 2013 in Birmingham (ASRM: a guideline 2013). Two of these studies were conducted by Cobo et al. in 2008 and in 2010. The first study observed a survival rate of 96.7% for the vitrified/warmed oocytes and that there was no difference in fertilization rates (76.3% and 82.2%), day 2 cleavage (94.2% and 97.8%), day 3 cleavage (80.8% and 80.5%), and blastocyst formation (48.7% and 47.5%) for vitrified and fresh oocytes, respectively (Cobo et al., 2008). The follow-up study further validated that vitrified oocytes compare equally to their fresh counter parts by reporting that the proportion of top-quality embryos obtained either by inseminated oocyte (30.8 versus 30.8% for Day-2; and 36.1 versus 37.7% for Day-3, respectively) or by cleaved embryos (43.6 versus 43.8% for Day-2 and 58.4 versus 60.7% for Day-3, respectively) was similar between groups of fresh versus vitrified donor sibling oocytes (Cobo et al., 2010).

Further studies showed that the survival rate of vitrified/warmed oocytes was 98.7%. There was no statistical difference between the fertilization rate and good-quality embryo rate between fresh and vitrified oocytes (83.3% vs 79.2% and 52.0% vs 51.6%, respectively) (Rienzi et al., 2010); and no significant difference in fertilization rate for fresh (72.6 %) versus vitrified (71.0%) oocytes (Parmegiani et al., 2011)

In summary, the RCT studies found that 92.5% of vitrified oocytes survived warming, and that there were no significant differences in fertilization rates (74.2% vitrified vs. 73.3% fresh), implantation rates (39.9% vs. 40.9%) and pregnancy rates per transfer (55.4% vs. 55.6%) between groups, with a mean of 1.7 embryos transferred (ASRM, 2013).

1.2.8 Maternal age and oocyte vitrification success

It is well known that the efficacy of oocytes declines with the increase in female age (Cimadomo et al., 2018), and this concept is no different for vitrified oocytes. There are no comparative trials evaluating success with cryopreserved versus fresh oocytes by female age, however, several studies using slow-freeze protocols suggest that success rates are lower with advanced maternal age (ASRM; a guideline 2013).

It was shown by a study conducted in Italy, using vitrified/warmed oocytes, that with woman over the age of 38 faced lower implantation rates (6.5% vs. 10.9%) and pregnancy rates (10.1% vs. 18.7%) compared to younger women. However, the survival rate of vitrified/warmed oocytes did not differ among the different ages (Borini et al., 2010). A similar study also reported lower implantation rates (16.7%, 11.6%, and 10.8%); pregnancy rates per thaw cycle (24.3%, 18.9%, and 16.1%); and pregnancy rates per embryo transfer (27.7%, 21.4%, and 17.6%) in women 34 years, 35–38 years, and over 38 years, respectively (Bianchi et al., 2012).

Lastly, with regards to the success of the vitrification process, it was reported that women who wish to vitrify their oocytes past the age of 40 will face significantly lower survival rates as well as a CPR of 22.2% (Ubaldi et al., 2010). However, in summary one can deduct that vitrified oocytes behave much the same as their fresh counterparts when impeded by the negative outcomes of age (Cimadomo et al., 2018).

1.2.9 Clinical application for oocyte vitrification

The clinical applications for oocyte vitrification include fertility preservation, especially for patients who struggle with cancer, social reasons for women who find relationships later on within their reproductive years, donor programs, patients at high risk of ovarian hyper-stimulation syndrome (OHSS), oocyte accumulation for poor responders and lastly, storage of surplus oocyte storage for patients who cannot afford embryo vitrification (Cobo & Diaz, 2011).

The primary clinical application of the use of donor oocyte cycles are for patients with premature ovarian failure. However, since the rise in popularity and use of donor oocytes, many women opt for donor cycles when faced with age-related fertility issues, such as the diagnosis of AMA, (Argyle et al., 2016). Vitrification of oocytes, opposed to slow freezing, still remains the gold standard (Cobo et al., 2015), and more recent studies have shown that vitrified donor cycles compare very well when compared to fresh donor cycles. There is still a need for fresh donor cycles, since there is still insufficient knowledge with regards to running an oocyte bank successfully and efficiently.

In other words, oocyte banks are still in their 'teething phase' with regards to their efficiency; this could be due to their only recent proliferation and existence. Another reason could be due to premature reliability on vitrification protocols. A successful oocyte vitrification/warming protocol and process is dependent on the skill of the embryologist and can have a significant effect on the survival rates and other outcomes of the oocyte vitrification program success (Cobo et al., 2015). Conversely, vitrification cycles are often very successful and present few or no clinical disadvantages when compared to fresh cycles (Doyle et al., 2017).

1.2.10 The drive for oocyte freezing

During the past three decades, the commercialized world has experienced a trend of women conceiving their first-born within their later reproductive years. Put simply: women are delaying childbearing (Devine et al., 2015). A study reported some staggering results of a 150% increase of women giving birth to their first-born between the ages of 35 and 39. The first-birth rate for women aged 40–44 years increased 5%, while the average overall first-birth age climbed from 21.4 years in 1970 to 25.4 years in 2013, across all races (Hodes-Wertz et al., 2013), further elaborating this shift delaying childbearing.

This trend of delaying motherhood has reportedly been caused by various educational, professional, personal, financial pursuits, and/or circumstances. The most popular reason for delaying childbearing was from women who said they did not have a partner (88%), which was then followed by women who did not conceive earlier due to career related reasons (24%) (Bretherick et al., 2010).

This trend of delayed childbearing does not, however, exclude the eminent fact that there is an unavoidable age-related decline in fertility, where advanced maternal age (AMA) is associated with chromosomal abnormalities and increased chances of down-syndrome and abortion (Cimadomo et al., 2018). Another dilemma, however,

arises; financial strain of the vitrification program versus the chances of success. There is still little known of the adverse effects of using vitrified oocytes within the offspring born however, it has been shown that the success of vitrified oocytes compares well against their fresh counterparts (Hodes-Wertz et al., 2013).

The conundrum of opposing ideals has left women with a troublesome social-financial-reproductive-dilemma, subsequently resulting in the increased demand for oocyte vitrification.

1.2.11 Ova donation

Vitrified donor oocytes cycles serve as an advantage to the patient in various ways. This includes the vast improvement with regards to the logistical task of synchronizing cycles of the donor and recipient, which can often prove to be difficult (Cobo et al., 2015). It also shortens waiting lists for recipients needing donors; it reduces the cost in terms of travelling as recipients need only to be concerned of their financial budget for an embryo transfer (ET).

Furthermore, with regards to the success of donor oocyte cycles, a recent study showed that there was almost a 100% chance of pregnancy after 3 or 4 cycles using donor oocytes (Cobo et al., 2015). This study elaborated on how the chances of pregnancy increase rapidly within cycles where there were one to 25 oocytes, while slightly decreasing from 25 to 40 oocytes, then plateauing when reaching number of oocytes succeeding 40; all while maintaining a cumulative live birth rate (CLBR) of 97.3% (Cobo et al., 2015). This validates the effectiveness of a donor oocyte program by highlighting the superior quality of donor oocytes.

1.3 ART incubators

It has been said that: “Embryo incubators can be considered the heart of any in IVF laboratory” and understanding the advantages and disadvantages of these incubators is absolutely crucial in obtaining optimal results in any IVF laboratory (Meintjies, 2012). Incubation equipment has advanced substantially since the onset of ART treatments in the past. There are essentially three categories of incubators available: large water-jacketed and direct heat incubators, smaller benchtop incubators, and time lapse incubators; the latter two are more commonly used in laboratories today.

1.3.1 CO_2 incubators (large and benchtop incubators)

Large incubators are considered to be inefficient incubators that were replaced by smaller, and more convenient, bench-top incubators since their introduction into the ART field. The concept behind the introduction of these bench- top incubators was based upon the rational that uninterrupted culture should be executed as best as possible. Smaller incubators with separated incubation chambers meant that taking one patient’s embryos out of the incubation chamber did not interrupt culture conditions in others; whereas with the larger incubators, one door was used to access all embryos in culture and thus causing unwanted fluctuations within the embryo incubation environment. The concept of passive heat reservoirs allows for faster temperature recovery. The turnaround time

for equilibration of embryo culture environment parameters within benchtop incubators have also been reported to be quicker than their larger counterparts (Cattt & Henman, 2000). It was reported that the implantation rate (IR) was increased from 10% to 14% and the pregnancy rate from 19% to 32% when culturing human embryos in a benchtop incubator (Meintjies, 2012). This study's results were concluded to be advantageous due to the more rapid recovery rate exhibited in benchtop incubators, compared to their larger counterparts. Furthermore, another practical example of the advantage of benchtop incubators was reported whereby the temperature recovery was approximately 5 min in an MINC incubator (benchtop incubator) compared with roughly 30 min for a standard, water-jacketed incubator after a single door opening (Fujiwara et al., 2006).

When one applies the logic to the concept of 'the smaller the incubator, the faster the gas-phase recovery' with the fastest recovery to be expected from the top-load, bench-top incubators, it makes sense that this concept has been validated. However, this is not always the case, as a larger incubator with an infrared CO_2 sensor can have a faster CO_2 recovery time than a smaller incubator with a thermo-conductivity CO_2 sensor (Zhang et al., 2010). Therefore, no matter the set up or type of incubator present within human IVF applications, the number of patients per incubator should be limited to reduce risk in the case of incubator malfunction, to decrease the likelihood of sample confusion, and to maintain the most optimum culture conditions by reducing the number of door openings per day (Zhang et al., 2010).

Since the development of benchtop incubators and their favor over their predecessors, new technology has subsequently been developed. Time-lapse incubators were officially commercially available first in Sweden in 2008, then shortly after being introduced by the European Society of Human Reproduction (Leung et al., 2016). Multiple integrated Time-lapse systems (TLS) are available on the market today, however, the dispute regarding the functionality, necessity and role of such systems are still under heavy debate (Kovacs, 2014).

1.4 Time-lapse systems

1.4.1 Introduction of TLS

The debate regarding the functionality of time-lapse (TL) incubators within an IVF laboratory originated from the cost and lack of clinical data to support claims of effective embryo selection via morphokinetic evaluation and analysis (Armstrong et al., 2015; Chen et al., 2017). Today TL incubators boast an array of benefits, solidifying its functionality within the lab. However, with the rising costs due to upgrades and advances in technology, the use of these complex machines is yet to be commonly integrated within the IVF community.

The most obvious advantage of TLS over conventional benchtop incubators (as well as larger incubators) is that there is no need to open the incubator to evaluate a static morphology grading of the embryo. This is beneficial since there is no disturbance within the highly sensitive embryo microenvironment. Secondly, static morphology grading/analysis may also be misleading. This is due to the fact that the development of embryos can be rapid and ever-changing. A static evaluation of an embryo on day 2 might yield a 'good quality embryo', however the grading on day 3 may be vastly different. This, to some extent, can be avoided using a morphokinetic evaluation

as more trends can be seen and a more accurate prediction can be made (Wong et al., 2010; Basile et al., 2015). Lastly, when evaluating embryos statically, it is more challenging to ensure that evaluation of each embryo occurs around the same time. It is crucial for time to be standardized as the timing of the development is relevant for analysis. TLS eliminate this issue and are thus superior to static evaluation with regards to the above mentioned.

The single most valuable asset of TL imaging is the access to large amounts of data from the non-invasive observation of embryogenesis (Milewski et al., 2015). This technology allows observation of embryo development through repeated multiple image acquisitions. Furthermore, this allows various observations of events occurring between conventional static morphological evaluations which are used without TL image viewing (Ciray et al., 2015). This concept of having multiple viewing points of the embryo development is defined as 'morphokinetic' evaluation (Ciray et al., 2015; Milewski et al., 2015). These observations of embryo development include absolute and comparable time-points (as seen in Table 1) for important embryo growth 'check-points'. The time-points are comparable and can be used to design laboratory specific algorithms or development models, which in turn can be used to predict future trends within embryo development. This insight is essential to aid the selection of embryos that will most likely result in a pregnancy (Ciray et al., 2015). Notably, these models are based on the population of the practicing laboratory and therefore should yield patient population accurate outcomes.

1.4.2 Annotation considerations

The annotation of embryo development, automatic or manual (done by an embryologist), requires standardization (Ciray et al., 2015). There are various guidelines available with most only differing slightly with abbreviation variants. Furthermore, with regards to annotation, automatic systems are also available but are not commonly used. This is due to the fact that embryo development presents with extremely diverse and complex anomalies, which make it difficult for an algorithm alone to follow and annotate. Various morphokinetic evaluation models exist (Meseguer et al., 2011; Basile et al., 2014; Desai et al., 2014; Rubio et al., 2014; Petersen et al., 2016), however, laboratory models must be followed with caution. Laboratories showcase prominent individuality; therefore, a one-model-fits-all approach will not be sufficient. Notably, since it was recommended that further research needed to be done regarding time-lapse implementation due to the limitations of only retrospective studies available around 2015 (Ciray et al., 2015), the call for a randomized control trial was sparked by the publications which reported that 'deviant' morphokinetic profiled blastocysts still yielded live births (LR) (Stetcher et al., 2014). Regarding this matter, in conclusion, the superior option for accuracy when using an annotation model is to design one's own according to the individual patient population.

1.4.3 The role of TLS in ART

The role of TLS within an IVF laboratory is vast. The study of embryo morphokinetics has resulted in the identification of different kinetic markers (Basile et al., 2015). These markers have predominantly been associated with embryo viability (Wong et al., 2010; Yang et al., 2015), blastulation (Dal Canto et al., 2012), implantation (Mesenguer et al., 2011; Dal Canto et al., 2012; Basile et al., 2014), pregnancy (Scott et al., 2007) and live birth

rates (Vernon et al., 2011). Further possible benefits include being an alternative to pre-implantation genetic testing for aneuploidy (PGT-a), reducing the time to pregnancy and reducing/lowering the occurrence or chance of miscarriage (Pribenszky et al., 2017). The notion of TLS aiding in reducing the use of PGT-a testing is based on the theory that morphokinetic evaluation assists in de-selecting chromosomally abnormal embryos, which therefor may render the need for PGT-a redundant (Campbell et al., 2013; Zhan et al., 2016; Desai et al., 2018). Regarding the time to pregnancy, TLS may aid in reducing this time owing to the benefits of selecting an embryo that may have an increased potential for implantation, pregnancy and live birth; all while having the largest chance of being chromosomally normal and reducing the chances of miscarriage (Desai et al., 2018).

Conclusively, benefits are copious when considering the integration of a TLS within an IVF laboratory, however there is still debate questioning the necessity of TLS when compared to their cheaper conventional benchtop counterparts (Armstrong et al., 2015; Chen et al., 2017). Notably, TLS are also excellent training tools for training embryologists as well as for practitioners in the field of IVF, when compared to conventional benchtops. Although few studies have suggested a call for more RCTs validating TLS, a decision surrounding the need for a TLS is one to be made based on individualized evaluation of the laboratory, staff and cost versus benefit analysis.

1.5 Morphokinetics

1.5.1 Introduction to annotation

As mentioned before, TLS generate vast amounts of data. This data is collected and interpreted as absolute time points, which represent a dynamic morphokinetic evaluation of the development of human embryos. The time points or 'check points' (as seen in Table 1) represent different uses and may vary among laboratories (Montag et al., 2011).

Table 1: Morphokinetic nomenclature (Basile et al., 2015; Ciray et al., 2015; Vitrolife: A guide on definitions for morphokinetics, 2019)

Timing	Meaning
t0	Time to IVF or mid-time of micro-injection (ICSI/PICSI/IMSI)
tPB2	The second polar body (PB2) detachment or extrusion
tPN	Fertilization status confirmed via visibility of pronuclei (PN)
tPNa	Appearance of individual PN
tPNf	Time of PN fading/disappearance
tZ	Time of PN scoring (not examined within this study)
t2 to t9	Timings for two to nine discrete cells/blastomeres
t9+	Nine or more discrete blastomeres
tSC	First evidence of compaction
tMf/p	End of compaction process, 'f' corresponds to fully compacted and 'p' corresponds to partial compaction (not examined within this study)
tSB	Initiation of blastulation
tB	Time to full blastocyst
tEB	Time to expanded blastocyst
tHB	Time at blastocyst hatching
tDead	Time of degeneration
ECC1 (t2 – tPB2)	Embryo cell cycle 1
ECC2 (t4 – 2)	Embryo cell cycle 2
ECC3 (t8 – t4)	Embryo cell cycle 3
s2 (t4 – t3)	Synchronization of cell divisions
s3 (t8 – t5)	Synchronization of cleavage pattern
dcom (tM – tSC)	Compaction
dB (tB – tSB)	Blastulation
dexp (tHB – tEB)	Blastocyst expansion

It is absolutely essential to ensure that annotation of these time-points is standardized within embryologists' annotating as well as compared to external clinics. This vast amount of data should be collected in the same manner, otherwise it will not be possible to be compared to, and validated, by outside sources. Thus, the time-points mentioned in Table 1, their definitions and a guide on how to grade/annotate them exists (Figure 2).

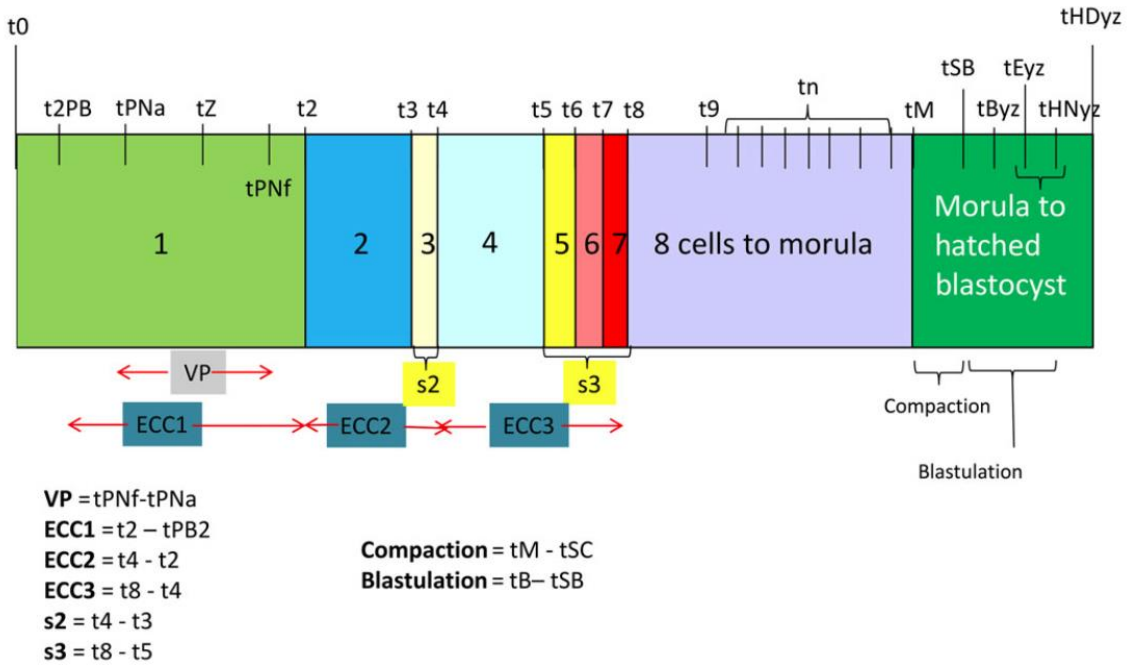


Figure 2: Graphic representation of a 'normal' morphokinetic monitoring of human embryogenesis (Ciray et al., 2015)

Time, appearance, fading/disappearance and cell/episode or number are represented by a 't', 'a', 'f' and a 'n' respectively (Basile et al., 2015; Ciray et al., 2015; Vitrolife: A guide on definitions for morphokinetics, 2019;). The process of annotation may become a time-consuming process, especially if done manually. However, as mentioned, it is essential to ensure proper and accurate annotation of morphokinetic time-points. It has therefore been suggested that during the process of annotating each separate episode or event, one should rewind and forward time-lapse images to before and after the event under speculation. This will aid in making sure the event is annotated correctly (Ciray et al., 2015).

1.5.2 Time points

t0: This is the time at which insemination occurs in conventional IVF. For ICSI/IMSI/PICSI, where the time of the sperm injection is recorded, per oocyte but otherwise, it is the mid-time point from when injection begins and ends for that patient's cohort of oocytes (Ciray et al., 2015). In order to standardize t0 for IVF when compared to ICSI it is suggested that tPNf is used as t0 for both insemination methods (Vitrolife: A guide on definitions for morphokinetics). All times from the start point are recorded in hours post insemination/t0.

tPB2: This is the time when the second polar body (PB2) is extruded. This is annotated at the first frame in which PB2 appears completely detached from the oolemma (Ciray et al., 2015). The extrusion of the PB2 is not always observable, and this may be due to the orientation of the oocyte within the well of the time-lapse slide. It may also be influenced by how well the oocyte was cleaned (denuded), which could cause visual obstructions.

tPNa: This is the time whereby pronuclei (PN) are visualized and thereby fertilization status is confirmed (Vitrolife: A guide on definitions for morphokinetics). It is suggested to annotate fertilization (2PN) directly before fading of pronuclei (tPNf) as no additional observational dynamic changes are predicted to occur. This will aid in grading the fertilization status accurately and ensuring if the fertilization was normal (2PN) or abnormal (1PN, 3PN) (Ciray et al., 2015).

tPNf: This is the time when both (or the last) PN disappear (Ciray et al., 2015).

t2: This is the time of the first cell cleavage, or mitosis. t2 is the first frame at which the two blastomeres are completely separated by individual cell membranes, as seen in Figure 3 and Figure 4 (Basile et al., 2015; Ciray et al., 2015).

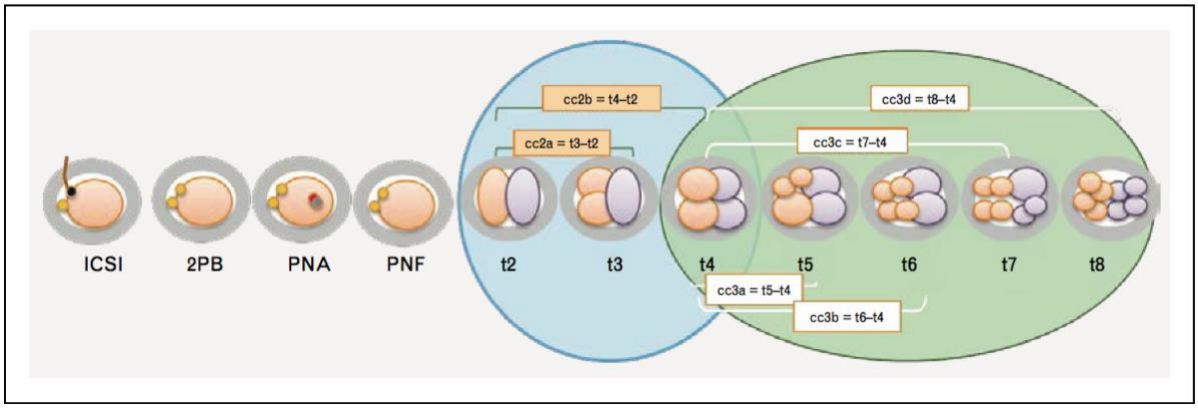


Figure 3: Graphic representation of kinetic variables till eight cell-stage (Basile et al., 2015)

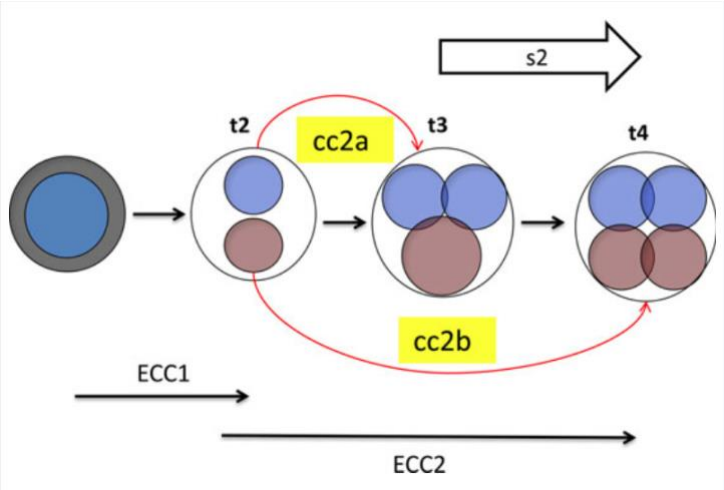


Figure 4: Schematic representation of the second cell cycle (ECC2) and s2 (Ciray et al.,2015)

It has been suggested that grading of this event should be done with precision since there are various manners in which a cell may cleave. Cleaving cells may appear to be divided, however may in fact be in a distorted cytoplasm movement (DCM) episode, as seen in Figure 5(6) (Yang et al., 2015).

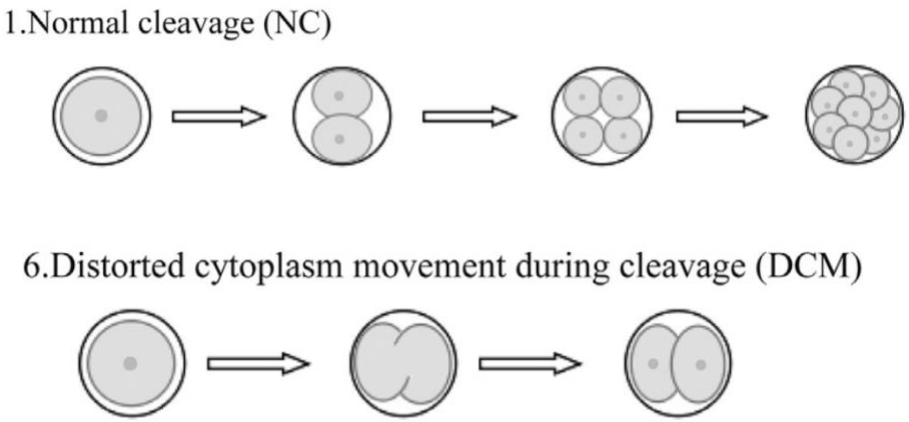


Figure 5: Schematic representation of (1) normal cleavage and (6) distorted cytoplasm movement (DCM) (adapted from: Yang et al., 2015)

t3: This is the first observation of three discrete cells. Notably, t3 marks the commencement of the second episode of cleavage and second cell cycle, as seen in Figure 4 (Ciray et al., 2015).

t4 – t8: This is identified as the third cell cycle (ECC3) (Figure6).

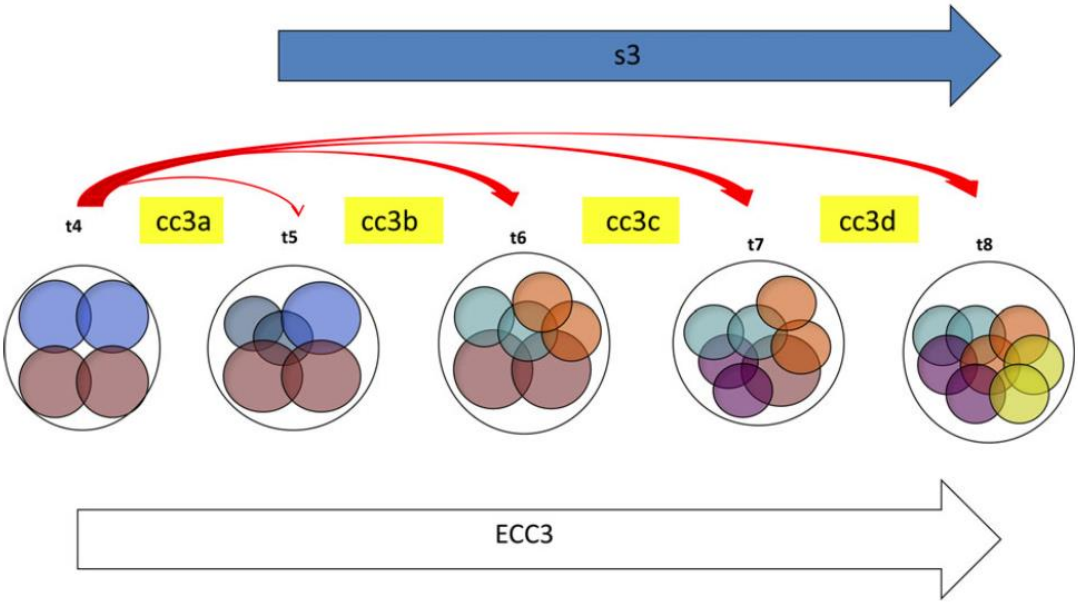


Figure 6: Schematic representation of the third cell cycle (ECC3) Ciray et al., 2015)

tSC: This is the first frame in which indication of compaction is apparent; the initial frame where any (two) cells begin to compact, is witnessed (Ciray et al., 2015). The exact timing of commencement of compaction may be challenging to observe due to the increased number of cells and the type of compaction (partial or complete).

tM: This denotes the completion of the compaction process and thus observable compaction is complete and a morula forms. Notably, the morula can be completely or partially compacted. During partial compaction, there may be excluded matter or fragments within the embryo which do not form part of the compaction (Ciray et al., 2015). The level and time of compaction has been described to be related with blastulation and quality (Ivec et al., 2011).

tSB: This is the initiation/start of blastulation. The first frame when initiation of a cavity formation is observed (Vitrolife: A guide on definitions for morphokinetics, 2019).

tB: This is the full blastocyst. The last frame before the zona pellucida starts to thin (Ciray et al., 2015).

tEB: This is the initiation of expansion. The first frame when the zona pellucida is half of its original thickness (Vitrolife: A guide on definitions for morphokinetics, 2019).

tHB: This is the first witness of signs of hatching within the blastocyst (Vitrolife: A guide on definitions for morphokinetics, 2019). Hatching blastocysts is a process whereby the blastocyst 'breaks free' from it's zona pellucida casing. This process usually takes place within the uterus, *in vivo*, before i3mplantation.

1.5.3 Irregular cleavage events

Rapid cleavage

Rapid cleavage was first reported in 2011 whereby a study stated that embryos dividing from one cell directly to three cells had a negative impact on implantation rate (Ciray et al., 2015). Rapid cleavage is also known as direct cleavage (DC) and direct uneven cleavage (DUC) and can occur at different stages of embryogenesis during different cell cycles (Rubio et al., 2012; Basile et al., 2015). Rapid cleavage is defined as a division from one cell to three or more blastomeres, as seen in Figure 7 (Yang et al., 2015). DUCs have been reported to appear in approximately 14% of all embryos and they were noted to be one of the highest embryo de-selection parameters, since they compromise implantation competence (Rubio et al., 2012).

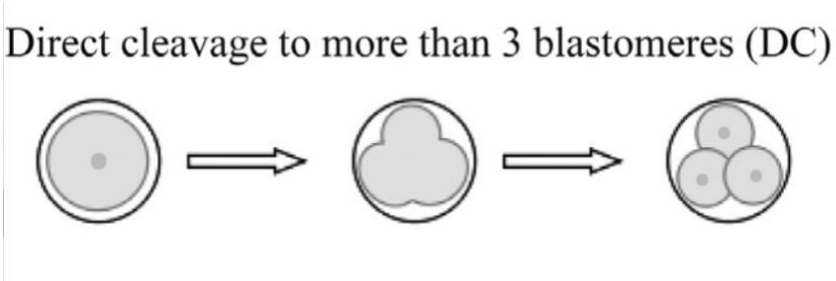


Figure 7: Schematic representation of a DC or also known as a DUC (Yang et al., 2015)

The occurrence of rapid cleavages within embryogenesis may be associated with faults in cell cycle mechanisms, which results in early cytokinesis (Ciray et al., 2015). Irregular cleavage patterns can occur at any cell stage as mentioned before, however are predominantly classified throughout early cleavage embryo stage of development (Rubio et al., 2012).

It has been reported that the stage at which a DUC occurs, as well as if it occurs singularly or in multiples can affect the normality of the embryo differently. If a single DUC occurs during the ECC1 (known as DUC1), it is unlikely to retain any chromosomally normal blastomeres, as seen in Figure 8. However, if the DUC occurs during the ECC2 (DUC2), the embryo may have the potential to correct the abnormal blastomeres (Scudellari, 2014). In other words, the sooner on in the cell cycle the DUC occurs, the more detrimental to the embryo the abnormality will be (Yang, 2015).

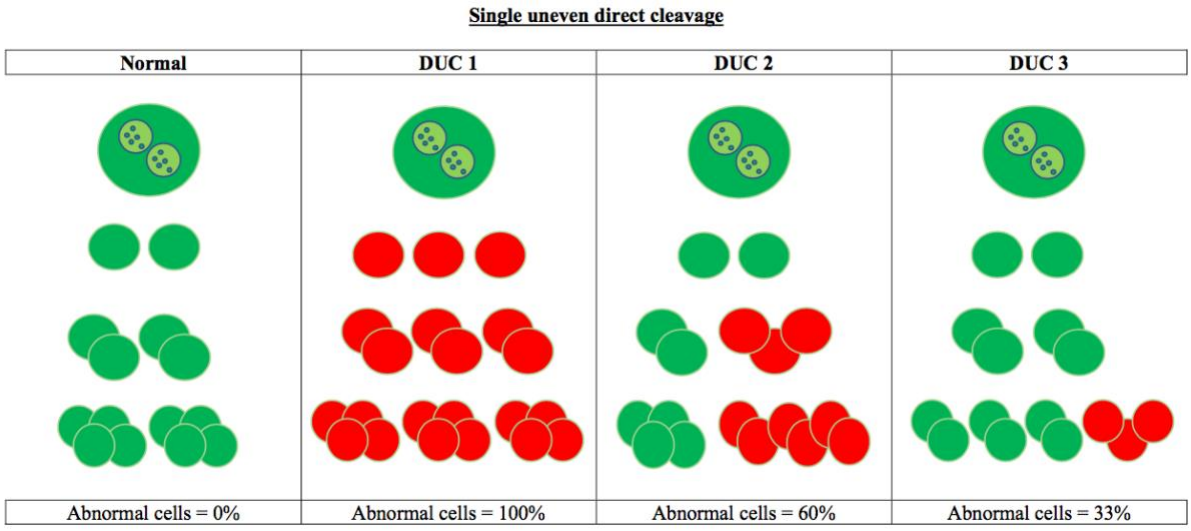


Figure 8: Schematic representation of single DUC anomalies (Adaped from: Scudellari, 2014; Yang, 2014)

Furthermore, similar conclusions may be drawn for multiple DUC divisions. The least chromosomal damage via DUC divisions occurs later on in the embryo development, during ECC3, as seen in Figure 9.

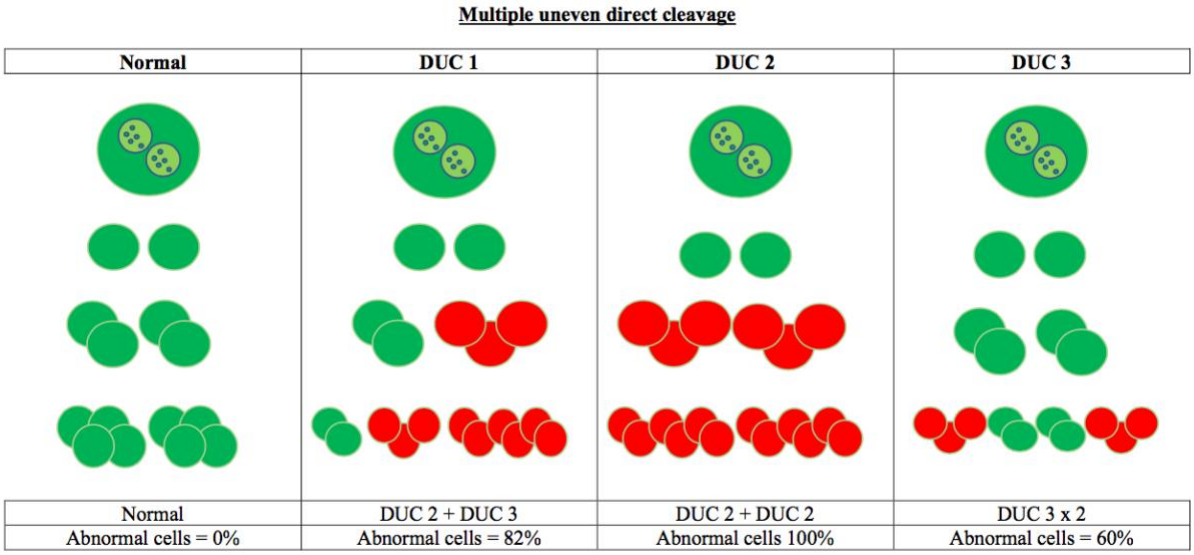


Figure 9: Schematic representation of multiple DUC anomalies (Adapted from: Scudellari, 2014; Yang, 2014)

Cell fusion

Cell fusion occurs independently of compaction (Ciray et al., 2015). It is described as a reduction in the number of cells of an embryo during its development due to the merging, or fusion, of cells giving the appearance of a reversed cleavage event (Yang et al., 2015). This event is identified as a cell fusion and not a reverse cleavage or fragmentation by the witnessing of a nucleus within the cells involved, before manifestation of this event. It is also noticeable from the fusion of cells throughout compaction forgoing morula establishment. In an observational study of 1698 zygotes, cell fusion was observed in 10% of all embryos (Ciray et al., 2015).

Embryo rolling

Embryo rolling, also observed as DCM (Figure 5), permits the imaging of blastomeres moving on or around themselves without dividing (Yang et al., 2015). DCM events may be an indication of poor embryo development and implantation potential, however, are not recorded commonly within laboratories (Ciray et al., 2015).

1.6 TL time-point comparisons in the literature

A one of the most promising tools that TLSs offer is that of patient population specific prediction models. These models are derived from exact time points using TL imagery, plotted and summarized into a concise manner in which one can reference future embryo developments. Table 2 illustrates a summary of various studies with exact TL time points for various embryo development stages; the most common ranging from tPNf to t5 or t2 to tEB.

Many studies emphasize the importance of kinetic embryo grading using TL imaging, most making note of the significance of early cleavage development (Wong et al., 2010; Meseguer et al., 2011; Chalwa et al., 2014; Chamayou et al., 2015; Milewski et al., 2015). Two studies further emphasized the need for scoring of early cleavage events using morphokinetics over static evaluation, stating that such events were connected with embryo quality and implantation rate (Lemmen et al., 2008; Montag et al., 2011). Another study in support of TLS recorded that TL imaging could be used to exclude embryos that would have been recorded as viable using static grading, however, also stating that this is due to erratic or abnormal divisions which need more research regarding their exact effect on clinical outcomes (Kirkegaard et al., 2013).

Time lapse and morphokinetic evaluation offer a unique opportunity to compare patients' groups, treatments and interventions in ART.

Fresh vs vitrified/warmed oocytes

De Gheselle et al. (2019) examined an overall delay (Table 2) in timings with regards to fresh versus vitrified/warmed oocytes, whereby the delay of 1.27h overall was exhibited within the vitrified/warmed oocyte population. It was further reported by the same study that a decrease in fertilization within the delayed vitrified/warmed oocyte population also existed. Cobo et al. (2017) reported a similar trend of delay within the vitrified/warmed oocyte population. (Table 2), however, less statistically significant differences were observed; although population sizes were larger. Chamayou et al. (2015) examined the difference between fresh versus vitrified/thawed oocytes and found a significant overall delay within the vitrified/warmed population.

Blastulation vs non blasulation

Milewski et al. (2015) recorded a delayed time within embryos that did blastulate, compared to embryos that did not.

Transferred vs not transferred

Desai et al. (2014) also found a trend of delayed times for embryos that were not transferred compared to embryos that were.

Implanted and KID+ vs not implanted and KID-

Meseguer et al. (2011) recorded a delay in timings within the population of embryos that did not achieve implantation, when compared to the population that achieved successful implantation (as seen in Table 2). Desai

et al. (2014) compared embryos that had known implantation data (KID+) versus embryos that had known non-implantation data (KID-) and found a delay in TL timings within the KID- arm (Table 2).

"Normal" and euploid vs "not normal" and aneuploid

Chawla et al. (2015) found a trend of delayed times within 'non-normal' oocytes when compared to their 'normal' counterparts. Furthermore, Campbell et al. (2013) compared embryos that exhibited euploidy versus embryos that exhibited single and multiple levels of aneuploidy and found that there was a delay in timings within the aneuploidy arms, showing statistical significance for blastulation timings (Table 2).

ICSI vs IVF

Kim et al. (2017) showed a statistical difference between the timings between ICSI and IVF whereby ICSI exhibited shorter times for time points tPNf, t2 and t5. These results, however, did not affect pregnancy rates between ICSI and IVF. Bodri et al. (2015) found similar results whereby IVF had statistically delayed timings for tPNf to t3 of embryo development compared to ICSI.

From Table 2 it is clear that in some studies many time points were not included in the study. The rationale behind the diminished amounts of TL time points being recorded in some studies was due to the fact that sequential media was in use and therefore timings from day 3 were not consistent and therefore avoided. Another reason included the difficulty of grading embryos past t5, where the smaller sized blastomeres become difficult to differentiate between fragmentations and cells (Meseguer et al., 2011). It was initially thought that morphokinetic gradings of early cleavage rates (t2 to t5) were sufficient to predict embryo quality and possible clinical outcome, however, it was concluded that timings past t5 may in fact be more indicative of embryo viability; albeit the ambiguity in grading past t5 (Meseguer et al., 2011).

As elaborated through the details of Table 2, one can perceive the discrepancies between different populations with regards to TL timings, even though the overall conclusion of different data populations may be similar. An example of this being that two different laboratories may both show trends of delayed timings for vitrified/warmed oocytes when compared to their fresh counterparts, however the specific TL timings may not be comparable between the respective laboratories. It has therefore been strongly suggested that in order to be able to predict possibilities of embryo development within a laboratory it is in the best interest of laboratories to collect TL image information and first establish baseline kinetics within their own population setting (Desai et al., 2014).

Table 2: A summary of studies comparing different study populations for specific TL time points (hours)

		Time lapse time points (hours)											
Study	Method	tPNf	t2	t3	t4	t5	t8	t9+	tSC	tSB	tB	tEB	tHB
De Gheselle et al,, 2019	Mean fresh oocyte TL timings	23,87 (p<0.001)	26,67 (p=0.004)	36,05 (p=0.004)	39,17 (p=0.001)	47,09 (p<0.001)	57,28 (p<0.001)	68,39 (p<0.001)	86,50 (p=0.002)	97,95 (p=0.013)	106,90 (p=ns)	109,90 (p=ns)	
	Mean vitrified / warmed oocyte TL timings	26,18	28,51	38,81	42,36	51,52	64,57	76,73	90,57	102,09			
	Difference (%)	9,7	6,9	7,7	8,1	9,4	12,7	12,2	4,7	4,2			
Cobo et al,, 2017	Mean fresh oocyte TL timing		27,7 (p<0.01)	37,8 (p<0.01)	40,2 (p<0.01)	50,5 (p<0.01)			86,6 (p<0.01)		103,4 (p=ns)	114,4 (p=ns)	114,9 (p=ns)
	Mean vitrified / warmed oocyte TL timing		28,7	38,9	41,4	51,7							
Chamayou et al,, 2015)	Mean fresh oocytes	26,1 (p=0.001)	29,0 (p=0.007)	39,4 (p=0.014)	41,5 (p=0.002)								
	Mean vitrified / warmed	23,6	26,9	37,1	39,2								
Milewski et al,, 2015	Median embryos that blastulated		26,2	37,8	39,2	53,6							
	Median embryos that did not blastulated		30,1	38,5	42,2	50,3							

Desai et al,, 2014	Mean blastocysts transferred	24,8 ± 2,6 (p=0.001)	27,2 ± 3,6 (p<0.001)	37,6 ± 5,5 (p=ns)	40,0 ± 5,4 (p=0.003)	52,0 ± 6,3 (p=ns)	62,1 ± 9,8 (p=<0.001)	73,5 ± 10,3 (p<0.001)	93,9 ± 9,8 (p<0.001)	100,2 ± 7,4 (p<0.001)	105,2 ± 6,3 (p<0.001)	110,0 ± 5,6 (p<0.001)	
Desai et al,, 2014	Mean known implantation data (KID+)	24,1 ± 2,5 (p<0.001)	26,8 ± 3,8 (p=0.02)	36,5 ± 4,7 (p=0.004)	39,3 ± 3,7 (p=ns)	51,0 ± 4,8 (p=0.02)	59,6 ± 9,1 (p=0.02)	72,3 ± 11,7 (p=ns)	90,5 ± 8,9 (p=ns)	98,1 ± 7,0 (p=ns)	102,9 ± 6,8 (p=ns)	109,9 ± 6,4 (p=ns)	
	Mean KID-	26,2 ± 2,7	28,5 ± 4,2	40,1 ± 6,8		54,0 ± 6,2	63,9 ± 9,8						
Chawla et al,, 2014	Mean normal embryos	24,5 ± 4,3 (p<0.05)	28,3 ± 7,2 (p<0.05)	38,7 ± 7,0 (p=ns)	40,5 ± 7,2 (p=ns)	52,3 ± 8,6 (p<0.05)							
	Mean abnormal	25,8 ± 5,6	30,6 ± 9,7			50,1±9,6							
Campbell etl al,, 2013	Median timings for euploidy	20,8 (p=ns)	23,2 (p=ns)	31,1 (p=ns)		43,7 (p=ns)	52,6 (p=ns)		74,1 (p=0.02)	91,7 (p=0.006)	101,2 (p=0.01)	104,5 (p=ns)	107,5 (p=ns)
	Multiple aneuploidy								85,1	101,9			
Herrero et al,, 2013	Median implanted embryos		27,5	39,3		54,6	61,8		85,1		108,1		
Meseguer et al,, 2011	Mean implanted embryos		25,6 (p=0.022)	37,4 (p=0.002)	38,2 (p=0.004)	52,3 (p<0.001)							
	Mean not implanted		26,7	38,4	40,0	52,6							
Kim et al,, 2017	Mean ICSI	24,3 ± 3,9 (p<0.001)	27,0 ± 4,5 (p<0.001)	36,5 ± 5,7 (p=ns)	38,7 ± 5,8 (p=ns)	48,7 ± 7,9 (p=0.005)	58,5 ± 11,2 (p=ns)	70,7 ± 13,2 (p=ns)	91,0 ± 11,8 (p=ns)	104,7 ± 11,2 (p=ns)	113,8 ± 10,8 (p=ns)	121,7 ± 12,0 (p=ns)	
	Mean IVF	52,2 ± 4,2	28,1 ± 4,8			49,9 ± 8,8							
Bodri et al,, 2015	Mean ICSI	22,6 ± 2,9 (P<0.001)	25,3 ± 3,1 (p<0.001)	36,4 ± 4,1 (p=0.005)	37,8 ± 4,6 (p=ns)	50,7 ± 7,0 (p=ns)	58,8 ± 9,4 (p=ns)	72,6 ± 10,0 (p=ns)		104 ± 10,5 (p=ns)	114,5 ± 13,0 (p=ns)		
	Mean IVF	24,1 ± 3,4	26,7 ± 3,4	37,7 ± 4,5									

RESEARCH QUESTION

With the rapid progression in technology within the ART field of medicine, TLS is an extremely unique and promising tool for improving embryo selection. Improvement of embryo selection generated from the vast amount of data available will only transpire the more time-lapse images that are annotated with data, which is standardized, to produce clinic-specific embryo kinetic models for prediction of success. The more models of embryo selection we create, the more we may understand whether an optimal morphokinetic profile exists.

This study will be focusing on establishing the profile value ranges of embryo development timings of fresh oocytes for Wijnland Fertility Clinic. The aim is to create the profile value for morphokinetic time frames, similar to the graphic seen in Figure 2. The study will include two sub-investigations, one to compare these timings with vitrified/warmed oocytes and a second, to compare fresh and vitrified/warmed sibling oocytes of patients who had both fresh and vitrified oocytes within the same treatment cycle.

OBJECTIVE AND AIM

Primary aim

The primary aim of this study was to establish the normative values using TLS technology for the time points of embryo development of embryos originating from fresh oocytes at the Wijnland Fertility Clinic. These established normative values were then compared to the developmental TL time points of vitrified/warmed oocytes to ascertain any significant differences between fresh and vitrified/warmed oocyte morphokinetics. The study included autologous oocytes as well as donor oocytes.

Secondary aim

The secondary aim of this study was to investigate the embryo development TL time points of sibling oocytes of patients having both fresh and vitrified oocytes used for treatment in the same insemination cycle.

"Normative values" is defined in this study as: morphokinetic time point values from a heterogeneous group of patients adhering to the inclusion and exclusion criteria of the study and specifically from the Wijnland Fertility Clinic, Stellenbosch.

HYPOTHESIS

Null Hypothesis H0

Embryos originating from fresh and vitrified oocytes will have similar embryo developmental time points as observed with time-lapse embryo incubation.

Alternative Hypothesis H1

Embryos originating from vitrified oocytes will have altered, inferior, embryo developmental time points as observed with time-lapse embryo incubation.

CHAPTER 2

MATERIALS AND METHODS

2.1 Study population, sample and sampling method

This was a retrospective analysis study conducted at the Wijnland Fertility Clinic (Stellenbosch, South Africa) from 2018 to 2019, on all ART cycles with fresh and vitrified/warmed oocytes (autologous and donor) between the years 2013-2017, sorted according to inclusion and exclusion criteria. All fertility patients who received donor oocytes gave written consent (see Appendix M). Only standard time-lapse generated embryo development records were used for the study and patient information was kept strictly confidential.

The approximate population size of the database consisting only of embryo development records between the aforementioned years, is ± n=5000 oocytes, of which ± n= 200 is vitrified oocytes.

2.2 Study design

The study design is schematically presented in Figure 10.

The study consisted of two major categories of data: fresh vs. vitrified/warmed (donor and autologous) and the explorative sibling study. The explorative sibling study data was examined separately to the primary objective of this study, however, was included within the primary objective data population. Both the primary and secondary objective of this study was evaluated with morphokinetc parameters.

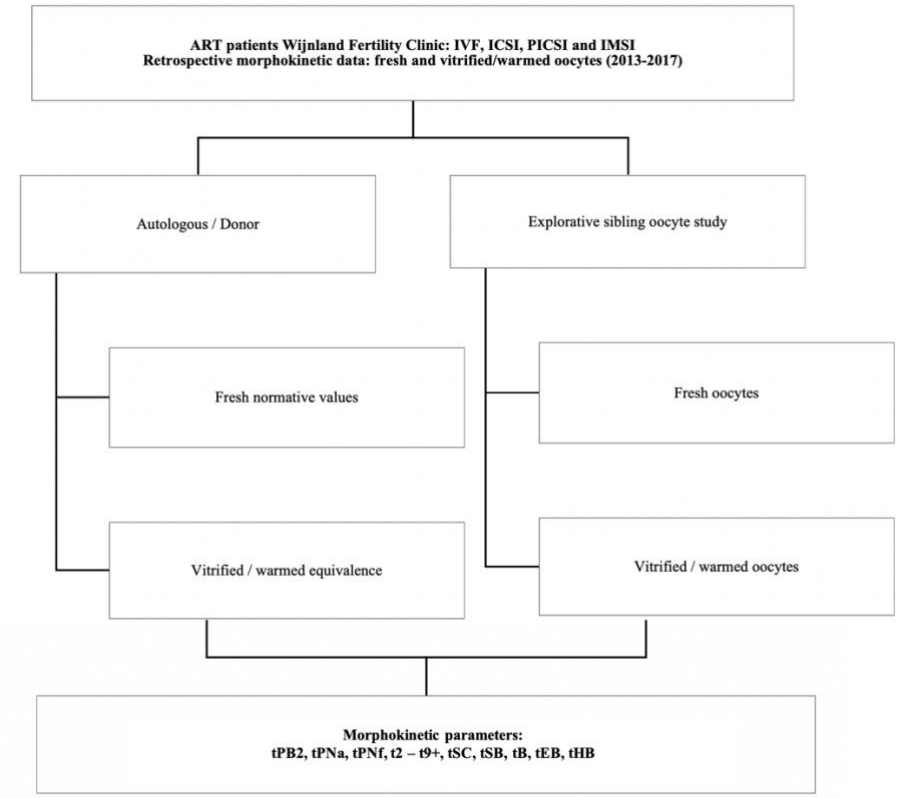


Figure 10: Study design

2.3 Data management and statistical analysis

The database from Wijnland Fertility Clinic of approximately 5000 individual oocytes tracked over time from fertilization to blastulation stage was used to compare fresh versus vitrified/warmed oocytes. All embryo development data was sorted according to fresh or vitrified origin.

Part I

The first part of the study was the analysis [descriptive data] of a large number of fresh oocytes (>3500) and the timing results (time points and time duration) for this subgroup was used to establish normative values (5% and 95% conditional percentiles) across the age range of women.

Part II

The second part of the study investigated the possible statistical differences between the fresh and vitrified/warmed oocyte morphokinetic TL time points. Traditional Quantile regression analysis was initially done, but for a more clinical useful outcome, an equivalence test was done. For the equivalence test an equivalence margin of 5% and 10% was used. The equivalence margin defines the range of values for which – in this case – the TL time points are "close enough" to be considered equivilant [Walker et al., 2010]. *"In practical terms, the margin is the maximum clinically accepted difference that one is willing to accept…."* [Walker et al., 2010].

Part III

The third part of the study consisted of a subgroup of women who had both fresh and vitrified/warmed oocytes fertilized within the same cycle. These participants provide a unique natural experiment for comparing the performance of fresh versus vitrified/warmed oocytes of the same cohort. A matched pair analysis was performed and the 95% confidence intervals of the difference in time were used to assess significant differences.

For the analysis, data was acquired from the standard, routine data files and database of Wijnland Fertility Clinic. Relevant medical/laboratory data only, was transferred to a Microsoft Excel spreadsheet specifically designed for the analyses (Appendix N).

A statistician from the Biostatistics Department of the South African Medical Research Council was consulted and the following appropriate statistical methods were used in the final analysis (Appendix P).
Descriptive statistics such as proportions, percentages for categorical variables and mean, medians, 25th and 75th quartiles were calculated for the continuous variables, especially the time variables for each of the oocyte groups. Too characterize the distribution of the different time epochs, the 2,5, 10, 25, 50, 75, 90, 97,5 percentiles were estimated. The 2,5th and 97,5th can be considered as the normal range for a particular group.

For the comparison of the time points between the fresh (normative) and vitrified/warmed oocyte subgroups (ICSI only) two approaches were used:

Quantile (median) regression model (Stats v15) was used to see if the **difference in median times** was statistically significant (CI:95%) and $p<0.05$ was regarded as statistically significant.

The two one sided test (TOST) test (Schuirmann, 1987) was used to assess **equivalence** based on the 90% confidence interval for the median difference in time points between the two groups. Two sets of equivalence margins were established a prior. The margins were defined in terms of the median time of the fresh (normal) oocytes and a 5% and 10% margin were specified. This confidence interval was estimated using quantile regression in Stats v15 (equivalence was regarded as $p<0.05$).

With regards to the literature where TL timings of fresh versus vitrified/warmed oocyte populations were available, there was a trend of a ±10% difference in timings (Desai et al., 2014; Chamayou et al., 2015; De Gheselle et al., 2019). Therefore, a 5% (Cobo et al., 2017) and 10% (De Gheselle et al., 2019) level was chosen in accordance to relative literature.

With regards to the exploratory sibling study, the method used included:

Matched comparison was done in a small study were both normal and frozen oocyte were used in the same fertilization attempt to control for confounders. The numbers of attempts that were found were small and simple descriptive statistics were done.

2.7 Methods

2.7.1 Data collection

Data was obtained from the existing medical records from Wijnland Fertility. The data collection sheet can be seen in Appendix N.

2.7.2 ART procedures

The following standard, routine procedures were used in this retrospective study (Appendices A-L).

Ovarian stimulation

The three phases of follicular stimulation, estrogen supplementation and luteal phase support were conducted according to the Wijnland Fertility ovarian stimulation standard operating procedure (SOP) – (Appendix A).

Oocyte retrieval

A standard oocyte retrieval procedure according to the SOP of Wijnland Fertility Clinic was used (Appendix B). Ova are collected via a process called 'aspiration' which is performed by the fertility specialist on duty. This process involves retrieving ova from the women's ovaries and aspirated follicular fluids are then examined to pick up and collect all cumulus oocyte complexes (COC) present. The found COCs are then further examined, as presented in Appendix C.

Semen Preparation

Semen was processed using the standard, routine protocols for gradient centrifugation. The standard protocols for semen preparation are presented in Appendix D.

Fertilization/insemination process

Mature MII oocytes were inseminated using the standard protocols for IVF, ICSI and IMSI. These 4 procedure's SOPs can be found in Appendix E and F.

Embryo culture

Standard embryo culture methods were used and are presented in Appendix G and 3. Different media was used over the 5-year period. 2013 – 2014 Global Total was used, while SAGE 1-step was used from late 2014 onwards.

Embryo evaluation

Standard morphokinetic evaluation for quality and morphology were annotated using EmbryoScope™ technology along with the clinic's embryo development sheet (Appendix H; 1-5).

Embryo transfer

A standard embryo transfer procedure was followed (Appendix I). In general, one embryo was transferred using a standard embryo transfer method.

Oocyte vitrification/thawing

The standard operating manual supplied by CryoTech™ and Kitazato™ was used (Appendix J and K; 6 - 9).

Consent forms

The relevant consent forms from Wijnland Fertility were used, as seen in Appendix M.

2.7.3 Inclusion criteria

All IVF, ICSI and IMSI treated patients (autologous and donor oocytes) during the time frame of the study: 2013 – 2017

- All available data on fresh and vitrified oocyte cycles
- All female recipient ages
- All donor ages
- Cryopreserved donor spermatozoa
- All male diagnoses
- Same sex couples
- Surrogacy couples

2.7.4 Exclusion criteria

- Oocytes with missing data points
- Oocytes with irregular divisions, data points after the irregular division occurred
- Irregular cleavages (reverse cleavage (RC))
- arrested embryos
- Erratic division
- Degenerated embryos
- Abnormal fertilization (1PN, 3PN or 4PN)
- Oocytes that did not fertilize, rescue-ICSI (RICSI)
- Germinal vesicle (GV) oocytes
- Missing data due to electricity outages

- Missing data due to services of the TL incubator
- Day 3 vitrification
- System tests and test run slides
- Embryos that did not reach blastocyst stage

The effect of sexually transmitted infections on embryo development is yet unknown, thus known positive infections are excluded to reduce statistical noise in establishing normative values. In addition, in donor oocytes treatment cycles, all gamete donors are tested for HIV and infectious diseases and are not accepted as donors when they test positive. This is in accordance with the National Health Act of 2003.

CHAPTER 3

RESULTS

The Wijnland Fertility Clinic patient population was assessed in order to extrapolate a normative data range (see definition under "Secondary aim" in Chapter 2) using the fresh oocyte population of embryos. The data range was collected based on a collection of various time points during the development of the mentioned embryos using a TLS. Vitrified/warmed oocyte population embryos were also examined and compared to the normative range in order to determine if there was a significant difference in the morphokinetic development of fresh versus vitrified/warmed oocytes in terms of time points. A subpopulation of sibling oocytes was also examined in order to determine if there was, if any found from the primary outcome, a similar difference between homogenous oocyte cohorts.

3.1 Study population

3.1.1 Estimated patient population

Prior to the approval of this study by the Health Research Ethics Council (HREC) the available data from the years 2013 to 2017 included an approximate sample population of n=±5000 oocytes, of which n=±200 were vitrified/warmed oocytes.

3.1.2 Exact patient population

As seen in Table 3, the exact population size before exclusion criteria were applied was n=5131.

The data was categorized into oocyte history (fresh or vitrified/warmed) and oocyte source (autologous or donor). The subpopulation of sibling oocytes was examined separately, and an exact population size was determined after the refined total population of n=2120 was concluded, and therefore a data usage rate was not calculated.

Table 3: Summary of the oocyte population sizes before exclusion criteria was applied

Raw data population			
			Total
Oocyte history	Vitrified/warmed	184	**5131**
	Other	101	
	"Unknown"	15	
	"Blank"	20	
	Fresh	4811	
Oocyte source	Autologous	4310	
	Donor	786	
	"Unknown"	15	
	"Blank"	20	
Final population size			
			Total
Oocyte history	Vitrified/warmed	179	**2120**
	Fresh	1941	
Oocyte source	Autologous	4310	
	Donor	786	
	Sibling oocytes	57	**57**

3.1.3 Refined patient population

The refined patient population was extrapolated post HREC approval and after exclusion criteria were applied. The data was managed in two steps: first, to obtain all data from the TLS export to obtain exact population sizes (Table 3) and second, to analyze each data point individually in order to apply the exclusion criteria. Upon the detailed inspection of the data, the following was recorded and subsequently excluded: irregular cleavages (reverse cleavage (RC)), arrested embryos, erratic division, degenerated embryos, abnormal fertilization (1PN, 3PN or 4PN), oocytes that did not fertilize, rescue-ICSI (RICSI), germinal vesicle (GV) oocytes, missing data due to electricity outages, missing data due to services of the TL incubator, day 3 vitrification, system tests and test run slides. Raw data included data points where the cell was either blank or had an "unknown" value. If these cells could not be repaired by examining each case individually these data points were also excluded.

The refined population size decreased dramatically with a data usage rate of 41,3% (n=2120), as seen in Figure 11. From Table 3 and Figure 11 it is clear the majority of the oocytes in the study population was fresh and autologous.

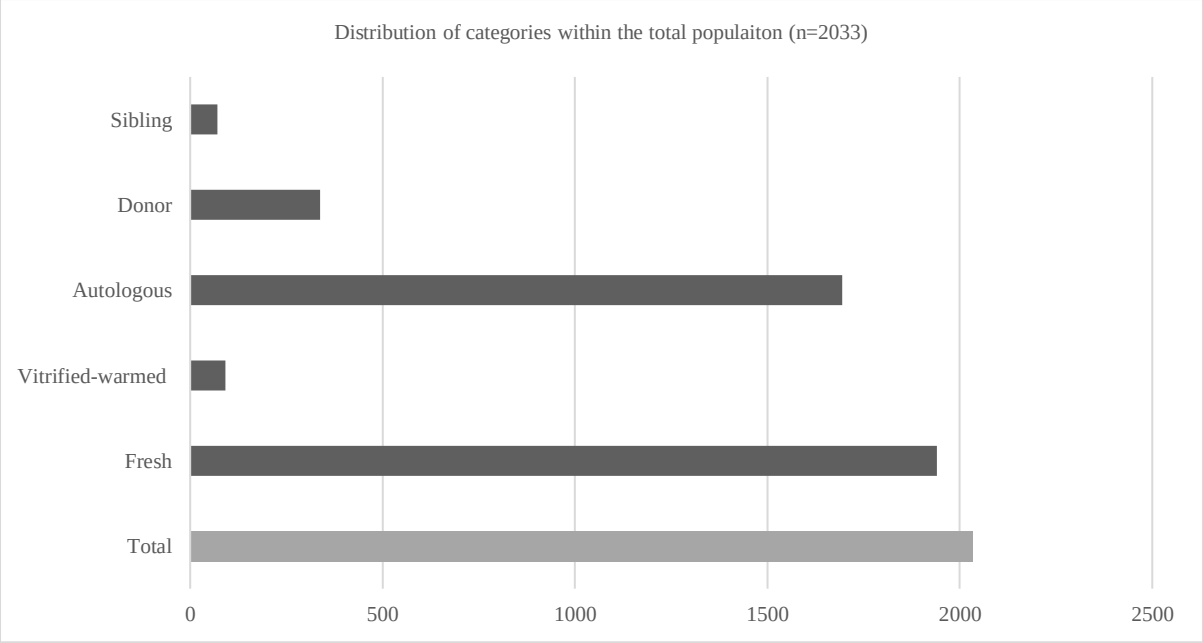


Figure 11: Graph of the distribution of categories within the refined population (n=2120)

A. Descriptive statistics

3.2 Fresh oocyte population

3.2.1 Centile values (hours) for time points for the fresh oocyte population (normative range)

The normative range was primarily formulated by estimating the relevant centiles for the morphokinetic development time points of relevant cell stages within the fresh oocyte population. The normal range was considered as the two centiles that contain 95% of the underlying population, thus the 2,5th and 97,5th percentile was recorded as such values. The confidence interval of these two estimates reflect the uncertainty around the estimate, however due to the large sample size (n=2120) this uncertainty was negligible.

The analysis of each time point contained 95% of the normative populations that exhibited time points specific to a cell stage (PN, tPNa, tPNf etc.,) between the 2,5th and 97,5th percentile in hours. These values are presented in Table 4. A median value (50% centile) was also recorded for each time point (Table 4), in order to easily compare this normative range to existing literature.

Table 4: Presentation of centile values for the normative time points (hours) for each TL event of the fresh oocyte population

		Centiles (hours)		
TL event	**Observations**	**2,5 %**	**50 % (Median)**	**97,5%**
tPB2	1349	1,88	3,68	7,67
tPNa	1382	4,78	7,33	12,70
tPNf	1408	18,81	23,10	30,51
t2	1415	21,53	25,80	33,83
t3	1413	30,73	36,83	46,78
t4	1412	32,08	37,65	49,94
t5	1407	39,04	49,63	64,01
t6	1413	42,36	50,89	67,74
t7	1409	43,71	52,40	72,92
t8	1413	44,48	54,51	82,36
t9	1413	52,18	68,52	90,12
tSC	1413	62,00	83,50	106,79
tSB	1395	84,07	97,25	120,81
tB	1317	91,20	105,37	134,56
tEB	960	98,28	111,84	139,64
tHB	137	104,64*	114,40	148,54*

*Lower (upper) confidence limit held at minimum (maximum) of sample.

3.2.2 Centile values (hours) for the duration between each time point (centile of difference) for the fresh oocyte population (normative range)

The centiles of difference were defined as the duration between each time point (hours). The centiles of difference were estimated in the same manner as with the centiles for each time point, however, were calculated using the difference between each time point (Table 5). The duration of any given embryo at each time point was considered as the two centiles that contain 95% of the underlying population, thus the 2.5th and 97.5th percentile was recorded as such values; as with the estimates of each time point (Table 4).

Table 5: Presentation of centile values for the normative duration between time points (hours) (centile of difference) for each TL event of the fresh oocyte population

	Centiles (hours)			
Variable	**Observations**	**2,5 %**	**50 % (Median)**	**97,5%**
t2 to t3	1415	8,44	11,01	13,79
VP duration	1380	10,75	15,51	21,79
ECC2 duration	1348	17,51	21,86	28,78
S2 duration	1412	0,00	0,50	7,19
t3 to t4	1413	0,00	0,50	7,19
t4 to t5	1412	0,50	12,25	17,34
t5 to t6	1407	0,00	0,75	12,97
t6 to t7	1413	0,00	1,00	14,00
t7 to t8	1409	0,00	1,25	17,50
t8 to t9	1413	0,00	13,50	23,69
t9+ duration	1413	0,00	13,26	37,76
ECC3 duration	1409	11,51	16,01	38,47
S3 duration	1404	0,75	3,75	25,21
tSC to tSB	1413	3,50	13,50	35,04
tSB to tB	1395	3,75	8,33	21,77
tB to tEB	1317	3,00	8,00	20,44
tEB to tHB	960	0,00	5,37	19,02
tHB duration	137	0,00	2,98	15,75

A summary of the median times (hours) for the duration between time points for fresh oocytes are graphically displayed in Figure 12 (Appendix U). This graphic representation of the centiles of difference allows a visual representation of the time (hours) any given embryo will spend at each cell stage.

Figure 13 (Appendix U) gives a further graphic representation of the cell cycle durations of the embryos within the normative range.

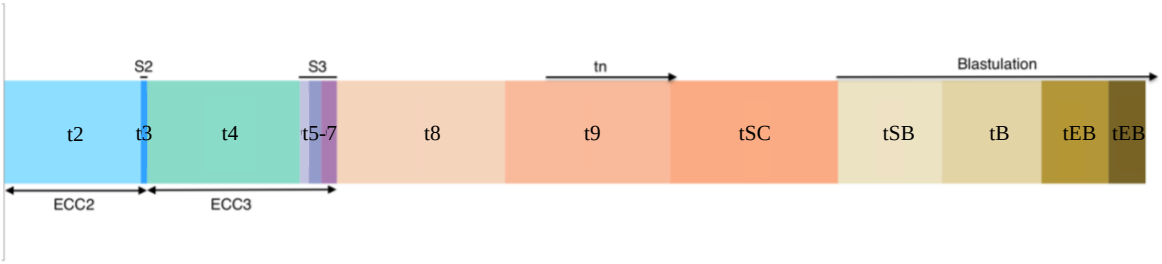


Figure 12: Diagram presenting median fresh embryo cell stage time durations (hours) for the fresh oocyte (normative) population

Figure 13 (Appendix U) gives a further graphic representation of the cell cycle durations of the embryos from the fresh oocytes within the normative range.

Figure 13: Median fresh embryo cell cycle time duration (hours) for the normative population

3.2.3 Centile values (hours) for time points for the fresh oocyte population (normative range) for the different insemination methods (ICSI, IVF and IMSI)

The fresh oocyte population consisted of the following insemination methods: ICSI (n=982; 69,30%), IMSI (n=226; 15,95), and IVF (n=209; 14,75%). The results are presented in Figure 16 and Table 5.

The only notable observed differences between time points for insemination method were within the IVF arm. Differentiation was observed from t9 onwards (Figure 14 and Table 6), where it was observed that embryos that were fertilized by IVF had a shorter median time point (hours) than their ICSI and IMSI counterparts, respectively (t9: 67,86 vs 68,43 and 69,58; tSC: 81,52 vs 83,13 and 86,58; tSB: 94,99 vs 97,32 and 99,39; tB: 103,86 vs 105,3 and 107,39). It is also interesting to note that IMSI time points were generally later than the IVF and ICSI time points.

The delayed IVF median time point (hours) did, however, accelerate when reaching the blastocyst stage. At tB, median time points plateaued (103,86 vs ICSI 105,3 and IMSI 107,39) and increased from tEB (111,53 vs ICSI 111,71 and IMSI 112,27) to tHB (115,82 vs ICSI 113,9 and IMSI 113,51), as seen in Table 6 and Figure 14.

Table 6: Presentation of centile values for the normative time points (hours) for each TL event of the fresh oocyte population according to insemination method (IVF, ICSI, IMSI)

	ICSI	IMSI	IVF
TL events	**Centile median (50%) (hours)**		
tPB2	3,56	3,49	4,40
tPNa	7,21	7,66	7,20
tPNf	23,05	23,62	23,01
t2	25,68	26,25	25,55
t3	36,74	37,21	36,80
t4	37,49	38,00	37,89
t5	49,67	50,05	49,13
t6	50,92	51,18	50,31
t7	52,42	52,42	52,08
t8	54,58	54,40	54,22
t9	68,43	69,58	67,86
tSC	83,13	86,58	81,52
tSB	97,32	99,39	94,99
tB	105,30	107,39	103,86
tEB	111,71	112,27	111,53
tHB	113,90	113,51	115,82

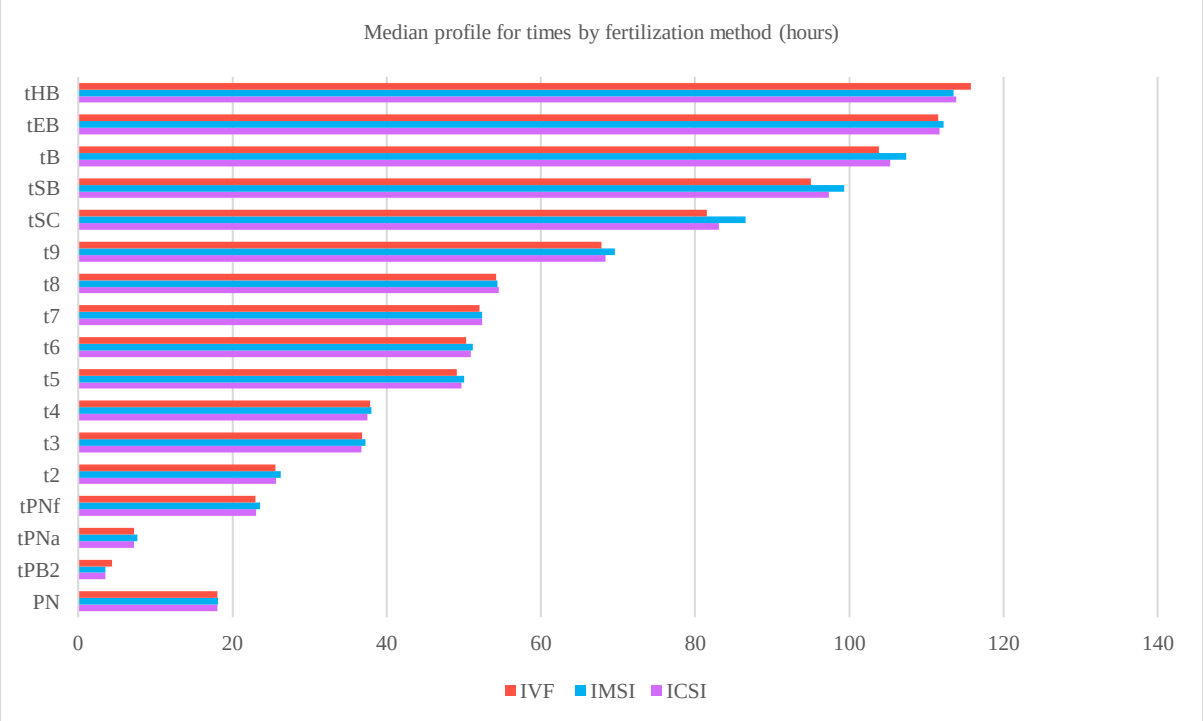


Figure 14: Bar graph showing the median values (hours) of each time point recorded for the fresh oocyte population according to insemination method (IVF, IMSI, ICSI)

3.2.4 Centile values (hours) the duration between each time point (centile of difference**)** for the fresh oocyte population (normative range) for the different insemination methods (IVF, ICSI, IMSI)

The centiles of difference (time (hours) between each time point) by insemination method was calculated in the same manner as centiles of difference for the entire normative range population. The median of each insemination method was recorded, as seen in Table 7 and Figure 15 and 16.

Table 7: Presentation of centile values for the normative duration between time points (hours) (centile of difference) for each TL event of the fresh oocyte population for the respective insemination methods (IVF, ICSI, IMSI)

Variables	**ICSI**	**IMSI**	**IVF**
	Median centiles of difference (50%) (hours)		
t2 to t3	11,02	11,13	11,00
VP duration	15,51	15,51	15,51
ECC2 duration	22,00	22,52	20,69
S2 duration	0,50	0,50	0,50
t3 to t4	0,50	0,50	0,50
t4 to t5	12,20	12,26	11,75
t5 to t6	0,75	0,74	0,50
t6 to t7	1,00	1,00	0,82
t7 to t8	1,25	1,25	1,25
t8 to t9	13,50	13,39	13,26
t9+ duration	12,93	15,50	12,50
ECC3 duration	16,25	16,10	15,51
S3 duration	2,00	3,75	3,51
tSC to tSB	13,76	12,70	13,24
tSB to tB	8,50	8,49	8,00
tB to tEB	8,00	8,00	8,50
tEB to tHB	5,50	4,76	6,25
tHB duration	3,00	1,43	0,75

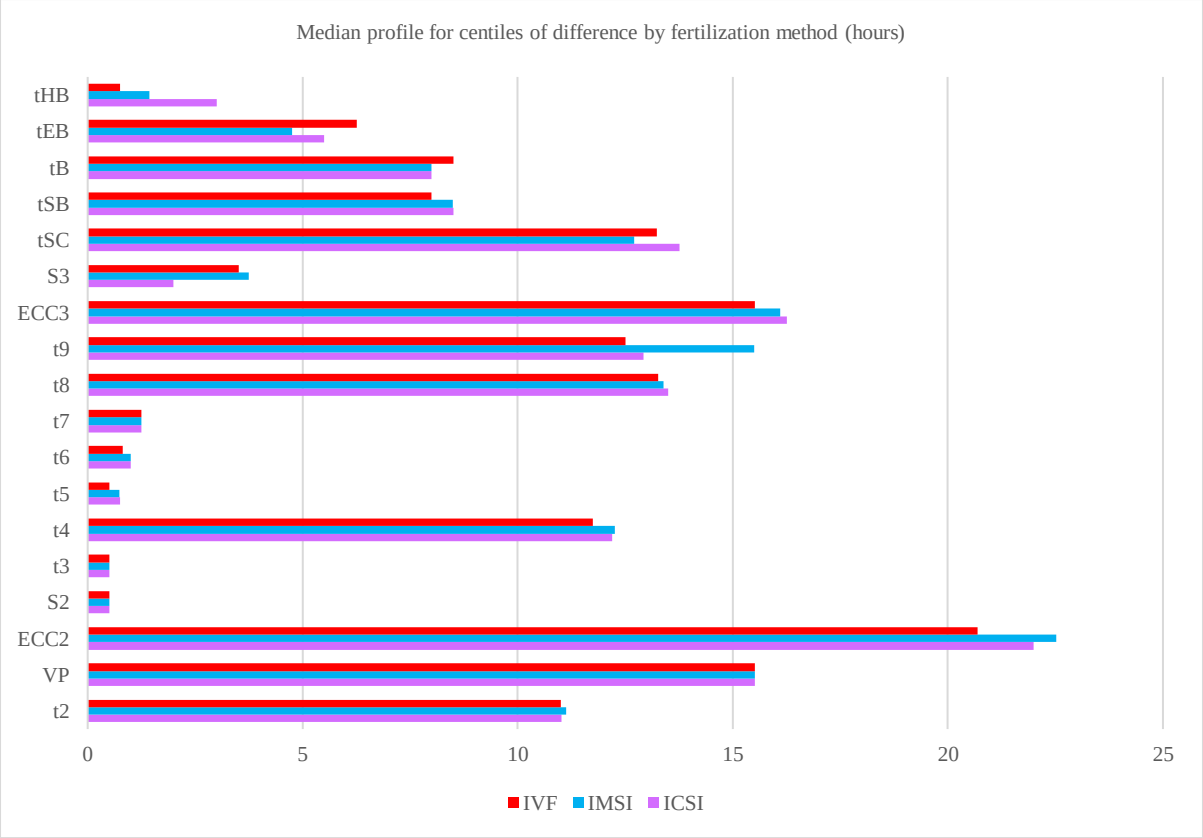


Figure 15: Bar graph showing centile values for the normative duration between time points (hours) (centile of difference) for each TL event of the fresh oocyte population for the respective insemination methods (IVF, ICSI, IMSI)

The observations made for the centiles of difference by insemination method was that IVF fertilized oocytes had shorter duration times during early cleavage to compaction when compared to ICSI and IMSI (Figure 16), respectively (t4: 11,75 vs 12,20 and 12,26 and 11,75; t6: 0,82 vs 1,00 and 1,00; t8: 13,26 vs 13,50 and 13,39; t9: 12,50 vs 12,93 and 15,50). It was observed, however, that the IVF population did exhibit longer duration times during the blastulation stages of embryo development (tB: 8,50 vs 8,00 ICSI and IMSI; tEB: 6,25 vs 5,50 ICSI and 4,76 IMSI). It was also noted that the IMSI population exhibited longer duration times for ECC2 (22,52 vs 22,00 ICSI and 20,69 IVF), t9 (15,50 vs 12,93 ICSI and 12,50 IVF). Lastly, it was observed that ICSI duration for tHB was the longest (3,00 vs 1,43 IMSI and 0,75 IVF), however this could be due to the fact that ICSI observation numbers for duration of tHB was considerably larger compared to IMSI and IVF, respectfully (n=93 vs n=16 and n=19).

Figure 16 shows a graphic representation of the time spent at each cell stage during embryo development (Appendix U) for the different insemination methods – IVF, ICSI and IMSI respectively.

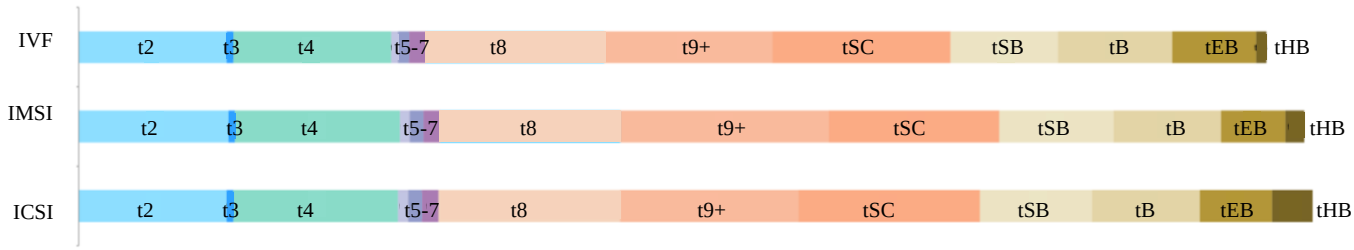


Figure 16: Diagram presenting median fresh embryo cell stage time durations (hours) for the normative population by insemination method (IVF, ICSI, IMSI)

3.3 Vitrified/warmed oocyte population

The total population for the vitrified/warmed oocyte category was n=179 (n=115 autologous and n=64 donor oocytes) (Table 8).

Table 8: Summary of vitrified/warmed oocyte population statistics

Category	Frequency	Percent
Autologous	115	64,25
Donor	64	35,75
N=179		

3.3.1 Centile values (hours) for time points for the vitrified/warmed oocyte population

Due to the small population size (n=179) the range of centiles were equivalent to the minimum and maximum values observed and this was true for all time points. For example, the minimum value recorded for t2=20,25 and therefore became the 2,5% centile for that time point.

The values are presented in Table 9.
A median value (50% centile) was also recorded for each time point in order to easily compare this normative range to existing literature.

Table 9: Presentation of centile values for the time points (hours) for each TL event of the vitrified/warmed oocyte population

		Centiles (hours)		
TL event	**Observations**	**2,5 %**	**50 % (Median)**	**97,5%**
tPB2	108	1,84	3,98	10,23
tPNa	110	4,14	8,37	15,50
tPNf	89	18,76	24,58	42,55
t2	83	20,25	27,98	46,07
t3	77	27,51	38,58	70,69
t4	73	32,63	40,78	85,58
t5	71	34,32	51,12	92,14
t6	69	37,18	53,79	94,03
t7	66	42,97	55,41	86,97
t8	61	43,28	61,74	86,97
t9	61	49,15	70,50	96,63
tSC	53	70,54	91,64	114,52
tSB	46	87,58	104,07	125,62
tB	40	95,69	112,34	150,95
tEB	29	98,88	116,28	163,30
tHB	6	114,14	121,80	139,48

3.3.2 Centile values (hours) for the duration between each time point (centile of difference) for the vitrified/warmed oocyte population,

The centiles of difference were defined as the duration between each time point (hours). Only the 50% centile value – the median – is presented (Table 10).

Table 10: Presentation of centile values for the duration between time points (hours) (centile of difference) for each TL event of the vitrified/warmed oocyte population

TL event	Observations	50% (Median)(hours)
t2 to t3	75	11,27
t3 to t4	67	0,75
t4 to t5	67	11,50
t5 to t6	64	1,25
t6 to t7	64	1,25
t7 to t8	59	2,00
t8 to t9	60	9,19
t9+ duration	51	21,76
tSC to tSB	46	11,50
tSB to tB	40	8,88
tB to tEB	29	7,75
tEB to tHB	6	4,38
tHB duration	6	3,90

3.4 Fresh vs vitrified/warmed oocyte population (ICSI insemination only)

It is important to note that for the analysis of this population, only ICSI insemination oocytes for fresh and vitrified/warmed oocytes were used.

3.4.1 Centile values (hours) for time points for the fresh vs vitrified/warmed oocyte population

When comparing fresh and vitrified/warmed oocyte sourced embryos for the median centile for time points (hours), the total fresh population oocytes had shorter duration times (vitrified/warmed were therefore delayed) from t2 to tHB, when compared to the vitrified/warmed oocyte population (t2: 25,8 vs 28,0; t3: 36,8 vs 38; t4: 37,7 vs 40,8; t5: 49,6 vs 51,1; t6: 50,9 vs 53,8; t7: 52,4 vs 55,4; t8: 54,5 vs 61,7; t9+: 68,5 vs 70,5; tSC: 83,5 vs 91,6; tSB: 97,2 vs 104,1; tB: 105,4 vs 112,3; tEB: 111,8 vs 116,3; tHB: 114,4 vs 121,8), as seen in Table 11 and Figure 17.

Table 11: Presentation of centile values for the time points (hours) for each TL event of the fresh vs vitrified/warmed oocyte population

	Median time (hours)		
TL event	**Fresh**	**Vitrified/warmed (ICSI only)**	**Vitrified/warmed delay (Yes/No)**
t2	25,8	28,0	Yes
t3	36,8	38,6	Yes
t4	37,7	40,8	Yes
t5	49,6	51,1	Yes
t6	50,9	53,8	Yes
t7	52,4	55,4	Yes
t8	54,5	61,7	Yes
t9+	68,5	70,5	Yes
tSC	83,5	91,6	Yes
tSB	97,2	104,1	Yes
tB	105,4	112,3	Yes
tEB	111,8	116,3	Yes
tHB	114,4	121,8	Yes

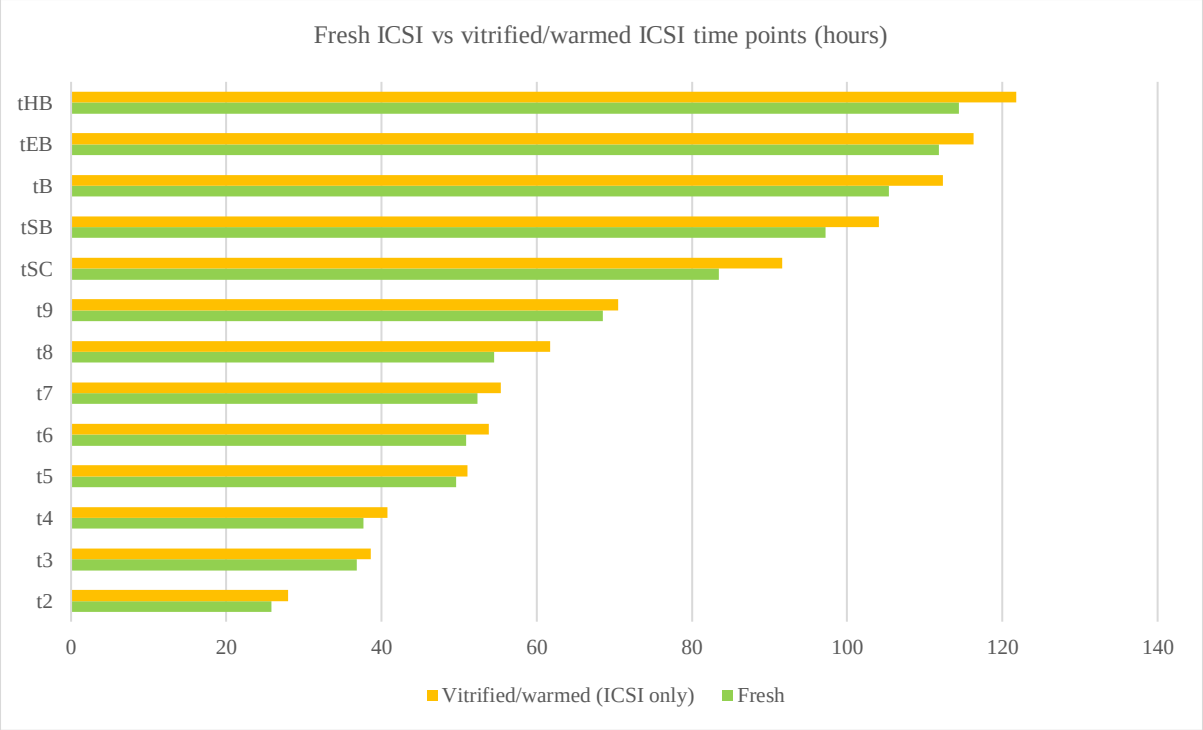


Figure 17: Bar graph showing median values for the time points (hours) for the fresh oocyte population vs the vitrified/warmed oocyte population

3.4.2 Centile values (hours) for the duration between each time point (centile of difference) for the fresh vs vitrified/warmed oocyte population

When comparing fresh and vitrified/warmed oocyte sourced embryos for the median centile of difference (hours), the overall fresh population oocytes had shorter duration times (vitrified/warmed were therefore delayed) from t2 to t3, t5 to t7 and t9, when compared to the vitrified/warmed oocyte population (t2: 11,02 vs 11,27; t3: 0,5 vs 0,75; t5: 0,75 vs 1,25; t6: 1,00 vs 1,25; t7: 1,25 vs 2,00; t9: 12,93 vs 21,76), as seen in Table 12, Figure 18 and Figure 19.

Figure 19 shows a graphic representation of the time spent at each cell stage during embryo development (Appendix U) for the fresh vs vitrified/warmed oocyte groups.

Table 12: Fresh vs vitrified/warmed oocyte source duration between time points (hours) (centiles of difference)

TL events	ICSI Fresh	ICSI Vitrified/warmed	Vitrified Delayed (Yes/No)
t2 to t3	11,02	11,27	Yes
VP duration	15,51	16,26	Yes
ECC2 duration	22,00	24,01	Yes
S2 duration	0,50	0,75	Yes
t3 to t4	0,50	0,75	Yes
t4 to t5	12,20	11,50	No
t5 to t6	0,75	1,25	Yes
t6 to t7	1,00	1,25	Yes
t7 to t8	1,25	2,00	Yes
t8 to t9	13,50	9,19	No
t9+ duration	12,93	21,76	Yes
ECC3 duration	16,25	17,26	Yes
S3 duration	2,00	8,13	Yes
tSC to tSB	13,76	11,50	No
tSB to tB	8,50	8,88	Yes
tB to tEB	8,00	7,75	No
tEB to tHB	5,50	4,38	No
tHB duration	3,00	3,90	Yes

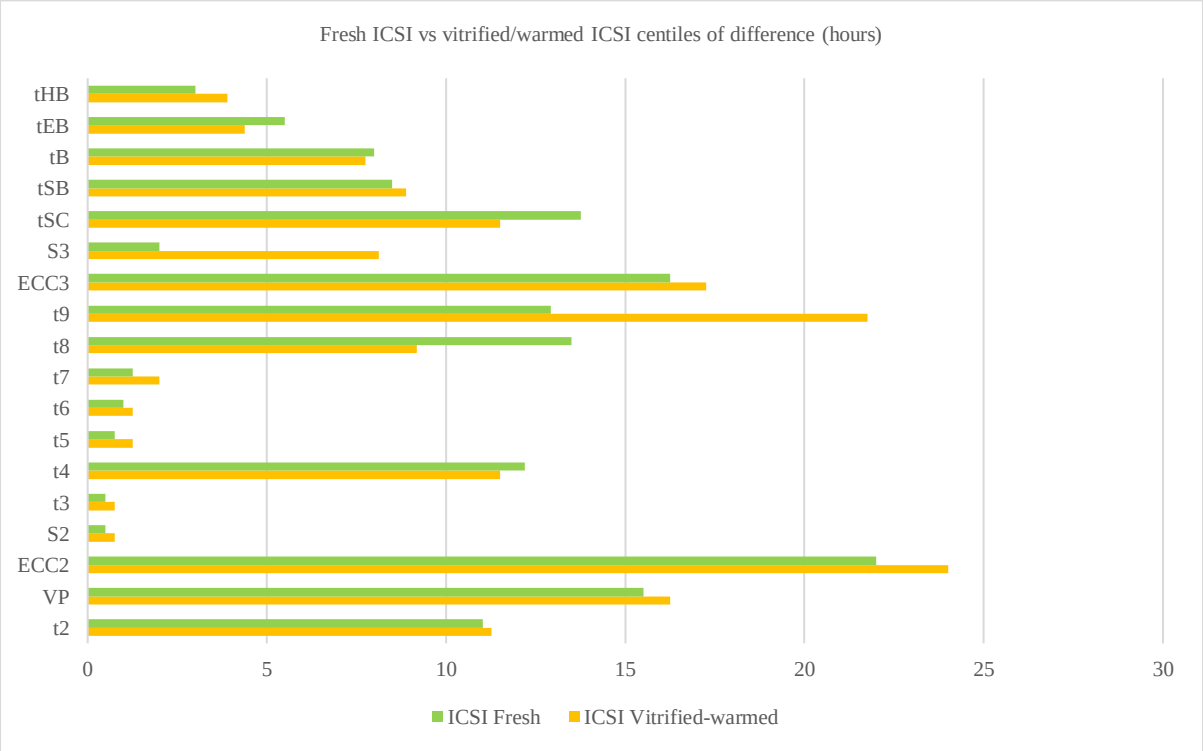


Figure 18: Bar graph showing the median duration between time points (hours) (centiles of difference) recorded for the fresh oocyte population vs the vitrified/warmed oocyte population

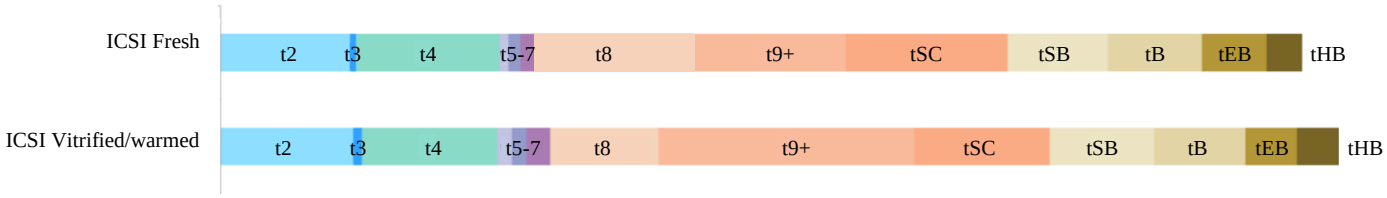


Figure 19: Diagram presenting median embryo cell stage time durations (hours) for the ICSI fresh oocyte population vs the vitrified-warmed oocyte population

B. Statistical data

The descriptive data showed clearly that fresh and vitrified/warmed oocyte populations have different time point timings as well as time durations from one time point to the next.

The clinical implication and significance of this result needs to be further explored.
A statistical analysis of the data was done to establish any significant differences between the two groups – fresh and vitrified/warmed morphokinetic information using TL.

Two statistical approaches were investigated: a) a Quantile (median) regression analysis and b) a two one-sided test (TOST) to test for equivalence.

Statistical analysis for the fresh vs vitrified/warmed comparison was done only on **ICSI** inseminated oocyte groups and also only for **TL time points.**

3.4.3 Quantile (median) regression analysis

A traditional comparative **quantile [median] regression analysis** was conducted in order to determine if there was a significant difference in time point values (hours) between fresh and vitrified/warmed oocytes at the 95% confidence level (CI). The results are presented in Table 12. This analysis was performed on ICSI only cycles (n=996).

It was found that most time point values were significantly different ($p<0.05$) when comparing fresh vs vitrified/warmed oocytes. For all time points tested the vitrified/warmed times were significantly longer and there was therefore a delay in development and reaching the specific time point for the vitrified/warmed oocyte group (Table 13). There was however no statistical difference in time point values for t5 ($p=0.068$; 95% CI) and t9 ($p=0.106$; 95% CI), although these time points were still delayed for the vitrified/warmed population.

Table 13: Presentation of the quantile median regression analysis comparing for significant difference in time points between fresh and vitrified/warmed oocytes groups (95% CI, ICSI only cycles)

Time point	Median Fresh	Median vitrified/warmed oocytes	Coefficient of Difference	p-value (95% CI)
tPB2	3,56	4,00	0,44	p<0.001
tPNa	7,21	8,38	1,17	p<0.001
tPNf	23,05	24,58	1,53	p<0.001
t2	25,69	27,98	2,29	p<0.001
t3	36,74	38,58	1,84	p=0.001
t4	37,52	40,78	3,26	p<0.001
t5	49,67	51,12	1,45	p=0.068
t6	50,93	53,79	2,86	p<0.001
t7	52,43	55,41	2,98	p=0.001
t8	54,60	61,74	7,14	p<0.001
t9+	68,44	70,50	2,06	p=0.106
tSC	83,13	91,64	8,51	p<0.001
tSB	97,32	104,46	7,14	p<0.001
tB	105,34	112,51	7,17	p=0.001
tEB	111,71	116,28	4,57	p=0.008
tHB	113,90	122,43	8,53	p=0.013

3.4.4 Two one-sided test (TOST) to test for equivalence

A Two one-sided test (TOST) to test was then done in order to test for equivalence in time point values [hours] between fresh and vitrified/warmed oocytes. Equivalence margins of 5% and 10% were decided on and tested for equivalence. Equivalence defines a range of values for which efficacies are close enough to be considered equivalent (Walker et al., 2010). A 90% CI is used to test against and if equivalence is established, it yields $p<0.05$ significance.

The results are presented in Table 14. This analysis was performed on ICSI only cycles (n=996).

From Table 14 it is clear that at the 5% level, none of the time point values is equivalent. This result is similar to the quantile regression analysis – showing that the time points between fresh and vitrified/warmed oocyte groups are significantly different. A 5 % level is however quite strict and equivalence at 10% was also tested. This resulted in several time point values now being equivalent. However, 9 of the time points still showed non-equivalence and were still significantly different. They included: tPB2, tPNa, t2, t4, t8, tSC, tSB and tB.

Table 14: Two one-sided test (TOST) to test for equivalence for TL time points in fresh versus vitrified/warmed oocyte populations,

Time Point	Median Fresh	Median Vitrified / warmed	5% Lower	5% Upper	10% Lower	10% Upper	90% CI Lower	90% CI Upper	Equivalence at 5%	Equivalence at 10%
tPB2	3,56	4,00	-0,178	0,178	-0,356	0,356	0,237	0,646	No	No
tPNa	7,21	8,38	-0,361	0,361	-0,721	0,721	0,829	1,153	No	No
tPNf	**23,05**	**24,58**	**-1,153**	**1,153**	**-2,305**	**2,305**	**0,854**	**2,210**	**No**	**Yes**
t2	25,69	27,98	-1,285	1,285	-2,569	2,569	1,590	2,980	No	No
t3	**36,74**	**38,58**	**-1,837**	**1,837**	**-3,674**	**3,674**	**0,988**	**2,700**	**No**	**Yes**
t4	37,52	40,78	-1,876	1,876	-3,752	3,752	2,314	4,223	No	No
t5	**49,67**	**51,12**	**-2,484**	**2,484**	**-4,967**	**4,967**	**0,134**	**2,758**	**No**	**Yes**
t6	**50,93**	**53,79**	**-2,547**	**2,547**	**-5,093**	**5,093**	**1,663**	**4,075**	**No**	**Yes**
t7	**52,43**	**55,41**	**-2,622**	**2,622**	**-5,243**	**5,243**	**1,537**	**4,426**	**No**	**Yes**
t8	54,60	61,74	-2,730	2,730	-5,460	5,460	4,999	9,293	No	No
t9+	**68,44**	**70,50**	**-3,422**	**3,422**	**-6,844**	**6,844**	**-0,041**	**4,170**	**No**	**Yes**
tSC	83,13	91,64	-4,157	4,157	-8,313	8,313	5,394	11,618	No	No
tSB	97,32	104,46	-4,866	4,866	-9,732	9,732	4,346	9,928	No	No
tB	105,34	112,51	-5,267	5,267	-10,534	10,534	3,640	10,687	No	No
tEB	**111,71**	**116,28**	**-5,586**	**5,586**	**-11,171**	**11,171**	**1,588**	**7,561**	**No**	**Yes**
tHB	113,90	122,43	-5,695	5,695	-11,390	11,390	3,086	13,979	No	No

The equivalence levels were calculated by determining the lower and upper confidence limit (90% CI) and then comparing them with the predefined theoretical equivalence margins (5% and 10%). If the confidence interval with the limits (5% or 10%) turned out to be completely included in the theoretical range, it was decided in favor of the hypothesis of equivalence.

This was the case whenever both the value of the lower 90% CI was larger than the lower limits of 5% or 10% and the upper 90% CI were smaller than the upper limits of 5% and 10%. In other words, if the 5% or 10% equivalence margin ‘engulfed’ the 90% CI, equivalence was accepted.

At the 5% level of equivalence it was found that no time points showed equivalence ($p<0.05$; 90%CI; 5%). This indicated that there was a significant delay for all time points within the vitrified/warmed oocyte population, when compared to the fresh oocyte population ($p<0.05$; 90%CI; 5%). At the 10% level of equivalence there were heterogeneous results regarding equivalence and non-equivalence, due to the broader testing level of 10%, compared to the stricter level of 5%. It was found at the 10% level that there was significant non-equivalence for time points tPB2, tPNa, t2, t4, t6, t8, tSC, tSB, tB and tHB ($p<0.05$; 90%CI; 10%). This indicated that for the times stated for non-equivalence there was a delay in timings within the vitrified/warmed oocyte population. Conversely, also at the 10% level, it was found that there was significant equivalence for time points tPNf, t3, t5, t7, t9+ and tEB ($p<0.05$; 90%CI; 10%). This indicated that for the time points stated there was no statistically significant difference in timings with regards to the fresh and vitrified/warmed oocyte population.

3.5 Exploratory study: sibling oocyte comparison

3.5.1 Population

Due to the small population of this study (n=57) the study was classified as exploratory. Seven patients were included in this study, which resulted in the population size of n=57 oocytes (n=37 Fresh and n=20 vitrified/warmed), as seen in Table 15. This population consisted of n=6 autologous patient's oocytes and n=1 donor oocytes. All seven patients included within this study included frozen and fresh oocytes that were used within the same cycle; defining them (fresh and frozen) as sibling oocytes.

Table 15: Sibling oocyte numbers showing the distribution of the oocyte population,

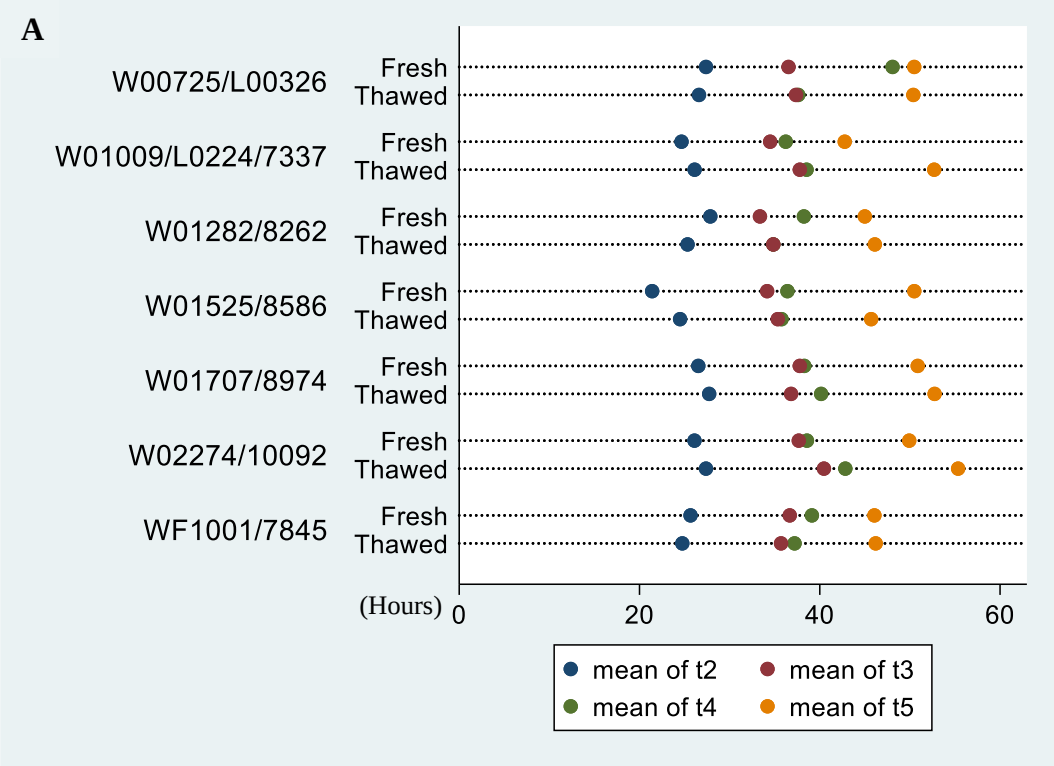
	Oocyte history		
Patient ID	**Fresh**	**Vitrified/warmed**	**Total**
W00725/L00326	5	2	7
W01009/L0224/7337	10	3	13
W01282/8262	4	1	5
W01525/8586	1	3	4
W1707/8974	3	3	6
W02274/10092	8	2	10
WF1001/7845	6	6	12
Total	**37**	**20**	**57**

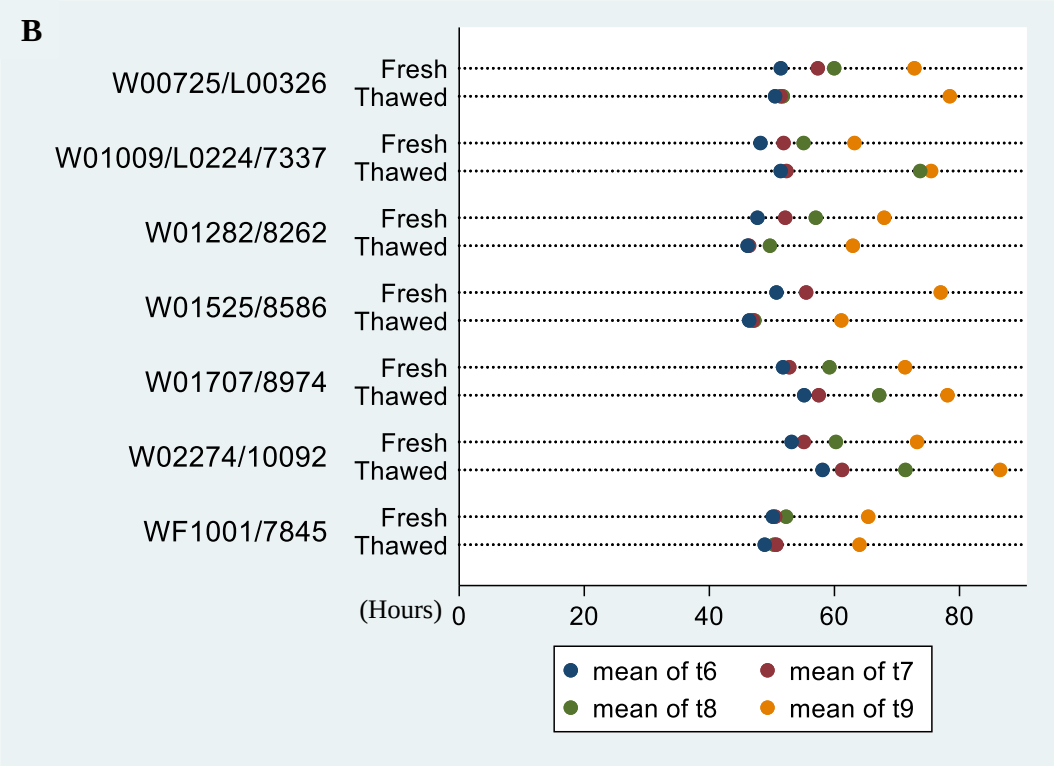
3.5.2 Sibling comparison

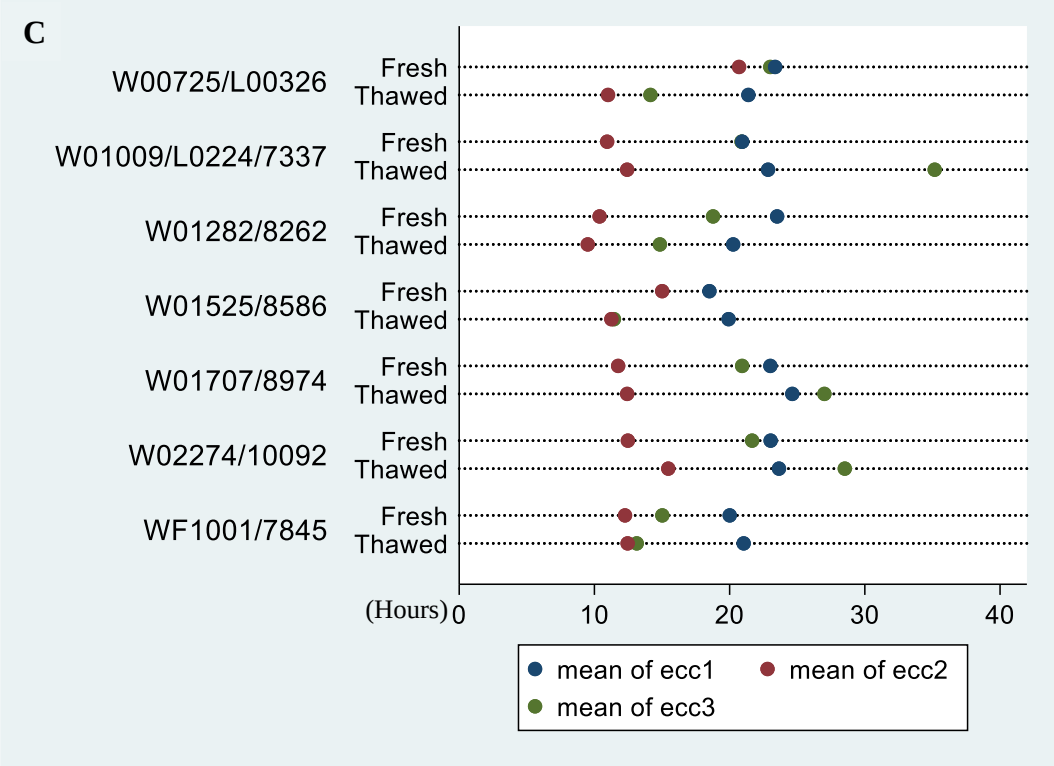
Figure 20 A-F represents dot plot graphs of mean times for each patient (n=7) for fresh and vitrified/warmed time points.

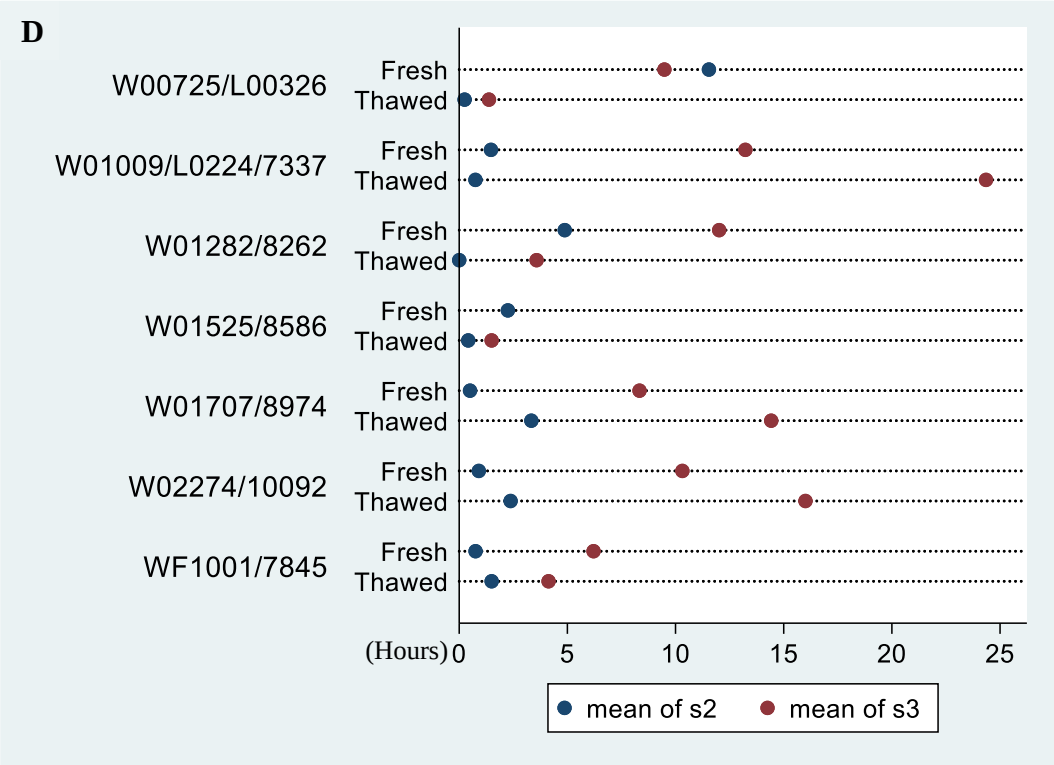
It was observed that for 4 patients (W01009/L0224/7337, W01282/8262, W01525/8586 and W02274/10092) t3 was longer for the vitrified/warmed oocyte population when compared to the fresh oocyte population. In the other 3 patients the opposite observation was noted.

It is clear from these results that there was no consistent pattern observed between the fresh and vitrified-warmed oocytes. It was recorded that select patients had longer times for time points and for others the opposite occurred. Thus, the difference in times between fresh and vitrified-warmed oocytes from the same cohort was considered as random.









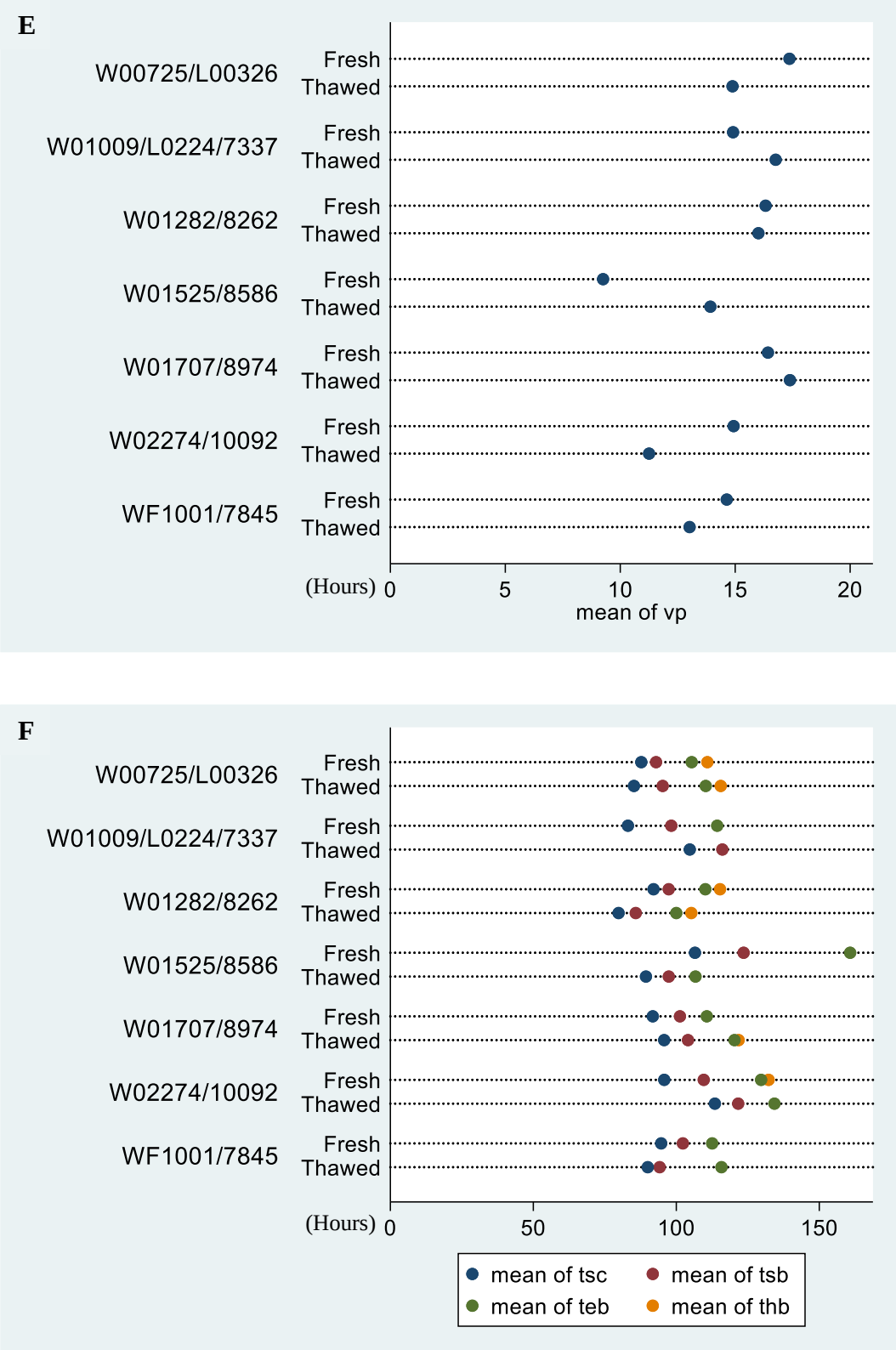


Figure 20: Sibling oocyte comparison of fresh and vitrified/warmed oocyte populations for duration of all time points during embryo development

3.5.3 Matched design linear regression model

A **matched design linear regression model** adjusting for clustering of values within patients was also used to estimate the mean differences between fresh and frozen oocytes in the sibling oocyte study.

The results are represented in Table 16.

There was no significant difference for any of the time points analysed (p>0,05). This result is different form the result of the main study – but can be explained due the very small number of oocytes included in the study.

Table 16: Presentation of the results of a matched design linear regression model adjusting for clustering of values within patients showing in a sibling oocyte study comparing fresh vs vitrified/warmed TL morphokinetic time points (hours)

Time point (hours)	Fresh mean (hours)	Vitrified/warmed (hours)	P value
t2	25,91	25,22	p=0.920
t3	35,98	36,72	p=0.391
t4	39,38	38,12	p=0.547
t5	47,02	49,24	p=0.307
t6	50,20	50,86	p=0.651
t7	53,18	52,39	p=0.650
t8	56,91	58,11	p=0.794
t9	68,45	71,04	p=0.503
tSC	90,45	94,14	p=0.477
tSB	101,59	101,88	p=0.952
tEB	118,44	114,12	p=0.450

CHAPTER 4

DISCUSSION

The main objective of this study was to determine a normative profile range for TL time points and then compare this profile to a vitrified/warmed oocyte counterpart population; analyzing via the use of TL imaging. Secondly, to perform a fresh vs vitrified/warmed oocyte comparison within a sibling population. The sibling study was exploratory due to the small population size of n=57 oocytes (7 patients).

It is well known that there are vast benefits of TLS within a laboratory setting. One such benefit highlighted throughout this study includes the ability to use TL data extracted from precise time points for different cell stages and divisions in order to collect and identify a population specific representation of morphokinetic embryo development trends and behaviors (Desai et al., 2014). Benefits of creating a laboratory specific baseline kinetic profile include aiding in possible predictions for future embryo developments (Chamayou et al., 2015; Cobo et al., 2017).

In this study, the fresh oocyte range was defined as the normative range, due to the exclusion criteria rendering the sample of embryos examined of better quality when compared to the entire Wijnland Fertility embryo population. Only embryos that made it to blastocyst stage were selected for the analysis, so that the best performing embryos were selected. With this process of selection for the normative range in mind, one can possibly assume that a compelling comparison can be made with literature that did not necessarily test for fresh versus vitrified/warmed oocytes. Literature exists that exhibits a morphokinetic comparison between euploidy and aneuploidy (Campbell et al., 2013) and implantation versus non-implantation (Meseguer et al., 2011; Herrero et al., 2013; Desai et al., 2014), among many other comparisons. The trend within such comparative studies is that the favorable outcome is associated with good blastocyst and embryo quality and this is the assumption that could be made regarding the normative range embryo population (good quality embryos) from this study. Perhaps the normative range, although not comparing for implantation rate (for example) could follow similar trends to the embryos that favored implantation and therefore similar trends may be assumed.

4.1 Fresh oocyte population

4.1.1 Time point analyses

Descriptive results for the fresh oocyte group for TL timings and/or profiles seem not to differ hugely from that available in the literature. There are, however, minor differences within timings for various time points that exist. One would expect small discrepancies among timings for morphokinetic embryo development, however, the general trend should follow existing morphokintetic profiles; which is evident within this study. The fact that there are small discrepancies further validates the need for laboratory specific baseline kinetic models, as suggested by Desai et al. (2014). In terms of the comparison of the time points for the fresh oocyte category to the literature timings found in Table 1, similar trends are exhibited across the studies listed.

In a study performed by Desai et al. (2014) embryos were selected based on known implantation data (KID+) and subsequently compared to embryos that where known to have non-implantation data (KID-). It can be expected that if using implantation rate as outcome, embryos that fell into the KID+ arm would be of better quality and subsequently have faster developmental time-points and more compact cleavage stage patterns, when compared to the KID- arm. When comparing the median normative values from the fresh oocyte group in the current study (Table 4 and 11) to that of Desai et al. (2014), there are similarities with the KID+ arm to the hour for time points tPNf, t2, and t3. The normative time points have faster times than the KID+ arm for t4 and t5, then subsequently lagging behind for time points t8 to blastulation. It must be stressed that the similar trends in our study of the normative values with the KID+ arm does not imply a similar outcome in terms of increased or decreased implantation rate. However, it can be assumed that because the trends are similar that the normative range population is comparable on a clinical setting, with other populations. Yet again, this also stresses the need for each laboratory to establish a baseline kinetic model, because of heterogeneous patient populations.

Similarly, when comparing the normative range (Table 4) to the study conducted by Campbell et al. (2013) (Table 2), there is an overall time lag within the normative range when compared to the mean euploidy rate arm time points. The only time point that was found to be similar to the nearest hour was for t3. Within the mean euploidy arm, however, there was a statistically significant trend of euploidy embryos being faster at compaction and blastulation when compared to the single and multiple aneuploidy arm. This by no means indicates that if there is a delay within the vitrified/warmed oocyte group during compaction and blasulation that they are more likely to be aneuploid, however, it does highlight the need for such a study (fresh versus vitrified/warmed) to be conducted; and again to ensure laboratories establish a baseline kinetic model.

4.1.2 Time difference (duration) analyses

In this dissertation, an infographic histogram graph model (durations) similar to that of Ciray et al. (2015) was used to create a visual representation of the Wijnland Fertility Clinic's 'baseline kinetic' profile or defined in this study as the normative profile. The aim of this model was to represent a summary of the top performing embryos within the TL population. The model was applied to the fresh oocyte-, fresh oocyte- according to insemination, vitrified/warmed oocyte- and vitrified/warmed oocyte-population for ICSI (Figures 12, 13, 16 and 19). For the

fresh oocyte population, it was observed that embryos spent the longest time at a stable phase of no divisions at t4, t8, t9 and tSC. A considerable amount of time was also spent stable at t2 (Figure 12). These findings correspond more or less to that of Ciray et al. (2015) where a similar model was used.

4.1.3 Insemination method analyses

Although it was not part of the study design and aim, morphokinetic information for different ART insemination methods was also analyzed.

The majority of the literature supports the notion that in general; ICSI fertilized oocytes develop faster than their IVF counterparts (Bodri et al., 2015; Kim et al., 2017). From our study, Figure 16 shows an opposing outcome, indicating that IVF yielded faster timings. Kim et al. (2017) recorded a lag in IVF fertilized oocytes until plateauing and catching up to their ICSI counterparts from t9+ to tEB. Bodri et al. (2015) also found a statistically significant lag in IVF fertilized oocytes from tPNf to t3. The opposite can be seen in Figure 17 whereby timings are relatively similar until t9+ to tEB where IVF is possibly considerably faster than ICSI and IMSI. It was noted that ICSI inseminated oocytes bypasses a specific process that is included in conventional IVF, which results in a differentiation process that is 1 hour faster on average until the 6-cell stage, however, not affecting pregnancy related outcomes (Kim et al., 2017).

A possible reason for the inverse trend occurring for the IVF fertilized oocytes within this study could possibly be attributed to the fact that within Wijnland Fertility Clinic the majority of cycles are ICSI. Subsequently, this could have skewed the IVF data due to the smaller sample size for the IVF category. Another possible reason could be due to the nature of the IVF patient population. Patients that are eligible for IVF within Winland Fertility Clinic are generally good prognosis patients, compared to poorer prognosis patients (especially male factor) for ICSI. This could have resulted in better quality embryos and thus tighter early cleavage timings, which is associated with higher chances of blastulation (Wong et al., 2010), a positive effect on embryo developmental potential (Yang et al., 2018) and higher chances of implantation (Meseguer et al., 2011).

4.2 Fresh vs vitrified/warmed oocyte population (ICSI insemination only)

It was very clear that when the time point data for the vitrified/warmed oocyte group from the current study was compared to that of fresh oocyte group, the former's time points were all delayed. These results were similar to that of the literature.

Three studies, included in Table 2, also examined the difference between fresh and vitrified/warmed oocytes (Chamayou et al., 2015; Cobo et al., 2017; De Gheselle et al., 2019). Their findings were similar to that of the current study – also exhibiting a lag trend within the vitrified/warmed group when compared to the fresh group.

Differences in time duration between time points was also different for the vitrified/warmed oocyte group as displayed in Figure 19. There was a trend towards the vitrified/warmed group lagging in development overall and specifically within the early cleavage stage. These findings are synonymous with the available literature (Chamayou et al., 2015; Milewski et al., 2015; Montjean et al., 2015; Cobo et al., 2017; De Gheselle et al., 2019) where vitrified/warmed oocytes where observed to lag behind their fresh oocyte counterparts by ±1 hour from t2 to blastulation (Cobo et al., 2017) or an average delay of 1,27 hours (De Gheselle et al., 2019). Notably, the longest delayed time point for the vitrified/warmed oocyte category was t9+. This trend was also found within a study done by De Gheselle et al., as seen in Table 2.

4.2.1 Time point (ICSI only) analyses

After observing the differences between the fresh and vitrified/warmed oocyte groups in the descriptive data analysis, a statistical analysis was done to determine if the differences were statistically significant. We observed that there was a constant trend of vitrified/warmed oocyte embryos lagging behind their fresh counterparts.

Initially, a traditional quantile (median) regression analysis was performed (Table 13) set at 95% CI. It was found that all time points, except two (t5 and t9), had vitrified/warmed oocyte population timings that were statistically different to their fresh counterparts. The vitrified/warmed oocyte group's time points were delayed (longer). Although t5 and t9 also showed delayed time points for the vitrified/warmed oocyte group, it was not statistically significant.

This outcome was similar to that reported in the literature (Chamayou et al., 2015; Milewski et al., 2015; Montjean et al., 2015; Cobo et al., 2017;;De Gheselle et al., 2019;). Several studies similar to the current study, however compared significantly different timings between fresh and vitrified/warmed groups with pregnancy outcomes such as implantation- and pregnancy-rates. Literature regarding these comparisons found that there were no significant differences in implantation- and pregnancy-rates, in spite of albeit the significant changes within the TL timings (Cobo et al., 2017; De Gheselle et al., 2019).

Owing to the fact that the comparison of the significant TL timing differences between fresh and vitrified/warmed oocytes could not be compared to pregnancy outcome data, due to sensitive data restrictions (as discussed in

'study limitations'), it was decided that a refined testing method for significant timing differences be executed (Table 14). A Two one-sided test (TOST) to test for equivalence was used to test at a 90% CI, and at certain levels of acceptance, if fresh and vitrified/warmed oocyte timings would be equivalent or not, i,e, if the vitrified/warmed population was the same as the fresh oocyte population. It was decided that a 5% level of equivalence be primarily tested, due to 5% being one of the most statistically significant (and most strict) version of this testing method (Walker et al., 2010). This level of equivalence was chosen in order to determine if there were any vitrified/warmed oocyte time points that were equivalent to their fresh counterparts, at a small margin (strict level) analysis. Subsequently, a 10% level of equivalence was carried out in order to see if at a larger margin of analysis, and greater possibility for difference, if there were any vitrified/warmed oocyte time points that presented equivalence to their fresh counterparts. This method of equivalence testing was set at a significance value of $p<0.05$, showing significant outcomes regardless of equivalence or not.

Notably, with regards to the literature where TL timings of fresh versus vitrified/warmed oocyte populations are available, there was a trend of a ±10% difference in timings (Desai et al., 2014; Chamayou et al., 2015; De Gheselle et a., 2019). De Gheselle et al. (2019), specifically, had a percentage difference between fresh and vitrified/warmed oocyte populations ranging from 4,2% to 12,7% (Table 2). It was therefore decided that a 10% level for testing equivalence would suffice.

With regards to the test for equivalence at the 5% level, it was found that no timings were statistically significant. This finding supported the initial findings that vitrified/warmed oocytes in general lag behind their fresh counterparts, showing significance within most time points. Due to the fact that at the 5% level there were no time points that exhibited equivalence. This trend was similarly found within the literature (De Gheselle et al., 2019; Cobo et al., 2017; Chamayou et al., 2015; Milewski et al., 2015; Montjean et al., 2015) (Table 2), however not following the more heterogeneous nature of the various findings. Due to the fact that most of the literature exhibited some time points that showed equivalence between the fresh and vitrified/warmed oocyte populations, we assumed that this margin of analysis was possibly too narrow; albeit somewhat mirroring the general trend of lagging vitrified/warmed oocytes.

With regards to the test for equivalence at the 10% level, the findings were more heterogeneous when compared to the 5% level. It was found that several vitrified/warmed time points showed significant equivalence to their fresh counterparts. De Gheselle et al. (2019) found that when comparing fresh and vitrified/warmed oocyte populations, there were significant time lags within the vitrified/warmed arm for time points tPNf to tSB, therefore excluding significant differences for time points tB, tEB and tHB. Cobo et al. (2017) found that there was significant difference for the same comparison whereby they found that vitrified/warmed time points t2 to t5 and tSC were slower than their fresh counterparts. Lastly, Chamayou et al. (2015) found statistically significant differences whereby the vitrified/warmed oocyte group lagged behind their fresh counterparts for time points tPNf, t2-4.

With regards to this study, it was found that for the vitrified/warmed oocytes the time points tPB2, tPNa, t2, t4, t8, tSC, tSB and tHB were statistically non-equivalent ($p<0.05$) when compared to their fresh counterparts. These findings yield a more accurate emulation of the literature trends for time point lags, when compared to the 5% level of equivalence.

An assumption could be drawn that the 10% level for testing for equivalence was perhaps of more clinical use compared to the 5% level, albeit no pregnancy outcomes were tested. One could make this assumption due to the proximity of this study's trends to that of the trends of the results from the literature mentioned above. In addition to this, it could also be assumed that even though there were statistically significant differences within the fresh and vitrified/warmed oocyte populations at 10%, one therefor questions the clinical significance of this difference. It could be assumed that this difference is not clinically significant, as within the literature (Cobo et al., 2017; De Gheselle et a., 2019), due to the fact that the overall time difference between fresh and their vitrified/warmed counterparts is 8,53-hours (Table 13). Practically, this time difference would not hinder the outcomes of an embryo transfer on day 5, as the embryo within the vitrified/warmed oocyte population would reach its endpoint on the same day as its fresh counterpart.

4.3 Clinical implications

Three possible clinical outcomes were identified within this study: 1) the validation of ova banks, 2) validation of oocyte pooling protocols, and 3) possible indication for delayed insemination for vitrified/warmed oocytes.

Due to the findings within various literature, no statistically significant differences in pregnancy rate (De Gheselle et al., 2019 and Cobo et al., 2017), implantation rate (Cobo et al., 2017) and positive embryo development outcomes (Chamayou et al., 2015) where recorded, albeit the findings of significant differences within vitrified/warmed oocyte populations exhibiting developmental delays compared to their fresh counterparts. It can therefore be deducted that vitrified/warmed oocyte in fact do compare to their fresh counterparts in terms of pregnancy outcomes.

The same deduction could be made within the fresh and vitrified/warmed oocytes compared within this study, owing to the fact that their significantly different time points are synonymous with the literature trends of lagging vitrified/warmed oocytes compared to fresh oocytes. The validation of the use of donor gamete banks, ova banks in this case, is imperative to achieve. Donor ova were primarily used in fresh cycles before the establishment of a robust oocyte vitrification protocol. With the notion of vitrified/warned oocytes being comparable to their fresh counterparts at a clinical level, this is a massive advantage for the use and growth of ova banks. Notably, this validation of the donor oocyte program can only be relied upon to a certain labor-dependent point; where the validation of the program becomes dependent on an embryologist. An embryologist requires proper and sufficient training in order to perform vitrification and thawing of oocytes, successfully.

Secondly, the validation of oocyte pooling for poor prognosis patients is also imperative and has massive application potentials. As mentioned with the validation of oocyte banking, the fact that vitrified/warmed oocytes compare well to their fresh counterparts at a clinical level, promotes the go-ahead for campaigning egg pooling for patients with poor ovarian response or low ovarian reserves. It is, however, imperative that such patients be well counselled regarding their detailed potential they possess to preserve their fertility to obtain at least one healthy live birth.

Lastly, and possibly the most practical, this study suggests that there may be a benefit to delayed insemination at ICSI for vitrified/warmed oocytes. This assumption was made due to the fact that there was a significant difference in timings whereby the vitrified/warmed oocyte group exhibited delays at tPB2, tPNa and t2 ($p<0.05$), as seen in Table 14. This was synonymous with the literature, as discussed, where the most prolific delay within the vitrified/warmed oocyte group was during the cleavage stages of development (De Gheselle et al., 2019; Cobo et al., 2017; Chamayou et al., 2015).

It is furthermore suggested that based on the timing of cleavage stage development, predictions of embryo viability (Wong et al., 2010) and short-term embryo developments (Herrero et al., 2013) can be made using TL imaging, also as discussed before. Notably, it has been suggested that the crucial developments during early cleavage (Mesenguer et al., 2011) can be influenced by chromosomal alternations causing delayed DNA replications or

delayed oocyte stabilization post thawing (Cobo et al., 2017). The delayed early cleavage divisions exhibited within this study is unlikely due to delayed DNA replications, which is primarily caused by alternations within the embryo culture. Fresh and vitrified/warmed oocytes were incubated using the same incubators and the same culture media. Additionally, there is evidence that oocyte vitrification does not increase the incidence of aneuploidy (Mullen et al., 2004), further validating the exclusion of delayed DNA replications as the reason for delayed cleavage stage development within vitrified/warmed oocytes.

The most likely cause of the different TL timings within fresh and vitrified/warmed oocytes may be due to the effects that vitrification has on the oocyte's ability to 'bounce back' after thawing (Cobo et al., 2017). Vitrification of an oocyte halts the cell on a cellular metabolic level. Due to the nature of this cellular cessation, it is speculated that reactivating the cell processes post thawing may require energy expenditure, which their fresh counterparts do not exhibit. This energy 'cost', due to the vitrification/thawing process presenting obstacles for the thawed oocyte to reactivate itself, places subsequent stress on the mitochondrial cells of the thawed oocyte (Dumollard et al., 2009; Cobo et al., 2017). Mitochondrial stress could cause dysfunction, which has been reported to be responsible for embryo arrest *in vitro* (Thouas et al., 2004; Cobo et al., 2017) and Studies have found that these mitochondrial alternations are temporary, whereby normal function was recorded after 3-4 hours of culture (Nohales-Corcoles et al., 2016; Cobo et all., 2017).

One can therefore theorize that temporary dysfunctional mitochondria within vitrified/warmed oocytes may be correlated to delayed embryo development, when compared to fresh oocytes. One could therefor surmise a delay in insemination of vitrified/warmed oocytes, in order to allow the cell structures to fully reactivate and reboot in order to operate at full capacity. It was suggested by Cobo et al. (2017) that a delay of 1 hour till time of ICSI could be beneficial.

4.4 Sibling oocyte study

Owing to the small sample size if this exploratory study, no conclusion can be made with regards to a trend within the fresh and vitrified/warmed sibling oocytes. The results of this exploratory study were deemed random and no trends were found.

4.5 Limitations of study

- The primary limitation was the retrospective nature of this study. The disadvantage of retrospective studies in general include inferior level of evidence compared with prospective studies, prone to selection bias, prone to recall bias or misclassification bias and prone to lack of standardization of data collection.

- Another major limitation of this study was the lack of correlation of the kinetic time points and significant differences to pregnancy outcomes, such as IR, PR and LBR. When this study was conceived, access by Wijnland Fertiliy Clinic to pregnancy data was limited. The pregnancy data was deemed sensitive by the management of Wijnland Fertility. With only three private IVF clinics within the Western Cape, the field of infertility is extremely competitive. An assumption can be made that this was perhaps the reason that the access to the data in question was limited.

- Another limitation to this study was the small sample size for the sibling oocyte exploratory study. This limitation, however, was not reliant on the premise of this paper nor was it reliant on the retrospective nature of this study.

- Furthermore, due to the exclusion criteria used in order to obtain a normative range, embryos with DUC were excluded. This limitation has conflicting roles, one being that the exclusion of embryos with DUCs was essential to obtain a normative range that was representative for good quality blastocysts with possible positive pregnancy potentials, and two being the actual limitation whereby DUCs should have been included in a separate analysis and then compared to the normative range / baseline kinetic model.

- The population that was tested was also not homogenous. Female age was not taken into account, which could have possibly skewed the results for the baseline kinetic model. In addition to this, male and female diagnosis was also not taken into account, yielding a similar disadvantage of possible skewing of the data due to increased heterogeneity.

CONCLUSION

In conclusion, this study showed that there was a statistically significant overall delay within the timings for vitrified/warmed oocytes when compared to their fresh counterparts. This trend was exhibited within various testing methods. These testing methods included a quantile (median) regression model set at 95% CI and a Two one-sided test (TOST) test for equivalence analyzed at 5%- and 10%-levels of equivalence set at a 90% CI ($p<0.05$). The most statistically significant findings within this study include the delayed vitrified/warmed oocyte time points for tPNa, t2, t4, t8, tSC, tSB and tHB ($p<0.05$). The most significant clinical finding of this study was the assumption that vitrified/warmed oocytes undergo mitochondrial stress post warming and requires 2-3 hours of culture in order to reboot the cellular machinery to full operating potential. As a result of this assumption it was suggested that vitrified/warmed oocytes exhibit a 1-hour insemination delay in order to give opportunity for mitochondrial recovery post warming.

Another crucial finding was that there was a total delay in the vitrified/warmed oocyte population of 8,53-hours, which could lead to the assumption that even though there was a statistically significant lag exhibited within the vitrified/warmed oocyte population, this is most probably not of clinical significance. This assumption was based on the fact that with the 8,53-hour delay, fresh and vitrified/warmed oocytes will be eligible for blastocyst transfer on the same day, resulting in no practical difference in treatment.

Lastly with regards to the exploratory sibling oocyte study, there were consistent patterns observed between the fresh and vitrified-warmed oocytes, and therefore, no conclusion was drawn.

Future recommendations for this study include the imperative inclusion of pregnancy data in order to correlate the findings to more tangible and accurate clinical outcomes. In addition, it would be recommended to perform an analysis whereby the population for the normative range is more homogeneous. This could be achieved in one of two ways: 1) to stratify the data according to female age or male and female diagnoses, or 2) to analyze a donor oocyte population in order to illuminate the confounding factors of female age and diagnosis.

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APPENDICES

Appendices A – L

WIJNLAND
Fertility

STANDARD OPERATION PROCEDURES

ASSISTED REPRODUCTIVE TREATMENT

A. Ovarian stimulation

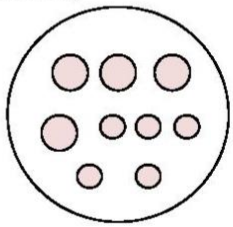
1. **Follicular stimulation:**
   a. Day 3 of menstrual cycle until trigger day with either HMG or Recombinant FSH
   b. LH suppresion with recombinant gonadotropin-releasing hormone (GnRH) antagonist from either day 8 of menstrual cycle or leading follicle of 14mm, which ever comes first, until trigger day
   c. Ovulation trigger when leading follicle ≥18mm with Recombinant HCG
2. **Estrogen supplementation:**
   a. Oral estrogen of 2mg per day according to prescription
3. **Luteal phase support:**
   a. Vaginal progesterone suppositories

B. Oocyte Retrieval

Acronyms:

- OPU = oocyte pick up
- COC = cumulus-oocyte-complex
- GT = Life Global Total medium range (IVF Online™)
- QA = Quinns Advantage culture medium range (Sage™)
- 1S = 1-Step with SPS or HSA culture medium (Sage™)

1. **Preparation procedure**
   a. Fertilization petri dish:
      i. Appropriate Fertilization medium in drops with tissue culture oil overlay
      ii. Label with patient identifier
      iii. Incubate overnight
   b. EmbryoSlide®:
      i. According to Technote (VitrolifeTM)
      ii. Incubate ≥4 hours
   c. Start patient and embryo documentation



2. **OPU**
   a. Prepare test tube with buffered culture medium and warm to 37°C
   b. Recieve follicular fluid from aspiration done in theatre
   c. Identify COCs and aspirate with pipette into warm culture medium tube
   d. Place all COCs in fertilization dish after completion of COCs OPU

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ASSISTED REPRODUCTIVE TREATMENT

C. COC stripping for sperm injection

1. **Preparation procedure**
   a. Strip petri dish:
      i. Appropriate drops of hyaluronidase supplemented buffered culture medium
      ii. Appropriate buffered culture drops with oil overlay
      iii. Warm to 37°C
      iv. Label with patient identifier
2. **Stripping**
   a. Use appropriate stripping pipette or micropipettor
   b. Count number of initial COCs to correspond to final oocyte count
   c. Check oocyte maturity
   d. Transfer oocytes to fertilization dish into unused drops and replace to incubator
   e. Note maturity on patient documentation

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STANDARD OPERATION PROCEDURES

ASSISTED REPRODUCTIVE TREATMENT

D. Sperm preparation

1. **Preparation procedure**
   a. Processing tubes
      i. Aliquot appropriate amount of selected gradient(s) solution(s) in tube
      ii. Aliquot appropriate sperm wash/fertilization medium in tube
      iii. Warm to 35°C
      iv. Label both tubes with patient identifier
2. **Sperm processing**
   a. Fresh semen sample
      i. Allow complete liquefaction
      ii. Warm to 35°C
      iii. Evaluate on wet preparation slide of semen and document semen parameters
      iv. Aliquot appropriate amount of semen onto prepared gradient solution
   b. Frozen semen/biopsy tissue sample
      i. Retrieve appropriate patient sample straws from dewar
      ii. Allow complete thawing of straw(s)
      iii. Warm to 35°C
      iv. Evaluate and note sperm parameters on wet preparation slide
      v. Aliquot whole thawed sample onto prepared gradient/wash solution
   c. Centrifugation and wash
      i. Centrifuge at 300-450g for 10-25min
      ii. Discard supernatant
      iii. Place sperm pellet into prepared sperm wash/fertilization medium tube
      iv. Centrifuge at 300-450g for 10min
      v. Discard supernatant
      vi. Resuspend sperm pellet
      vii. Evaluate on wet preparation slide and document semen parameters
   d. Insemination
      a. IVF
         i. Place prepared sperm sample in incubator for equilibration
      b. Sperm injection
         ii. Place prepared sperm sample on bench until injection procedure

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STANDARD OPERATION PROCEDURES

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E. Standard IVF Insemination

1. **Preparation procedure**
   a. Retrieve fertilization dish with patient COCs from incubator
      i. Check patient identifier – double witness
   b. Retrieve prepared sperm sample tube with sperm sample from incubator
      ii. Check patient identifier – double witness
2. **Sperm insemination**
   a. Retrieve sperm with pipettor
      i. Exact concentration of sperm to be used may be calculated
   b. Release sperm into drops with COCs
      i. Work under microscope
   c. Replace dish into incubator
   d. Document on patient form
3. **Inseminated COC stripping**
   a. Retrieve fertilization dish from incubator
   b. Identify COCs
   c. Retrieve COCs with pipettor
   d. Use appropriate stripping pipette or micropipettor
   e. Count number of initial COCs to correspond to final oocyte count
   f. Check oocyte maturity
   g. Transfer oocytes to fertilization dish into unused drops and replace to incubator
   h. Note maturity on patient documentation

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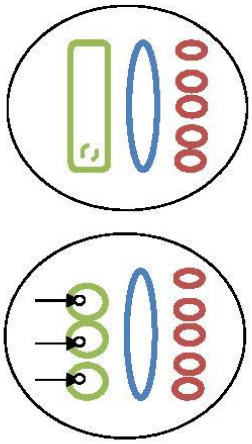
F. Intra Cytoplasmic Sperm Injection (ICSI) and variations (PICSI,IMSI)

1. **Preparation procedure**
   a. ICSI/PICSI/IMSI dish:
   b. Appropriate drop(s) of buffered culture medium for gametes,
      i. Appropriate drop(s) of PVP supplemented medium for manipulation
      ii. Cover with oil overlay
      iii. Label with patient identifier
   c. Manipulator:
      i. Load and set micropipettes for holding and injection
      ii. Warm heated stage to 37°C
      iii. Set up light configurations of microscope



2. **Sperm injection**
   a. Retrieve prepared sperm sample tube with sperm sample from bench
      i. Check patient identifier – double witness
      ii. Load sufficient sperm into allocated sperm drop in ICSI dish with pipet
   b. Retrieve fertilization dish with patient oocytes from incubator
      i. Check patient identifier – double witness
   c. Select individual sperm for injection of individual oocytes
   d. Load oocytes according to selected sperm
      a. Inject all oocytes with single selected sperm
      b. Replace injected oocytes into fertilization dish into new drop
   e. Document injection details onto patient form

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G. Standard Embryo Culture

1. **Preparation procedure**
   a. Embryo culture dish:
      i. Appropriate drops of embryo culture medium for oocyte wash and culture
      ii. Cover with oil overlay
      iii. Label with patient identifier
2. **Inseminated oocyte loading**
   a. Retrieve fertilization dish with patient oocytes from incubator
      i. Check patient identifier – double witness
   b. Wash oocytes in embryo culture wash drops
   c. Allocate single oocytes to embryo culture drops
   d. Replace patient embryo culture dish to incubator
3. **Daily embryo culture and grading**
   a. Retrieve embryo culture dish from incubator
   b. Place embryo culture dish on heated ICSI manipulator stage
   c. Evaluate embryo development according to Istanbul Consensus embryo scoring method *(see References)*
   d. Replace patient embryo culture dish to incubator
   e. Document embryo evaluation on patient form
   f. Complete daily from oocyte to blastocyst stage until final status has been decided
      i. Allocate and document viable embryos for transfer, cryopreservation and non-viable embryos for disposal
   g. Remove embryo culture dish from incubator after completion of embryo culture and allocations

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H. Time-lapse Embryo Culture

1. **Preparation procedure**
   a. EmbryoSlide® dish:
      i. Use appropriate embryo culture medium and pipettor
      ii. Prepare appropriate number of slide(s) according to number of oocytes retrieved
      iii. Prepare slides according to Vitrolife™ EmbryoSlide® preparation Technote
      iv. Cover with oil overlay
      v. Label with patient identifier
      vi. Equilibrate in incubator for ≥4 hours
   b. Initiate patient file on EmbryoViewer® station with patient details
2. **Inseminated oocyte loading**
   a. Retrieve EmbryoSlide® dish from incubator
      i. Check patient identifier – double witness
      ii. Remove bubbles from wells
   b. Retrieve fertilization dish with patient oocytes from incubator
      iii. Check patient identifier – double witness
   c. Wash oocytes in embryo culture wash wells
   d. Allocate all oocytes to single embryo culture wells
   e. Load EmbryoSlide® into EmbryoScope® according to manufacturer instruction manual
   f. Allocate EmbryoSlide® to patient
3. **Embryo annotation and grading**
   a. Annotate embryos according to Vitrolife™ EmbryoScope® embryo annotation Technote
   b. Complete daily from oocyte to blastocyst stage until final status has been decided
      i. Allocate and document viable embryos for transfer, cryopreservation and non-viable embryos for disposal
   c. Remove and end EmbryoSlide® from EmbryoScope® after completion of embryo culture and allocations

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I. Embryo transfer

1. **Preparation procedure**
   a. Embryo transfer dish:
      i. Appropriate embryo culture medium for transfer with pipettor
      ii. Appropriate embryo culture medium for wash
      iii. Label with patient identifier
      iv. Equilibrate in incubator for ≥2 hours
   b. Identify, select and allocate embryo for transfer
   c. Retrieve embryo culture dish/EmbryoSlide® from incubator
      i. Check patient identifier – double witness
   d. Retrieve embryo transfer dish from incubator
      i. Check patient identifier – double witness
   e. Transfer selected embryo with pipet to transfer well/drop in prepared transfer dish
2. **Transfer procedure**
   a. Retrieve prepared embryo transfer dish with selected embryo loaded
      i. Check patient identifier – double witness
   b. Rinse syringe with embryo culture wash medium
   c. Connect transfer catheter with transfer syringe
   d. Aspirate embryo from embryo transfer medium into transfer catheter
   e. Hand loaded embryo transfer catheter to clinician for embryo transfer procedure
   f. Retrieve emptied embryo transfer catheter from clinician
   g. Place embryo catheter tip into empty well and disconnect syringe from catheter
      a. Check released embryo transfer medium for embryo retention
   h. Reload embryo in case of retention adn repeat transfer procedure

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J. Oocyte & Embryo Cryopreservation

1. **Preparation procedure**
   a. Vitrification dish:
      i. Wait for vitrification medium to reach room/ambient temperature
      ii. Prepare Repro Plate/Vitri Plate with appropriate vitrification mediums according to Kitazato/Cryotec instruction leaflet according to sample type *(See Appendices)*
      iii. Use pipettor
      iv. Label with patient identifier
   b. Storage device:
      i. Select appropriate Cryotop®/Cryotec®storage coloured device
      ii. Label with patient and straw identifiers
      iii. Check patient identifier – double witness
   c. Storage dewar:
      i. Find open storage goblet and allocate to patient samples
2. **Sample loading and vitrification procedure**
   a. Retrieve fertilization dish/embryo culture dish/EmbryoSlide® from incubator
      i. Check patient identifier – double witness
      ii. Identify selected patient oocyte/embryo for vitrification
   b. Retrieve oocyte/embryo from culture drop/well with pipet
   c. Load into vitrification medium according to Kitazato/Cryotec instruction leaflet *(See Appendices)*
   d. Follow vitrification procedures according to Kitazato/Cryotec instruction leaflet *(See Appendices)*
3. **Storage procedure**
   a. Retrieve allocated goblet from storage dewar
   b. Place into liquid nitrogen container with patient vitrification device loaded with samples
      i. Check patient identifier – double witness
   c. Place storage devices into goblet under level of liquid nitrogen
      i. Load all devices until goblet is full
   d. Retrieve filled goblet from liquid nitrogen and replace to original space in dewar
      i. Check patient identifier – double witness
   e. Document storage details on patient form

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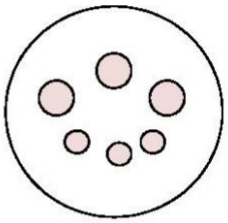
K. Oocyte/Embryo thawing

1. **Preparation procedure**
   a. Fertilization/Embryo culture petri dish:
      i. Appropriate fertilization/embryo culture medium in drops
      ii. Cover with tissue culture oil overlay
      iii. Label with patient identifier
      iv. Equilibrate for ≥4hours
   b. Document on patient form
   c. Prepare thawing medium according to Kitazato®/Cryotech® product insert



   d. Thawing dish:
      i. Wait for thawing medium to reach room/ambient temperature
      ii. Prepare Repro Plate/Thaw Plate with appropriate thawing mediums according to Kitazato®/Cryotec® instruction leaflet according to sample type *(See Appendices)*
      iii. Use pipettor
      iv. Label with patient identifier
   e. Storage dewar:
      i. Identify allocated storage goblet with patient samples
      ii. Check patient identifier – double witness
      iii. Retrieve goblet from dewar and place directly under liquid nitrogen level
   f. Storage device:
      i. Identify and retrieve storage device with selected patient sample in liquid nitrogen
      ii. Check patient identifier – double witness
      iii. Replace goblet to original space in dewar, if applicable, with remaining patient storage devices
2. **Thawing**
   a. Thaw sample according to Kitazato®/Cryotech® product insert
   b. Note survival on patient documentation

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L. Appendices

1. **Vitrolife™ instructions for EmbryoSlide® preparation for embryo culture in the EmbryoScope®**
2. **Vitrolife™ instructions for embryo annotations for embryo grading in the EmbryoScope®**
3. **Istanbul consensus for embryo grading during standard embryo culture**
    a. **Istanbul consensus for fertilization check during standard embryo culture**
    b. **Istanbul consensus for embryo grading at cleavage stage during standard embryo culture**
4. **Gardner blastocyst grading system for blastocyst grading during standard embryo culture**
5. **Vitrolife™ instructions for blastocyst grading annotations in the EmbryoScope®**
6. **Kitazato™ instructions for oocyte vitrification and thawing with the Cryotop® method**
7. **Kitazato™ instructions for embryo vitrification and thawing with the Cryotop® method**
8. **Cryotech™ instructions for vitrification with the Cryotop® method**
9. **Cryotech™ instructions for thawing with the Cryotop® method**

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1. Vitrolife™ instructions for EmbryoSlide® preparation for embryo culture in the EmbryoScope®

TechNOTE

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Preparation of EmbryoSlide Culture Dishes

The EmbryoSlide® culture dish is specifically designed for the individual culture of up to 12 embryos in the EmbryoScope™ time-lapse incubator. The dish also contains wells designed for rinsing.
The EmbryoSlide culture dishes are designed for easy and stable handling, and are made of culture-tested polystyrene. They are delivered in sterile, single pouches.

Vitrolife recommends preparation of EmbryoSlide culture dishes on the day before use. Prepare the dishes with cold medium and on a non-heated surface to avoid evaporation. The procedure described below requires less than 1.5 minutes per dish.

General characteristics of the EmbryoSlide culture dish

The embryos are incubated in individual microwells in a small (25 µl) volume of culture medium under a confluent oil cover.

Each well carries a number from 1-12 for identification under a stereo microscope. Each well number corresponds to the well identification number in the EmbryoViewer® software.

Two rinsing wells are available at each end of the dish. These special wells can be used during embryo handling (identified as A-D).

There is a slight variation in how much the temperature decreases in the microwells (approx. 0.6°C) and the rinsing wells (approx. 0.7°C). Both measurements have been performed on a 37°C heating plate over a period of two minutes. This represents normal dish handling time.

Each batch of EmbryoSlide+ culture dishes must pass our stringent MEA testing procedure before being released for sale. This is part of the Vitrolife quality assurance.

Preparation for use on the next day

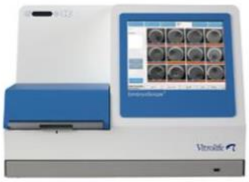
Prepare the EmbryoSlide culture dishes on the day before use. Prepare one dish at a time to minimise the handling time of each dish.

The EmbryoSlide culture dishes should be prepared with cold medium and oil on a non-heated workbench to avoid evaporation of the medium during preparation.

When they have been prepared, the culture dishes must equilibrate overnight before loading embryos into the microwells.

Use a stereo microscope to control the process.

The recommended procedure for preparing the culture dishes is outlined on the next page.



TECHNOTE: Preparation of EmbryoSlide culture dishes, Vitrolife, v.11 INT, JUNE 2018

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TechNOTE

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Step	Action
	**Remove the culture dish from the pouch.** **Prepare the dishes with cold culture medium and oil on a non-heated workbench to avoid evaporation.** **Prepare one dish at a time to minimise the handling time of each dish.**
	**Fill all microwells with a small amount of culture medium*** **Use a micropipette.** Slightly overfill the microwell to create a convex meniscus.
	**Immediately fill all needed wells, including the rinsing wells, with 25 µL of culture medium*. Use a standard pipette.**
	**Immediately load 1.4 mL of culture oil* into the reservoir** It is important to apply the oil overlay quickly to avoid evaporation of medium. Make sure that all wells, including the rinsing wells, are covered with a confluent oil layer to eliminate evaporation of medium. Add an additional 25µL of culture oil per well not filled with medium.
	**Push up larger bubbles with a micropipette and remove them** **Cover with the lid and equilibrate overnight.** **Remove any bubbles that may have formed.** **Load embryos into the center of microwells. Use a micropipette.**
	**Place the dish in the EmbryoScope incubator.**

*Vitrolife recommends using G-TL medium, designed specifically for continuous culture with time-lapse technology and OVOIL™ 100% paraffin culture oil for complete control of your culture system. Vitrolife products are produced under highly controllec processes.

Refer to the TECHNOTE *Additional notes on EmbryoSlide® culture dish preparation* for further recommendations about optimal handling of the EmbryoSlide culture dish.

TECHNOTE: Preparation of EmbryoSlide culture dishes, Vitrolife, v.11 INT, JUNE 2018

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TECHNOTE

Vitrolife

Additional notes for EmbryoSlide® culture dish preparation

This TECHNOTE describes additional procedures and information related to the handling and preparation of EmbryoSlide® culture dishes.

The handling of EmbryoSlide culture dishes is described in the TECHNOTE "Preparation of EmbryoSlide® culture dishes".

EmbryoSlide culture dishes: preparation for use on the same day

Although preparation of EmbryoSlide culture dishes is recommended one day before use, there may be circumstances requiring preparation of a culture dish for use on the same day.

The procedure follows essentially the one described in the TECHNOTE "Preparation of EmbryoSlide® culture dishes" except that the use of pre-warmed and pre-gassed/equilibrated medium is mandatory.

Culture dishes prepared with pre-gassed/equilibrated medium should be re-equilibrated after preparation for another 2-4 hours before embryos are loaded in the micro-wells. This serves mainly to stabilize the temperature.

Removal of occasional air bubbles

Usually the above method of filling does not produce air bubbles but all wells need to be carefully checked.

If air bubbles are present after preparation remove all bubbles in the well and in the oil layer immediately. However, small bubbles and bubbles in the micro-well can be more easily removed after equilibration.

- If air bubbles are present at the interface between the medium and the oil they should be removed immediately with a standard pipette containing media.

  By capillary effect the bubbles will aspirate into the pipette tip when this is placed close to the air bubble

- If air bubbles are present at the bottom of the micro-well or small bubbles are sticking to the side of the well it is recommended to incubate the EmbryoSlide culture dish in an incubator for 1-2 hours as this will cause the bubbles to grow and to round up for easier removal.

  Once the bubbles have rounded up simply touching them with a micro pipette tip will cause them to swim up and they can be easily removed without dragging oil into the micro-well.

The EmbryoSlide® culture dish

TECHNOTE: Additional notes for EmbryoSlide® culture dish preparation, Vitrolife, v.3 INT, AUGUST 2015

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2. Vitrolife™ instructions for embryo annotations for embryo grading in the EmbryoScope®

TechNOTE

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Consistent annotation for better evaluation – a guide on definitions for morphokinetics

Annotations constitute the base on which embryo evaluation can be performed using time-lapse monitoring in the IVF clinic.
The embryo developmental events that can be detected with time-lapse technology are immense. Events relevant for annotation ideally reflect embryonic potential in the specific clinical setting. Therefore it is important to define which events are relevant for the evaluation of embryos in your clinical setting.

Annotations should be objective and definitions should be the same across operators in order to perform meaningful evaluations.
This technote describes definitions of variables most commonly used in embryo assessment with time-lapse. These definitions will help you attain consistent annotations and thereby objective evaluations in your clinic and further streamline the understanding of embryo developmental events within the clinic and beyond the clinic.

Evaluation of embryos with KIDScore models require only few annotations, however this technote describes a more extensive selection of variables.

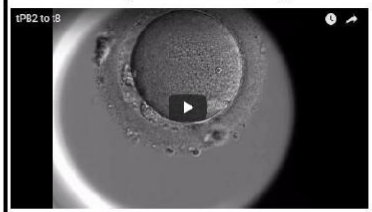
Time-lapse assessment

The first step on the way to reach consistency of annotations within a clinic is to agree on definitions of each annotated variable.
Time-lapse facilitates a more precise and objective method of embryo assessment than with static embryo monitoring. This is due to the continuous monitoring provided by time-lapse technology.

This continuous monitoring allows you to visually detect changes in embryo stages and morphology in a precise manner.

Morphokinetics – assessing embryo stages

With time-lapse the exact time that an embryo transits into a new stage can be determined with precision.
To do this, visual differences from one image to the next should be registered as annotations.
With morphokinetic variables, annotating the first time that an embryo is observed to be in a certain stage ensures a consistent and objective annotation strategy.



Annotation of fertilization events and blastomere cleavages: tB2, tPNa, tPNf, t2, t3, t4, t5, t6, t7 and t8

Variables from tPB2 to t8 represent distinct events that are detectable by differences from one image to the next.
To annotate those, the first image for which the stage is observed is annotated.
tPB2; time of extrusion of 2nd Polar Body: annotate at the first image in which the 2nd polar body is observed.
tPNa; time of ProNuclei appearance: annotate at the first image in which all pronuclei can be observed.
tPNf; time of ProNucei fading: annotate at the first image in which all pronuclei have faded.
t2-t8; time of cleavage to 2 etc cells: annotate at the first image in which a distinct separation of cell membranes can be observed, i.e. mark the exact time that the embryo progresses into another developmental stage.

The video to the left illustrates the definitions of morphokinetic variables from tPB2 to t8. View the full video at www.vitrolife.com

TECHNOTE: Making consistent annotations for better evaluation, Vitrolife A/S, v.2, NOVEMBER 2017

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TechNOTE

Vitrolife

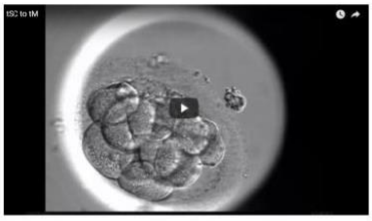
Annotation of morula and blastocyst formation

Morula and blastocyst formation are both processes that are not observed as instantaneous occurrences but rather observed as reached through gradual, subtle changes.

In order to reach consistency when annotating developmental steps during morulation and blastulation, definitions are based on distinctive features during the gradual processes.

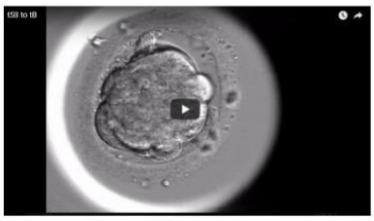
time of Starting Compaction (tSC): the first time that membranes between some of the blastomeres of the future morula are no longer distinct.

time of Morula (tM): the first image in which a compacted morula includes all the blastomeres that will later take part in the formation of the blastocyst. This solves the question of how to handle partial compactions as excluded cells can be accounted for



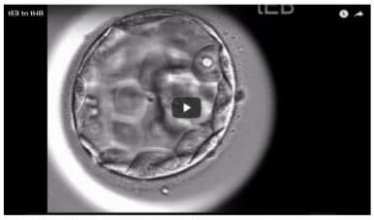
time of Starting Blastulation (tSB): the first time that a sign of cavity formation is observed. As the blastocoel cavity grows during blastulation, going back in the image sequence from a definite blastocyst stage can be helpful to attain this annotation.

time of Full Blastocyst (tB): the last image before expansion starts. This is recognised as the last image before the zona pellucida is pushed by the growing blastocyst. This is a very distinct hallmark during blastocyst development and therefore easy to annotate precisely and consistently.



time of Expanding Blastocyst (tEB): blastocyst expansion can go on for several hours and therefore a defined characteristic during this process is necessary to obtain accuracy during embryo analysis. Importantly, this should be informative on another level than previous parameters as otherwise annotation would be dispensable. Therefore, we characterize tEB as the time at which the blastocyst has expanded so much that the zona pellucida has reached half of its original thickness, which can be measured and thus represents a truly objective assessment.

time of Hatching Blastocyst (tHB): the first image at which a sign of hatching is observed.



View the full videos at www.vitrolife.com

Note that for some variables precise and consistent annotation is easier if the video sequence is followed backwards in time, i.e. from a time of definite observation to the exact time of first observation.
This is especially helpful for variables which occur gradually and hence do not evoke extensive changes between consecutive images such as e.g. time of ProNuclear appearance (tPNa) and time of Starting Blastulation (tSB).
The above definitions reflect time-lapse annotations as recommended by Vitrolife and to some extend based on the definitions of Ciray et al., 2014: Hum Reprod 29(12): 2650-2660

TECHNOTE: Making consistent annotations for better evaluation, Vitrolife A/S, v.2, NOVEMBER 2017

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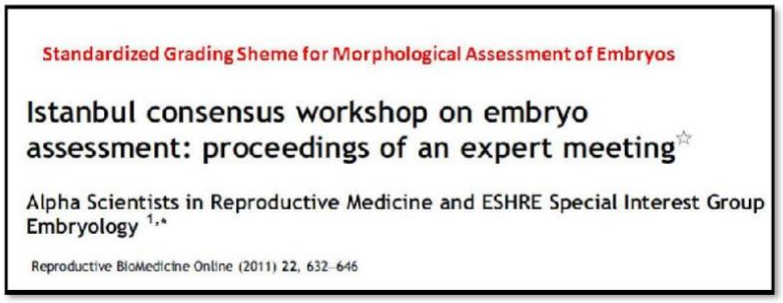
3. Istanbul consensus for embryo grading during standard embryo culture


Standardized Grading Sheme for Morphological Assessment of Embryos

Istanbul consensus workshop on embryo assessment: proceedings of an expert meeting☆

Alpha Scientists in Reproductive Medicine and ESHRE Special Interest Group Embryology [1,*]

Reproductive BioMedicine Online (2011) 22, 632–646

a. Istanbul consensus for fertilization check during standard embryo culture

Fertilization check

The optimal fertilized oocyte should be spherical, and have two polar bodies, with two centrally located, juxtaposed pronuclei that are even sized, with distinct membranes. The pronuclei should have equivalent numbers and size of NPBs that are ideally equatorially aligned at the region of membrane juxtaposition.

It was agreed that both pronuclear size and location should be assessed at fertilization check (Table IV). The consensus was that the following features of pronuclei are severely atypical: widely separated pronuclei; pronuclei of grossly different sizes; micronuclei. The presence of sER disks should be assessed as part of the fertilization check (if IVF, rather than ICSI was performed). Normally fertilized oocytes in which sER disks are observed should not be transferred.

The consensus was that at present, there is insufficient evidence to support a prognostic value for the observation of a peripheral cytoplasmic translucency in the fertilized oocyte (a 'halo').

The decision to perform a second Day 1 assessment is at the discretion of the laboratory, and may be either a syngamy or an early cleavage assessment (Table IV). The purpose of the second assessment can be for either quality control (syngamy) or prognostic (early cleavage) reasons, which will define the assessment time selected.

b. Istanbul consensus for embryo grading at cleavage stage during standard embryo culture

Table VI
Consensus scoring system for cleavage-stage embryos (in addition to cell number).

Grade	Rating	Description
1	Good	• <10% fragmentation
		• Stage-specific cell size
		• No multinucleation
2	Fair	• 10–25% fragmentation
		• Stage-specific cell size for majority of cells
		• No evidence of multinucleation
3	Poor	• Severe fragmentation (>25%)
		• Cell size not stage specific
		• Evidence of multinucleation

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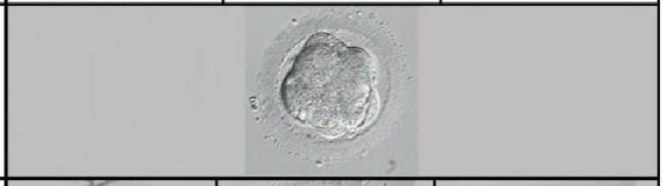
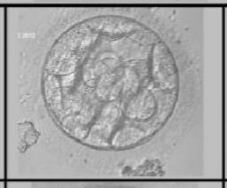
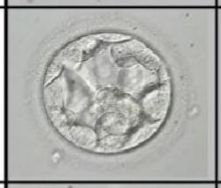
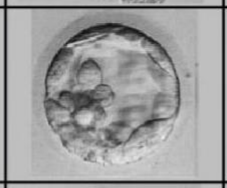
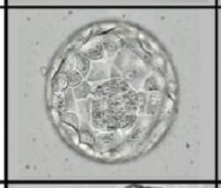
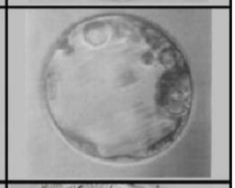
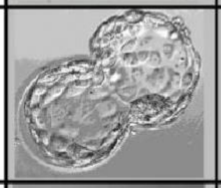
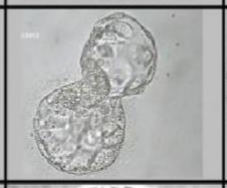
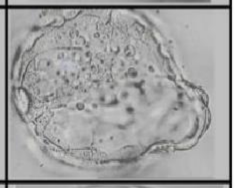
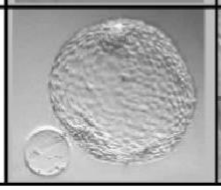
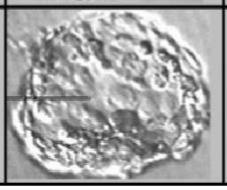
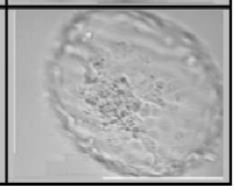
4. Gardner blastocyst grading system for blastocyst grading during standard embryo culture

Inner Cell Mass (ICM)	*A* Numerous and tightly packed	*B* Several and loosely packed cells	*C* Few cells
Trophectoderm (TE)	*A* Many tightly packed cells organised into epithelium	*B* Several cells organised into loose epithelium	*C* Few cells
Morula			
Early Blastocyst			
Blastocyst			
Expanded Blastocyst			
Hatching Blastocyst			
Fully Hatched Blastocyst			

5. Vitrolife™ instructions for blastocyst grading annotations in the EmbryoScope®

TECHNOTE

Vitrolife

Guidelines for blastocyst morphology grading with time-lapse

Grading blastocyst morphology with the use of time-lapse technology facilitates a more thorough evaluation because the complete course of development can be considered. This means that e.g. cells that are excluded during compaction or subsequent blastocyst formation can be accounted for. Similarly, fragments disturbing the visual impression of a blastocyst can be identified as all focal planes can be reviewed throughout embryo development.

Altogether, this means that a comprehensive impression of the blastocyst can be used as the basis for grading morphology. This should be utilized when grading ICM and TE and is necessary when using KIDScore D5.

Blastocyst grading for KIDScore D5

Time-lapse monitoring of embryos gives you a different level of information regarding development of both ICM and TE. This includes number of cells that each layer originates from and extrusion of cells during the compaction or expansion phase. This information must be taken into account when grading blastocysts for KIDScore D5 application.

To use KIDScore D5 a separate and independent grade from A to C must be given for both ICM and TE for each embryo reaching the blastocyst stage. Grade "A" defines cell layers with highest quality morphology whereas grade "C" defines cell layers with lowest quality morphology.

Definitions for each grade for both ICM and TE is defined below.

ICM grade	Description
A	Many tightly packed cells. Cell boundaries are not distinct and the layer is homogenous without vacuoles and debris.
B	Several cells and the layer can be less tightly packed. The layer can be less homogenous and few vacuoles or minor degenerations may be observed.
C	Very few cells that are loosely packed. Cells may be large and show distinct boundaries. The size of the ICM may differ in this group as a few big cells lead to an overall larger size. The larger size is, however, the result of poor compaction. The layer may show vacuoles, degenerated cells or independent cells. This grading group also covers cases where the ICM is not distinguishable.

TE grade	Description
A	Many flattened cells (often >40) forming a cohesive layer that lines the blastocoel cavity. The cells often contain clearly visible nuclei and the cytoplasm is homogenous.
B	Several (often > 20) cells. The layer is not completely cohesive and the shape of the cells varies within the layer. Cell cytoplasm may appear non-homogenous and it may be difficult to distinguish nuclei.
C	Very few cells which are often large and stretched over a large area. Cytoplasm often appears non-homogenous and vacuoles may be present.

For both layers the grade "N/A" is given to embryos in case the cell layer can not be evaluated.

TECHNOTE: Guidelines for blastocyst morphology grading with time-lapse, Vitrolife A/S, v.3, AUGUST 2016

WIJNLAND
Fertility

STANDARD OPERATION PROCEDURES

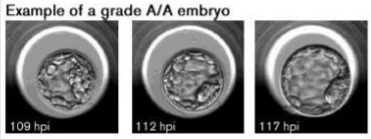
ASSISTED REPRODUCTIVE TREATMENT

TECHNOTE Vitrolife

Examples of blastocyst morphology grades with time-lapse

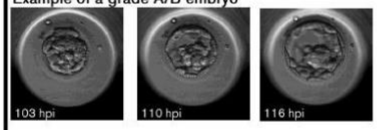
Below you can see the progression of some examples of blastocyst development with associated ICM/TE grades. A short description to illustrate the grade is given next to each embryo. Time is given in hours post insemination (hpi).

Example of a grade A/A embryo



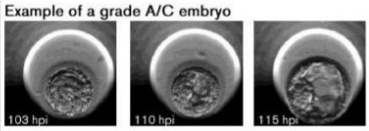
ICM is large, originates from many cells and ends up as a tightly compacted layer.
TE originates from many cells that end up forming a cohesive layer lining the blastocoel cavity.

Example of a grade A/B embryo



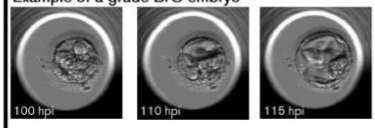
ICM is composed of many cells and is tightly compacted.
TE is composed of several cells but varies in size and cohesiveness.
Note: At 103 hpi the embryo shows a blastomere that is pushed into the perivitelline space and does not take part in blastocyst formation. At 116 hpi this blastomere is degenerated and appears as debris in the same position.

Example of a grade A/C embryo



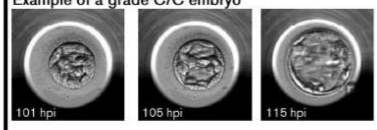
ICM is large and originates from many cells which are tightly compacted.
TE is composed of few, large cells and some are stretched over a long distance.

Example of a grade B/C embryo



ICM consists of several cells and is loosely compacted.
TE consists of very few and large cells.

Example of a grade C/C embryo



ICM is composed of few cells. Image 2 (105 hrs) shows a "bridge" that connects the two cell layers.
TE originates from few cells that are large and stretches over a long distance.
Note: The large cellular debris (fragmentation) pushed into the perivitelline space is not part of the actual blastocyst

For KIDScore D5 to work as intended, the guidelines described here should be followed and blastocyst morphology grades must be annotated between 115 and 120 hours after insemination.

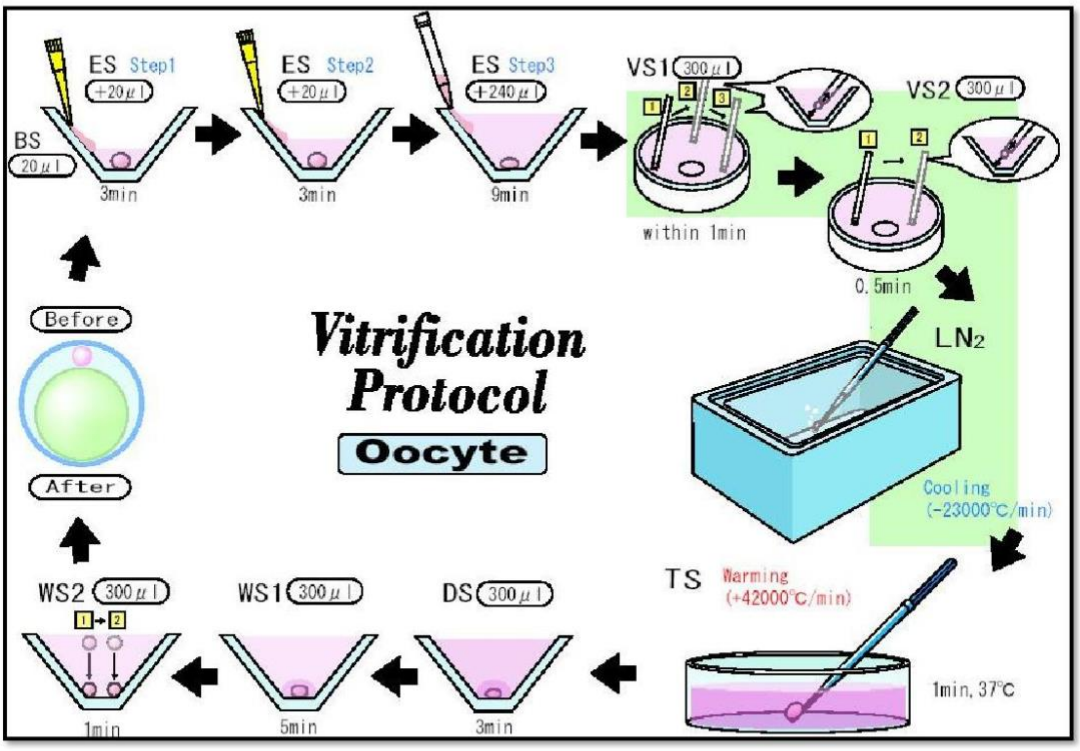
TECHNOTE: Guidelines for blastocyst morphology grading with time-lapse, Vitrolife A/S, v.3, AUGUST 2016

WIJNLAND
Fertility

STANDARD OPERATION PROCEDURES

ASSISTED REPRODUCTIVE TREATMENT

6. **Kitazato™ instructions for oocyte vitrification and thawing with the Cryotop® method**

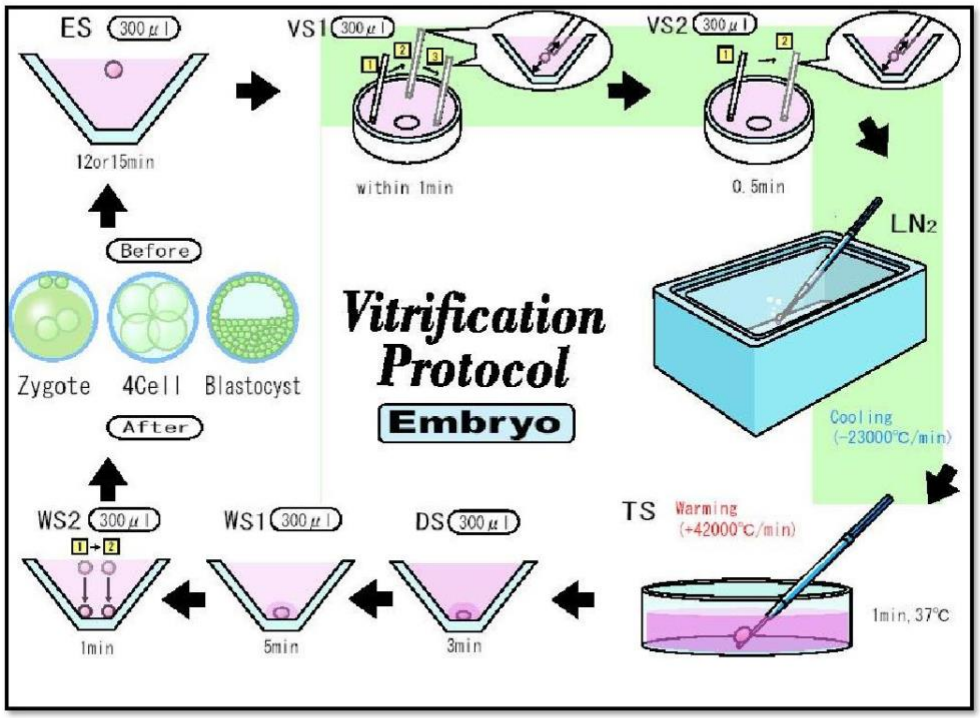


WIJNLAND
Fertility

STANDARD OPERATION PROCEDURES

ASSISTED REPRODUCTIVE TREATMENT

7. Kitazato™ instructions for embryo vitrification and thawing with the Cryotop® method



WIJNLAND
Fertility

STANDARD OPERATION PROCEDURES

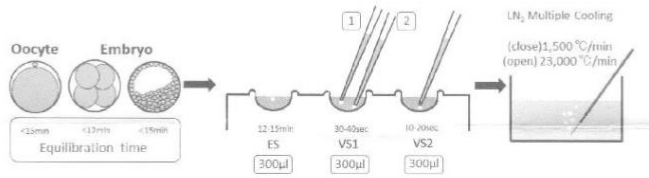
ASSISTED REPRODUCTIVE TREATMENT

8. Cryotech™ instructions for vitrification with the Cryotop® method

Cryotech®

For Oocytes and Embryos

VITRIFICATION SOLUTION SET (110) : For 10 times Uses



Contents:
-2 vials of 1.8 ml Equilibration Solution (ES).
-4 vials of 1.8 ml Vitrification Solution (VS).

Instructions:

Preparation
-The whole process should be performed under room temperature (25-27°C).
-Fill a nitrogen container.
-Compare the thickness of the zona pellucida with the perivitelline space and take note for oocytes.
-Important : Use a right diameter size of Pasteur pipette for oocytes and embryos (140-150 µm), and blastocysts (160-200 µm).

Equilibration of oocytes and embryos

1. Fill in the 1st well of a Vitri Plate with 300 µl of ES, and 300 µl of VS in both of the 2nd and 3rd well.
2. Put an oocyte/embryo on the surface of ES in the 1st well.
3. The oocyte/embryo will sink and begin to shrink and gradually return to the original size (maximum time is 15 min for oocytes and blastocysts, and 12 min for other stages of embryos).

Vitrification

Attention: The following steps must be taken in more than 25 sec and less than 90 sec.

4. Transfer the oocyte/embryo from the 1st well to the half depth of the 2nd well with VS. (Not with the minimum volume of ES at the first step) The oocyte/embryo immediately floats to the surface of VS while being washed.
5. After washing the inside wall of the pipette with fresh VS media, take only the oocyte/embryo and transfer it to the bottom of the well. Wait until the oocyte/embryo stops floating in VS.
6. Transfer the oocyte/embryo to the half depth of the 3rd well with VS, and mix the media by pipette around for 5 times.
7. Take only the oocyte/embryo at the tip of the pipette, and put it on the end of the Cryotec seat with a minimum volume of VS.
8. Immediately submerge the Cryotech into liquid nitrogen.
9. Place the cap, and store the Cryotec in a nitrogen tank.

Please leave the Cryotec in liquid nitrogen at all times.

Quality Control Tests:

-Solutions
Lot is labeled on the vial of themselves.

Successfully passed the following controls.
- Sterility : Sterility test .
- Endotoxin by KT methodology (Each component).
- Efficiency: survival of 50/50 mouse embryos and porcine oocytes.

Storage and stability:
Solutions and kits can be transported under the room temperature, and then must be kept in the fridge at 2-8°C until the expiration date.
Use before the expiration date indicated on the each of the labels.

Composition:

-Modified HEPES Bufferec MEM
-Hydroxy Propyl Cellulose
-Ethylene Glycol
-Dimethyl Sulfoxide
-Endotoxin free Trehalose

References:

- Kuwayama M. Highly efficient vitrification for cryopreservation of human oocytes and embryos: The CryoTop method. Theriogenology 67, 73-80, 2007.
- Cobo A, Kuwayama M. Comparison of concomitant outcome achieved with fresh and cryopreserved donor oocytes vitrified by the Cryotop method. Fertil Steril. 89(6): 1657-64, 2007.
- Antinori M, Licata E, Dani G, Cerusico F, Versaci C, Antinori S. Cryotop vitrification of human oocytes results in high survival rate and healthy deliveries. Reproductive Biomedicine Online 14 : 5-687, 2007.
- Vajta G, Kuwayama M. Improving cryopreservation systems. Theriogenology 65(1), 236-44, 2006.
- Kuwayama M. Highly efficient vitrification method for cryopreservation of human oocytes. Reproductive BioMedicine Online 11:300-308, 2005.
- Ushijima J, Kuwayama M. High survival rate of bovine oocytes matured in vitro following vitrification. J Reprod Dev. 50:685-96, 2004.
- Fukui Y, Kuwayama M. Effect of cryodevice type and donor's sexual maturity on vitrification of minke whale oocytes at germinal vesicle stage. Zygote 12, 333-338, 2004.
- Hochi S, Kuwayama M. Improved Survival of Vitrified In vivo-derived porcine embryos. J. Reprod. Develop. 50, 481-486, 2004.
- Esaki R, Kuwayama M. Cryopreservation of porcine embryos derived from in vitro-matured oocytes. biology of Reproduction. 71, 432-437, 2004.

Product only for in vitro use.

Ver. 2
Revised 1th AUG 2014

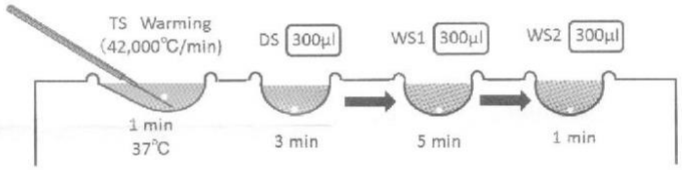
Cryotech Japan 2-5-5-8F Shinjuku, Shinjuku-ku, Tokyo 160-0022 JAPAN contact@cryotech-japan.jp http://cryotech-japan.jp/

9. Cryotech™ instructions for thawing with the Cryotop® method

Cryotech®

For Oocytes and Embryos

WARMING SOLUTION SET (205) : For 5 times Uses



Contents:
-5 vials of 1.8 ml Warming Solution (TS).
-1 vial of 1.8 ml Diluent Solution (DS).
-2 vials of 1.8 ml Washing Solution (WS).

Instructions:

Preparation
-The whole process should be made under room temperature (25-27℃).
-Important : Use a right diameter size of Pasteur pipette for oocytes and embryos (140-160 µm), and blastocysts (160-200 µm).

-Place a Warm plate and TS vial (with lid) in an incubator at 37℃ 3 hours before the use (overnight storage is preferable).

-Expose DS and WS vials to the room temperature air at least 1 hour before the use.

-Take a patient's cane out of a liquid nitrogen tank, and take off the cover cap and prop up the Cryotec to the inside wall of the cooling rack with liquid nitrogen foaming it against the wall.

Warming and dilution of CPAs

1. Take the Warm Plate out of the incubator and fill the second well with 300µl of DS.
2. Take the TS vial out of the incubator, and expel TS to the 1st square well.
3. Quickly (within 1 sec) put the Cryotec into the 1st square well with TS, and wait for 1 min.
4. Aspirate the oocyte/embryo and 3 mm long of TS into the pipette, and expel them most slowly to the bottom of the 2nd well (DS).
   Wait for 3 min.
5. While waiting, fill the 3rd(WS1) and 4th wells(WS2) with 300µl of WS each.
6. Aspirate the oocyte/embryo and 3 mm long of DS into the pipette, and expel them most slowly to the bottom of the 3rd well (WS1), and wait for 5 min.
7. Give a survival judgment at the end of this step if the shrunk oocyte/embryo is recovered or not.
8. Put the oocyte/embryo on the surface of the 4th well (WS2). When they sink and reach to the bottom, put them again on the surface of the same WS2 to wash for 2 times in total.
9. Put the oocyte/embryo in the droplet of the culture media for the recovery for ICSI and ET.

Note: 2 to 4 hours of culture for oocytes, and 3 hours for embryos.

Quality Control Tests:

-Solutions
Lot is labeled on the vial of itself.

Successfully passed the following controls.
- Sterility : Sterility test .
- Endotoxin by KT methodology (Each component).
- Efficiency: survival of 50/50 mouse embryos and porcine oocytes.

Storage and stability:

Solutions and kits can be transported under the room temperature, and then must be kept in the fridge at 2-8℃ until the expiration date.
Use before the expiration date indicated on the each of the labels.

Composition:

-Modified HEPES Buffered MEM
-Hydroxy Propyl Cellulose
-Endotoxin free Trehalose

References:
- Kuwayama M. Highly efficient vitrification for cryopreservation of human oocytes and embryos: The CryeTop method. Theriogenology 67: 73-80, 2007.
- Cobo A, Kuwayama M. Comparison of concomitant outcome achieved with fresh and cryopreserved donor oocytes vitrified by the Cryotop method. Fertil Steril. 89(4): 1657-64. 2007.
- Antinori M, Licata E, Dani G, Cerusico F, Versaci C, Antinori S. Cryotop vitrification of human oocytes results in high survival rate and healthy deliveries. Reproductive BioMedicine Online. 14: 5-867. 2007.
- Vajta G, Kuwayama M. Improving cryopreservation systems. Theriogenology 65(1): 236-44. 2006.
- Kuwayama M. Highly efficient vitrification method for cryopreservation of human oocytes. Reproductive BioMedicine Online. 11 300-308, 2005.
- Ushijima J, Kuwayama M. High survival rate of bovine oocytes matured in vitro following vitrification. J Reprod Dev. 50:685-96, 2004.
- Fukui Y, Kuwayama M. Effect of cryodevice type and donor's weight maturity on vitrification of minke whale oocytes at germinal vesicle stage. Zygote. 12: 353-364, 2004.
- Hochi S, Kuwayama M. Improved Survival of Vitrified In vivo-Derived Porcine Embryos. J. Reprod. Develop. 50, 461-469. 2004.
- Ezoki R, Kuwayama M. Cryopreservation of porcine embryos derived from in vitro- matured oocytes. Biology of Reproduction. 71: 452-457. 2004.

Product only for in vitro use.

Ver. 2
Revised 1th AUG 2014

Cryotech Japan 2-5-5-8F Shinjuku, Shinjuku-ku, Tokyo 160-0022 JAPAN contact@cryotech-japan.jp http://cryotech-japan.jp/

Appendix M: Wijnland Fertility Consent Forms

DONATION OF EGGS (Female Gametes)
AGREEMENT & CONSENT

I, _____ (Donor) I.D. Number _____,

hereby voluntarily consent to participate in the egg donation programme of WIJNLAND FERTILITEIT / FERTILITY clinic and declare the following:

1. I consent to the donation of my eggs to WIJNLAND FERTILITEIT / *FERTILITY* clinic on the condition that my identity are kept anonymous, the recipients identity is kept anonymous to myself and only profiling information given by me on application form, as stipulated in the National Health Act 2003 (No.61 of 2003) in Government Gazette, 12 March 2012 No.35099, is made known to the recipient(s) of my eggs, and my identity number can only be released to the Director of Health under strict confidentiality or by court order.
2. I understand that my donated eggs will be utilised, fresh or frozen, for fertility procedures with the aim of the conception of children to undisclosed recipients and have declared any previous egg donations at any other clinic to WIJNLAND FERTILITEIT / *FERTILITY* clinic.
3. I relinquish all parental rights and responsibilities for a child conceived through the use of my donated eggs.
4. I will inform the WIJNLAND FERTILITEIT / *FERTILITY* clinic of any changes in personal details (e.g. postal address; telephone numbers) until all donations and blood screening tests are completed.
5. I have not received any blood or blood related products per transfusion in the last 5 years.
6. I am not and have never been a drug addict or intravenous drug user.
7. I give permission that my blood is tested for sexually transmissible diseases, including HIV, Hepatitis and syphilis, before, during and 6 weeks after donation. All required blood screening tests are done by independent pathology laboratories.
8. I am informed of all test results and all information will be regarded as strictly confidential.
9. I have completed the separate application form which includes personal, profile, genetic history and social/ sexual history information truthfully and to the best of my knowledge.
10. I acknowledge that becoming an egg donor is subjected to a selection process, done only according to psychological screening results, medical conditions and genetic history; and the requirements are stated on the application form and discussed with me.
11. I acknowledge that all my donated eggs will be used or issued to recipients/patients of WIJNLAND FERTILITEIT / *FERTILITY* clinic until the legal limit of 6 live offspring has been confirmed born.
12. I undertake to maintain a healthy lifestyle and practice safe sexual activities for the whole period of egg donation, until donation is completed and all final blood screening tests are completed.
13. I acknowledge that remuneration for donation is only to compensate for time and effort, and my intention to donate eggs is not for financial gain.
14. I acknowledge that I have read this consent and had adequate opportunity to ask questions.

Signed at _____ on the _____ day of _____ 20 ____

Donor_____ (print name)_____

Scientist_____ (print name)_____

WIJNLAND
Ova Bank

AGREEMENT & CONSENT FOR USE OF DONOR OVA

I, (recipient/patient) I.D. Number ,
and (patient's partner) I.D. Number ,
treated by (doctor's name), hereby request WIJNLAND FERTILITY INC. PTY (LTD) to match, allocate and consent to the use of donor ova as required for fertility procedures under the following conditions stated below:

1. I/We understand that the donated ova (eggs) will be utilised in fertility procedures by WIJNLAND FERTILITY INC. PTY (LTD) clinic for the aim of conceiving a child/children for myself/ourselves.

2. I/We are aware that the fertility treatment with donated ova should be done at a fertility clinic for use in fertility treatment procedures done by a registered gynaecologist/fertility specialist for an optimal chance of success.

3. I/We unconditionally accept all parental rights and responsibilities for any child/children conceived through the use of the donated ova as if my/our own.

4. I/We understand that the release of information to the recipient(s) regarding the ova donor will be strictly in accordance with the National Health Act 61 of 2003 which states that the physical profile and screening test results about the ova donor are available to the recipient(s) but identity of the ova donor will remain anonymous; and access to any other information in the donor file may only be released by WIJNLAND FERTILITY INC. PTY (LTD) to the Director General of Health only by court order in terms of legislation.

5. The child/children conceived may have access to medical and other biographical information, except the identity of the gamete donor, after 18 years of age in accordance with the National Children's Act 41 of 2005.

6. I/We acknowledge that an ova donor may only donate to recipients until the legal limit of 6 live offspring has been confirmed born after which the specific ova donor may no longer donate as stipulated by the National Health Act 61 of 2003.

7. I/We have completed in full the donor ova application form truthfully and in full depicting profiling information for matching purposes to an ova donor according to my/our true features and have discussed the final ova donor choice with WIJNLAND FERTILITY INC. PTY (LTD) clinic and are completely satisfied.

8. I/We consent to psychological/psychometrical screening via questionnaires and/or interview/counselling as suggested by WIJNLAND FERTILITY INC. PTY (LTD) to optimise the matching process and fertility treatment with donor gametes.

9. I/We am/are liable for all screening, consultation and treatment costs of the ova donor and myself as stated on my account and is payable to the WIJNLAND FERTILITY INC. PTY (LTD) clinic on date of treatment.

9 Oewerpark, Rokewood Avenue, Die Boord, Stellenbosch. PO Box 637, Stellenbosch, 7599 / T: +27 (0)21 882 9666 / www.wijnlandfertility.co.za

WIJNLAND
Fertility

CONSENT TO DONATE ALL REMAINING OOCYTES TO SCIENTIFIC RESEARCH

I, _____ (full names of female genetic donor),

ID-number _____ , and my partner, _____

ID-number _____ ,referred by Dr _____hereby agree that all our remaining oocytes in culture or storage should not to be used for embryo transfer for ourselves, and give consent to the donation of all remaining oocytes for use in scientific research/analysis by WIJNLAND FERTILITY INC. PTY (LTD) and/or collaborators in human reproductive biology studies and on the condition that all oocytes are disposed of after completion of the research/analysis (by day 8 of embryo development) and not used for transfer with intention to treat.

We also understand and agree that scientific research in human reproduction are subject to ethical approval and therefore may be contacted should ethical committees request further consent for the use of the oocytes for research purposes.

I agree to pay in full all/any outstanding storage fees (calculated per month from last payment received) up to the date when WIJNLAND FERTILITY INC. PTY (LTD) receives this consent.

Signed at _____ (place) on the ___day of _____ 20____

Patient (genetic donor)_____(name in block letters) _____

Partner_____(name in block letters) _____

Witness _____(name in block letters) _____

E-mail: lab@wijnlandfertility.co.za (only scanned pdf document with original signatures accepted)
Fax: +27 86 566 1701
Post: PO Box 637, Stellenbosch, 7599
Delivery: 9 Oewerpark, Rokewood Ave, Die Boord, Stellenbosch, 7599

WIJNLAND
Fertility

CONSENT FOR THE FREEZING & STORAGE OF OOCYTES

CONSENT, TERMS AND CONDITIONS

I, _____(genetic donor patient) ID number _____

referred by _____(doctor), hereby request WIJNLAND FERTILITY INC. PTY (LTD) to freeze and store my oocytes (eggs) under the following conditions:

1. I undertake to the pay in full for all oocyte freezing fees and storage fees immediately to WIJNLAND FERTILITY INC. PTY (LTD);

2. I agree and consent to the discarding of all my oocytes in storage should I fail to pay the freezing fees or renewal of *pro rata* storage fees in full within six (6) months after these fees have been charged and ignore statements and/written warnings as issued during this time by WIJNLAND FERTILITY INC. PTY (LTD) to my personal mail and/or email address as provided by

   me _____ (the patient);

3. I will inform WIJNLAND FERTILITY INC. PTY (LTD) of any changes in personal contact details (e.g. postal address, telephone numbers, email) during the entire period of storage. My current contact information is as follow:

   Full Name and Surname: _____

   Contact Number: _____ E-mail:_____

4. Should I not be reached, I nominate my next of kin to be contacted and I will ensure that they are informed by me:

   Name (next of kin): _____ Relationship: _____

   Contact Number (next of kin): _____

   E-mail (next of kin): _____

5. I have been informed and understand that the fertilization potential of the oocytes may decline and/or may not survive the freezing and thawing procedures;

Patient: _____ Print Name: _____

6. I agree to do blood screening tests for sexually transmitted diseases, including HIV1&2 and Hepatitis B, at least 3 days before stimulation starts or in emergency, at latest 3 days before the aspiration procedure, and will send the reports directly to WIJNLAND FERTILITY INC. PTY (LTD), and understand that these costs are my own responsibility;

WIJNLAND
Fertility
T: +27 21 882 9666, F: +27 86 566 1701, E: lab@wijnlandfertility.co.za

STORAGE OF FROZEN SPERM
AGREEMENT & INFORMED CONSENT

I, _____ (patient) ID number _____
referred by _____ (doctor), hereby request WIJNLAND FERTILITY INC. PTY (LTD) to freeze and store my sperm under the following conditions:

1. I undertake to the pay in full for all freezing fees and annual storage fee immediately to WIJNLAND FERTILITY INC. PTY (LTD);
2. I,_____ consent to the discarding of all my sperm without warning if I fail to pay the freezing fees or renewal of *pro rata* annual storage fees in full within three (3) months after these fees have been charged;
3. I will inform WIJNLAND FERTILITY INC. PTY (LTD) of any changes in personal contact details (e.g. postal address, telephone numbers, email);
4. I have been informed and understand that the fertilization potential of the sperm may decline after the freezing procedure;
5. I understand that my sperm will only be utilised for fertility procedures involving myself and my wife/partner;
6. I will do blood screening tests for sexually transmitted diseases, including HIV1&2 and Hepatitis B, within a maximum of 72 hours at time of the freezing, and will send the original reports directly to WIJNLAND FERTILITY INC. PTY (LTD), and understand that these costs are my own responsibility;
7. In the event of my death, the stored sperm must be:

(Please mark and initial the selected option)		**Sign Initials**
	thawed and discarded	
	assigned to the care of my wife/partner: wife's/partner's name: _____ ID no. _____	
	used for scientific research	
	offered for donation by WIJNLAND FERTILITY INC. PTY (LTD)	

8. I acknowledge that if, at any time, I wish to have my frozen sperm discarded, and I must request such action in writing, give written consent by completing the appropriate forms of WIJNLAND FERTILITY INC. PTY (LTD) and agree to pay all outstanding fees in full.
9. Hereby, I declare that all my personal details are correct to my knowledge.

Signed at _____ on the _____ day of_____ 20 _____
Patient: _____ (print name) _____
Witness: _____ (print name) _____

Appendix N: Data Collection

Headings for data collection on Excel spreadsheet:

Patient Lab ID	Slide ID	Well #	Ova DOB	BMI	Ova source	Procedure	Diagnosis	Stimulation	Slide description	Aspirated oocytes

tPB2	tPNa	tPNf	T2	T3	T4	T5	T6	T7	T8	T9+	tSC

tM	tSB	tB	tEB	tHB	tDead	Grade	Day ET	ET	ET Grade	FR	BR

Appendix O: Consent from Wijnland Fertility Clinic

WIJNLAND
Fertility

TO WHOM IT MAY CONCERN

We, the partners of Wijnland Fertility, Stellenbosch, hereby give consent that **Mr. Dylan Ramsay (SU: 18170560; MSIN 0003972)** can use de-identified routine medical records of patients treated at the clinic for his MSc research study titled: *Time-lapse analysis and morphokinetic evaluation of fresh vs frozen oocytes, including donor and sibling oocyte cycles*.

We also give consent that the clinic's name be used in the final thesis document.

Dr. Johannes van Waart
DIRECTOR

Signed at Stellenbosch on 30/5/18

Lizanne van Waart
DIRECTOR

Signed at Stellenbosch on 30/05/18

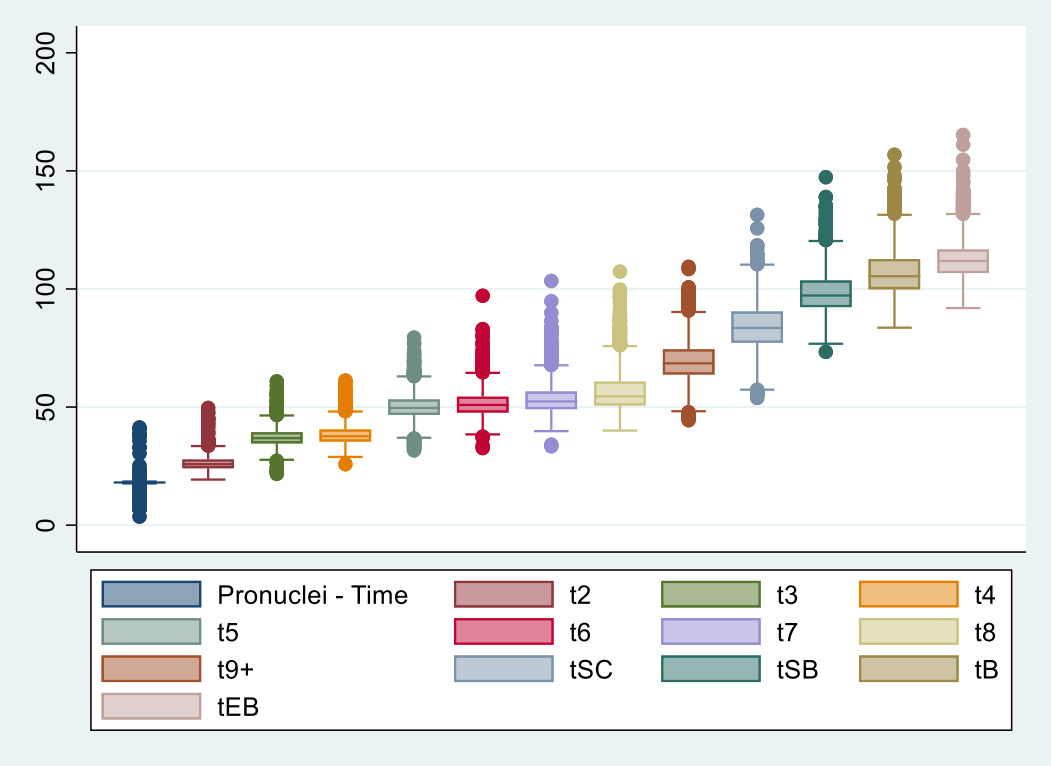
Appendix P: Raw data analysis

Dylan Ramsay
Statistical Report

TIME-LAPSE ANALYSIS AND MORPHOKINETIC EVALUATION OF FRESH VS FROZEN

NORMATIVE DATA / FRESH

Boxplots of major to epoch completion



Estimating the relevant centiles for each epoch,
The normal range is usually considered as the two centiles that contain 95% of the underlying population, thus the 2,5th and 97,5th percentile are such values

The confidence interval of these two estimates reflects the uncertainty around the estimate, With large sample size this uncertainty will be small,

These are the time points when an event appears/happens

tPN – fertilization 2PN

, centile pronucleitime, centile(2,5 10 25 50 75 90 97,5)

Variable	Obs	Percentile	Centile	-- Binom, Interp, -- [95% Conf,	Interval]
tPN	1,416	2,5	11,71704	10,84744	12,74675
	10		17,67328	17,48764	17,7838
	25		17,96989	17,96109	17,98241
	50		18,07023	18,05941	18,08008
	75		18,16995	18,15912	18,18469
	90		18,45162	18,37612	18,52759
	97,5		20,04942	19,18464	21,01652

- 95% of the normative populations will have pronuclei times between 11,72 and 20,05 hours
- The median time is 18,07 hours
- There is about 1 hour uncertainty around the normal values range +-

tPB2 - 2nd polar body time

```
 centile tpb2 , centile(2,5 10 25 50 75 90 97,5)

                                                      -- Binom, Interp, --
    Variable |       Obs  Percentile    Centile        [95% Conf, Interval]
-------------+-------------------------------------------------------------
   tpb2 |     1,349        2,5    1,878621        1,747183     1,95055
             |                10    2,470487        2,382918    2,509732
             |                25    3,003898        2,932023    3,077061
             |                50    3,676244        3,600218    3,753952 median
             |                75    4,438308        4,343156    4,519052
             |                90    5,463918        5,283665    5,641201
             |              97,5     7,67081        6,967186    10,29226
```

tPNa – appearance of individual PN

```
, centile tpna , centile(2,5 10 25 50 75 90 97,5)

                                                      -- Binom, Interp, --
    Variable |       Obs  Percentile    Centile        [95% Conf, Interval]
-------------+-------------------------------------------------------------
tPNa  |             1,382        2,5    4,779101        4,545912    4,957746
             |                10    5,604237        5,492331    5,738375
             |                25    6,365737        6,298915    6,457692
             |                50    7,328266        7,222892    7,410644 median
             |                75    8,572867        8,444857    8,694678
             |                90    10,05597        9,766147     10,3143
             |              97,5    12,72546        12,16551    15,89627
```

tpnf

```
, centile tpnf , centile(2,5 10 25 50 75 90 97,5)

                                                      -- Binom, Interp, --
    Variable |       Obs  Percentile    Centile        [95% Conf, Interval]
-------------+-------------------------------------------------------------
tpnf
               1,408            2,5    18,80977        18,60014    19,02263
             |                10    20,09869        19,88805    20,31714
             |                25    21,32061        21,12986    21,53668
             |                50    23,10079        22,90937    23,24465
             |                75     25,0702        24,87256    25,26781
             |                90    27,28597        26,94886    27,69202
             |              97,5    30,50125        29,95457    31,73508
```

t2 – 2 cell

```
, centile t2 , centile(2,5 10 25 50 75 90 97,5)

                                                      -- Binom, Interp, --
    Variable |       Obs  Percentile    Centile        [95% Conf, Interval]
-------------+-------------------------------------------------------------
          t2 |     1,415        2,5    21,53106        21,18154     21,7463
             |                10    22,77426        22,52309    22,92294
             |                25    24,06625        23,85651    24,25702
             |                50    25,80631        25,57795    25,99194
             |                75    27,84073        27,61491    28,07596
             |                90    30,10389        29,76121    30,59825
             |              97,5    33,83079        32,85279    35,19556
```

t3 – 3 cell

```
, centile t3   , centile(2,5 10 25 50 75 90 97,5)

                                                      -- Binom, Interp, --
    Variable |       Obs  Percentile    Centile        [95% Conf, Interval]
-------------+-------------------------------------------------------------
          t3 |     1,413        2,5    30,72778        29,99054    31,15676
             |                10     32,9613        32,70274    33,25535
```

	25	34,66541	34,48264	34,89741
	50	36,83479	36,63819	37,00595
	75	39,38374	39,09702	39,73939
	90	42,28449	41,6528	42,98257
	97,5	46,7763	45,74527	48,40661

T4- 4cell

V

, centile t4 , centile(2,5 10 25 50 75 90 97,5)

Variable	Obs	Percentile	Centile	-- Binom, Interp, -- [95% Conf, Interval]	
t4	1,412	2,5	32,08386	31,64858	32,26383
		10	33,71527	33,4983	33,91459
		25	35,38804	35,16077	35,64961
		50	37,65034	37,38637	37,88642
		75	40,48689	40,10779	40,70611
		90	43,79733	43,29107	44,45898
		97,5	49,94436	48,52423	52,50342

t5 – 5 cell

, centile t5 , centile(2,5 10 25 50 75 90 97,5)

Variable	Obs	Percentile	Centile	-- Binom, Interp, -- [95% Conf, Interval]	
t5	1,407	2,5	39,04088	38,00448	40,10802
		10	43,9259	43,53203	44,35087
		25	46,65523	46,36227	46,90569
		50	49,63217	49,36118	49,91914
		75	53,22787	52,71018	53,57125
		90	57,20222	56,53792	57,77342
		97,5	64,01048	63,25777	65,19362

t6 –6 cell

, centile t6 , centile(2,5 10 25 50 75 90 97,5)

Variable	Obs	Percentile	Centile	-- Binom, Interp, -- [95% Conf, Interval]	
t6	1,413	2,5	42,36309	41,72762	43,16763
		10	45,31452	44,89414	45,60786
		25	47,69254	47,50691	48,03322
		50	50,88729	50,61992	51,18335
		75	54,46142	54,05203	55,02767
		90	59,21673	58,39797	60,46045
		97,5	67,74127	65,72	69,532

t7- 7 cell

, centile t7 , centile(2,5 10 25 50 75 90 97,5)

Variable	Obs	Percentile	Centile	-- Binom, Interp, -- [95% Conf, Interval]	
t7	1,409	2,5	43,71275	43,14639	44,16367
		10	46,5974	46,11969	46,89955
		25	49,06442	48,67817	49,46021
		50	52,39317	52,07691	52,72995
		75	56,5644	56,0803	57,12609
		90	63,16761	61,98404	64,45321

```
             |                 97,5    72,91841        70,82324    74,46668
```

t8- 8cell

```
, centile t8   , centile(2,5 10 25 50 75 90 97,5)

                                                    -- Binom, Interp, --
    Variable |       Obs  Percentile    Centile        [95% Conf, Interval]
-------------+-------------------------------------------------------------
          t8 |     1,413        2,5    44,47904         44,1915    45,30334
             |                   10    47,57454        47,06793    48,11311
             |                   25    50,62387        50,28991     50,8913
             |                   50    54,51545        54,22075    54,97908
             |                   75    60,82463        59,93329    62,15186
             |                   90    69,28454        68,38207     70,7579
             |                 97,5    82,35905         79,5296    83,92926
```

t9 – 9 cell

```
, centile t9   , centile(2,5 10 25 50 75 90 97,5)

                                                    -- Binom, Interp, --
    Variable |       Obs  Percentile    Centile        [95% Conf, Interval]
-------------+-------------------------------------------------------------
          t9 |     1,413        2,5    52,19429        50,67439    53,31334
             |                   10    58,59972        57,76524    59,17685
             |                   25    63,78573        63,17427    64,31241
             |                   50    68,52028        68,12159     69,0991
             |                   75    74,46112        73,76468     75,0904
             |                   90    80,35463        79,26908     81,6501
             |                 97,5    90,11943        87,66999     91,9161
```

tSC - compation

```
, centile tsc   , centile(2,5 10 25 50 75 90 97,5)

                                                    -- Binom, Interp, --
    Variable |       Obs  Percentile    Centile        [95% Conf, Interval]
-------------+-------------------------------------------------------------
         tsc |     1,413        2,5    62,00282        60,31359    64,10399
             |                   10    70,35081        69,26795    71,44802
             |                   25    77,20207        76,62811    77,89792
             |                   50    83,49568        82,89625    84,40776
             |                   75    90,46778        89,61054    91,10587
             |                   90    97,49146        96,26914    98,53989
             |                 97,5    106,7959        104,9902    109,4304
```

tSB – initiation of blastulation

```
, centile tsb   , centile(2,5 10 25 50 75 90 97,5)

                                                    -- Binom, Interp, --
    Variable |       Obs  Percentile    Centile        [95% Conf, Interval]
-------------+-------------------------------------------------------------
```

				-- Binom, Interp, --	
Variable	Obs	Percentile	Centile	[95% Conf,	Interval]
tsb	1,395	2,5	84,07062	83,34872	84,80342
		10	88,35211	87,72522	88,89899
		25	92,2686	91,70976	92,63809
		50	97,24725	96,84585	97,62696
		75	103,5852	102,8728	104,2568
		90	111,3477	110,5848	112,5339
		97,5	120,8153	117,9226	122,9435

tB –full blastocyst

, centile tb , centile(2,5 10 25 50 75 90 97,5)

				-- Binom, Interp, --	
Variable	Obs	Percentile	Centile	[95% Conf,	Interval]
tb	1,317	2,5	91,19585	90,61646	92,14775
		10	95,71043	95,00769	96,26183
		25	99,8878	99,41438	100,4908
		50	105,3701	104,7719	106,1193
		75	112,6653	111,8573	113,4026
		90	121,955	120,241	123,427
		97,5	134,5572	132,1154	137,2673

tEB – expanded blastocyst

, centile teb , centile(2,5 10 25 50 75 90 97,5)

				-- Binom, Interp, --	
Variable	Obs	Percentile	Centile	[95% Conf,	Interval]
teb	960	2,5	98,27284	97,25319	99,41647
		10	102,5223	101,9986	103,0649
		25	106,68	106,2352	107,3137
		50	111,8383	111,4281	112,4622
		75	116,7176	116,0724	117,8767
		90	131,3342	128,8694	133,1207
		97,5	139,6419	137,8312	140,8876

tHB - hatching blastoccyst

, centile thb , centile(2,5 10 25 50 75 90 97,5)

				-- Binom, Interp, --	
Variable	Obs	Percentile	Centile	[95% Conf,	Interval]
thb	137	2,5	104,6359	97,4	106,3381*
		10	107,1367	106,0647	109,0193
		25	110,4807	110,0234	111,493
		50	114,4	113,0218	115,3965
		75	119,4714	116,3695	132,6821
		90	138,1818	135,865	140,6625
		97,5	148,5442	140,6146	152,4486*

* Lower (upper) confidence limit held at minimum (maximum) of sample

Centiles of difference between epochs (instant in time chosen as the origin)

t2 duration – time at 2 cell

```
, centile t2duration, centile(2,5 10 25 50 75 90 97,5)

                                                  -- Binom, Interp, --
    Variable |       Obs  Percentile    Centile        [95% Conf, Interval]
-------------+-------------------------------------------------------------
  t2duration |     1,411        2,5    8,435602         7,93343     9,00281
             |                   10    9,752496        9,506048    9,753323
             |                   25    10,26122        10,25343    10,50276
             |                   50      11,009        11,00369    11,25324
             |                   75    11,76679        11,75392     12,0038
             |                   90    12,76105        12,75355      13,004
             |                 97,5    13,87608        13,75395     14,2555
```

ECC1 (t2-tPB2)

```
, centile ecc1 , centile(2,5 10 25 50 75 90 97,5)

                                                  -- Binom, Interp, --
    Variable |       Obs  Percentile    Centile        [95% Conf, Interval]
-------------+-------------------------------------------------------------
        ecc1 |     1,348        2,5    17,50599        17,07819    17,94206
             |                   10    19,00581        18,75597     19,2529
             |                   25    20,25749        20,01679    20,50609
             |                   50    21,85689        21,75376    22,03278
             |                   75    23,90671        23,70287    24,09314
             |                   90    25,84069        25,50803    26,22894
             |                 97,5    28,77515        28,28993    29,43124
```

VP

```
, centile vp , centile(2,5 10 25 50 75 90 97,5)

                                                  -- Binom, Interp, --
    Variable |       Obs  Percentile    Centile        [95% Conf, Interval]
-------------+-------------------------------------------------------------
          vp |     1,380        2,5    10,75393        10,25279    11,25014
             |                   10    12,26706        12,15508     12,5049
             |                   25     13,9243        13,71386    14,01153
             |                   50    15,50515        15,25829    15,75448
             |                   75    17,40337        17,19958    17,64348
             |                   90    19,28668        19,00611    19,51188
             |                 97,5     21,7922        21,37423    23,01266
```

t3 duration – time as 3 cell

```
, centile t3duration , centile(2,5 10 25 50 75 90 97,5)

                                                  -- Binom, Interp, --
    Variable |       Obs  Percentile    Centile        [95% Conf, Interval]
-------------+-------------------------------------------------------------
  t3duration |     1,409        2,5           0               0           0
             |                   10           0               0           0
```

```
             |                  25    ,2500249        ,2499898    ,2500555
             |                  50    ,5001305        ,5000926    ,5001735
             |                  75    1,000176        ,7522902    1,000358
             |                  90    2,000696        1,751197    2,251015
             |                97,5    7,191232        5,751892    11,08377
```

t4 duration – time as 4 cell

```
, centile t4duration , centile(2,5 10 25 50 75 90 97,5)

                                                    -- Binom, Interp, --
    Variable |       Obs  Percentile    Centile        [95% Conf, Interval]
-------------+-------------------------------------------------------------
  t4duration |     1,403         2,5    ,5001689        ,2501873    1,000194
             |                   10    9,252899        8,753048    9,503801
             |                   25    10,83756        10,75306    11,00406
             |                   50    12,25367        12,00533    12,25922
             |                   75    13,50438        13,25894    13,72914
             |                   90     14,8106        14,54232    15,05986
             |                 97,5    17,34163        16,52553    18,10233
```

t5 duration – time as 5 cell

```
, centile t5duration   , centile(2,5 10 25 50 75 90 97,5)

                                                    -- Binom, Interp, --
    Variable |       Obs  Percentile    Centile        [95% Conf, Interval]
-------------+-------------------------------------------------------------
  t5duration |     1,405         2,5           0               0           0
             |                   10           0               0           0
             |                   25    ,2501074        ,2500792    ,2501447
             |                   50    ,7489239        ,5005385    ,7501825
             |                   75    1,500224        1,250585    1,500517
             |                   90    2,900203        2,502348    3,501048
             |                 97,5    12,96905        11,91214    13,84354
```

T6 duration – time as 6 cell

```
, centile t6duration   , centile(2,5 10 25 50 75 90 97,5)

                                                    -- Binom, Interp, --
    Variable |       Obs  Percentile    Centile        [95% Conf, Interval]
-------------+-------------------------------------------------------------
  t6duration |     1,412         2,5           0               0           0
             |                   10    ,0493656               0    ,2499279
             |                   25    ,5000481        ,4971122    ,5001072
             |                   50    1,000252        ,9972247    1,000371
             |                   75    2,000834        2,000268    2,250705
             |                   90    4,251707        3,751367     5,01666
             |                 97,5    14,00432        11,09513    16,65849
```

t7 duration – time as 7 cell

```
, centile t7duration   , centile(2,5 10 25 50 75 90 97,5)

                                                    -- Binom, Interp, --
    Variable |       Obs  Percentile    Centile        [95% Conf, Interval]
-------------+-------------------------------------------------------------
  t7duration |     1,408         2,5           0               0           0
             |                   10    ,2499438               0    ,2500035
             |                   25    ,5001528        ,5000997    ,5002427
             |                   50    1,250312        1,001919    1,250484
```

	75	3,25114	3,000688	3,505808
	90	9,765284	8,409948	11,75353
	97,5	17,59841	16,28702	18,92496

t8 duration - time as 8 cell

```
, centile t8duration   , centile(2,5 10 25 50 75 90 97,5)
```

Variable	Obs	Percentile	Centile	-- Binom, Interp, -- [95% Conf,	Interval]
t8duration	1,412	2,5	0	0	,2499926
		10	1,50038	1,00072	1,7537
		25	8,00322	7,004699	9,00296
		50	13,50411	13,14876	14,00289
		75	17,01049	16,75476	17,44627
		90	19,51446	19,18694	20,2391
		97,5	23,69316	22,75776	24,50741

t9 duration - time as 9cell

```
, centile t9duration   , centile(2,5 10 25 50 75 90 97,5)
```

Variable	Obs	Percentile	Centile	-- Binom, Interp, -- [95% Conf,	Interval]
t9duration	1,411	2,5	0	0	0
		10	3,001105	2,000926	3,751144
		25	7,752241	7,00344	8,252207
		50	13,25537	12,75403	13,75876
		75	20,60297	19,75625	21,50633
		90	27,23254	26,05896	28,50931
		97,5	37,76313	34,33241	40,48901

ECC3 duration - t8-t4

```
 centile ecc3   , centile(2,5 10 25 50 75 90 97,5)
```

Variable	Obs	Percentile	Centile	-- Binom, Interp, -- [95% Conf,	Interval]
ecc3	1,409	2,5	11,50526	11,25375	11,75384
		10	12,75369	12,5049	12,94731
		25	14,09458	13,93738	14,25661
		50	16,00854	15,7558	16,25537
		75	20,03571	19,50543	20,99884
		90	29,26047	27,65329	30,50937
		97,5	38,47464	36,52707	39,77985

S3 - t8-t5

```
, centile s3   , centile(2,5 10 25 50 75 90 97,5)
```

Variable	Obs	Percentile	Centile	-- Binom, Interp, -- [95% Conf,	Interval]
s3	1,404	2,5	,7501781	,6413965	,7502416

	10	1,250243	1,000502	1,250447
	25	2,00064	2,000304	2,004749
	50	3,750952	3,500848	4,000957
	75	8,754516	7,923053	10,00268
	90	17,8073	16,51772	18,75572
	97,5	25,21451	23,2976	28,62055

tSC – duration as compacted

```
, centile tscduration   , centile(2,5 10 25 50 75 90 97,5)
```

Variable	Obs	Percentile	Centile	-- Binom, Interp, -- [95% Conf,	Interval]
tscduration	1,395	2,5	3,500719	3,000743	3,69669
		10	5,752466	5,25198	6,251632
		25	9,25434	8,756324	9,562435
		50	13,50379	13,00382	13,75451
		75	19,25567	18,57046	20,00627
		90	26,71951	25,33501	27,6031
		97,5	35,03539	33,04772	37,07768

tSB – duration as blastocyst before full blastocyst

```
, centile tsbduration   , centile(2,5 10 25 50 75 90 97,5)
```

Variable	Obs	Percentile	Centile	-- Binom, Interp, -- [95% Conf,	Interval]
tsbduration	1,316	2,5	3,750989	3,388606	3,985465
		10	4,75456	4,504877	5,007238
		25	6,315583	6,251274	6,502544
		50	8,339596	8,047933	8,50302
		75	11,03527	10,75339	11,47543
		90	14,51754	13,78527	15,50465
		97,5	21,77319	20,52544	23,11072

tB – duration as full blastocyst before expanded blastocyst

```
, centile tbduration   , centile(2,5 10 25 50 75 90 97,5)
```

Variable	Obs	Percentile	Centile	-- Binom, Interp, -- [95% Conf,	Interval]
tbduration	960	2,5	3,000633	2,250749	3,250939
		10	4,501101	4,251136	4,50361
		25	5,75276	5,502969	6,004329
		50	8,008262	7,773135	8,255996
		75	10,46963	10,00631	10,7592
		90	13,72969	13,09511	14,5061
		97,5	20,44249	18,35715	23,05363

tEB – duration as expanded blastocyst

```
, centile tebduration   , centile(2,5 10 25 50 75 90 97,5)
```

Variable	Obs	Percentile	Centile	-- Binom, Interp, -- [95% Conf,	Interval]
tebduration	960	2,5	-,2525927	-1,046012	0
		10	0	0	,2126825
		25	2,001069	1,750574	2,410508
		50	5,377507	5,001597	5,847279
		75	9,493976	8,505212	9,955399
		90	13,2566	12,75551	14,00452
		97,5	19,01808	17,2566	22,68618

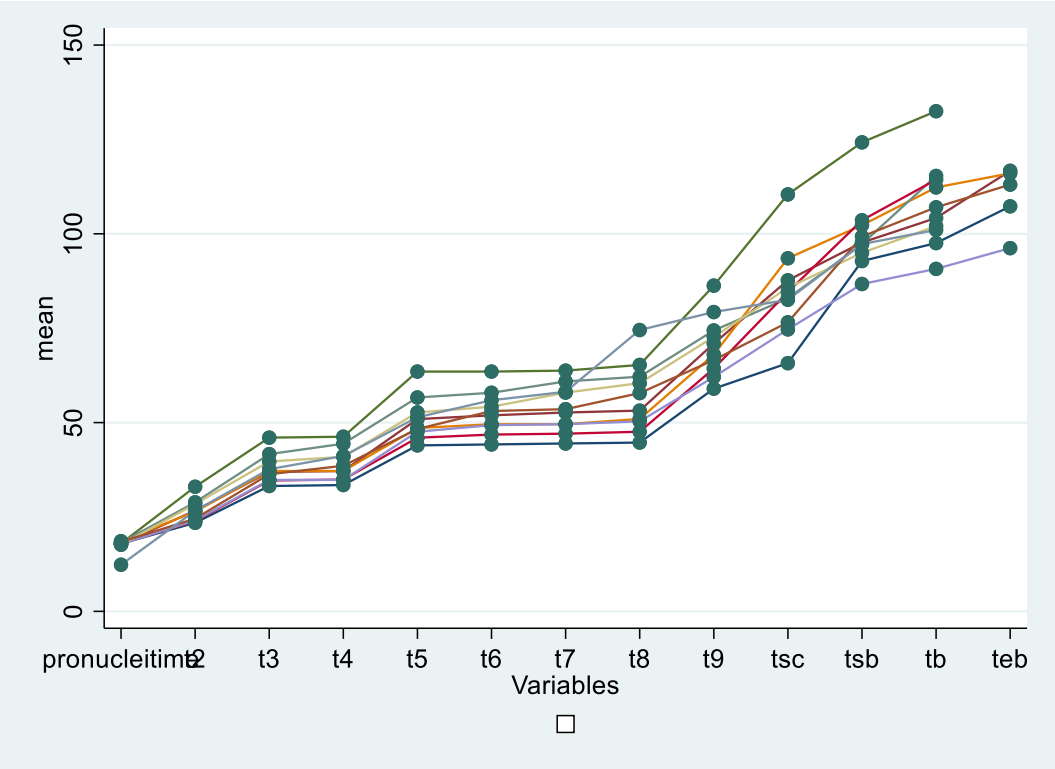
tHB – duration as hatched blastocyst

```
, centile thbduration   , centile(2,5 10 25 50 75 90 97,5)
```

Variable	Obs	Percentile	Centile	-- Binom, Interp, -- [95% Conf,	Interval]
thbduration	137	2,5	-3,550674	-10,80778	-,2929556*
		10	0	-,3482646	0
		25	,1249835	0	,9826714
		50	2,998741	1,5	3,304689
		75	5,500795	4,501056	7,503593
		90	10,41711	8,571926	12,22043
		97,5	15,74801	11,8657	30,79063*

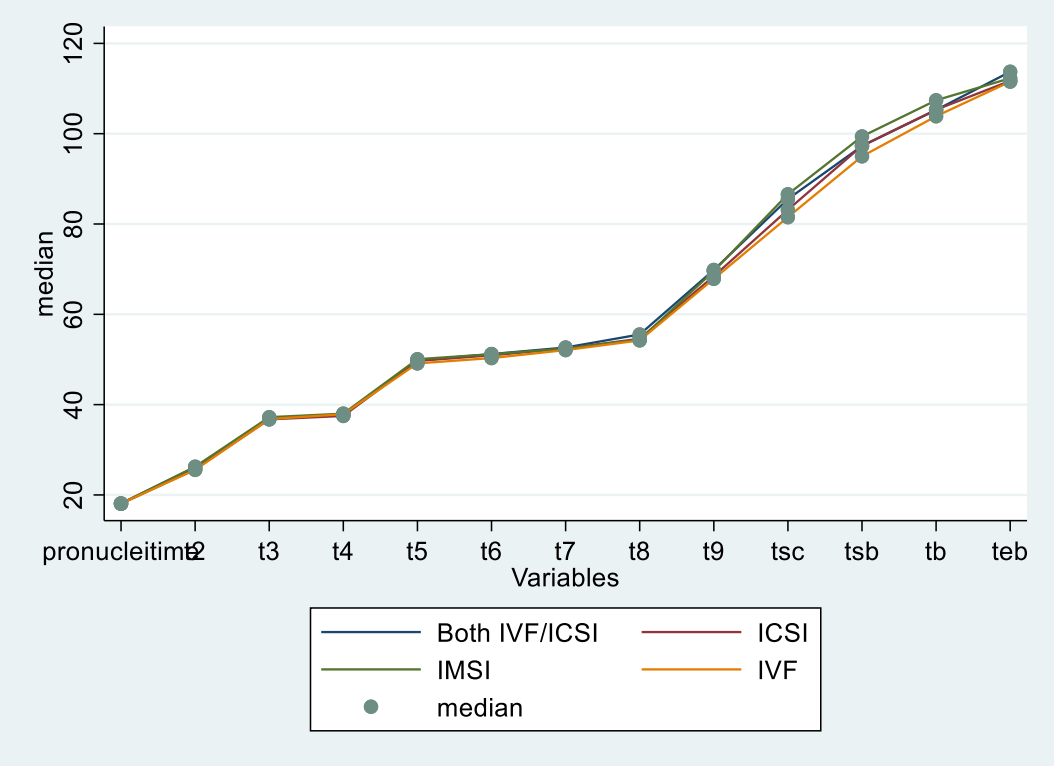
* Lower (upper) confidence limit held at minimum (maximum) of sample

```
Epoch time profile for 10 randomly selected cases
```



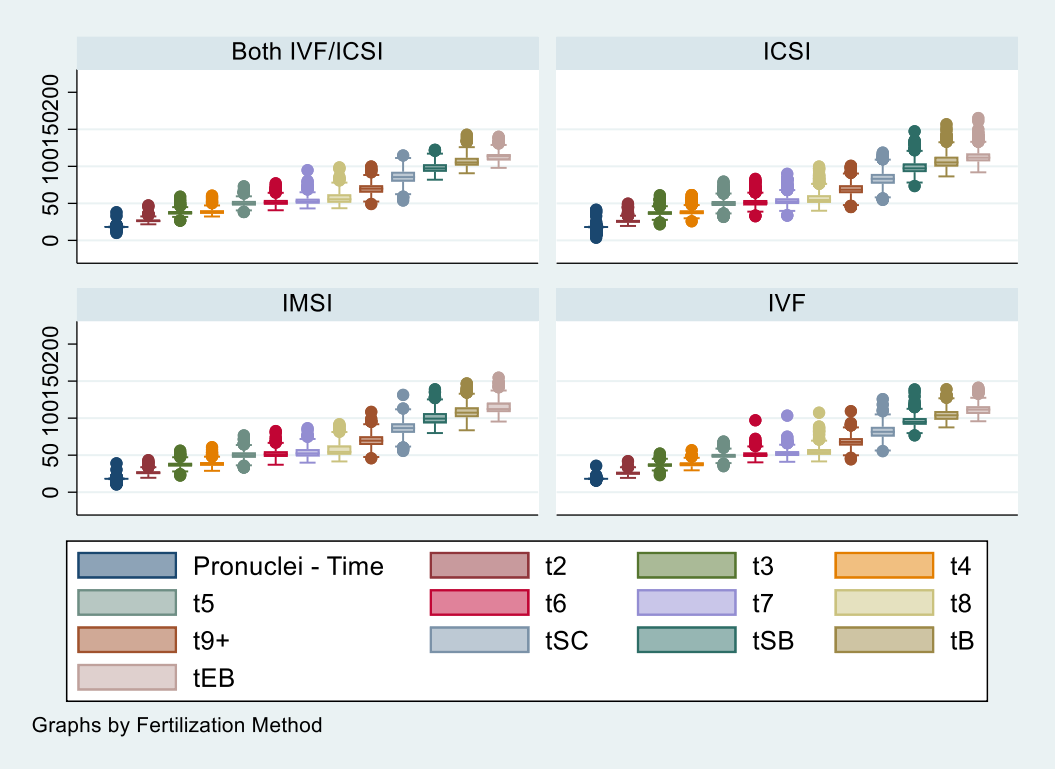

```
,
end of do-file
```

```
Median profile over epochs by fertilization method

encode fertilizationmethod, gen(nfert_meth)

profileplot pronucleitime  t2 t3 t4  t5 t6 t7 t8 t9 tsc tsb tb teb, by( nfert_meth ) median
```



- Some differentiation occurs only after t9 with IVF having slightly shorter median time

Boxplots of epoch times by fertilization type



Times by fertilization type

```
, tab nfert_meth

Fertilization |
       Method |      Freq,     Percent        Cum,
--------------+-----------------------------------
Both IVF/ICSI |         134        9,46        9,46  1    small
         ICSI |         848       59,84       69,30  2
         IMSI |         226       15,95       85,25  3
          IVF |         209       14,75      100,00  4
--------------+-----------------------------------
        Total |       1,417      100,00

foreach var of varlist pronucleitime tpb2 tpna tpnf t2  t3  t4  t5  t6 t7  t8  t9 tsc  tsb
tb  teb thb {
  2,   centile `var' if nfert_meth==1, centile(2,5 10 25 50 75 90 97,5)
  3, }
```

-- Binom, Interp, --

IVF/ICSI =1

tPN

```
   Variable |       Obs  Percentile    Centile        [95% Conf, Interval]
------------+-------------------------------------------------------------
pronucleit~e |       134        2,5    13,06906        10,08255    17,80757*
             |                   10    17,91354        17,79054    17,93704
             |                   25    17,98028        17,95435    18,01768
             |                   50    18,09784        18,06721    18,13353
             |                   75    18,21855        18,16961    18,29246
             |                   90    18,62443        18,29744    19,38764
             |                 97,5    20,99658        19,24394    37,99054*

* Lower (upper) confidence limit held at minimum (maximum) of sample
```

tPB2

Variable	Obs	Percentile	Centile	-- Binom, Interp, -- [95% Conf,	Interval]
tpb2	131	2,5	1,997103	1,613219	2,647347*
		10	2,714136	2,630139	2,833065
		25	3,151365	2,908814	3,302465
		50	3,710105	3,490832	3,971765
		75	4,505782	4,254636	5,053771
		90	5,870672	5,216317	16,71555
		97,5	20,99679	10,48886	25,23541*

* Lower (upper) confidence limit held at minimum (maximum) of sample

tPNa

Variable	Obs	Percentile	Centile	-- Binom, Interp, -- [95% Conf,	Interval]
tpna	134	2,5	4,665486	4,541341	5,180126*
		10	5,701911	5,119469	5,977659
		25	6,430929	6,126719	6,846588
		50	7,72642	7,391047	8,14146
		75	9,468632	8,628594	10,12991
		90	11,6249	10,30066	19,91334
		97,5	22,18705	19,64963	30,98799*

* Lower (upper) confidence limit held at minimum (maximum) of sample

tPNf

Variable	Obs	Percentile	Centile	-- Binom, Interp, -- [95% Conf,	Interval]
tpnf	134	2,5	19,36187	18,74088	19,93627*
		10	20,50614	19,8711	20,94218
		25	21,91328	21,10654	22,21527
		50	23,27667	22,7299	23,87899
		75	25,35944	24,80497	26,14257
		90	27,74849	26,39157	29,64697
		97,5	33,89765	29,2248	44,00654*

* Lower (upper) confidence limit held at minimum (maximum) of sample

t2

Variable	Obs	Percentile	Centile	-- Binom, Interp, -- [95% Conf,	Interval]
t2	134	2,5	21,99503	21,72797	22,22503*
		10	22,89895	22,2239	23,72128
		25	24,64619	23,88765	24,96145
		50	26,14525	25,41886	26,88755
		75	28,00894	27,48992	28,85337
		90	30,66452	29,303	32,31561
		97,5	36,18881	32,26679	47,25735*

* Lower (upper) confidence limit held at minimum (maximum) of sample

t3

Variable	Obs	Percentile	Centile	-- Binom, Interp, -- [95% Conf,	Interval]
t3	134	2,5	31,7257	26,60358	32,75933*
		10	33,50852	32,59901	34,55354
		25	35,30198	34,93014	35,92938
		50	37,00484	36,52363	37,91561
		75	39,70163	38,98722	40,72236
		90	42,79109	41,05689	45,89006
		97,5	51,39387	45,20515	59,01083*

* Lower (upper) confidence limit held at minimum (maximum) of sample

t4

Variable	Obs	Percentile	Centile	-- Binom, Interp, -- [95% Conf,	Interval]
t4	133	2,5	32,54851	32,22263	33,69594*
		10	34,49131	33,61839	35,41245
		25	35,98621	35,57982	36,65425
		50	37,72612	37,21064	38,60623
		75	40,6699	39,75391	41,52202
		90	44,27989	42,06761	50,61866
		97,5	56,83935	50,34765	60,43004*

* Lower (upper) confidence limit held at minimum (maximum) of sample

Variable	Obs	Percentile	Centile	-- Binom, Interp, -- [95% Conf,	Interval]
t5	132	2,5	40,94709	38,36161	44,24738*
		10	44,97241	44,23021	46,19201
		25	47,39732	46,48479	47,93139
		50	49,77083	48,76116	50,93398
		75	53,4171	52,21609	54,50384
		90	57,85472	55,17748	64,40845
		97,5	65,92883	64,28219	73,01492*

* Lower (upper) confidence limit held at minimum (maximum) of sample

Variable	Obs	Percentile	Centile	-- Binom, Interp, -- [95% Conf,	Interval]
t6	134	2,5	42,79945	40,64127	44,57794*
		10	45,69869	44,52625	46,89093
		25	48,20476	47,65794	48,91979
		50	51,19833	50,15342	52,20511
		75	54,64331	53,17705	55,91546
		90	58,67844	57,04022	66,90425
		97,5	71,32786	66,77604	77,01754*

* Lower (upper) confidence limit held at minimum (maximum) of sample

Variable	Obs	Percentile	Centile	-- Binom, Interp, -- [95% Conf,	Interval]
t7	134	2,5	44,32876	43,17659	46,11501*
		10	47,02906	45,8072	48,42876
		25	49,68729	48,63324	50,55084
		50	52,65652	51,52722	53,47446
		75	56,45789	55,20206	58,38799
		90	63,61689	58,99617	70,18386
		97,5	76,77036	70,12642	94,78995*

* Lower (upper) confidence limit held at minimum (maximum) of sample

Variable	Obs	Percentile	Centile	-- Binom, Interp, -- [95% Conf,	Interval]
t8	134	2,5	45,07818	43,42689	46,68534*
		10	47,90182	46,55428	49,39735
		25	50,85042	49,76858	52,11746
		50	55,53283	53,46426	56,94996
		75	62,1744	58,87806	65,36283
		90	71,05041	66,27828	77,47459
		97,5	85,07551	76,97195	98,54265*

* Lower (upper) confidence limit held at minimum (maximum) of sample

Variable	Obs	Percentile	Centile	-- Binom, Interp, -- [95% Conf,	Interval]
t9	134	2,5	56,55324	49,11099	59,35126*
		10	61,66104	59,21174	62,91454
		25	64,58098	63,47757	66,48167
		50	69,82052	68,30461	71,63268
		75	74,47306	73,15533	77,98408

```
             |                  90    80,52315        78,44363    87,27635
             |                97,5     95,4316        86,36304    99,77494*

* Lower (upper) confidence limit held at minimum (maximum) of sample

                                                    -- Binom, Interp, --
    Variable |       Obs  Percentile    Centile        [95% Conf, Interval]
-------------+-------------------------------------------------------------
         tSC |       134         2,5    58,49295        53,86217    68,41819*
             |                    10    73,52596        67,48752    76,59265
             |                    25    79,34985        77,76948     80,6309
             |                    50    85,50554        83,05533     88,2938
             |                    75    92,72779        90,98554    95,32056
             |                    90    99,69359         96,8938    104,9848
             |                  97,5     108,476        104,7584    114,6688*

* Lower (upper) confidence limit held at minimum (maximum) of sample

                                                    -- Binom, Interp, --
    Variable |       Obs  Percentile    Centile        [95% Conf, Interval]
-------------+-------------------------------------------------------------
         tSB |       132         2,5    85,12059        81,91349    88,46799*
             |                    10    89,25746          88,116     91,0974
             |                    25    92,70565        91,43411    94,10507
             |                    50    97,26524        96,47278    99,47866
             |                    75     102,828        101,4591    105,7397
             |                    90     112,114        108,6483    114,9962
             |                  97,5    119,6908        114,7518    122,2918*

* Lower (upper) confidence limit held at minimum (maximum) of sample


                                                    -- Binom, Interp, --
    Variable |       Obs  Percentile    Centile        [95% Conf, Interval]
-------------+-------------------------------------------------------------
          tB |       125         2,5    92,96252        90,68102    94,82433*
             |                    10    98,00701        94,05464    99,16714
             |                    25    101,0733        99,62428    102,6845
             |                    50    105,2478        104,2836    108,2474
             |                    75    111,6506        110,2731    114,6262
             |                    90    122,4435         115,415    127,3212
             |                  97,5    135,2114        125,0864    142,6876*

* Lower (upper) confidence limit held at minimum (maximum) of sample


                                    -- Binom, Interp, --
    Variable |       Obs  Percentile    Centile        [95% Conf, Interval]
-------------+-------------------------------------------------------------
         tEB |        90         2,5    101,7681        97,93288    104,7056*
             |                    10    105,5714        103,8377    106,4013
             |                    25    108,2488        106,3974    110,5194
             |                    50    113,7143        111,4021    114,9789
             |                    75      116,72        115,5097    119,0498
             |                    90    131,9599         118,692    133,3597
             |                  97,5    138,2596        132,6763    139,9257*

* Lower (upper) confidence limit held at minimum (maximum) of sample

                                                    -- Binom, Interp, --
    Variable |       Obs  Percentile    Centile        [95% Conf, Interval]
-------------+-------------------------------------------------------------
         tHB |         9         2,5    108,1582        108,1582    110,1246*
             |                    10    108,1582        108,1582    115,4827*
             |                    25    112,4459        108,1582    122,2942*
             |                    50    116,6868        110,5319    139,4467
             |                    75    138,3887        116,2289    149,3017*
             |                    90    149,3017        130,0395    149,3017*
             |                  97,5    149,3017        139,9035    149,3017*

* Lower (upper) confidence limit held at minimum (maximum) of sample
```

ICSI =2

```
, foreach var of varlist pronucleitime tpb2 tpna tpnf t2  t3  t4  t5  t6 t7  t8  t9 tsc  tsb
tb  teb thb {
  2,   centile `var' if nfert_meth==2, centile(2,5 10 25 50 75 90 97,5)
  3, }
```

Variable	Obs	Percentile	Centile	-- Binom, Interp, -- [95% Conf,	Interval]
pronucleit~e	847	2,5	10,72577	8,634423	11,97697
		10	17,48318	17,16295	17,70985
		25	17,95928	17,93905	17,97092
		50	18,05011	18,03572	18,06388
		75	18,14628	18,13464	18,16266
		90	18,36696	18,32309	18,48377
		97,5	19,03789	18,8319	20,61938

Variable	Obs	Percentile	Centile	-- Binom, Interp, -- [95% Conf,	Interval]
tpb2	819	2,5	1,910055	1,786888	2,004064
		10	2,444674	2,349983	2,497068
		25	2,961369	2,852279	3,047522
		50	3,560619	3,48293	3,616896
		75	4,27857	4,138727	4,384621
		90	5,154688	4,977837	5,359474
		97,5	7,478026	6,273222	9,093331

Variable	Obs	Percentile	Centile	-- Binom, Interp, -- [95% Conf,	Interval]
tpna	835	2,5	4,791146	4,516168	5,008005
		10	5,568351	5,359313	5,725691
		25	6,337473	6,24707	6,46003
		50	7,209845	7,090982	7,325948
		75	8,464993	8,191823	8,646675
		90	9,864652	9,577449	10,27878
		97,5	12,67881	11,9677	14,55715

Variable	Obs	Percentile	Centile	-- Binom, Interp, -- [95% Conf,	Interval]
tpnf	847	2,5	18,70871	18,29403	18,98166
		10	19,99915	19,79522	20,25078
		25	21,20025	21,0327	21,50535
		50	23,05097	22,7821	23,21885
		75	24,94868	24,71475	25,2614
		90	27,1701	26,74266	27,5411
		97,5	30,34325	29,51765	31,79405

Variable	Obs	Percentile	Centile	-- Binom, Interp, -- [95% Conf,	Interval]
t2	848	2,5	21,3472	21,01041	21,75565
		10	22,63974	22,3993	22,89211
		25	23,96308	23,74309	24,20907
		50	25,68341	25,50674	25,9677
		75	27,7239	27,42747	28,05518
		90	29,92372	29,5404	30,42696
		97,5	33,19248	32,54815	35,23519

Variable	Obs	Percentile	Centile	-- Binom, Interp, -- [95% Conf,	Interval]
t3	846	2,5	30,73249	30,05501	31,25907
		10	32,85906	32,47636	33,20895
		25	34,61375	34,35088	34,86293
		50	36,73802	36,52842	37,00672
		75	39,28861	38,9262	39,80051
		90	42,30264	41,54761	42,99159
		97,5	46,37101	45,0221	48,34297

Variable	Obs	Percentile	Centile	-- Binom, Interp, -- [95% Conf,	Interval]
t4	844	2,5	31,83071	31,22572	32,15823
		10	33,65826	33,33737	33,93916

				-- Binom, Interp, --	
		25	35,31504	35,06112	35,57942
		50	37,49494	37,17246	37,84252
		75	40,46025	39,95327	40,85565
		90	43,55701	42,98398	44,31218
		97,5	49,83668	47,73498	53,02114

Variable	Obs	Percentile	Centile	-- Binom, Interp, -- [95% Conf,	Interval]
t5	844	2,5	39,12767	36,78182	41,09681
		10	43,75554	43,40033	44,26916
		25	46,42685	46,09619	46,96611
		50	49,67242	49,30884	50,10254
		75	53,1712	52,61897	53,82532
		90	56,95945	56,08031	57,73717
		97,5	63,78496	61,9835	65,14138

Variable	Obs	Percentile	Centile	-- Binom, Interp, -- [95% Conf,	Interval]
t6	845	2,5	42,5068	41,79486	43,40747
		10	45,09388	44,65415	45,61414
		25	47,81268	47,3851	48,22244
		50	50,9251	50,6046	51,3145
		75	54,58354	53,8883	55,2426
		90	59,10217	58,00227	60,43438
		97,5	67,04757	64,92596	69,68289

Variable	Obs	Percentile	Centile	-- Binom, Interp, -- [95% Conf,	Interval]
t7	844	2,5	43,66718	42,83426	44,03781
		10	46,31816	45,55999	46,90953
		25	49,10011	48,76937	49,56847
		50	52,42665	52,07084	52,86405
		75	56,68542	56,11377	57,40155
		90	62,7881	61,59924	64,502
		97,5	72,93455	70,4277	75,21297

Variable	Obs	Percentile	Centile	-- Binom, Interp, -- [95% Conf,	Interval]
t8	846	2,5	44,34621	43,99565	45,30422
		10	47,48816	46,62009	48,09442
		25	50,58784	50,10608	51,02052
		50	54,58462	54,12905	55,25375
		75	60,95435	59,76367	62,3473
		90	68,95131	67,85938	70,05337
		97,5	79,70747	76,75016	83,7279

Variable	Obs	Percentile	Centile	-- Binom, Interp, -- [95% Conf,	Interval]
t9	846	2,5	52,35139	50,46316	53,82672
		10	58,34211	57,3212	59,0881
		25	63,6393	62,98032	64,32704
		50	68,43429	67,95198	69,18022
		75	74,35899	73,53834	75,09893
		90	79,814	78,7711	81,21432
		97,5	88,86959	87,19966	91,65978

Variable	Obs	Percentile	Centile	-- Binom, Interp, -- [95% Conf,	Interval]
tsc	847	2,5	62,92632	60,6437	64,73708
		10	69,67609	68,64815	71,08487
		25	76,741	75,6409	77,47353
		50	83,13009	82,26766	83,88895
		75	89,79131	88,93111	90,94071
		90	96,44537	94,97167	98,21033
		97,5	106,1252	103,473	108,9854

Variable	Obs	Percentile	Centile	-- Binom, Interp, -- [95% Conf,	Interval]
tsb	838	2,5	84,28949	83,24605	85,26753
		10	88,2808	87,5417	88,91415

```
             |                  25    92,13989        91,42222    92,64139
             |                  50    97,32137        96,77336    97,86182
             |                  75    103,7489        102,8907    104,8914
             |                  90    111,1234        109,8392    112,3106
             |                97,5    120,1432         115,996    122,5878

                                                    -- Binom, Interp, --
    Variable |       Obs  Percentile    Centile        [95% Conf, Interval]
-------------+-------------------------------------------------------------
          tb |       792        2,5    91,00546        90,07898    92,37047
             |                  10    95,71114         95,1085    96,36041
             |                  25    99,84627        98,93305    100,5587
             |                  50    105,3061        104,5439    106,2675
             |                  75    113,0783        112,0943    114,4926
             |                  90    122,1167        120,4614    123,7818
             |                97,5    133,8714        130,4004     138,244

                                                    -- Binom, Interp, --
    Variable |       Obs  Percentile    Centile        [95% Conf, Interval]
-------------+-------------------------------------------------------------
         teb |       593        2,5    97,97121        96,63942    99,67906
             |                  10     102,495        101,5035    103,0096
             |                  25    106,6272        105,7586    107,4449
             |                  50    111,7074        111,1086    112,4205
             |                  75    117,3013        115,9923    120,4031
             |                  90    131,1061        128,3827    133,5285
             |                97,5    139,6699         137,865    140,9237

                                                    -- Binom, Interp, --
    Variable |       Obs  Percentile    Centile        [95% Conf, Interval]
-------------+-------------------------------------------------------------
         thb |        93        2,5    104,6066        103,6198    105,5558*
             |                  10    106,8159        105,1156    109,3556
             |                  25    110,4592        109,3068    111,5181
             |                  50    113,8951        112,3048    115,3422
             |                  75    118,6799        115,6923    132,4612
             |                  90       136,9        132,4458    140,4414
             |                97,5    140,7259        138,6211    147,6184*

* Lower (upper) confidence limit held at minimum (maximum) of sample
```

IMSI +3

```
, foreach var of varlist pronucleitime tpb2 tpna tpnf t2  t3  t4  t5  t6 t7  t8  t9 tsc  tsb
tb  teb thb {
  2,   centile `var' if nfert_meth==3, centile(2,5 10 25 50 75 90 97,5)
  3, }

                                                    -- Binom, Interp, --
    Variable |       Obs  Percentile    Centile        [95% Conf, Interval]
-------------+-------------------------------------------------------------
pronucleit~e |       226        2,5    11,44812        11,27641     13,3614
             |                  10    17,22647        15,04672    17,78011
             |                  25    17,99193        17,95601    18,01597
             |                  50    18,10163        18,08171    18,11537
             |                  75    18,28015        18,19053    18,33458
             |                  90    18,57026        18,42439    18,83446
             |                97,5    21,12129        19,10231    29,91842

                                                    -- Binom, Interp, --
    Variable |       Obs  Percentile    Centile        [95% Conf, Interval]
-------------+-------------------------------------------------------------
        tpb2 |       225        2,5    1,662256        1,504522    1,883062
             |                  10    2,251496        2,052963    2,354861
             |                  25     2,73615        2,590319    2,857727
             |                  50    3,494328        3,196054    3,645897
             |                  75    4,277541        4,078668    4,478461
             |                  90    5,310867        5,007653    5,706175
             |                97,5    6,858447        5,862588    22,10026

                                                    -- Binom, Interp, --
    Variable |       Obs  Percentile    Centile        [95% Conf, Interval]
-------------+-------------------------------------------------------------
        tpna |       226        2,5    4,866272        4,143943    5,158602
             |                  10    5,880259        5,511453    6,098844
```

		25	6,508306	6,323873	6,8547
		50	7,658478	7,408628	8,031945
		75	8,946145	8,596203	9,247026
		90	10,2356	9,583794	10,66703
		97,5	11,96483	10,85596	25,42588

Variable	Obs	Percentile	Centile	-- Binom, Interp, -- [95% Conf,	Interval]
tpnf	226	2,5	18,98165	17,00619	19,59921
		10	20,33833	19,8058	20,59525
		25	21,53708	21,07342	21,98664
		50	23,62422	22,90091	23,94616
		75	25,26957	24,86596	25,89668
		90	27,72408	27,09605	28,94554
		97,5	32,3079	29,56596	37,78272

Variable	Obs	Percentile	Centile	-- Binom, Interp, -- [95% Conf,	Interval]
t2	226	2,5	21,67455	19,76309	22,49458
		10	23,11045	22,63466	23,51629
		25	24,3443	23,76209	24,75467
		50	26,25296	25,52131	26,77902
		75	28,20112	27,66444	28,97045
		90	31,24874	29,81526	31,90833
		97,5	34,86135	32,68298	41,05029

Variable	Obs	Percentile	Centile	-- Binom, Interp, -- [95% Conf,	Interval]
t3	224	2,5	28,83607	24,44794	31,92288
		10	33,32016	32,43001	33,6271
		25	34,88829	34,26652	35,44675
		50	37,21551	36,54079	37,88577
		75	39,87255	39,35829	40,81375
		90	43,31252	41,87226	45,14476
		97,5	48,20077	45,93376	53,17124

Variable	Obs	Percentile	Centile	-- Binom, Interp, -- [95% Conf,	Interval]
t4	226	2,5	32,51214	30,59678	33,15626
		10	33,84228	33,41263	34,28166
		25	35,71859	35,05607	36,05853
		50	38,00363	37,02453	38,77755
		75	40,7003	39,88696	42,0317
		90	45,14295	43,67103	46,45258
		97,5	50,68772	46,99953	57,1286

Variable	Obs	Percentile	Centile	-- Binom, Interp, -- [95% Conf,	Interval]
t5	224	2,5	35,30484	33,52419	39,27333
		10	44,42386	40,35301	45,02174
		25	46,49944	45,8671	47,27678
		50	50,05662	49,31547	50,82697
		75	53,65236	52,62335	54,67185
		90	59,55029	57,033	62,26759
		97,5	65,54404	63,48168	72,11693

Variable	Obs	Percentile	Centile	-- Binom, Interp, -- [95% Conf,	Interval]
t6	226	2,5	39,99203	37,52577	44,11208
		10	45,46959	44,80979	45,99868
		25	47,56021	46,77891	48,05058
		50	51,18074	50,36134	52,10513
		75	55,26842	54,08355	56,75455
		90	61,93137	59,07759	63,74425
		97,5	68,05969	64,63188	79,87672

Variable	Obs	Percentile	Centile	-- Binom, Interp, -- [95% Conf,	Interval]
t7	223	2,5	44,92334	41,55714	45,63017
		10	46,73537	46,1696	47,34794

		25	48,52507	48,0264	49,63911
		50	52,42332	51,55069	53,58327
		75	58,19454	55,90519	60,1504
		90	65,21512	62,9864	68,3998
		97,5	75,43989	69,24164	83,67575

Variable	Obs	Percentile	Centile	-- Binom, Interp, -- [95% Conf,	Interval]
t8	225	2,5	45,78084	42,01427	46,51915
		10	47,8415	46,95614	48,59765
		25	50,57168	49,59592	51,47718
		50	54,409	53,54542	56,04613
		75	62,97438	59,8382	65,86809
		90	73,77685	69,67064	76,94394
		97,5	83,74654	79,67042	90,06018

Variable	Obs	Percentile	Centile	-- Binom, Interp, -- [95% Conf,	Interval]
t9	225	2,5	49,08054	47,49396	52,24587
		10	58,08296	55,09828	59,89061
		25	63,63922	61,67514	65,10385
		50	69,57984	67,91351	70,60423
		75	75,79777	74,51531	78,03991
		90	83,16294	80,9754	86,39734
		97,5	92,93223	87,38075	98,99184

Variable	Obs	Percentile	Centile	-- Binom, Interp, -- [95% Conf,	Interval]
tsc	226	2,5	60,33683	57,1878	66,53599
		10	74,40971	69,76622	76,90442
		25	80,22938	78,73979	81,7612
		50	86,58285	84,43988	88,13701
		75	92,9935	91,10795	95,55959
		90	100,3509	98,10566	102,3762
		97,5	110,3433	105,9417	112,9555

Variable	Obs	Percentile	Centile	-- Binom, Interp, -- [95% Conf,	Interval]
tsb	219	2,5	83,3445	80,09135	85,51535
		10	89,71313	86,84339	91,11792
		25	93,26768	92,08626	94,51402
		50	99,38609	97,2418	101,6061
		75	106,8062	105,608	108,7569
		90	114,7397	112,16	117,7944
		97,5	127,7897	120,9713	135,5263

Variable	Obs	Percentile	Centile	-- Binom, Interp, -- [95% Conf,	Interval]
tb	199	2,5	90,12639	85,23342	94,79741
		10	96,4737	95,30845	98,45405
		25	101,2613	99,49228	103,1283
		50	107,3959	105,8399	109,0566
		75	114,5532	112,7114	116,9059
		90	127,9332	121,1026	132,8477
		97,5	138,6496	134,6185	146,3623

Variable	Obs	Percentile	Centile	-- Binom, Interp, -- [95% Conf,	Interval]
teb	128	2,5	99,18277	95,4	101,947*
		10	104,0176	101,3733	105,9004
		25	108,078	106,1209	109,4811
		50	112,2799	110,5443	113,9559
		75	120,7783	115,2002	127,1646
		90	136,0129	130,1433	137,3263
		97,5	147,1859	137,2677	154,6982*

* Lower (upper) confidence limit held at minimum (maximum) of sample

Variable	Obs	Percentile	Centile	-- Binom, Interp, -- [95% Conf,	Interval]

```
         thb |        16        2,5         97,4            97,4      108,21*
             |                  10    104,2734            97,4    109,9303*
             |                  25    109,5026         100,1512    113,2128
             |                  50     113,519         109,6948    116,2239
             |                  75    116,6929         113,8253    133,4006
             |                  90    129,9587         115,6497    135,6979*
             |                97,5    135,6979         121,3668    135,6979*

* Lower (upper) confidence limit held at minimum (maximum) of sample


IVF

foreach var of varlist pronucleitime tpb2 tpna tpnf t2  t3  t4  t5  t6 t7  t8  t9 tsc  tsb
tb  teb thb {
  2,   centile `var' if nfert_meth==4, centile(2,5 10 25 50 75 90 97,5)
  3, }

                                                        -- Binom, Interp, --
    Variable |       Obs  Percentile    Centile        [95% Conf, Interval]
-------------+-------------------------------------------------------------
pronucleit~e |       209        2,5    17,42071        15,79871    17,77188
             |                  10    17,91639        17,82393    17,95488
             |                  25    17,99413        17,97189    18,01365
             |                  50    18,08095        18,05941    18,10485
             |                  75    18,19514        18,16827    18,25341
             |                  90    18,47712        18,34696     18,8506
             |                97,5    21,10123        19,54863    26,03891

                                                        -- Binom, Interp, --
    Variable |       Obs  Percentile    Centile        [95% Conf, Interval]
-------------+-------------------------------------------------------------
        tpb2 |       174        2,5    2,288519        1,126412    2,753808
             |                  10    3,215719        2,805473    3,475804
             |                  25    3,842457        3,649918    4,014785
             |                  50    4,400599        4,184509    4,520174
             |                  75    5,288549        4,960497    5,581619
             |                  90    6,012335        5,774909    6,866863
             |                97,5     8,70618        7,018648    21,08507

                                                        -- Binom, Interp, --
    Variable |       Obs  Percentile    Centile        [95% Conf, Interval]
-------------+-------------------------------------------------------------
        tpna |       187        2,5    4,417882        4,161006    5,161414
             |                  10    5,492048        5,244021    5,807413
             |                  25    6,248642        5,964296     6,47749
             |                  50    7,202484        6,964903    7,539526
             |                  75    8,162912        7,962145    8,527661
             |                  90    9,452261        8,968455    10,15634
             |                97,5    11,52444        10,36319    28,52295

                                                        -- Binom, Interp, --
    Variable |       Obs  Percentile    Centile        [95% Conf, Interval]
-------------+-------------------------------------------------------------
        tpnf |       201        2,5    18,98527        17,15689    19,33836
             |                  10    19,85429        19,47879    20,48286
             |                  25     21,1327        20,82358    21,64908
             |                  50    23,00898        22,45281    23,29924
             |                  75    24,93172        24,23097    25,56362
             |                  90    26,95714        26,18712    28,24793
             |                97,5    30,05226         29,2007    34,61179

                                                        -- Binom, Interp, --
    Variable |       Obs  Percentile    Centile        [95% Conf, Interval]
-------------+-------------------------------------------------------------
          t2 |       207        2,5    21,22646        19,95253    21,73471
             |                  10    22,43563        22,05583    22,96741
             |                  25    23,72683        23,36087    24,39828
             |                  50    25,55441        25,18256    25,86951
             |                  75    27,73926        26,86779    28,25058
             |                  90    29,64364        28,99859    31,71048
             |                97,5    33,82876        32,31366    36,76309

                                                        -- Binom, Interp, --
    Variable |       Obs  Percentile    Centile        [95% Conf, Interval]
-------------+-------------------------------------------------------------
          t3 |       209        2,5    30,84221        26,17475    31,72431
```

				-- Binom, Interp, --	
		10	32,69953	32,24692	33,33248
		25	34,36051	33,67074	34,88777
		50	36,8	36,12016	37,15694
		75	38,77366	38,11446	39,76003
		90	41,54743	40,3782	43,2725
		97,5	45,50483	43,86468	49,42044

Variable	Obs	Percentile	Centile	-- Binom, Interp, -- [95% Conf,	Interval]
t4	209	2,5	31,4722	30,42708	32,62539
		10	33,40242	32,82142	33,87443
		25	35,0007	34,6138	35,88509
		50	37,89352	37,05769	38,35638
		75	40,2377	39,37367	40,94201
		90	43,54156	41,809	45,60084
		97,5	48,26587	45,95473	51,67955

Variable	Obs	Percentile	Centile	-- Binom, Interp, -- [95% Conf,	Interval]
t5	207	2,5	39,88516	36,46117	41,73013
		10	43,57837	42,52545	44,61891
		25	46,69178	46,0859	47,11653
		50	49,13397	48,3062	49,85676
		75	51,78311	51,10084	53,40564
		90	55,89623	54,57141	58,23778
		97,5	62,76011	59,37947	67,74954

Variable	Obs	Percentile	Centile	-- Binom, Interp, -- [95% Conf,	Interval]
t6	208	2,5	42,25489	40,67684	43,2993
		10	44,91731	43,68121	46,31817
		25	47,56702	47,11086	48,09447
		50	50,31409	49,17839	51,06401
		75	53,46196	52,10716	54,70899
		90	58,00122	55,98442	61,14656
		97,5	64,42745	62,07335	79,02624

Variable	Obs	Percentile	Centile	-- Binom, Interp, -- [95% Conf,	Interval]
t7	208	2,5	43,00987	41,28137	44,3912
		10	46,09735	44,93142	47,37871
		25	48,89777	48,11035	49,91078
		50	52,0859	51,14267	52,63359
		75	55,5175	54,30679	56,24889
		90	61,65765	58,65009	65,8361
		97,5	70,11003	66,59511	82,93953

Variable	Obs	Percentile	Centile	-- Binom, Interp, -- [95% Conf,	Interval]
t8	208	2,5	43,86943	42,64738	45,02445
		10	47,62172	45,8356	48,75494
		25	50,41362	49,51932	51,50407
		50	54,22892	53,01848	54,83843
		75	58,30616	56,91516	60,80901
		90	67,44382	63,49261	71,91985
		97,5	84,7823	77,58524	93,92373

Variable	Obs	Percentile	Centile	-- Binom, Interp, -- [95% Conf,	Interval]
t9	208	2,5	52,25802	45,85419	55,16644
		10	57,30981	55,92941	59,90818
		25	63,3057	61,86858	64,72602
		50	67,86119	66,74231	68,82683
		75	73,04967	71,17168	75,22457
		90	79,77714	77,29748	81,99583
		97,5	90,21629	85,74526	97,73751

Variable	Obs	Percentile	Centile	-- Binom, Interp, -- [95% Conf,	Interval]
tsc	206	2,5	62,08655	56,05916	64,98541

```
             |                  10    69,23443        65,95648    72,00365
             |                  25    75,25355        74,38077     76,8311
             |                  50    81,52046        79,92942    84,04922
             |                  75     88,3486        86,68246    89,94162
             |                  90    94,73694         91,3149    97,07586
             |                97,5    104,6719        98,35177     120,649

                                                    -- Binom, Interp, --
    Variable |       Obs  Percentile    Centile        [95% Conf, Interval]
-------------+-------------------------------------------------------------
         tsb |       206         2,5    83,86143        78,86147    85,53151
             |                  10    87,24281        85,83969    88,41738
             |                  25    91,14954        89,96731    92,35688
             |                  50    94,98866        93,68099    96,78891
             |                  75    100,1491        99,06314    101,6956
             |                  90    105,7019        103,4511    111,0016
             |                97,5    119,2408        115,0469    136,2321

                                                    -- Binom, Interp, --
    Variable |       Obs  Percentile    Centile        [95% Conf, Interval]
-------------+-------------------------------------------------------------
          tb |       201         2,5    90,96657        88,23376    92,16101
             |                  10    93,53199        92,45687    94,84434
             |                  25    97,88724          96,573    99,83503
             |                  50    103,8644        102,7056    105,6114
             |                  75    109,6839        108,2797     111,185
             |                  90    115,9513        113,1027    122,2081
             |                97,5    129,7781        125,8565    134,7527

                                                    -- Binom, Interp, --
    Variable |       Obs  Percentile    Centile        [95% Conf, Interval]
-------------+-------------------------------------------------------------
         teb |       149         2,5    97,32883        95,80839    99,41623
             |                  10    101,6211         99,4235    102,4978
             |                  25    105,6668        103,4359    106,8533
             |                  50    111,5296        109,6027    112,7065
             |                  75    116,0737         115,274    116,9301
             |                  90    121,8966         117,401    134,5177
             |                97,5    137,8458         134,708    141,1112

                                                    -- Binom, Interp, --
    Variable |       Obs  Percentile    Centile        [95% Conf, Interval]
-------------+-------------------------------------------------------------
         thb |        19         2,5    106,9576        106,9576    109,7318*
             |                  10    107,9868        106,9576     111,326*
             |                  25    111,2733        107,7559    115,6955
             |                  50    115,8262        111,3688    137,3814
             |                  75    138,3806        116,1082    151,2046
             |                  90    150,8449        137,8289    152,4486*
             |                97,5    152,4486        143,6723    152,4486*

* Lower (upper) confidence limit held at minimum (maximum) of sample
```

Differences between epochs by fertilization type

ICSI/IVF =1

```
, foreach var of varlist vp  t2duration ecc1 t3duration  t4duration  t5duration  t6duration
t7duration t8d
> uration ecc3  s3 t9duration  tscduration  tsbduration  tbduration tebduration  thbduration
{
  2,   centile `var' if nfert_meth==1, centile(2,5 10 25 50 75 90 97,5)
  3, }

                                                    -- Binom, Interp, --
    Variable |       Obs  Percentile    Centile        [95% Conf, Interval]
-------------+-------------------------------------------------------------
          vp |       134         2,5    5,314981       -,0007158    8,470382*
             |                  10     11,2561        8,388986    12,28157
             |                  25    13,50455        13,00387    14,25505
             |                  50    15,25488        14,75611    15,75517
             |                  75    17,53092        16,50088    18,23546
             |                  90     19,4221        18,49915    19,89585
             |                97,5    24,23415        19,79557    27,90885*
```

* Lower (upper) confidence limit held at minimum (maximum) of sample

Variable	Obs	Percentile	Centile	-- Binom, Interp, -- [95% Conf,	Interval]
t2duration	134	2,5	9,190329	0	9,753072*
		10	10,003	9,753011	10,25295
		25	10,50309	10,25366	10,75338
		50	11,009	11,00325	11,25459
		75	11,81616	11,51194	12,25382
		90	12,88445	12,25806	13,39504
		97,5	13,79156	13,29416	16,04903*

* Lower (upper) confidence limit held at minimum (maximum) of sample

Variable	Obs	Percentile	Centile	-- Binom, Interp, -- [95% Conf,	Interval]
ecc1	131	2,5	8,434624	7,548741	18,19242*
		10	19,2387	18,06394	19,43876
		25	20,00931	19,50698	20,91522
		50	22,00678	21,40808	22,50679
		75	23,81965	23,25704	24,56609
		90	25,82472	25,01013	27,40651
		97,5	29,36535	27,17554	33,41738*

* Lower (upper) confidence limit held at minimum (maximum) of sample

Variable	Obs	Percentile	Centile	-- Binom, Interp, -- [95% Conf,	Interval]
t3duration	133	2,5	0	0	0*
		10	0	0	0
		25	,2499996	,0320542	,2501299
		50	,5001619	,4999768	,5009863
		75	1,00016	,7501613	1,250379
		90	2,75159	1,269758	4,384502
		97,5	11,15779	3,940335	17,25616*

* Lower (upper) confidence limit held at minimum (maximum) of sample

Variable	Obs	Percentile	Centile	-- Binom, Interp, -- [95% Conf,	Interval]
t4duration	131	2,5	2,650223	0	7,939802*
		10	9,252586	7,811086	10,0032
		25	11,0035	10,25435	11,25385
		50	12,00521	11,637	12,50439
		75	13,41796	12,75687	13,76504
		90	14,25473	14,00416	15,23524
		97,5	16,46505	15,19269	17,35092*

* Lower (upper) confidence limit held at minimum (maximum) of sample

Variable	Obs	Percentile	Centile	-- Binom, Interp, -- [95% Conf,	Interval]
t5duration	132	2,5	0	0	0*
		10	0	0	,2499975
		25	,2501026	,2500479	,250208
		50	,7467936	,5001934	,7503291
		75	1,500344	1,000435	2,251493
		90	4,351285	2,541942	5,652474
		97,5	11,7103	5,609895	13,8192*

* Lower (upper) confidence limit held at minimum (maximum) of sample

Variable	Obs	Percentile	Centile	-- Binom, Interp, -- [95% Conf,	Interval]
t6duration	134	2,5	0	0	,249972*
		10	,2500635	,2499443	,2501645
		25	,5000692	,2502995	,5002866
		50	1,000249	,7502513	1,001082
		75	2,000758	1,500448	3,000815
		90	4,001452	3,005542	8,153939
		97,5	14,42939	7,15042	23,01379*

* Lower (upper) confidence limit held at minimum (maximum) of sample

Variable	Obs	Percentile	Centile	-- Binom, Interp, -- [95% Conf,	Interval]
t7duration	134	2,5	0	0	0*
		10	0	0	,2500342
		25	,5000492	,2500487	,5038156
		50	1,25026	,7503478	1,737823
		75	4,313973	2,489677	6,154398
		90	12,38213	8,668225	15,56643
		97,5	18,1622	15,52756	21,35826*

* Lower (upper) confidence limit held at minimum (maximum) of sample

Variable	Obs	Percentile	Centile	-- Binom, Interp, -- [95% Conf,	Interval]
t8duration	134	2,5	0	0	1,341031*
		10	3,249954	,9395292	5,062959
		25	8,690984	6,501637	10,50352
		50	14,18028	12,54235	15,15784
		75	17,45582	16,41749	18,28502
		90	20,40712	18,73997	21,2912
		97,5	24,51015	21,26648	34,27477*

* Lower (upper) confidence limit held at minimum (maximum) of sample

Variable	Obs	Percentile	Centile	-- Binom, Interp, -- [95% Conf,	Interval]
ecc3	133	2,5	11,1816	10,75334	11,99698*
		10	12,76772	11,98176	13,08811
		25	13,96113	13,25915	14,73896
		50	15,75791	15,26035	16,50629
		75	20,56609	18,16159	25,35295
		90	30,10247	26,05663	36,14444
		97,5	40,37795	34,64824	43,64124*

* Lower (upper) confidence limit held at minimum (maximum) of sample

Variable	Obs	Percentile	Centile	-- Binom, Interp, -- [95% Conf,	Interval]
s3	132	2,5	,5804888	,2501017	,7501905*
		10	1,074565	,7501866	1,500466
		25	1,875567	1,750374	2,087252
		50	3,251076	2,750682	4,501517
		75	9,442572	7,24885	15,26542
		90	18,62927	16,48797	21,72086
		97,5	30,90472	21,26226	33,36315*

* Lower (upper) confidence limit held at minimum (maximum) of sample

Variable	Obs	Percentile	Centile	-- Binom, Interp, -- [95% Conf,	Interval]
t9duration	134	2,5	0	0	0*
		10	3,375998	0	6,002068
		25	8,439996	6,56272	10,51533
		50	13,63014	12,05506	16,22843
		75	20,25586	18,74431	23,79259
		90	27,71193	24,74192	30,42974
		97,5	38,09141	30,12824	58,57456*

* Lower (upper) confidence limit held at minimum (maximum) of sample

Variable	Obs	Percentile	Centile	-- Binom, Interp, -- [95% Conf,	Interval]
tscduration	132	2,5	3,000728	1,775006	3,596855*
		10	5,251542	3,57225	6,29335
		25	7,814772	6,511003	9,253348
		50	12,05535	10,56119	13,20807
		75	16,75645	15,00419	19,24752
		90	22,60942	20,28554	24,64603

```
             |                 97,5     34,1259         24,3676    45,54474*

* Lower (upper) confidence limit held at minimum (maximum) of sample

                                                      -- Binom, Interp, --
    Variable |       Obs  Percentile    Centile        [95% Conf, Interval]
-------------+-------------------------------------------------------------
 tsbduration |       125        2,5     3,78893         3,00277    4,501766*
             |                   10    4,754336        4,453782    5,252799
             |                   25    5,874077        5,439025    6,513808
             |                   50    8,499474        7,252438    9,252484
             |                   75    11,23382        10,00273    12,50279
             |                   90    15,53452        13,50459    18,49369
             |                 97,5    21,23448        18,13114    34,42605*

* Lower (upper) confidence limit held at minimum (maximum) of sample

                                                      -- Binom, Interp, --
    Variable |       Obs  Percentile    Centile        [95% Conf, Interval]
-------------+-------------------------------------------------------------
  tbduration |        90        2,5    3,570103         3,25089     4,50116*
             |                   10    5,001276        3,870809    5,269949
             |                   25    6,250741        5,258417    6,778145
             |                   50    8,502576        7,251893    9,002476
             |                   75    10,63572        9,508248    12,41227
             |                   90    14,68091        12,35324    17,20732
             |                 97,5    20,95682        16,24666     21,6842*

* Lower (upper) confidence limit held at minimum (maximum) of sample

                                                      -- Binom, Interp, --
    Variable |       Obs  Percentile    Centile        [95% Conf, Interval]
-------------+-------------------------------------------------------------
 tebduration |        90        2,5    -9,55512        -22,5638   -,2415258*
             |                   10           0       -,3904583    ,4235617
             |                   25    1,718013        ,3826577    2,041813
             |                   50    5,251467         3,98887    6,236399
             |                   75    8,580322        6,715124    11,00664
             |                   90    13,40127         11,0054    21,64594
             |                 97,5    29,18634          15,737    31,73157*

* Lower (upper) confidence limit held at minimum (maximum) of sample

                                                      -- Binom, Interp, --
    Variable |       Obs  Percentile    Centile        [95% Conf, Interval]
-------------+-------------------------------------------------------------
 thbduration |         9        2,5   -2,000685       -2,000685   -,0890649*
             |                   10   -2,000685       -2,000685    ,3135529*
             |                   25    -,017894       -2,000685    4,470519*
             |                   50     2,25081       -,0330045    13,97955
             |                   75    11,12843        1,175045    14,51785*
             |                   90    14,51785        6,568113    14,51785*
             |                 97,5    14,51785        14,50511    14,51785*

* Lower (upper) confidence limit held at minimum (maximum) of sample


ICSI =2

,
, foreach var of varlist vp  t2duration ecc1 t3duration  t4duration  t5duration  t6duration
t7duration t8d
> uration ecc3  s3 t9duration  tscduration  tsbduration  tbduration tebduration  thbduration
{
  2,   centile `var' if nfert_meth==2, centile(2,5 10 25 50 75 90 97,5)
  3, }

                                                      -- Binom, Interp, --
    Variable |       Obs  Percentile    Centile        [95% Conf, Interval]
-------------+-------------------------------------------------------------
          vp |       834        2,5    11,09375         10,6956    11,41748
             |                   10     12,5037        12,16744    12,75487
             |                   25    13,94136        13,66977    14,19116
             |                   50     15,5065        15,25837    15,75531
             |                   75    17,43057         17,0285    17,75501
             |                   90    19,26942        19,00582    19,66409
```

		97,5	21,62782	21,25699	22,50658

Variable	Obs	Percentile	Centile	-- Binom, Interp, -- [95% Conf,	Interval]
t2duration	846	2,5	8,54655	8,252215	9,008294
		10	9,752545	9,506159	9,758227
		25	10,25896	10,2533	10,50292
		50	11,02199	11,0037	11,25352
		75	11,76954	11,75378	12,00387
		90	12,7564	12,62197	13,00397
		97,5	13,90939	13,50455	14,50564

Variable	Obs	Percentile	Centile	-- Binom, Interp, -- [95% Conf,	Interval]
ecc1	819	2,5	18,00559	17,6758	18,25501
		10	19,10896	18,76647	19,26267
		25	20,44396	20,25567	20,52792
		50	22,00565	21,75637	22,25705
		75	23,8603	23,63825	24,11121
		90	25,75748	25,44967	26,12572
		97,5	28,509	27,73583	29,2585

Variable	Obs	Percentile	Centile	-- Binom, Interp, -- [95% Conf,	Interval]
t3duration	843	2,5	0	0	0
		10	0	0	0
		25	,2500344	,249992	,2500727
		50	,5001197	,500061	,5001744
		75	,9979394	,7503347	1,00032
		90	1,999572	1,685453	2,250733
		97,5	6,244812	4,030406	10,96664

Variable	Obs	Percentile	Centile	-- Binom, Interp, -- [95% Conf,	Interval]
t4duration	841	2,5	,500105	,2500171	1,250386
		10	9,502163	9,003174	9,755843
		25	11,0032	10,75446	11,06834
		50	12,25449	12,01999	12,50343
		75	13,52283	13,26233	13,75523
		90	14,98914	14,61811	15,25426
		97,5	17,4927	16,50958	18,72779

Variable	Obs	Percentile	Centile	-- Binom, Interp, -- [95% Conf,	Interval]
t5duration	843	2,5	0	0	0
		10	0	0	,0424801
		25	,2501244	,2500828	,2502012
		50	,7501292	,5004184	,7502556
		75	1,500365	1,250668	1,503039
		90	2,75145	2,500714	3,506616
		97,5	13,22924	11,77949	14,00431

Variable	Obs	Percentile	Centile	-- Binom, Interp, -- [95% Conf,	Interval]
t6duration	846	2,5	0	0	0
		10	,2467421	0	,2499957
		25	,5000951	,4982728	,5001935
		50	1,000293	,9981866	1,000426
		75	2,000799	1,752944	2,250795
		90	4,001307	3,501062	4,753709
		97,5	15,96318	11,5421	17,98932

Variable	Obs	Percentile	Centile	-- Binom, Interp, -- [95% Conf,	Interval]
t7duration	843	2,5	0	0	0
		10	,2499615	,2474835	,2500506
		25	,5001964	,5001244	,5004062
		50	1,25031	1,000515	1,250516
		75	3,002029	2,751006	3,522333
		90	8,681504	7,254078	10,25287

		97,5	16,48534	15,50534	18,50504

Variable	Obs	Percentile	Centile	-- Binom, Interp, -- [95% Conf,	Interval]
t8duration	845	2,5	0	0	,2501395
		10	1,500278	1,000442	1,754238
		25	8,127362	6,752178	9,555123
		50	13,50433	13,00434	14,01417
		75	16,75952	16,50471	17,28719
		90	19,50592	19,00564	20,08009
		97,5	23,34473	22,7562	24,24959

Variable	Obs	Percentile	Centile	-- Binom, Interp, -- [95% Conf,	Interval]
ecc3	843	2,5	11,50408	11,25357	11,75506
		10	12,75375	12,504	13,00364
		25	14,09494	13,79324	14,5038
		50	16,25494	15,95142	16,50493
		75	20,06353	19,25755	21,57465
		90	28,99485	27,09581	30,50928
		97,5	36,48561	34,92088	39,8089

Variable	Obs	Percentile	Centile	-- Binom, Interp, -- [95% Conf,	Interval]
s3	843	2,5	,7503174	,7502328	,7617757
		10	1,250451	1,250201	1,49997
		25	2,000699	1,951285	2,250518
		50	3,751068	3,303188	4,001404
		75	8,752287	7,502519	10,50368
		90	17,53255	16,19158	19,00555
		97,5	24,25929	22,73622	27,22172

Variable	Obs	Percentile	Centile	-- Binom, Interp, -- [95% Conf,	Interval]
t9duration	846	2,5	0	0	0
		10	3,001431	1,342022	4,001215
		25	7,691441	6,637497	8,142545
		50	12,93035	12,00334	13,75465
		75	20,00589	18,52815	21,25802
		90	26,0806	25,27042	28,02981
		97,5	34,96596	32,51528	39,65531

Variable	Obs	Percentile	Centile	-- Binom, Interp, -- [95% Conf,	Interval]
tscduration	838	2,5	3,50144	2,770379	4,141283
		10	6,002136	5,502042	6,502059
		25	9,455516	8,93571	10,00268
		50	13,75566	13,30961	14,50555
		75	19,84993	19,03483	20,76035
		90	27,05046	25,75855	28,72974
		97,5	36,01641	33,13685	37,51833

Variable	Obs	Percentile	Centile	-- Binom, Interp, -- [95% Conf,	Interval]
tsbduration	791	2,5	3,710267	3,251158	4,165338
		10	5,001595	4,570339	5,251797
		25	6,502058	6,253783	6,75224
		50	8,501934	8,182304	8,752002
		75	11,25358	10,75356	11,75367
		90	14,5277	13,7541	15,68008
		97,5	22,86983	20,27592	24,08854

Variable	Obs	Percentile	Centile	-- Binom, Interp, -- [95% Conf,	Interval]
tbduration	593	2,5	2,961568	2,250708	3,24522
		10	4,251367	3,978013	4,501517
		25	5,502851	5,252145	6,001573
		50	8,002266	7,537607	8,253095
		75	10,41511	10,00431	11,00327
		90	13,50876	12,75815	14,87876

```
             |                97,5    20,76979        18,10394    23,92501

                                                    -- Binom, Interp, --
    Variable |       Obs  Percentile    Centile        [95% Conf, Interval]
-------------+-------------------------------------------------------------
 tebduration |       593        2,5   -,2501335       -4,136023           0
             |                   10           0               0     ,250156
             |                   25    2,252445        1,778017    2,778635
             |                   50    5,501788        5,001491     6,00178
             |                   75     9,50246        8,371944    10,25299
             |                   90    13,08301        12,50823    14,00194
             |                97,5    17,83399        16,64303    22,70867

                                                    -- Binom, Interp, --
    Variable |       Obs  Percentile    Centile        [95% Conf, Interval]
-------------+-------------------------------------------------------------
 thbduration |        93        2,5    -,500219       -4,001225          0*
             |                   10           0       -,1469954           0
             |                   25    ,5000822               0    1,053715
             |                   50    3,000907        2,001916    3,791012
             |                   75    6,376986        4,708295    8,750779
             |                   90     10,6407        8,750678    12,52199
             |                97,5    15,70735        11,47062        28,3*

* Lower (upper) confidence limit held at minimum (maximum) of sample


IMSI =3


,
, foreach var of varlist vp  t2duration ecc1 t3duration  t4duration  t5duration  t6duration
t7duration t8d
> uration ecc3  s3 t9duration  tscduration  tsbduration  tbduration tebduration  thbduration
{
  2,   centile `var' if nfert_meth==3, centile(2,5 10 25 50 75 90 97,5)
  3, }

                                                    -- Binom, Interp, --
    Variable |       Obs  Percentile    Centile        [95% Conf, Interval]
-------------+-------------------------------------------------------------
          vp |       226        2,5     10,7515         9,76651    11,50394
             |                   10    12,25338        11,67485    12,75379
             |                   25     14,0064        13,33852    14,47388
             |                   50    15,50882        15,20367     16,0074
             |                   75    17,58136        17,00718    18,03526
             |                   90    19,71604          18,756    21,03132
             |                97,5    24,08958        21,71158    25,48745

                                                    -- Binom, Interp, --
    Variable |       Obs  Percentile    Centile        [95% Conf, Interval]
-------------+-------------------------------------------------------------
  t2duration |       224        2,5    2,254538        ,0235683    9,003331
             |                   10    9,627639        9,252706     10,0026
             |                   25    10,25337        10,00435    10,50453
             |                   50    11,13326        11,00325    11,50333
             |                   75    12,00854        11,75404    12,25673
             |                   90    13,25396        12,74957    13,75401
             |                97,5    14,69922        13,75902    15,76792

                                                    -- Binom, Interp, --
    Variable |       Obs  Percentile    Centile        [95% Conf, Interval]
-------------+-------------------------------------------------------------
        ecc1 |       225        2,5    17,83199        17,01725    18,75591
             |                   10    19,58764        19,14162    20,00639
             |                   25    20,79204        20,34841    21,22465
             |                   50    22,51748        22,00883    23,00934
             |                   75    24,50707        24,04298    25,25905
             |                   90    27,02339        26,06271    27,82901
             |                97,5     30,9823        28,42217    32,49238

                                                    -- Binom, Interp, --
    Variable |       Obs  Percentile    Centile        [95% Conf, Interval]
-------------+-------------------------------------------------------------
  t3duration |       224        2,5           0               0           0
             |                   10           0               0           0
```

		25	,2499701	,0079765	,2500605
		50	,5001401	,5000132	,500304
		75	1,000148	,7503111	1,250324
		90	2,083953	1,750639	4,160813
		97,5	9,757962	5,233267	14,1626

Variable	Obs	Percentile	Centile	-- Binom, Interp, -- [95% Conf,	Interval]
t4duration	224	2,5	,4062748	0	,7752828
		10	8,377944	1,113818	9,753923
		25	11,00424	10,50354	11,30704
		50	12,25684	12,00336	12,50671
		75	13,75857	13,25473	14,01565
		90	15,29383	14,63195	16,14053
		97,5	18,60502	16,50518	20,2315

Variable	Obs	Percentile	Centile	-- Binom, Interp, -- [95% Conf,	Interval]
t5duration	224	2,5	0	0	0
		10	0	0	0
		25	,2500832	,2499918	,2509166
		50	,7401257	,5002519	,750826
		75	1,500592	1,250485	1,988553
		90	3,749835	2,502658	8,599733
		97,5	12,98138	10,7061	17,00793

Variable	Obs	Percentile	Centile	-- Binom, Interp, -- [95% Conf,	Interval]
t6duration	223	2,5	0	0	0
		10	0	0	,2410888
		25	,4996964	,2500922	,500133
		50	1,000239	,7502691	1,25032
		75	2,250349	1,750473	2,750709
		90	5,90256	3,782654	7,908542
		97,5	12,3585	8,757613	20,16253

Variable	Obs	Percentile	Centile	-- Binom, Interp, -- [95% Conf,	Interval]
t7duration	223	2,5	0	0	0
		10	0	0	,2500003
		25	,4999872	,2501003	,5002169
		50	1,250222	1,000243	1,750309
		75	3,501195	2,754377	4,2994
		90	11,90368	6,517425	14,28803
		97,5	21,68124	16,49447	29,66965

Variable	Obs	Percentile	Centile	-- Binom, Interp, -- [95% Conf,	Interval]
t8duration	225	2,5	0	0	,500183
		10	1,250397	,7501714	2,500699
		25	7,002285	4,813178	9,073956
		50	13,38864	12,32805	14,25492
		75	17,2076	16,26033	18,0051
		90	21,00722	18,98185	21,68946
		97,5	24,62027	22,2572	27,96692

Variable	Obs	Percentile	Centile	-- Binom, Interp, -- [95% Conf,	Interval]
ecc3	225	2,5	11,50567	11,00522	12,01335
		10	12,55062	12,25355	13,01414
		25	14,25759	13,69265	14,636
		50	16,09896	15,51181	16,91302
		75	20,86072	19,50552	23,25859
		90	30,18982	26,7405	34,96326
		97,5	38,94173	37,2762	49,76675

Variable	Obs	Percentile	Centile	-- Binom, Interp, -- [95% Conf,	Interval]
s3	223	2,5	,2501839	,2499017	,5152936
		10	,9970532	,7501908	1,248365

		25	2,000421	1,500146	2,294442
		50	3,750936	3,281383	4,758701
		75	10,75273	8,003503	13,69844
		90	18,61221	15,75454	21,56401
		97,5	27,9631	22,25299	38,73882

Variable	Obs	Percentile	Centile	-- Binom, Interp, -- [95% Conf,	Interval]
t9duration	225	2,5	0	0	0
		10	3,401412	0	5,501608
		25	9,503678	7,502424	10,93543
		50	15,50467	13,75585	17,18458
		75	23,41143	21,10206	25,01709
		90	31,41582	27,99265	37,01515
		97,5	42,75945	39,60644	45,21857

Variable	Obs	Percentile	Centile	-- Binom, Interp, -- [95% Conf,	Interval]
tscduration	219	2,5	2,750986	1,348384	3,739366
		10	5,003613	4,023321	6,501717
		25	9,006054	7,754025	10,00618
		50	12,75395	11,75138	13,76457
		75	18,75614	17,24104	21,51613
		90	27,76509	23,35631	30,49716
		97,5	35,43165	31,36174	43,18646

Variable	Obs	Percentile	Centile	-- Binom, Interp, -- [95% Conf,	Interval]
tsbduration	199	2,5	3,5	1,509328	4,25113
		10	4,752201	4,306408	5,266562
		25	6,502237	5,751722	7,00182
		50	8,497594	7,779895	9,002457
		75	11,00719	10,25307	11,50365
		90	14,00955	12,2585	17,79874
		97,5	22,8864	19,21355	30,07979

Variable	Obs	Percentile	Centile	-- Binom, Interp, -- [95% Conf,	Interval]
tbduration	128	2,5	2,057657	1,750438	3,163195*
		10	3,97251	3,015153	5,251046
		25	6,000256	5,251719	6,753072
		50	8,005408	7,502183	8,502631
		75	9,916193	9,002642	11,25434
		90	13,00618	11,66372	15,46565
		97,5	20,3062	15,04827	26,77659*

* Lower (upper) confidence limit held at minimum (maximum) of sample

Variable	Obs	Percentile	Centile	-- Binom, Interp, -- [95% Conf,	Interval]
tebduration	128	2,5	-,2520623	-8,520542	0*
		10	0	0	,249238
		25	1,500612	,2501095	2,306991
		50	4,76214	3,378594	5,696117
		75	7,862645	6,752656	9,502871
		90	12,02901	9,686162	14,73097
		97,5	16,65573	14,46448	20,56617*

* Lower (upper) confidence limit held at minimum (maximum) of sample

Variable	Obs	Percentile	Centile	-- Binom, Interp, -- [95% Conf,	Interval]
thbduration	16	2,5	-3	-3	0*
		10	-,9	-3	1,094653*
		25	,500039	-2,15942	1,382611
		50	1,434174	,7673688	4,24708
		75	4,766118	1,485737	10,39072
		90	8,706949	3,611636	11,51452*
		97,5	11,51452	6,885679	11,51452*

* Lower (upper) confidence limit held at minimum (maximum) of sample

```
IVF =4
,
, foreach var of varlist vp  t2duration ecc1 t3duration  t4duration  t5duration  t6duration
t7duration t8d
> uration ecc3  s3 t9duration  tscduration  tsbduration  tbduration tebduration  thbduration
{
  2,   centile `var' if nfert_meth==4, centile(2,5 10 25 50 75 90 97,5)
  3, }
```

Variable	Obs	Percentile	Centile	-- Binom, Interp, -- [95% Conf,	Interval]
vp	186	2,5	11,17195	2,753071	12,00395
		10	12,50451	12,11276	12,77448
		25	13,75548	13,25441	14,37241
		50	15,50502	15,12146	16,00494
		75	17,25524	16,75496	17,75488
		90	18,75638	18,09713	19,50196
		97,5	21,68293	19,75568	25,53154

Variable	Obs	Percentile	Centile	-- Binom, Interp, -- [95% Conf,	Interval]
t2duration	207	2,5	8,426034	1,201526	9,002969
		10	9,45444	9,122564	9,752489
		25	10,2543	10,00516	10,50324
		50	11,00354	10,75366	11,25326
		75	11,75359	11,50479	12,00366
		90	12,51517	12,25392	12,97821
		97,5	13,54184	13,0129	17,32061

Variable	Obs	Percentile	Centile	-- Binom, Interp, -- [95% Conf,	Interval]
ecc1	173	2,5	15,00557	1,481649	16,99852
		10	17,96819	17,21429	18,25983
		25	19,25612	18,75958	19,75714
		50	20,6876	20,39445	21,25643
		75	22,75733	22,24463	23,50773
		90	24,75935	24,25794	25,94254
		97,5	28,91759	26,18149	30,41237

Variable	Obs	Percentile	Centile	-- Binom, Interp, -- [95% Conf,	Interval]
t3duration	209	2,5	0	0	0
		10	0	0	0
		25	,250049	,2497756	,2501099
		50	,5001411	,5000299	,5002725
		75	1,250203	1,000215	1,500405
		90	2,500612	1,751696	3,000952
		97,5	11,31937	3,87234	17,57535

Variable	Obs	Percentile	Centile	-- Binom, Interp, -- [95% Conf,	Interval]
t4duration	207	2,5	,5537933	0	3,138962
		10	9,210454	6,906226	9,742911
		25	10,25358	9,898961	10,75515
		50	11,75355	11,50861	12,25338
		75	13,11419	12,86427	13,50424
		90	14,27719	13,74958	14,89754
		97,5	17,13158	15,10932	22,33927

Variable	Obs	Percentile	Centile	-- Binom, Interp, -- [95% Conf,	Interval]
t5duration	206	2,5	0	0	0
		10	0	0	,2479503
		25	,250072	,2500181	,250156
		50	,5009507	,5001984	,7501426
		75	1,250257	1,000219	1,25185
		90	2,250788	1,750858	3,250851
		97,5	11,97273	7,022999	32,79413

Variable	Obs	Percentile	Centile	-- Binom, Interp, -- [95% Conf,	Interval]
t6duration	209	2,5	0	0	0
		10	0	0	,2499607
		25	,2521389	,2500685	,5000651
		50	,8249733	,7501326	1,250242
		75	2,125719	1,501062	2,751426
		90	4,490246	3,42909	7,186101
		97,5	10,37462	8,645068	19,04535

Variable	Obs	Percentile	Centile	-- Binom, Interp, -- [95% Conf,	Interval]
t7duration	208	2,5	0	0	0
		10	,2500078	0	,2502493
		25	,5002152	,5000466	,7500825
		50	1,250383	1,000376	1,74684
		75	3,251042	2,500795	3,751168
		90	10,98954	4,255451	12,97272
		97,5	18,16559	15,95618	19,19647

Variable	Obs	Percentile	Centile	-- Binom, Interp, -- [95% Conf,	Interval]
t8duration	208	2,5	0	0	,2500104
		10	1,000325	,3001517	3,000744
		25	8,069829	6,122173	10,51251
		50	13,25936	12,35679	14,25417
		75	17,50628	16,50452	18,13558
		90	19,25576	18,73588	20,35081
		97,5	24,32943	21,3592	33,07387

Variable	Obs	Percentile	Centile	-- Binom, Interp, -- [95% Conf,	Interval]
ecc3	208	2,5	11,5202	10,68363	12,00318
		10	12,7368	12,05241	13,04951
		25	14,00554	13,50566	14,25763
		50	15,5061	15,11502	16,00513
		75	18,0833	17,26662	21,17584
		90	28,35252	23,98719	32,13372
		97,5	39,25898	33,51723	58,33162

Variable	Obs	Percentile	Centile	-- Binom, Interp, -- [95% Conf,	Interval]
s3	206	2,5	,5437961	,5000491	,922745
		10	1,000332	1,000131	1,250519
		25	2,065431	1,750614	2,469729
		50	3,512338	3,000837	4,251153
		75	7,25223	5,746911	10,32339
		90	15,82966	13,6098	19,69402
		97,5	35,33007	20,44993	49,464

Variable	Obs	Percentile	Centile	-- Binom, Interp, -- [95% Conf,	Interval]
t9duration	206	2,5	0	0	0
		10	2,111533	0	4,249392
		25	6,252713	5,501512	8,16589
		50	12,50437	10,17048	13,5265
		75	20,3891	18,06035	21,76113
		90	27,01592	23,59018	29,93078
		97,5	32,31391	30,62968	43,76005

Variable	Obs	Percentile	Centile	-- Binom, Interp, -- [95% Conf,	Interval]
tscduration	206	2,5	3,501786	2,762295	3,883504
		10	6,001959	4,286302	6,506002
		25	9,752682	8,294055	10,5031
		50	13,23759	12,00358	14,72952
		75	17,51178	16,25398	20,46639
		90	26,37162	22,0746	28,32272
		97,5	33,11445	29,70436	37,6573

Variable	Obs	Percentile	Centile	-- Binom, Interp, -- [95% Conf,	Interval]
tsbduration	201	2,5	3,750983	3,047034	3,836446
		10	4,257631	4,00149	5,053939
		25	6,002167	5,75144	6,50136
		50	8,002689	7,291702	8,502152
		75	10,66826	9,670187	11,75341
		90	14,90487	12,68376	16,81769
		97,5	20,88816	19,03181	32,62871

Variable	Obs	Percentile	Centile	-- Binom, Interp, -- [95% Conf,	Interval]
tbduration	149	2,5	4,007194	1,801784	4,566988
		10	5,204218	4,586406	5,501925
		25	6,252997	5,751933	7,003909
		50	8,50285	8,137932	9,003192
		75	10,75341	9,753187	12,25432
		90	14,25405	12,80418	16,62532
		97,5	22,0025	16,66232	33,72029

Variable	Obs	Percentile	Centile	-- Binom, Interp, -- [95% Conf,	Interval]
tebduration	149	2,5	-,5002599	-48,36109	0
		10	0	0	,5001811
		25	1,926563	1,000294	3,555609
		50	6,251624	5,001506	7,728485
		75	10,63374	8,907602	12,60291
		90	15,75464	13,65206	18,32868
		97,5	23,45855	18,42655	34,14687

Variable	Obs	Percentile	Centile	-- Binom, Interp, -- [95% Conf,	Interval]
thbduration	19	2,5	-10,80778	-10,80778	-1,090745*
		10	-5,5	-10,80778	3,83e-09*
		25	0	-6,690702	,3637577
		50	,7502475	6,94e-09	3,169081
		75	3,550175	1,157455	10,98064
		90	5,251372	3,339773	30,79063*
		97,5	30,79063	5,220765	30,79063*

* Lower (upper) confidence limit held at minimum (maximum) of sample

,,
end of do-file

,

FROZEN OOCYTES

There are 179 records in the excel file but there is a lot of missing data!

Data management
Converted all the times with non-numeric characters to numeric data, Some of the times have lots of missing values and hence reduces the information available to compare with fresh,

, destring pronucleitime tpb2 tpna vp tpnf t2 t2duration ecc1 t3 t3duration t4 t4duration ecc2 s2 t5 t5duration t6 t6duration t7 t7duration t8 t8duration ecc3 s3 t9 t9duration tsc tscduration tsb tsbduration tb tbduration blastulation teb tebduration thb thbduration, replace force float

pronucleitime: contains nonnumeric characters; replaced as float
(22 missing values generated)
tpb2: contains nonnumeric characters; replaced as float
(69 missing values generated)
tpna: contains nonnumeric characters; replaced as float
(67 missing values generated)
vp: contains nonnumeric characters; replaced as float
(89 missing values generated)
tpnf: contains nonnumeric characters; replaced as float
(88 missing values generated)
t2: contains nonnumeric characters; replaced as float
(95 missing values generated) (Thus more than half of the records are missing for this time)
t2duration: contains nonnumeric characters; replaced as float
(103 missing values generated)
ecc1: contains nonnumeric characters; replaced as float
(99 missing values generated)
t3: contains nonnumeric characters; replaced as float
(101 missing values generated)
t3duration: contains nonnumeric characters; replaced as float
(111 missing values generated)
t4: contains nonnumeric characters; replaced as float
(104 missing values generated)
t4duration: contains nonnumeric characters; replaced as float
(111 missing values generated)
ecc2: contains nonnumeric characters; replaced as float
(113 missing values generated)
s2: contains nonnumeric characters; replaced as float
(111 missing values generated)
t5: contains nonnumeric characters; replaced as float
(107 missing values generated)
t5duration: contains nonnumeric characters; replaced as float
(114 missing values generated)
t6: contains nonnumeric characters; replaced as float
(109 missing values generated)
t6duration: contains nonnumeric characters; replaced as float
(115 missing values generated)
t7: contains nonnumeric characters; replaced as float
(113 missing values generated)
t7duration: contains nonnumeric characters; replaced as float
(120 missing values generated)
t8: contains nonnumeric characters; replaced as float
(118 missing values generated)
t8duration: contains nonnumeric characters; replaced as float
(119 missing values generated)
ecc3: contains nonnumeric characters; replaced as float
(120 missing values generated)
s3: contains nonnumeric characters; replaced as float
(121 missing values generated)
t9: contains nonnumeric characters; replaced as float
(118 missing values generated)
t9duration: contains nonnumeric characters; replaced as float
(128 missing values generated)
tsc: contains nonnumeric characters; replaced as float
(126 missing values generated)
tscduration: contains nonnumeric characters; replaced as float
(133 missing values generated)
tsb: contains nonnumeric characters; replaced as float
(133 missing values generated)
tsbduration: contains nonnumeric characters; replaced as float
(139 missing values generated)
tb: contains nonnumeric characters; replaced as float

```
(139 missing values generated)
tbduration: contains nonnumeric characters; replaced as float
(150 missing values generated)
blastulation: contains nonnumeric characters; replaced as float
(139 missing values generated)
teb: contains nonnumeric characters; replaced as float
(150 missing values generated)
tebduration: contains nonnumeric characters; replaced as float
(173 missing values generated)
thb: contains nonnumeric characters; replaced as float
(173 missing values generated)
thbduration: contains nonnumeric characters; replaced as float
(173 missing values generated)


, tab oocyte source

     Oocyte |
     Source |      Freq,     Percent        Cum,
------------+-----------------------------------
 Autologous |        115       64,25       64,25
      Donor |         64       35,75      100,00
------------+-----------------------------------
      Total |        179      100,00

, tab fertilization method

Fertilization |
       Method |      Freq,     Percent        Cum,
--------------+-----------------------------------
Both IVF/ICSI |          2        1,14        1,14
         ICSI |        166       94,32       95,45
      Unknown |          8        4,55      100,00
--------------+-----------------------------------
        Total |        176      100,00



, tab diagnosis

                Diagnosis |      Freq,     Percent        Cum,
--------------------------+-----------------------------------
              Anovulation |          5        2,79        2,79
              Azoospermia |          3        1,68        4,47
            Endometriosis |         16        8,94       13,41
              Male factor |         57       31,84       45,25
                    Other |         12        6,70       51,96
                      PCO |          2        1,12       53,07
Premature Ovarian Failure |         49       27,37       80,45
           Single Female |          9        5,03       85,47
  Unexplained Infertility |         26       14,53      100,00
--------------------------+-----------------------------------
                    Total |        179      100,00



, summarize age

    Variable |        Obs        Mean    Std, Dev,       Min        Max
-------------+---------------------------------------------------------
         age |        179    38,83743    4,763903      31,03      50,54



, tab1 oocytehistory oocytesource oocytesaspirated selection well

-> tabulation of oocyte history ????

     Oocyte |
    History |      Freq,     Percent        Cum,
------------+-----------------------------------
      Other |         22       12,29       12,29
     Thawed |        157       87,71      100,00
------------+-----------------------------------

      Total |        179      100,00
```

-> tabulation of oocyte source

Oocyte Source	Freq,	Percent	Cum,
Autologous	115	64,25	64,25
Donor	64	35,75	100,00
Total	179	100,00	

-> tabulation of oocytes aspirated

Oocytes Aspirated	Freq,	Percent	Cum,
0	16	8,94	8,94
1	9	5,03	13,97
2	9	5,03	18,99
3	5	2,79	21,79
4	11	6,15	27,93
5	17	9,50	37,43
6	15	8,38	45,81
7	25	13,97	59,78
8	8	4,47	64,25
9	15	8,38	72,63
10	15	8,38	81,01
12	24	13,41	94,41
16	10	5,59	100,00
Total	179	100,00	

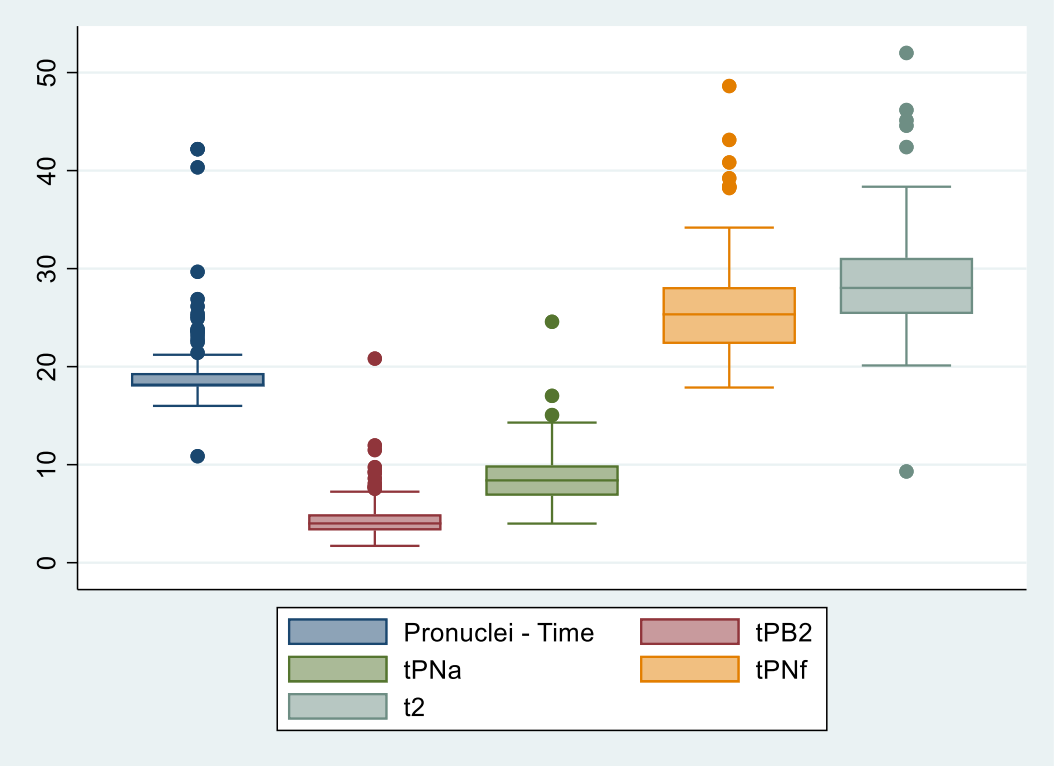
-> tabulation of selection

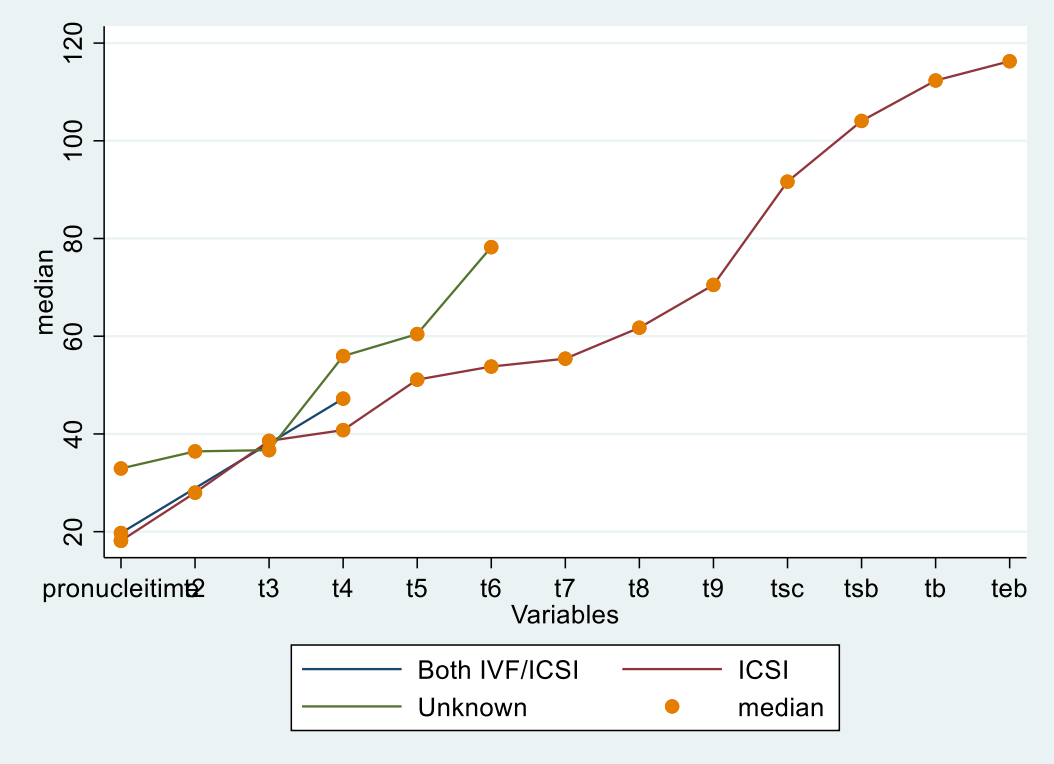
Selection	Freq,	Percent	Cum,
Avoid	149	83,24	83,24
Freeze	17	9,50	92,74
Transfer	13	7,26	100,00
Total	179	100,00	

-> tabulation of well

Well	Freq,	Percent	Cum,
1	16	8,94	8,94
2	22	12,29	21,23
3	20	11,17	32,40
4	16	8,94	41,34
5	18	10,06	51,40
6	23	12,85	64,25
7	18	10,06	74,30
8	11	6,15	80,45
9	9	5,03	85,47
10	12	6,70	92,18
11	6	3,35	95,53
12	8	4,47	100,00
Total	179	100,00	

Normal range for frozen oocytes





- Only icsi seems a viable subgroup to do

Please note that when the sample size gets too small the normal range is equivalent to the minimum and maximum values observed, This happens for all the times in the frozen subgroup except for pronuclei time,

2PN

```
, centile pronucleitime, centile(2,5 10 25 50 75 90 97,5)

                                                      -- Binom, Interp, --
    Variable |       Obs  Percentile    Centile        [95% Conf, Interval]
-------------+-------------------------------------------------------------
pronucleit~e |       157        2,5    17,57568        11,28845     17,6054
             |                   10    17,82194        17,63037    17,92028
             |                   25    18,00095        17,94805    18,03684
             |                   50    18,15463        18,07105    18,17875
             |                   75    19,38299        18,47857    20,16574
             |                   90    22,55134        20,59525    24,69346
             |                 97,5    30,20739        24,97255    42,19238


, centile tPB2 , centile(2,5 10 25 50 75 90 97,5)

                                                      -- Binom, Interp, --
    Variable |       Obs  Percentile    Centile        [95% Conf, Interval]
-------------+-------------------------------------------------------------
        tpb2 |       110        2,5    1,840413        1,719633    2,608855*
             |                   10    2,900397         2,54873    3,166566
             |                   25    3,295808        3,183256    3,551687
             |                   50    4,006835        3,807735    4,154617
             |                   75      4,9489        4,424869    6,026668
             |                   90    7,664934        6,568001    9,202271
             |                 97,5    11,59826        8,659984    20,82378*

* Lower (upper) confidence limit held at minimum (maximum) of sample


, centile tPNa , centile(2,5 10 25 50 75 90 97,5)

                                                      -- Binom, Interp, --
    Variable |       Obs  Percentile    Centile        [95% Conf, Interval]
-------------+-------------------------------------------------------------
        tpna |       112        2,5      4,1428        3,990016    5,596817*
             |                   10    6,13532        5,414742    6,527819
             |                   25    6,817542        6,604293    7,272599
             |                   50    8,398308         7,82551    8,898409
             |                   75    9,928959        9,480681    11,40694
             |                   90    12,65041        11,47043    14,00045
             |                 97,5    15,40367        13,86419    24,57565*

* Lower (upper) confidence limit held at minimum (maximum) of sample


, centile tPNf , centile(2,5 10 25 50 75 90 97,5)

                                                      -- Binom, Interp, --
    Variable |       Obs  Percentile    Centile        [95% Conf, Interval]
-------------+-------------------------------------------------------------
        tpnf |        91        2,5    18,77542        17,86992    19,55217*
             |                   10     20,1244        19,35531    21,13904
             |                   25    22,31623        21,04692    23,62893
             |                   50    25,33866        24,15476    26,25855
             |                   75    28,12082         26,8809    30,57781
             |                   90    33,17128        30,11716    38,94737
             |                 97,5    42,44003        38,32312    48,62604*

* Lower (upper) confidence limit held at minimum (maximum) of sample


, centile t2 , centile(2,5 10 25 50 75 90 97,5)

                                                      -- Binom, Interp, --
```

Variable	Obs	Percentile	Centile	[95% Conf, Interval]	
t2	84	2,5	20,2831	9,306869	22,38646*
		10	22,78491	21,63219	24,12725
		25	25,27337	24,03609	26,40902
		50	28,0323	26,68001	29,31586
		75	31,09874	30,11904	33,10954
		90	35,26701	32,63363	44,62469
		97,5	46,04629	38,95554	52*

* Lower (upper) confidence limit held at minimum (maximum) of sample

, centile **t3** , centile(2,5 10 25 50 75 90 97,5)

				-- Binom, Interp, --	
Variable	Obs	Percentile	Centile	[95% Conf, Interval]	
t3	78	2,5	27,54018	26,46447	29,92525*
		10	31,36833	29,03308	33,14105
		25	33,42507	32,87478	35,85816
		50	38,24212	36,41514	39,64184
		75	42,12107	39,99386	43,69827
		90	44,50182	43,53308	54,66067
		97,5	69,94003	48,12271	99*

* Lower (upper) confidence limit held at minimum (maximum) of sample

, centile **t4** , centile(2,5 10 25 50 75 90 97,5)

				-- Binom, Interp, --	
Variable	Obs	Percentile	Centile	[95% Conf, Interval]	
t4	75	2,5	32,63824	32,41719	33,29757*
		10	34,04436	32,81061	35,68211
		25	37,15124	35,57318	39,4874
		50	40,92032	39,64835	42,68587
		75	44,8222	43,38837	48,34242
		90	51,37336	46,86716	63,52898
		97,5	83,42	54,49933	122,3*

* Lower (upper) confidence limit held at minimum (maximum) of sample

, centile **t5** , centile(2,5 10 25 50 75 90 97,5)

				-- Binom, Interp, --	
Variable	Obs	Percentile	Centile	[95% Conf, Interval]	
t5	72	2,5	34,32996	33,91541	39,17459*
		10	40,34617	35,29735	43,99572
		25	46,08572	42,56997	48,21897
		50	51,20226	48,4422	53,24294
		75	57,9324	53,51218	60,2458
		90	61,53765	59,84626	81,77646
		97,5	91,1975	65,23658	122,3*

* Lower (upper) confidence limit held at minimum (maximum) of sample

, centile **t6** , centile(2,5 10 25 50 75 90 97,5)

				-- Binom, Interp, --	
Variable	Obs	Percentile	Centile	[95% Conf, Interval]	
t6	70	2,5	37,25228	34,96718	42,71627*

```
             |                  10    44,23711        40,65753    47,56961
             |                  25    49,02724        47,08648    52,05952
             |                  50    53,81518        52,44337        56,4
             |                  75     61,0757        57,53776    65,80972
             |                  90    67,59259        64,12224    78,69195
             |                97,5     93,0825        72,69821       122,3*

* Lower (upper) confidence limit held at minimum (maximum) of sample
```

```
, centile t7   , centile(2,5 10 25 50 75 90 97,5)

                                                    -- Binom, Interp, --
    Variable |       Obs  Percentile    Centile        [95% Conf, Interval]
-------------+-------------------------------------------------------------
          t7 |        66        2,5    42,97508        42,55188    46,05141*
             |                  10    47,53115         43,1826    49,16186
             |                  25    50,55155        48,71184    53,29209
             |                  50    55,40757        53,52664    60,23941
             |                  75    62,64599        60,72834    67,48778
             |                  90    70,72417        64,81298    85,53853
             |                97,5    86,97287        74,22329    87,21191*

* Lower (upper) confidence limit held at minimum (maximum) of sample
```

```
, centile t8   , centile(2,5 10 25 50 75 90 97,5)

                                                    -- Binom, Interp, --
    Variable |       Obs  Percentile    Centile        [95% Conf, Interval]
-------------+-------------------------------------------------------------
          t8 |        61        2,5    43,28133        42,80273    47,38192*
             |                  10    48,83275        44,13837    51,46539
             |                  25    53,49826        49,88319    56,48717
             |                  50    61,74266        56,83509    64,45026
             |                  75    68,15014        64,72725    71,97328
             |                  90    75,85691         70,5805    84,48899
             |                97,5    86,96645        80,78132    89,85878*

* Lower (upper) confidence limit held at minimum (maximum) of sample
```

```
, centile t9   , centile(2,5 10 25 50 75 90 97,5)

                                                    -- Binom, Interp, --
    Variable |       Obs  Percentile    Centile        [95% Conf, Interval]
-------------+-------------------------------------------------------------
          t9 |        61        2,5    49,14782        48,72115    56,46293*
             |                  10    56,90566          51,212    62,06856
             |                  25     64,3508        61,16725    68,61017
             |                  50    70,50475        68,68029    72,97436
             |                  75    76,14907        73,10492     83,8827
             |                  90     86,1395        81,28525    92,57434
             |                97,5     96,6271        89,13436    101,4692*

* Lower (upper) confidence limit held at minimum (maximum) of sample
```

```
, centile tSC   , centile(2,5 10 25 50 75 90 97,5)

                                                    -- Binom, Interp, --
    Variable |       Obs  Percentile    Centile        [95% Conf, Interval]
-------------+-------------------------------------------------------------
         tsc |        53        2,5    70,54125        67,89182    79,23354*
             |                  10    80,07103        75,15978    83,43299
             |                  25    84,89738        80,85397    89,20027
             |                  50    91,63601        88,92916     94,8443
             |                  75    98,14961        94,66539    103,8953
```

```
             |                  90     107,142        102,4824    113,7502
             |                97,5    114,5184        108,9408     114,959*

* Lower (upper) confidence limit held at minimum (maximum) of sample

, centile tSB   , centile(2,5 10 25 50 75 90 97,5)

                                                    -- Binom, Interp, --
    Variable |       Obs  Percentile    Centile        [95% Conf, Interval]
-------------+-------------------------------------------------------------
         tsb |        46        2,5    87,57508        87,56339    90,98987*
             |                  10     91,40892        87,59191     97,53237
             |                  25     98,40294        95,14093     101,8611
             |                  50     104,0663        101,3141      108,586
             |                  75     112,8185        108,4555     114,8784
             |                  90     116,3971        113,5397     125,1289
             |                97,5     125,6205        117,1525     125,9619*

* Lower (upper) confidence limit held at minimum (maximum) of sample



, centile tB   , centile(2,5 10 25 50 75 90 97,5)

                                                    -- Binom, Interp, --
    Variable |       Obs  Percentile    Centile        [95% Conf, Interval]
-------------+-------------------------------------------------------------
          tb |        40        2,5    95,68578        95,62644    98,12508*
             |                  10     98,27536        95,99567     103,1599
             |                  25     104,9416        100,1983     110,0949
             |                  50     112,3412         108,104     116,4959
             |                  75     121,6841        115,2546     128,8302
             |                  90     131,9377        124,8483     149,0658
             |                97,5     150,9507         132,419     151,3116*

* Lower (upper) confidence limit held at minimum (maximum) of sample



, centile tEB   , centile(2,5 10 25 50 75 90 97,5)

                                                    -- Binom, Interp, --
    Variable |       Obs  Percentile    Centile        [95% Conf, Interval]
-------------+-------------------------------------------------------------
         teb |        29        2,5    98,87723        98,87723    109,2092*
             |                  10      108,888        98,87723    112,4591*
             |                  25     112,5828        109,2269     115,3306
             |                  50     116,2819        113,8836     129,1453
             |                  75      133,485        121,1114     139,6897
             |                  90     140,3597        134,0069     163,3045*
             |                97,5     163,3045        139,7247     163,3045*

* Lower (upper) confidence limit held at minimum (maximum) of sample



, centile tHB   , centile(2,5 10 25 50 75 90 97,5)

                                                    -- Binom, Interp, --
    Variable |       Obs  Percentile    Centile        [95% Conf, Interval]
-------------+-------------------------------------------------------------
         thb |         6        2,5    114,1377        114,1377       114,7*
             |                  10     114,1377        114,1377    120,5835*
             |                  25     114,6185        114,1377    127,8829*
             |                  50     121,8031        114,2018     139,2011
             |                  75     137,3947        118,7324     139,479*
             |                  90      139,479        123,7546     139,479*
             |                97,5      139,479        137,0413     139,479*

* Lower (upper) confidence limit held at minimum (maximum) of sample
```

For ICSI only

```
, foreach var of varlist pronucleitime tpb2 tpna tpnf t2  t3  t4  t5  t6 t7  t8  t9 tsc  tsb
tb  teb
```

```
> thb {
  2,   centile `var' if nfert_meth==2, centile(2,5 10 25 50 75 90 97,5)
  3, }
```

Variable	Obs	Percentile	Centile	-- Binom, Interp, -- [95% Conf,	Interval]
pronucleit~e	149	2,5	17,53235	10,98323	17,60503
		10	17,80677	17,6052	17,91765
		25	17,99155	17,93817	18,02635
		50	18,1316	18,05292	18,17052
		75	19,06768	18,39113	19,80606
		90	20,86175	20,31802	23,74033
		97,5	26,33443	23,76345	40,08985

Variable	Obs	Percentile	Centile	-- Binom, Interp, -- [95% Conf,	Interval]
tpb2	108	2,5	1,837806	1,719633	2,606363*
		10	2,894874	2,537364	3,165496
		25	3,29577	3,178345	3,546303
		50	3,983414	3,789342	4,136872
		75	4,918841	4,41776	5,921157
		90	7,273516	5,996714	8,858557
		97,5	10,22898	8,173513	20,82378*

* Lower (upper) confidence limit held at minimum (maximum) of sample

Variable	Obs	Percentile	Centile	-- Binom, Interp, -- [95% Conf,	Interval]
tpna	110	2,5	4,14066	3,990016	5,588866*
		10	6,092574	5,353082	6,523973
		25	6,795027	6,565012	7,248993
		50	8,374381	7,647792	8,822986
		75	9,830137	9,44711	11,33207
		90	12,53433	11,41223	14,054
		97,5	15,50167	13,86984	24,57565*

* Lower (upper) confidence limit held at minimum (maximum) of sample

Variable	Obs	Percentile	Centile	-- Binom, Interp, -- [95% Conf,	Interval]
tpnf	89	2,5	18,75832	17,86992	19,51193*
		10	20,0829	19,33888	21,0409
		25	22,25902	20,91892	23,59853
		50	24,58214	24,11858	26,21129
		75	28,01254	26,72199	29,16731
		90	31,99978	28,96872	39,04504
		97,5	42,55482	38,33508	48,62604*

* Lower (upper) confidence limit held at minimum (maximum) of sample

Variable	Obs	Percentile	Centile	-- Binom, Interp, -- [95% Conf,	Interval]
t2	83	2,5	20,25057	9,306869	22,38371*
		10	22,75916	21,61056	24,11152
		25	25,17536	23,93809	26,40259
		50	27,97561	26,6414	29,21916
		75	31,09705	29,95911	32,60054
		90	34,93636	32,09151	44,67647
		97,5	46,0725	39,07879	52*

* Lower (upper) confidence limit held at minimum (maximum) of sample

Variable	Obs	Percentile	Centile	-- Binom, Interp, -- [95% Conf,	Interval]
t3	77	2,5	27,51259	26,46447	29,92207*
		10	31,31982	28,94058	33,11246
		25	33,42348	32,84109	35,57432
		50	38,58172	36,3732	39,66057
		75	42,14212	40,08642	43,73549
		90	44,65078	43,54821	55,13897
		97,5	70,68516	48,12694	99*

* Lower (upper) confidence limit held at minimum (maximum) of sample

Variable	Obs	Percentile	Centile	-- Binom, Interp, -- [95% Conf,	Interval]
t4	73	2,5	32,62596	32,41719	33,27377*
		10	33,91948	32,79448	35,61815
		25	37,13413	35,40246	39,44303
		50	40,78466	39,60842	42,48388
		75	44,68239	43,14585	47,08088
		90	50,48912	45,16586	63,85808
		97,5	85,58	52,78494	122,3*

* Lower (upper) confidence limit held at minimum (maximum) of sample

Variable	Obs	Percentile	Centile	-- Binom, Interp, -- [95% Conf,	Interval]
t5	71	2,5	34,3174	33,91541	39,11303*
		10	40,28891	35,1558	43,91261
		25	45,93588	42,33443	48,16432
		50	51,11914	48,38631	53,20039
		75	57,90486	53,4998	59,98702
		90	61,57754	59,7637	82,60222
		97,5	92,14	65,28294	122,3*

* Lower (upper) confidence limit held at minimum (maximum) of sample

Variable	Obs	Percentile	Centile	-- Binom, Interp, -- [95% Conf,	Interval]
t6	69	2,5	37,17856	34,96718	42,67747*
		10	44,04936	40,46518	47,50016
		25	48,96571	47,08508	51,95586
		50	53,79412	52,38448	56,23824
		75	60,83838	57,50412	64,88685
		90	67,55431	62,67272	79,05044
		97,5	94,025	68,32758	122,3*

* Lower (upper) confidence limit held at minimum (maximum) of sample

Variable	Obs	Percentile	Centile	-- Binom, Interp, -- [95% Conf,	Interval]
t7	66	2,5	42,97508	42,55188	46,05141*
		10	47,53115	43,1826	49,16186
		25	50,55155	48,71184	53,29209
		50	55,40757	53,52664	60,23941
		75	62,64599	60,72834	67,48778
		90	70,72417	64,81298	85,53853
		97,5	86,97287	74,22329	87,21191*

* Lower (upper) confidence limit held at minimum (maximum) of sample

Variable	Obs	Percentile	Centile	-- Binom, Interp, -- [95% Conf,	Interval]
t8	61	2,5	43,28133	42,80273	47,38192*
		10	48,83275	44,13837	51,46539
		25	53,49826	49,88319	56,48717
		50	61,74266	56,83509	64,45026
		75	68,15014	64,72725	71,97328
		90	75,85691	70,5805	84,48899
		97,5	86,96645	80,78132	89,85878*

* Lower (upper) confidence limit held at minimum (maximum) of sample

Variable	Obs	Percentile	Centile	-- Binom, Interp, -- [95% Conf,	Interval]
t9	61	2,5	49,14782	48,72115	56,46293*
		10	56,90566	51,212	62,06856
		25	64,3508	61,16725	68,61017
		50	70,50475	68,68029	72,97436
		75	76,14907	73,10492	83,8827
		90	86,1395	81,28525	92,57434

```
             |                 97,5     96,6271        89,13436    101,4692*

* Lower (upper) confidence limit held at minimum (maximum) of sample

                                                      -- Binom, Interp, --
    Variable |       Obs  Percentile    Centile        [95% Conf, Interval]
-------------+-------------------------------------------------------------
         tSC |        53        2,5    70,54125        67,89182    79,23354*
             |                  10    80,07103        75,15978    83,43299
             |                  25    84,89738        80,85397    89,20027
             |                  50    91,63601        88,92916     94,8443
             |                  75    98,14961        94,66539    103,8953
             |                  90     107,142        102,4824    113,7502
             |                97,5    114,5184        108,9408     114,959*

* Lower (upper) confidence limit held at minimum (maximum) of sample

                                                      -- Binom, Interp, --
    Variable |       Obs  Percentile    Centile        [95% Conf, Interval]
-------------+-------------------------------------------------------------
         tSB |        46        2,5    87,57508        87,56339    90,98987*
             |                  10    91,40892        87,59191    97,53237
             |                  25    98,40294        95,14093    101,8611
             |                  50    104,0663        101,3141     108,586
             |                  75    112,8185        108,4555    114,8784
             |                  90    116,3971        113,5397    125,1289
             |                97,5    125,6205        117,1525    125,9619*

* Lower (upper) confidence limit held at minimum (maximum) of sample

                                                      -- Binom, Interp, --
    Variable |       Obs  Percentile    Centile        [95% Conf, Interval]
-------------+-------------------------------------------------------------
          tB |        40        2,5    95,68578        95,62644    98,12508*
             |                  10    98,27536        95,99567    103,1599
             |                  25    104,9416        100,1983    110,0949
             |                  50    112,3412         108,104    116,4959
             |                  75    121,6841        115,2546    128,8302
             |                  90    131,9377        124,8483    149,0658
             |                97,5    150,9507         132,419    151,3116*

* Lower (upper) confidence limit held at minimum (maximum) of sample




                                                      -- Binom, Interp, --
    Variable |       Obs  Percentile    Centile        [95% Conf, Interval]
-------------+-------------------------------------------------------------
         tEB |        29        2,5    98,87723        98,87723    109,2092*
             |                  10     108,888        98,87723    112,4591*
             |                  25    112,5828        109,2269    115,3306
             |                  50    116,2819        113,8836    129,1453
             |                  75     133,485        121,1114    139,6897
             |                  90    140,3597        134,0069    163,3045*
             |                97,5    163,3045        139,7247    163,3045*

* Lower (upper) confidence limit held at minimum (maximum) of sample

                                                      -- Binom, Interp, --
    Variable |       Obs  Percentile    Centile        [95% Conf, Interval]
-------------+-------------------------------------------------------------
         tHB |         6        2,5    114,1377        114,1377       114,7*
             |                  10    114,1377        114,1377    120,5835*
             |                  25    114,6185        114,1377    127,8829*
             |                  50    121,8031        114,2018    139,2011
             |                  75    137,3947        118,7324     139,479*
             |                  90     139,479        123,7546     139,479*
             |                97,5     139,479        137,0413     139,479*

* Lower (upper) confidence limit held at minimum (maximum) of sample
```

ICSI durations

```
, foreach var of varlist vp  t2duration ecc1 t3duration  t4duration  t5duration  t6duration
t7duration t8duration ecc3  s3 t9duration  tscduration  tsbduration  tbduration tebduration
thbduration {
  2,   centile `var' if nfert_meth==2, centile(2,5 10 25 50 75 90 97,5)
  3, }
```

Variable	Obs	Percentile	Centile	-- Binom, Interp, -- [95% Conf, Interval]	
vp	89	2,5	10,0654	8,752907	12,50045*
		10	12,75421	12,0037	13,25494
		25	14,09933	13,2547	15,24542
		50	16,25574	15,75141	17,25897
		75	19,33125	17,76622	20,26167
		90	22,26245	20,26001	28,03233
		97,5	33,3299	25,26996	39,01143*

* Lower (upper) confidence limit held at minimum (maximum) of sample

Variable	Obs	Percentile	Centile	-- Binom, Interp, -- [95% Conf, Interval]	
t2duration	75	2,5	,7501538	,7500005	1,379664*
		10	1,650191	1,064443	2,627088
		25	9,752951	2,071349	10,75251
		50	11,26721	10,79855	12,00104
		75	12,50289	12,0035	13,25952
		90	14,00728	13,25357	21,2675
		97,5	32,39265	15,62719	47*

* Lower (upper) confidence limit held at minimum (maximum) of sample

Variable	Obs	Percentile	Centile	-- Binom, Interp, -- [95% Conf, Interval]	
ecc1	80	2,5	16,30511	15,77978	19,14976*
		10	19,29642	18,54576	20,28288
		25	21,46548	20,04244	22,50713
		50	24,00748	23,04601	24,79469
		75	26,9253	25,58311	28,19189
		90	30,26641	27,71583	36,82019
		97,5	42,47683	31,40357	48,70425*

* Lower (upper) confidence limit held at minimum (maximum) of sample

Variable	Obs	Percentile	Centile	-- Binom, Interp, -- [95% Conf, Interval]	
t3duration	67	2,5	0	0	,2499589*
		10	,2500045	0	,2501132
		25	,4978953	,2500685	,5002497
		50	,7504567	,5002883	1,49895
		75	8,502707	1,689619	12,61892
		90	14,00792	11,43615	21,75555
		97,5	27,48369	15,85754	37,24565*

* Lower (upper) confidence limit held at minimum (maximum) of sample

Variable	Obs	Percentile	Centile	-- Binom, Interp, -- [95% Conf, Interval]	
t4duration	67	2,5	0	0	,0160023*
		10	,4502307	0	1,250571
		25	3,250907	,8765998	10,08045
		50	11,50283	10,16701	13,00293
		75	13,75721	13,03241	15,11575
		90	15,55776	14,78587	18,13615
		97,5	20,81751	16,29849	22,50942*

* Lower (upper) confidence limit held at minimum (maximum) of sample

Variable	Obs	Percentile	Centile	-- Binom, Interp, -- [95% Conf, Interval]	

				-- Binom, Interp, --	
Variable	Obs	Percentile	Centile	[95% Conf,	Interval]
t5duration	64	2,5	0	0	,234283*
		10	,2499408	0	,254428
		25	,500261	,2501612	,7502515
		50	1,251835	,7503291	2,000538
		75	4,939197	2,000696	12,37025
		90	13,5059	10,14033	18,20892
		97,5	20,06729	14,15937	21,00715*

* Lower (upper) confidence limit held at minimum (maximum) of sample

				-- Binom, Interp, --	
Variable	Obs	Percentile	Centile	[95% Conf,	Interval]
t6duration	64	2,5	0	0	0*
		10	0	0	,4829306
		25	,5001175	,250045	,7503266
		50	1,250242	,7504303	1,750482
		75	3,938846	1,751426	7,265368
		90	13,35774	6,202583	19,05415
		97,5	20,53875	17,87157	22,00734*

* Lower (upper) confidence limit held at minimum (maximum) of sample

				-- Binom, Interp, --	
Variable	Obs	Percentile	Centile	[95% Conf,	Interval]
t7duration	59	2,5	0	0	0*
		10	,2500333	0	,5000309
		25	,7501283	,252567	1,250504
		50	2,002693	1,250513	2,76535
		75	6,001658	2,77999	13,99791
		90	15,33457	9,479202	19,50808
		97,5	19,97506	15,9075	20,44163*

* Lower (upper) confidence limit held at minimum (maximum) of sample

				-- Binom, Interp, --	
Variable	Obs	Percentile	Centile	[95% Conf,	Interval]
t8duration	60	2,5	0	0	1,00029*
		10	1,025426	,2144206	1,986642
		25	2,654752	1,678505	5,084771
		50	9,19274	5,232293	12,62773
		75	14,56208	13,02724	18,73472
		90	21,25555	16,73396	27,31967
		97,5	29,01848	24,88694	30,75938*

* Lower (upper) confidence limit held at minimum (maximum) of sample

				-- Binom, Interp, --	
Variable	Obs	Percentile	Centile	[95% Conf,	Interval]
ecc3	59	2,5	7,751596	5,5	12,04296*
		10	12,16328	10,09307	14,05392
		25	15,36796	12,76148	16,24878
		50	17,25573	16,25583	21,11731
		75	28,26661	21,50869	31,09182
		90	35,47294	30,35438	39,00182
		97,5	43,14518	35,50456	46,51481*

* Lower (upper) confidence limit held at minimum (maximum) of sample

				-- Binom, Interp, --	
Variable	Obs	Percentile	Centile	[95% Conf,	Interval]
s3	58	2,5	,2346805	0	1,099051*
		10	1,463438	,5420442	2,251707
		25	2,500795	2,190703	4,251478
		50	8,127325	4,254062	16,00411
		75	19,44351	16,0786	21,92081
		90	24,45381	21,18604	30,02018
		97,5	32,22731	28,62273	34,22212*

* Lower (upper) confidence limit held at minimum (maximum) of sample

-- Binom, Interp, --

Variable	Obs	Percentile	Centile	[95% Conf, Interval]	
t9duration	51	2,5	0	0	7,50252*
		10	8,372323	0	9,894436
		25	10,65355	8,792969	16,04856
		50	21,75683	15,05431	23,08849
		75	27,25735	23,04197	32,42749
		90	34,19434	30,74247	41,99523
		97,5	43,99759	34,92492	45,26312*

* Lower (upper) confidence limit held at minimum (maximum) of sample

Variable	Obs	Percentile	Centile	-- Binom, Interp, -- [95% Conf,	Interval]
tscduration	46	2,5	3,505787	3,400733	4,532403*
		10	4,676709	3,657085	6,768173
		25	7,388117	5,493234	9,243957
		50	11,50402	9,002989	14,76369
		75	17,57629	14,51123	20,89539
		90	23,65683	19,83572	35,67123
		97,5	37,94036	25,38644	39,51593*

* Lower (upper) confidence limit held at minimum (maximum) of sample

Variable	Obs	Percentile	Centile	-- Binom, Interp, -- [95% Conf,	Interval]
tsbduration	40	2,5	3,507328	3,501079	4,01955*
		10	4,045258	3,539965	6,501903
		25	6,75199	4,415354	7,637646
		50	8,877673	7,211503	10,50316
		75	12,00511	9,985911	14,25917
		90	14,6103	13,21204	35,63372
		97,5	37,65991	15,00542	38,04789*

* Lower (upper) confidence limit held at minimum (maximum) of sample

Variable	Obs	Percentile	Centile	-- Binom, Interp, -- [95% Conf,	Interval]
tbduration	29	2,5	3,250782	3,250782	4,924362*
		10	4,751543	3,250782	6,001989*
		25	6,25193	4,933872	7,153104
		50	7,752351	6,677652	11,083
		75	13,22686	8,3482	17,30057
		90	17,75552	14,70539	29,79589*
		97,5	29,79589	17,3243	29,79589*

* Lower (upper) confidence limit held at minimum (maximum) of sample

Variable	Obs	Percentile	Centile	-- Binom, Interp, -- [95% Conf,	Interval]
tebduration	6	2,5	,250095	,250095	2,663049*
		10	,250095	,250095	3,227848*
		25	2,313205	,250095	7,029458*
		50	4,375268	,5251764	22,55024
		75	13,12681	3,155478	24*
		90	24	5,871598	24*
		97,5	24	11,28304	24*

* Lower (upper) confidence limit held at minimum (maximum) of sample

Variable	Obs	Percentile	Centile	-- Binom, Interp, -- [95% Conf,	Interval]
thbduration	6	2,5	0	0	1,316202*
		10	0	0	3,132693*
		25	1,125371	0	8,707044*
		50	3,900451	,1500495	16,63064
		75	15,81793	2,612189	16,75567*
		90	16,75567	5,524024	16,75567*
		97,5	16,75567	15,65891	16,75567*

```
* Lower (upper) confidence limit held at minimum (maximum) of sample

,
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,,
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,
```

SIBLING COMPARISON

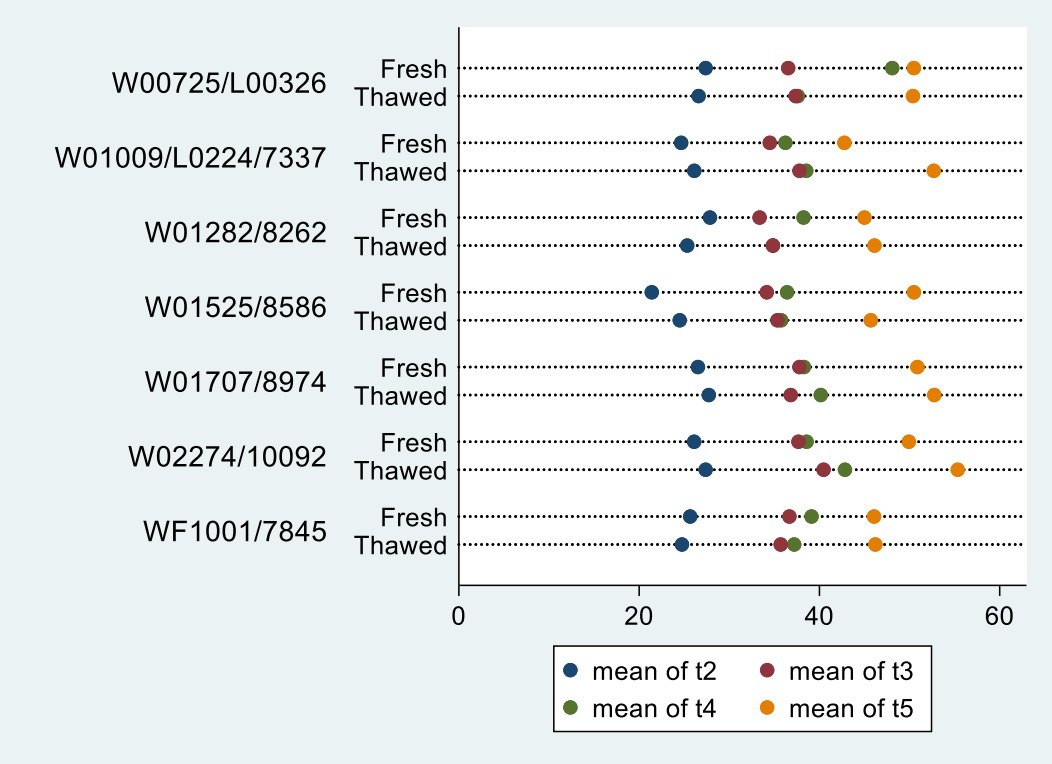
, tab patientid oocytehistory

Patient ID	Oocyte History: Fresh	Thawed	Total
W00725/L00326	5	2	7
W01009/L0224/7337	10	3	13
W01282/8262	4	1	5
W01525/8586	1	3	4
W01590/8499	0	4	4
W01593/8969	4	0	4
W01707/8974	3	3	6
W01772/9355	0	1	1
W01820/9235	4	0	4
W02274/10092	8	2	10
WF1001/7845	6	6	12
Total	45	25	70

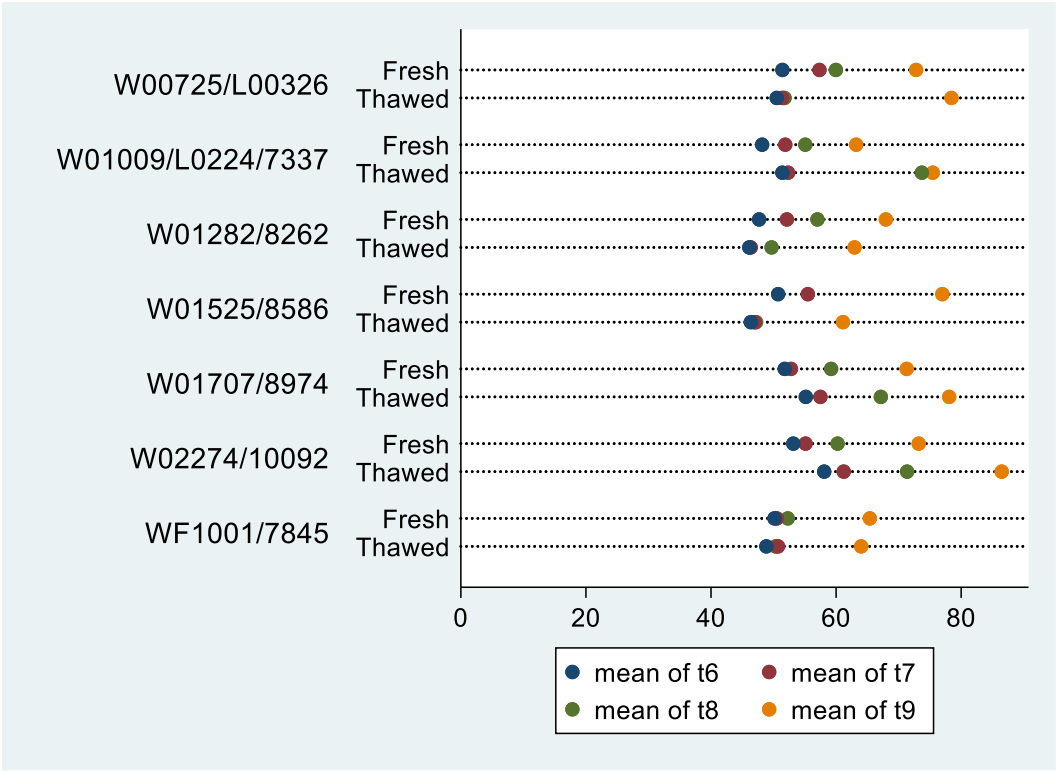
- There are 4 patients with only a single type of oocyte, Therefore they cannot be used to contrast fresh versus frozen within the same patient
- Numbers of patient and number of oocytes are very small – thus this analysis can only be considered **exploratory**

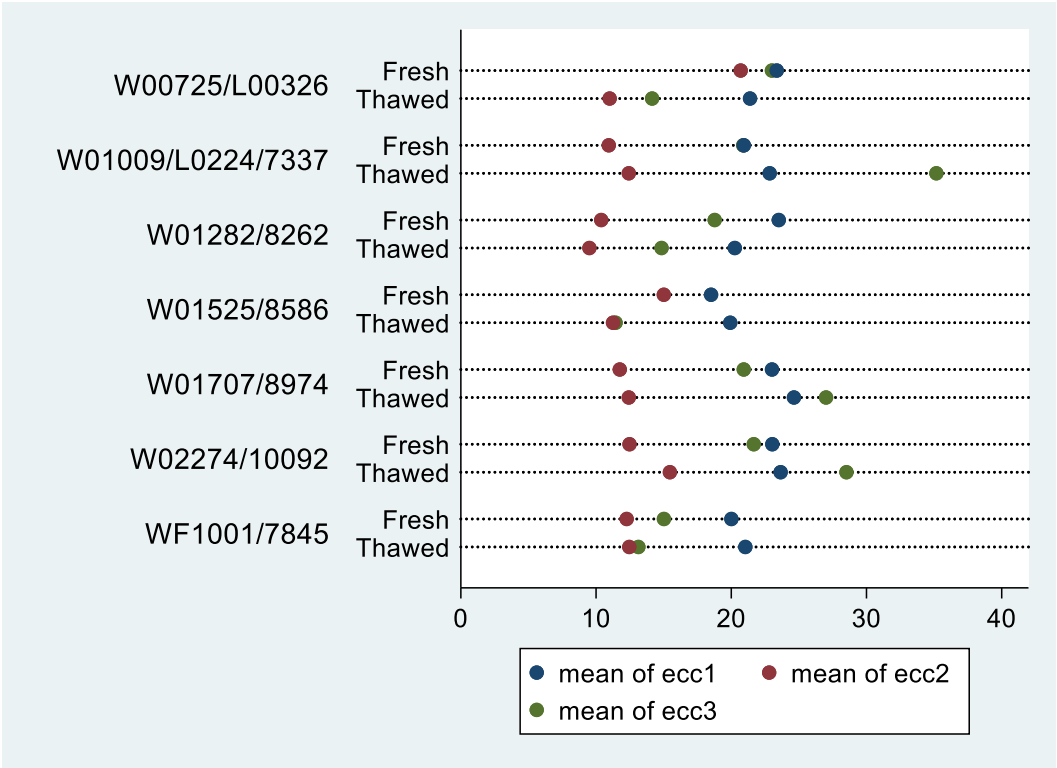
Dot plot of mean times for each patient by occyte history (frozen , fresh)

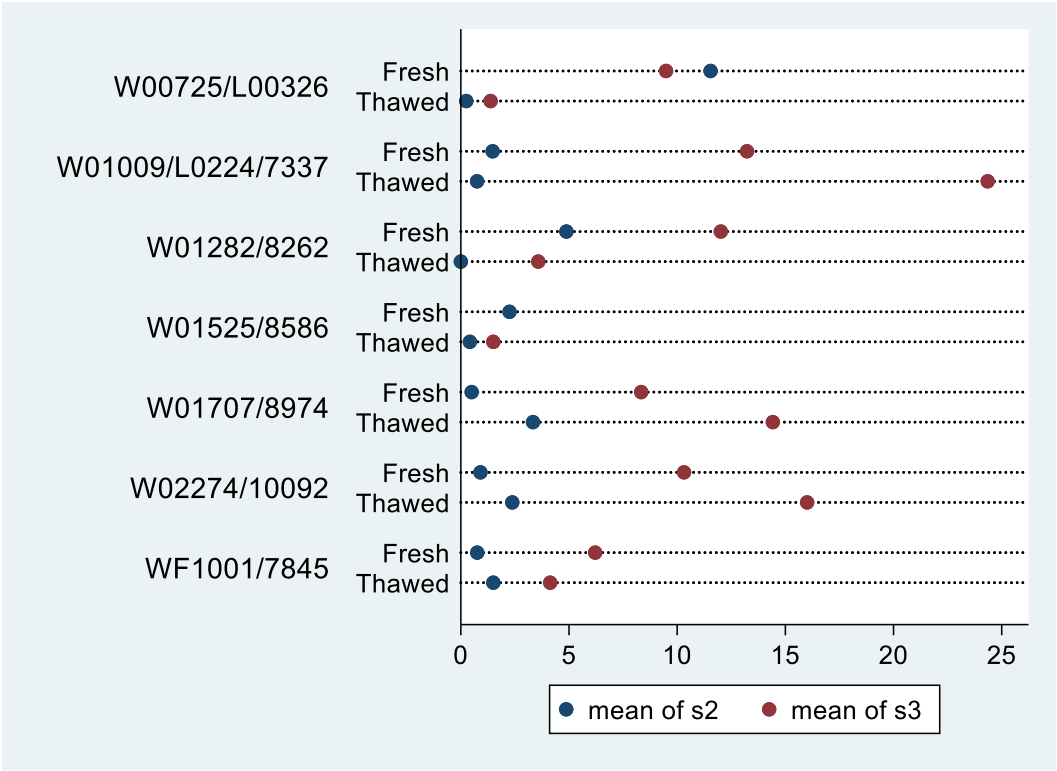
graph dot (mean) t2 t3 t4 t5 if include==1, over(oocytehistory) over(patientid)

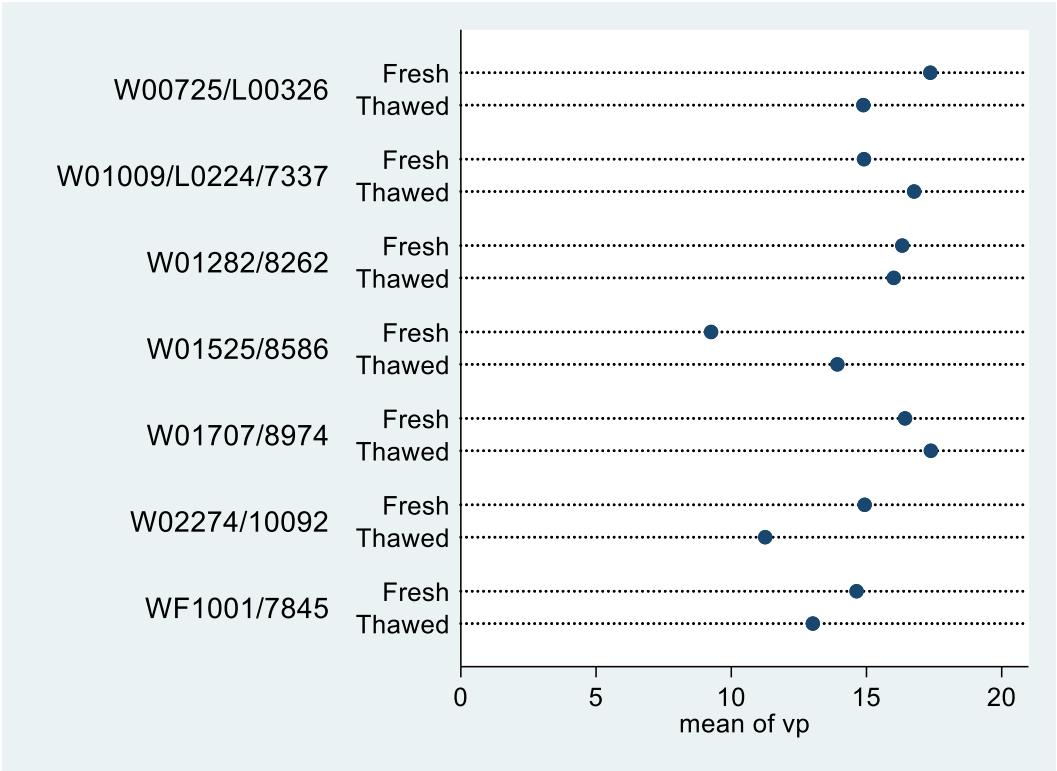


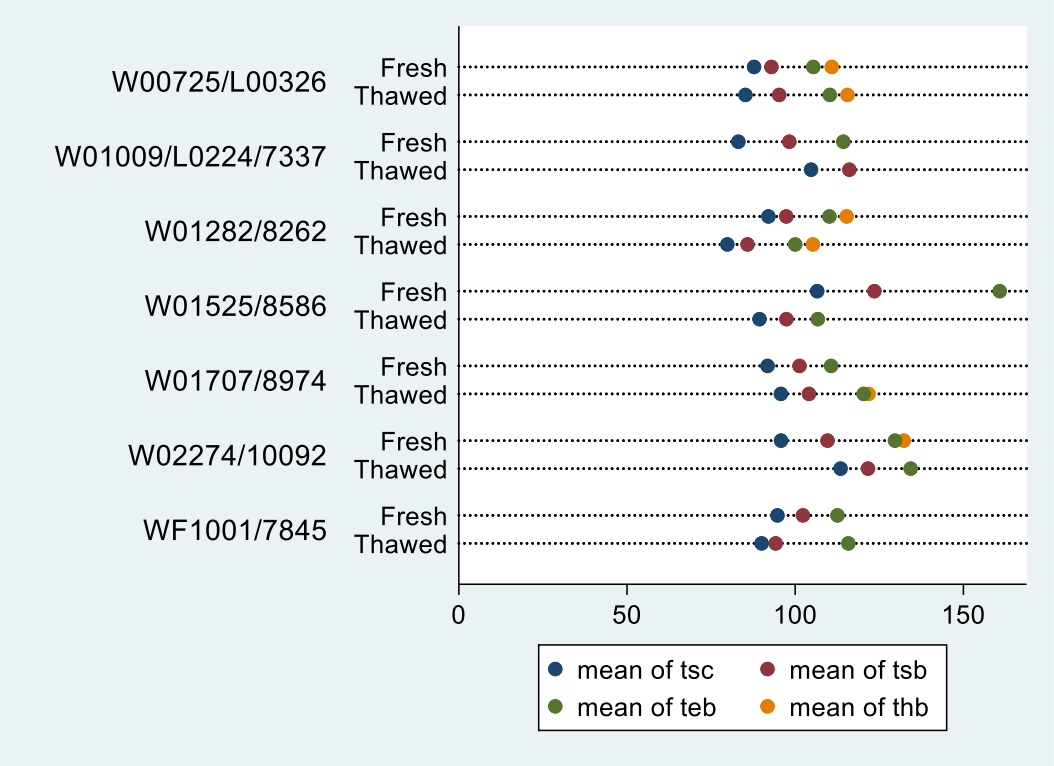
- There is no consistent pattern, For some patients fresh have longer times and for some vice versa, Thus difference in times between oocyte type is random,
- For t3 four patients frozen id longer than fresh giving 3 patients with the opposite, 4:3 in the 7 patients is the closest you can get to 50-50 split,
- The trend within patients is fairly consistent – thus if fresh is longer than frozen it carries through to the later durations











Estimating the mean difference between fresh and frozen using the **matched design**
Linear regression model adjusting for clustering of values within patient

Time points

```
, xi: regress t2 i,oocytehistory if include==1, vce(cluster patientid)

i,oocytehistory   _Ioocytehis_1-2     (_Ioocytehis_1 for oocy~y==Fresh omitted)

Linear regression                               Number of obs     =         57
                                                F(1, 6)           =       0,01
                                                Prob > F          =     0,9199
                                                R-squared         =     0,0001
                                                Root MSE          =     2,9375

                                (Std, Err, adjusted for 7 clusters in patientid)
------------------------------------------------------------------------------
             |               Robust
          t2 |      Coef,   Std, Err,      t    P>|t|     [95% Conf, Interval]
-------------+----------------------------------------------------------------
_Ioocytehis_2 |  -,0694767   ,6627916    -0,10   0,920    -1,691269    1,552316
       _cons |   25,91133   ,5092781    50,88   0,000     24,66517    27,15749
```

- Estimate time difference between frozen and fresh for t2 is -,06?? (95%CI: -1,69 to 1,55) p=,920
- _cons of 25,91 is the mean of t2 in the fresh oocytes and then mean of the frozen oocytes is slight less as indicated in the previous bullet,

```
, xi: regress t3 i,oocytehistory if include==1, vce(cluster patientid)
i,oocytehistory   _Ioocytehis_1-2     (_Ioocytehis_1 for oocy~y==Fresh omitted)

Linear regression                               Number of obs     =         56
                                                F(1, 6)           =       0,86
                                                Prob > F          =     0,3908
                                                R-squared         =     0,0071
```

```
                                            Root MSE          =     4,2721

                              (Std, Err, adjusted for 7 clusters in patientid)
------------------------------------------------------------------------------
             |               Robust
          t3 |      Coef,   Std, Err,      t    P>|t|     [95% Conf, Interval]
-------------+----------------------------------------------------------------
_Ioocytehis_2 |   ,7416968   ,8021243     0,92   0,391    -1,221031    2,704424
       _cons |   35,98439   ,6957541    51,72   0,000     34,28194    37,68683
------------------------------------------------------------------------------

, xi: regress t4 i,oocytehistory if include==1, vce(cluster patientid)
i,oocytehistory   _Ioocytehis_1-2     (_Ioocytehis_1 for oocy~y==Fresh omitted)

Linear regression                               Number of obs     =         53
                                                F(1, 6)           =       0,41
                                                Prob > F          =     0,5467
                                                R-squared         =     0,0056
                                                Root MSE          =     8,3253

                              (Std, Err, adjusted for 7 clusters in patientid)
------------------------------------------------------------------------------
             |               Robust
          t4 |      Coef,   Std, Err,      t    P>|t|     [95% Conf, Interval]
-------------+----------------------------------------------------------------
_Ioocytehis_2 |  -1,264317   1,979849    -0,64   0,547    -6,108833    3,580199
       _cons |   39,37845   1,688627    23,32   0,000     35,24652    43,51037
------------------------------------------------------------------------------

, xi: regress t5 i,oocytehistory if include==1, vce(cluster patientid)
i,oocytehistory   _Ioocytehis_1-2     (_Ioocytehis_1 for oocy~y==Fresh omitted)

Linear regression                               Number of obs     =         54
                                                F(1, 6)           =       1,25
                                                Prob > F          =     0,3070
                                                R-squared         =     0,0225
                                                Root MSE          =     7,1059

                              (Std, Err, adjusted for 7 clusters in patientid)
------------------------------------------------------------------------------
             |               Robust
          t5 |      Coef,   Std, Err,      t    P>|t|     [95% Conf, Interval]
-------------+----------------------------------------------------------------
_Ioocytehis_2 |   2,216414   1,985402     1,12   0,307    -2,641688    7,074517
       _cons |   47,01818   1,538749    30,56   0,000       43,253    50,78337
------------------------------------------------------------------------------

, xi: regress t6 i,oocytehistory if include==1, vce(cluster patientid)
i,oocytehistory   _Ioocytehis_1-2     (_Ioocytehis_1 for oocy~y==Fresh omitted)

Linear regression                               Number of obs     =         54
                                                F(1, 6)           =       0,23
                                                Prob > F          =     0,6513
                                                R-squared         =     0,0036
                                                Root MSE          =     5,3184

                              (Std, Err, adjusted for 7 clusters in patientid)
------------------------------------------------------------------------------
             |               Robust
          t6 |      Coef,   Std, Err,      t    P>|t|     [95% Conf, Interval]
-------------+----------------------------------------------------------------
_Ioocytehis_2 |    ,655098   1,378002     0,48   0,651    -2,716752    4,026948
       _cons |   50,20374   ,9788642    51,29   0,000     47,80855    52,59894
------------------------------------------------------------------------------

, xi: regress t7 i,oocytehistory if include==1, vce(cluster patientid)
i,oocytehistory   _Ioocytehis_1-2     (_Ioocytehis_1 for oocy~y==Fresh omitted)

Linear regression                               Number of obs     =         55
                                                F(1, 6)           =       0,23
                                                Prob > F          =     0,6497
                                                R-squared         =     0,0035
                                                Root MSE          =     6,4742

                              (Std, Err, adjusted for 7 clusters in patientid)
------------------------------------------------------------------------------
             |               Robust
```

```
           t7 |      Coef,   Std, Err,      t    P>|t|     [95% Conf, Interval]
--------------+----------------------------------------------------------------
_Ioocytehis_2 |  -,7886997   1,650577    -0,48   0,650    -4,827517    3,250117
        _cons |   53,17941   ,9409948    56,51   0,000     50,87688    55,48194
------------------------------------------------------------------------------

, xi: regress t8 i,oocytehistory if include==1, vce(cluster patientid)
i,oocytehistory   _Ioocytehis_1-2     (_Ioocytehis_1 for oocy~y==Fresh omitted)

Linear regression                               Number of obs     =         55
                                                F(1, 6)           =       0,07
                                                Prob > F          =     0,7935
                                                R-squared         =     0,0029
                                                Root MSE          =     10,797

                               (Std, Err, adjusted for 7 clusters in patientid)
------------------------------------------------------------------------------
             |               Robust
          t8 |      Coef,   Std, Err,      t    P>|t|     [95% Conf, Interval]
-------------+----------------------------------------------------------------
_Ioocytehis_2 |   1,195911   4,369671     0,27   0,794    -9,496289    11,88811
        _cons |   56,90786   1,399119    40,67   0,000     53,48434    60,33138
------------------------------------------------------------------------------

, xi: regress t9 i,oocytehistory if include==1, vce(cluster patientid)
i,oocytehistory   _Ioocytehis_1-2     (_Ioocytehis_1 for oocy~y==Fresh omitted)

Linear regression                               Number of obs     =         56
                                                F(1, 6)           =       0,51
                                                Prob > F          =     0,5030
                                                R-squared         =     0,0136
                                                Root MSE          =     10,781

                               (Std, Err, adjusted for 7 clusters in patientid)
------------------------------------------------------------------------------
             |               Robust
          t9 |      Coef,   Std, Err,      t    P>|t|     [95% Conf, Interval]
-------------+----------------------------------------------------------------
_Ioocytehis_2 |   2,590795    3,63703     0,71   0,503    -6,308696    11,49029
        _cons |   68,44502   2,136962    32,03   0,000     63,21607    73,67398
------------------------------------------------------------------------------
```

DURATION

```
, xi: regress ecc1 i,oocytehistory if include==1, vce(cluster patientid)
i,oocytehistory   _Ioocytehis_1-2     (_Ioocytehis_1 for oocy~y==Fresh omitted)

Linear regression                               Number of obs     =         54
                                                F(1, 6)           =       0,05
                                                Prob > F          =     0,8231
                                                R-squared         =     0,0010
                                                Root MSE          =     2,8136

                               (Std, Err, adjusted for 7 clusters in patientid)
------------------------------------------------------------------------------
             |               Robust
        ecc1 |      Coef,   Std, Err,      t    P>|t|     [95% Conf, Interval]
-------------+----------------------------------------------------------------
_Ioocytehis_2 |  -,1811131   ,7756308    -0,23   0,823    -2,079013    1,716787
        _cons |   22,11847   ,5775804    38,30   0,000     20,70518    23,53176
------------------------------------------------------------------------------

, xi: regress ecc2 i,oocytehistory if include==1, vce(cluster patientid)
i,oocytehistory   _Ioocytehis_1-2     (_Ioocytehis_1 for oocy~y==Fresh omitted)

Linear regression                               Number of obs     =         53
                                                F(1, 6)           =       0,24
                                                Prob > F          =     0,6407
                                                R-squared         =     0,0032
                                                Root MSE          =     7,0234
```

```
                              (Std, Err, adjusted for 7 clusters in patientid)
------------------------------------------------------------------------------
             |               Robust
         ecc2 |      Coef,   Std, Err,      t    P>|t|     [95% Conf, Interval]
--------------+---------------------------------------------------------------
_Ioocytehis_2 |  -,8080109   1,644756    -0,49   0,641    -4,832584    3,216563
        _cons |   13,08028   1,445829     9,05   0,000     9,542469     16,6181
------------------------------------------------------------------------------

, xi: regress ecc3 i,oocytehistory if include==1, vce(cluster patientid)
i,oocytehistory   _Ioocytehis_1-2     (_Ioocytehis_1 for oocy~y==Fresh omitted)

Linear regression                               Number of obs     =         51
                                                F(1, 6)           =       0,01
                                                Prob > F          =     0,9247
                                                R-squared         =     0,0003
                                                Root MSE          =     9,4377
                              (Std, Err, adjusted for 7 clusters in patientid)
------------------------------------------------------------------------------
             |               Robust
         ecc3 |      Coef,   Std, Err,      t    P>|t|     [95% Conf, Interval]
--------------+---------------------------------------------------------------
_Ioocytehis_2 |  -,3433983   3,485334    -0,10   0,925    -8,871703    8,184906
        _cons |   20,33304   ,9576373    21,23   0,000     17,98978    22,67629
------------------------------------------------------------------------------

, xi: regress vp i,oocytehistory if include==1, vce(cluster patientid)
i,oocytehistory   _Ioocytehis_1-2     (_Ioocytehis_1 for oocy~y==Fresh omitted)

Linear regression                               Number of obs     =         57
                                                F(1, 6)           =       0,96
                                                Prob > F          =     0,3655
                                                R-squared         =     0,0196
                                                Root MSE          =     2,7317

                              (Std, Err, adjusted for 7 clusters in patientid)
------------------------------------------------------------------------------
             |               Robust
          vp |      Coef,   Std, Err,      t    P>|t|     [95% Conf, Interval]
--------------+---------------------------------------------------------------
_Ioocytehis_2 |  -,7940657    ,811317    -0,98   0,366    -2,779287    1,191155
        _cons |   15,32236   ,4286641    35,74   0,000     14,27346    16,37126
------------------------------------------------------------------------------

, xi: regress s2 i,oocytehistory if include==1, vce(cluster patientid)
i,oocytehistory   _Ioocytehis_1-2     (_Ioocytehis_1 for oocy~y==Fresh omitted)

Linear regression                               Number of obs     =         53
                                                F(1, 6)           =       0,87
                                                Prob > F          =     0,3871
                                                R-squared         =     0,0147
                                                Root MSE          =     7,0119

                              (Std, Err, adjusted for 7 clusters in patientid)
------------------------------------------------------------------------------
             |               Robust
          s2 |      Coef,   Std, Err,      t    P>|t|     [95% Conf, Interval]
--------------+---------------------------------------------------------------
_Ioocytehis_2 |  -1,733682   1,859447    -0,93   0,387    -6,283584     2,81622
        _cons |   3,121729   1,640372     1,90   0,106    -,8921177    7,135575
------------------------------------------------------------------------------

, xi: regress s3 i,oocytehistory if include==1, vce(cluster patientid)
i,oocytehistory   _Ioocytehis_1-2     (_Ioocytehis_1 for oocy~y==Fresh omitted)

Linear regression                               Number of obs     =         53
                                                F(1, 6)           =       0,61
                                                Prob > F          =     0,4640
                                                R-squared         =     0,0093
                                                Root MSE          =     9,5567

                              (Std, Err, adjusted for 7 clusters in patientid)
------------------------------------------------------------------------------
             |               Robust
          s3 |      Coef,   Std, Err,      t    P>|t|     [95% Conf, Interval]
--------------+---------------------------------------------------------------
_Ioocytehis_2 |  -1,889754   2,417038    -0,78   0,464    -7,804033    4,024526
```

```
       _cons |   10,28928   1,196068     8,60   0,000     7,362608    13,21595
------------------------------------------------------------------------------

, xi: regress tsc i,oocytehistory if include==1, vce(cluster patientid)
i,oocytehistory   _Ioocytehis_1-2     (_Ioocytehis_1 for oocy~y==Fresh omitted)

Linear regression                               Number of obs     =         57
                                                F(1, 6)           =       0,58
                                                Prob > F          =     0,4767
                                                R-squared         =     0,0303
                                                Root MSE          =     10,136
(Std, Err, adjusted for 7 clusters in patientid)
```

Timepoints again???

```
------------------------------------------------------------------------------
             |               Robust
         tsc |      Coef,   Std, Err,      t    P>|t|     [95% Conf, Interval]
-------------+----------------------------------------------------------------
_Ioocytehis_2 |   3,685095   4,856713     0,76   0,477    -8,198854    15,56904
       _cons |   90,65698   2,714583    33,40   0,000     84,01464    97,29933
------------------------------------------------------------------------------

, xi: regress tsb i,oocytehistory if include==1, vce(cluster patientid)
i,oocytehistory   _Ioocytehis_1-2     (_Ioocytehis_1 for oocy~y==Fresh omitted)

Linear regression                               Number of obs     =         56
                                                F(1, 6)           =       0,00
                                                Prob > F          =     0,9521
                                                R-squared         =     0,0002
                                                Root MSE          =     10,722

                             (Std, Err, adjusted for 7 clusters in patientid)
------------------------------------------------------------------------------
             |               Robust
         tsb |      Coef,   Std, Err,      t    P>|t|     [95% Conf, Interval]
-------------+----------------------------------------------------------------
_Ioocytehis_2 |   ,2859981   4,568769     0,06   0,952    -10,89338    11,46537
       _cons |   101,5853   2,667923    38,08   0,000     95,05716    108,1135
------------------------------------------------------------------------------

, xi: regress teb i,oocytehistory if include==1, vce(cluster patientid)
i,oocytehistory   _Ioocytehis_1-2     (_Ioocytehis_1 for oocy~y==Fresh omitted)

Linear regression                               Number of obs     =         37
                                                F(1, 6)           =       0,65
                                                Prob > F          =     0,4501
                                                R-squared         =     0,0274
                                                Root MSE          =     12,631

                             (Std, Err, adjusted for 7 clusters in patientid)
------------------------------------------------------------------------------
             |               Robust
         teb |      Coef,   Std, Err,      t    P>|t|     [95% Conf, Interval]
-------------+----------------------------------------------------------------
_Ioocytehis_2 |  -4,323163   5,352723    -0,81   0,450     -17,4208    8,774479
       _cons |   118,4363   4,991224    23,73   0,000     106,2232    130,6494
------------------------------------------------------------------------------
```

Comment

- In none of the times were there any indication of effect of frozen on the times,
- Small study
- The hypothesis tested in the regression models is that of different times for frozen, We found no difference but one cannot conclude equivalence since that is a different hypothesis,
- You can only say that in this exploratory sample of 7 patients the differences in time between frozen and fresh oocytes were random,

95% MEDIAN REGRESSION ANALYSIS

ALL

```
, foreach var of varlist pronucleitime tpb2 tpna tpnf t2  t3  t4  t5  t6 t7  t8  t9 tsc  tsb
tb
>  teb thb {
  2,   qreg `var' group, quantile(50) level(95)
  3,   *margins, at(group=(0 1))
,   }

Median regression                                   Number of obs =      1,573
  Raw sum of deviations 616,0256 (about 18,074328)
  Min sum of deviations 615,4392                    Pseudo R2     =     0,0010

------------------------------------------------------------------------------
pronucleit~e |      Coef,   Std, Err,      t    P>|t|     [95% Conf, Interval]
-------------+----------------------------------------------------------------
       group |   ,0844174   ,0182382     4,63   0,000     ,0486437    ,1201911
       _cons |   18,07021   ,0057619  3136,15   0,000     18,05891    18,08151
------------------------------------------------------------------------------

Median regression                                   Number of obs =      1,459
  Raw sum of deviations 861,0283 (about 3,6963178)
  Min sum of deviations 858,4033                    Pseudo R2     =     0,0030

------------------------------------------------------------------------------
        tpb2 |      Coef,   Std, Err,      t    P>|t|     [95% Conf, Interval]
-------------+----------------------------------------------------------------
       group |   ,3354284   ,1242625     2,70   0,007      ,091676    ,5791809
       _cons |   3,676244     ,03412   107,74   0,000     3,609314    3,743173
------------------------------------------------------------------------------

Median regression                                   Number of obs =      1,494
  Raw sum of deviations 1217,331 (about 7,3683253)
  Min sum of deviations 1208,349                    Pseudo R2     =     0,0074

------------------------------------------------------------------------------
        tpna |      Coef,   Std, Err,      t    P>|t|     [95% Conf, Interval]
-------------+----------------------------------------------------------------
       group |   1,086313   ,2064332     5,26   0,000     ,6813832    1,491243
       _cons |   7,329371   ,0565214   129,67   0,000     7,218501    7,440241
------------------------------------------------------------------------------

Median regression                                   Number of obs =      1,499
  Raw sum of deviations 1844,787 (about 23,155426)
  Min sum of deviations 1826,594                    Pseudo R2     =     0,0099

------------------------------------------------------------------------------
        tpnf |      Coef,   Std, Err,      t    P>|t|     [95% Conf, Interval]
-------------+----------------------------------------------------------------
       group |   2,237471   ,3877379     5,77   0,000     1,476904    2,998039
       _cons |   23,10119    ,095534   241,81   0,000      22,9138    23,28859
------------------------------------------------------------------------------

Median regression                                   Number of obs =      1,499
  Raw sum of deviations 1892,536 (about 25,874121)
  Min sum of deviations 1874,423                    Pseudo R2     =     0,0096

------------------------------------------------------------------------------
          t2 |      Coef,   Std, Err,      t    P>|t|     [95% Conf, Interval]
-------------+----------------------------------------------------------------
       group |   2,282675   ,4070348     5,61   0,000     1,484255    3,081094
       _cons |   25,80631   ,0963541   267,83   0,000     25,61731    25,99532
------------------------------------------------------------------------------

Median regression                                   Number of obs =      1,491
  Raw sum of deviations 2349,581 (about 36,850527)
  Min sum of deviations 2346,823                    Pseudo R2     =     0,0012

------------------------------------------------------------------------------
          t3 |      Coef,   Std, Err,      t    P>|t|     [95% Conf, Interval]
-------------+----------------------------------------------------------------
       group |   1,746933   ,4922031     3,55   0,000      ,781448    2,712418
       _cons |   36,83479   ,1125778   327,19   0,000     36,61396    37,05562
------------------------------------------------------------------------------

Median regression                                   Number of obs =      1,487
```

```
  Raw sum of deviations 2548,995 (about 37,759234)
  Min sum of deviations 2516,259                    Pseudo R2     =     0,0128

------------------------------------------------------------------------------
          t4 |      Coef,   Std, Err,      t    P>|t|     [95% Conf, Interval]
-------------+----------------------------------------------------------------
       group |   3,264669   ,5698302     5,73   0,000     2,146911    4,382426
       _cons |   37,65565   ,1279737   294,25   0,000     37,40462    37,90668
------------------------------------------------------------------------------

Median regression                                 Number of obs =      1,479
  Raw sum of deviations 3328,757 (about 49,669782)
  Min sum of deviations 3322,473                  Pseudo R2     =     0,0019

------------------------------------------------------------------------------
          t5 |      Coef,   Std, Err,      t    P>|t|     [95% Conf, Interval]
-------------+----------------------------------------------------------------
       group |   1,653201   ,7642442     2,16   0,031     ,1540815     3,15232
       _cons |   49,63217    ,168622   294,34   0,000      49,3014    49,96293
------------------------------------------------------------------------------

Median regression                                 Number of obs =      1,483
  Raw sum of deviations 3459,949 (about 50,980027)
  Min sum of deviations 3437,633                  Pseudo R2     =     0,0064

------------------------------------------------------------------------------
          t6 |      Coef,   Std, Err,      t    P>|t|     [95% Conf, Interval]
-------------+----------------------------------------------------------------
       group |   2,948957   ,7092847     4,16   0,000     1,557647    4,340266
       _cons |   50,88729   ,1540987   330,23   0,000     50,58501    51,18956
------------------------------------------------------------------------------

Median regression                                 Number of obs =      1,475
  Raw sum of deviations 3886,869 (about 52,537271)
  Min sum of deviations 3867,587                  Pseudo R2     =     0,0050

------------------------------------------------------------------------------
          t7 |      Coef,   Std, Err,      t    P>|t|     [95% Conf, Interval]
-------------+----------------------------------------------------------------
       group |   3,015519   ,8289924     3,64   0,000     1,389388    4,641651
       _cons |   52,39317   ,1753584   298,78   0,000     52,04919    52,73715
------------------------------------------------------------------------------

Median regression                                 Number of obs =      1,474
  Raw sum of deviations 5063,995 (about 54,612091)
  Min sum of deviations 5022,905                  Pseudo R2     =     0,0081

------------------------------------------------------------------------------
          t8 |      Coef,   Std, Err,      t    P>|t|     [95% Conf, Interval]
-------------+----------------------------------------------------------------
       group |   7,227214   1,242304     5,82   0,000     4,790338     9,66409
       _cons |   54,51545   ,2527227   215,71   0,000     54,01972    55,01119
------------------------------------------------------------------------------

Median regression                                 Number of obs =      1,474
  Raw sum of deviations 5112,576 (about 68,660553)
  Min sum of deviations 5105,273                  Pseudo R2     =     0,0014

------------------------------------------------------------------------------
          t9 |      Coef,   Std, Err,      t    P>|t|     [95% Conf, Interval]
-------------+----------------------------------------------------------------
       group |   1,984464   1,222191     1,62   0,105    -,4129576    4,381885
       _cons |   68,52028    ,248631   275,59   0,000     68,03257    69,00799
------------------------------------------------------------------------------

Median regression                                 Number of obs =      1,466
  Raw sum of deviations  6181,41 (about 83,923056)
  Min sum of deviations 6117,587                  Pseudo R2     =     0,0103

------------------------------------------------------------------------------
         tsc |      Coef,   Std, Err,      t    P>|t|     [95% Conf, Interval]
-------------+----------------------------------------------------------------
       group |   8,140324   1,817895     4,48   0,000     4,574368    11,70628
       _cons |   83,49568   ,3456525   241,56   0,000     82,81766    84,17371
------------------------------------------------------------------------------

Median regression                                 Number of obs =      1,441
```

```
  Raw sum of deviations 5195,889 (about 97,348355)
  Min sum of deviations 5144,219                    Pseudo R2     =     0,0099

------------------------------------------------------------------------------
         tsb |      Coef,   Std, Err,      t    P>|t|     [95% Conf, Interval]
-------------+----------------------------------------------------------------
       group |   7,212165   1,703879     4,23   0,000     3,869812    10,55452
       _cons |   97,24725   ,3044288   319,44   0,000     96,65007    97,84442
------------------------------------------------------------------------------

Median regression                                 Number of obs =      1,357
  Raw sum of deviations  5519,58 (about 105,56599)
  Min sum of deviations 5483,417                  Pseudo R2     =     0,0066

------------------------------------------------------------------------------
          tb |      Coef,   Std, Err,      t    P>|t|     [95% Conf, Interval]
-------------+----------------------------------------------------------------
       group |   7,137972   1,989425     3,59   0,000     3,235285    11,04066
       _cons |   105,3701   ,3415605   308,50   0,000     104,7001    106,0402
------------------------------------------------------------------------------

Median regression                                 Number of obs =        989
  Raw sum of deviations  3858,13 (about 112,03987)
  Min sum of deviations 3837,527                  Pseudo R2     =     0,0053

------------------------------------------------------------------------------
         teb |      Coef,   Std, Err,      t    P>|t|     [95% Conf, Interval]
-------------+----------------------------------------------------------------
       group |   4,436407   1,798244     2,47   0,014     ,9075866    7,965228
       _cons |   111,8455   ,3079282   363,22   0,000     111,2412    112,4497
------------------------------------------------------------------------------

Median regression                                 Number of obs =        143
  Raw sum of deviations 577,3394 (about 114,76014)
  Min sum of deviations 570,6745                  Pseudo R2     =     0,0115

------------------------------------------------------------------------------
         thb |      Coef,   Std, Err,      t    P>|t|     [95% Conf, Interval]
-------------+----------------------------------------------------------------
       group |   8,027658   3,225051     2,49   0,014     1,651953    14,40336
       _cons |      114,4   ,6606085   173,17   0,000      113,094     115,706
------------------------------------------------------------------------------
```

```
ICSI,
,   foreach var of varlist pronucleitime tpb2 tpna tpnf t2  t3  t4  t5  t6 t7  t8  t9 tsc
tsb  t
> b  teb thb {
  2,   qreg `var' group if fertilizationmethod=="ICSI", quantile(50) level(95)
  3,   *margins, at(group=(0 1))
,   }

Median regression                                       Number of obs =        996
  Raw sum of deviations 407,1218 (about 18,054461)
  Min sum of deviations 406,6169                        Pseudo R2     =     0,0012

------------------------------------------------------------------------------
pronucleit~e |      Coef,   Std, Err,      t    P>|t|     [95% Conf, Interval]
-------------+----------------------------------------------------------------
       group |   ,0814897    ,018156     4,49   0,000     ,0458612    ,1171182
       _cons |   18,05011   ,0070224  2570,37   0,000     18,03633    18,06389
------------------------------------------------------------------------------

Median regression                                       Number of obs =        927
  Raw sum of deviations 498,6355 (about 3,5937189)
  Min sum of deviations 494,7235                        Pseudo R2     =     0,0078

------------------------------------------------------------------------------
        tpb2 |      Coef,   Std, Err,      t    P>|t|     [95% Conf, Interval]
-------------+----------------------------------------------------------------
       group |   ,4413803   ,1201845     3,67   0,000     ,2055143    ,6772463
       _cons |   3,560619   ,0410224    86,80   0,000     3,480111    3,641126
------------------------------------------------------------------------------

Median regression                                       Number of obs =        945
  Raw sum of deviations 750,2846 (about 7,2830534)
  Min sum of deviations 740,7368                        Pseudo R2     =     0,0127

------------------------------------------------------------------------------
        tpna |      Coef,   Std, Err,      t    P>|t|     [95% Conf, Interval]
-------------+----------------------------------------------------------------
       group |   1,171087   ,2085288     5,62   0,000     ,7618523    1,580321
       _cons |   7,209845   ,0711454   101,34   0,000     7,070224    7,349467
------------------------------------------------------------------------------

Median regression                                       Number of obs =        936
  Raw sum of deviations 1166,938 (about 23,135415)
  Min sum of deviations 1150,082                        Pseudo R2     =     0,0144

------------------------------------------------------------------------------
        tpnf |      Coef,   Std, Err,      t    P>|t|     [95% Conf, Interval]
-------------+----------------------------------------------------------------
       group |   1,531166   ,3912677     3,91   0,000     ,7633005    2,299032
       _cons |   23,05097    ,120651   191,05   0,000      22,8142    23,28775
------------------------------------------------------------------------------

Median regression                                       Number of obs =        931
  Raw sum of deviations 1189,458 (about 25,851282)
  Min sum of deviations 1171,536                        Pseudo R2     =     0,0151

------------------------------------------------------------------------------
          t2 |      Coef,   Std, Err,      t    P>|t|     [95% Conf, Interval]
-------------+----------------------------------------------------------------
       group |   2,284657   ,4206748     5,43   0,000     1,459074     3,11024
       _cons |   25,69095   ,1256061   204,54   0,000     25,44444    25,93745
------------------------------------------------------------------------------

Median regression                                       Number of obs =        923
  Raw sum of deviations 1480,922 (about 36,801186)
  Min sum of deviations 1476,906                        Pseudo R2     =     0,0027

------------------------------------------------------------------------------
          t3 |      Coef,   Std, Err,      t    P>|t|     [95% Conf, Interval]
-------------+----------------------------------------------------------------
       group |    1,84142   ,5392633     3,41   0,001      ,783093    2,899748
       _cons |    36,7403   ,1557562   235,88   0,000     36,43462    37,04598
------------------------------------------------------------------------------

Median regression                                       Number of obs =        917
  Raw sum of deviations 1604,934 (about 37,683111)
```

```
  Min sum of deviations 1573,869                    Pseudo R2     =     0,0194

------------------------------------------------------------------------------
          t4 |      Coef,   Std, Err,      t    P>|t|     [95% Conf, Interval]
-------------+----------------------------------------------------------------
       group |   3,268878   ,5628089     5,81   0,000     2,164332    4,373425
       _cons |   37,51578   ,1587953   236,25   0,000     37,20414    37,82743
------------------------------------------------------------------------------

Median regression                                 Number of obs =        915
  Raw sum of deviations 2098,802 (about 49,685473)
  Min sum of deviations 2093,385                  Pseudo R2     =     0,0026

------------------------------------------------------------------------------
          t5 |      Coef,   Std, Err,      t    P>|t|     [95% Conf, Interval]
-------------+----------------------------------------------------------------
       group |   1,446404   ,7924884     1,83   0,068    -,1089062    3,001715
       _cons |   49,67274   ,2207555   225,01   0,000     49,23949    50,10599
------------------------------------------------------------------------------

Median regression                                 Number of obs =        914
  Raw sum of deviations 2144,224 (about 51,086308)
  Min sum of deviations 2124,216                  Pseudo R2     =     0,0093

------------------------------------------------------------------------------
          t6 |      Coef,   Std, Err,      t    P>|t|     [95% Conf, Interval]
-------------+----------------------------------------------------------------
       group |   2,869014   ,7467786     3,84   0,000      1,40341    4,334618
       _cons |    50,9251   ,2051839   248,19   0,000     50,52242    51,32779
------------------------------------------------------------------------------


Median regression                                 Number of obs =        910
  Raw sum of deviations 2433,861 (about 52,632555)
  Min sum of deviations 2415,741                  Pseudo R2     =     0,0074

------------------------------------------------------------------------------
          t7 |      Coef,   Std, Err,      t    P>|t|     [95% Conf, Interval]
-------------+----------------------------------------------------------------
       group |   2,981352   ,9003867     3,31   0,001     1,214271    4,748433
       _cons |   52,42734   ,2424825   216,21   0,000     51,95144    52,90323
------------------------------------------------------------------------------

Median regression                                 Number of obs =        907
  Raw sum of deviations  3098,44 (about 54,929337)
  Min sum of deviations 3059,987                  Pseudo R2     =     0,0124

------------------------------------------------------------------------------
          t8 |      Coef,   Std, Err,      t    P>|t|     [95% Conf, Interval]
-------------+----------------------------------------------------------------
       group |   7,146366    1,27634     5,60   0,000     4,641435    9,651297
       _cons |    54,5963   ,3309998   164,94   0,000     53,94668    55,24591
------------------------------------------------------------------------------

Median regression                                 Number of obs =        907
  Raw sum of deviations 3116,058 (about 68,644957)
  Min sum of deviations  3108,21                  Pseudo R2     =     0,0025

------------------------------------------------------------------------------
          t9 |      Coef,   Std, Err,      t    P>|t|     [95% Conf, Interval]
-------------+----------------------------------------------------------------
       group |   2,064437   1,275735     1,62   0,106     -,439306    4,568181
       _cons |   68,44031   ,3308429   206,87   0,000       67,791    69,08962
------------------------------------------------------------------------------

Median regression                                 Number of obs =        900
  Raw sum of deviations 3777,648 (about 83,358787)
  Min sum of deviations 3706,177                  Pseudo R2     =     0,0189

------------------------------------------------------------------------------
         tsc |      Coef,   Std, Err,      t    P>|t|     [95% Conf, Interval]
-------------+----------------------------------------------------------------
       group |   8,505921   1,905607     4,46   0,000      4,76596    12,24588
       _cons |   83,13009   ,4624342   179,77   0,000     82,22251    84,03767
------------------------------------------------------------------------------

Median regression                                 Number of obs =        884
```

```
  Raw sum of deviations 3183,368 (about 97,549109)
  Min sum of deviations 3134,061                    Pseudo R2     =     0,0155

------------------------------------------------------------------------------
         tsb |      Coef,   Std, Err,      t    P>|t|     [95% Conf, Interval]
-------------+----------------------------------------------------------------
       group |   7,137105   1,684095     4,24   0,000     3,831805    10,44241
       _cons |   97,32231   ,3841663   253,33   0,000     96,56832    98,07629
------------------------------------------------------------------------------

Median regression                                 Number of obs =        832
  Raw sum of deviations  3451,31 (about 105,63158)
  Min sum of deviations 3414,903                  Pseudo R2     =     0,0105

------------------------------------------------------------------------------
          tb |      Coef,   Std, Err,      t    P>|t|     [95% Conf, Interval]
-------------+----------------------------------------------------------------
       group |   7,163575   2,163678     3,31   0,001     2,916652     11,4105
       _cons |   105,3445   ,4744177   222,05   0,000     104,4133    106,2757
------------------------------------------------------------------------------

Median regression                                 Number of obs =        622
  Raw sum of deviations  2509,82 (about 111,99442)
  Min sum of deviations 2487,975                  Pseudo R2     =     0,0087

------------------------------------------------------------------------------
         teb |      Coef,   Std, Err,      t    P>|t|     [95% Conf, Interval]
-------------+----------------------------------------------------------------
       group |   4,574492   1,722102     2,66   0,008     1,192632    7,956352
       _cons |   111,7074   ,3718457   300,41   0,000     110,9771    112,4376
------------------------------------------------------------------------------

Median regression                                 Number of obs =         99
  Raw sum of deviations 372,1796 (about 114,13766)
  Min sum of deviations 364,1368                  Pseudo R2     =     0,0216

------------------------------------------------------------------------------
         thb |      Coef,   Std, Err,      t    P>|t|     [95% Conf, Interval]
-------------+----------------------------------------------------------------
       group |   8,532569   3,374816     2,53   0,013     1,834494    15,23064
       _cons |   113,8951   ,8308222   137,09   0,000     112,2461     115,544
------------------------------------------------------------------------------
```

,

TESTING FOR EQUIVALENCE

This is done by a using the 2-sided 90% confidence interval, One checks if the upper of lower CI boundary is within the margins of equivalence – if they are then the times are equivalent if they are not then they are non equivalent,
For equivalence p<,05

Group is the indicator for fresh =0 or warmed=1
, tab group oocytehistory

group	Oocyte History: Fresh	Other	Thawed	Total
0	1,417	0	0	1,417
1	0	22	157	179
Total	1,417	22	157	1,596

In your tables you report the median times, We will therefore test the equivalence of the medians using quantile regression and estimate the median difference with 90% confidence intervals, We then use the limits to test,

You will have to work out what the limits are as you have specified them to me,
I have not done that

, qreg **t2** group, quantile(50) level(90)

Median regression — Number of obs = 1,499
Raw sum of deviations 1892,536 (about 25,874121)
Min sum of deviations 1874,423 — Pseudo R2 = 0,0096

t2	Coef,	Std, Err,	t	P>|t|	[90% Conf,	Interval]
group	2,282675	,4217911	5,41	0,000	1,58846	2,976889
_cons	25,80631	,0998473	258,46	0,000	25,64198	25,97065

- The median difference is 2,28 and the 90% CI is 1,59 to 2,98,
- If these two values lie within the margins of equivalence the two methods are equivalent, else they are not, Hypothesis of equivalence
- The difference if 2,28 is actually significantly different p<,001 but this is not the hypothesis of interest but you can comment on it, Hypothesis of difference

Median of the two groups from the model above – same as your table

, margins, at(group=(0 1))

Adjusted predictions — Number of obs = 1,499
Model VCE : IID

Expression : Linear prediction, predict()

1,_at : group = 0

2,_at : group = 1

	Delta-method: Margin	Std, Err,	z	P>|z|	[95% Conf,	Interval]
_at 1	25,80631	,0998473	258,46	0,000	25,61062	26,00201
2	28,08899	,4098027	68,54	0,000	27,28579	28,89219

,

Printout for list including t2 above (did t2 above first to setup the analysis)

, foreach var of varlist pronucleitime tpb2 tpna tpnf t2 t3 t4 t5 t6 t7 t8 t9 tsc tsb tb
> teb thb {
2, qreg `var' group, quantile(50) level(90)

```
  3,   margins, at(group=(0 1))
  4,   }

Median regression                                   Number of obs =      1,573
  Raw sum of deviations 616,0256 (about 18,074328)
  Min sum of deviations 615,4392                    Pseudo R2     =     0,0010

------------------------------------------------------------------------------
pronucleit~e |      Coef,   Std, Err,      t    P>|t|     [90% Conf, Interval]
-------------+----------------------------------------------------------------
       group |   ,0844174   ,0183744     4,59   0,000     ,0541763    ,1146584
       _cons |   18,07021    ,005805  3112,89   0,000     18,06066    18,07977
------------------------------------------------------------------------------

Adjusted predictions                              Number of obs     =      1,573
Model VCE    : IID

Expression   : Linear prediction, predict()

1,_at        : group           =           0

2,_at        : group           =           1

------------------------------------------------------------------------------
             |            Delta-method
             |     Margin   Std, Err,      z    P>|z|     [95% Conf, Interval]
-------------+----------------------------------------------------------------
         _at |
          1  |   18,07021    ,005805  3112,89   0,000     18,05883    18,08159
          2  |   18,15463   ,0174333  1041,37   0,000     18,12046     18,1888
------------------------------------------------------------------------------



Median regression                                   Number of obs =      1,459
  Raw sum of deviations 861,0283 (about 3,6963178)
  Min sum of deviations 858,4033                    Pseudo R2     =     0,0030

------------------------------------------------------------------------------
        tpb2 |      Coef,   Std, Err,      t    P>|t|     [90% Conf, Interval]
-------------+----------------------------------------------------------------
       group |   ,3354284   ,1269509     2,64   0,008     ,1264799    ,5443769
       _cons |   3,676244   ,0348582   105,46   0,000     3,618871    3,733617
------------------------------------------------------------------------------

Adjusted predictions                              Number of obs     =      1,459
Model VCE    : IID

Expression   : Linear prediction, predict()

1,_at        : group           =           0

2,_at        : group           =           1

------------------------------------------------------------------------------
             |            Delta-method
             |     Margin   Std, Err,      z    P>|z|     [95% Conf, Interval]
-------------+----------------------------------------------------------------
         _at |
          1  |   3,676244   ,0348582   105,46   0,000     3,607923    3,744564
          2  |   4,011672   ,1220714    32,86   0,000     3,772416    4,250928
------------------------------------------------------------------------------



Median regression                                   Number of obs =      1,494
  Raw sum of deviations 1217,331 (about 7,3683253)
  Min sum of deviations 1208,349                    Pseudo R2     =     0,0074

------------------------------------------------------------------------------
        tpna |      Coef,   Std, Err,      t    P>|t|     [90% Conf, Interval]
-------------+----------------------------------------------------------------
       group |   1,086313   ,1994412     5,45   0,000     ,7580579    1,414569
       _cons |   7,329371    ,054607   134,22   0,000     7,239495    7,419248
------------------------------------------------------------------------------
```

```
Adjusted predictions                            Number of obs     =      1,494
Model VCE    : IID

Expression   : Linear prediction, predict()

1._at        : group           =           0

2._at        : group           =           1

------------------------------------------------------------------------------
             |            Delta-method
             |     Margin   Std. Err.      z    P>|z|     [95% Conf. Interval]
-------------+----------------------------------------------------------------
         _at |
          1  |   7.329371    .054607   134.22   0.000     7.222344    7.436399
          2  |   8.415685   .1918199    43.87   0.000     8.039725    8.791645
------------------------------------------------------------------------------
```

```
Median regression                               Number of obs =      1,499
  Raw sum of deviations 1844.787 (about 23.155426)
  Min sum of deviations 1826.594                Pseudo R2     =     0.0099

------------------------------------------------------------------------------
        tpnf |      Coef.   Std. Err.      t    P>|t|     [90% Conf. Interval]
-------------+----------------------------------------------------------------
       group |   2.237471   .4020478     5.57   0.000     1.575752    2.899191
       _cons |   23.10119   .0990598   233.20   0.000     22.93815    23.26423
------------------------------------------------------------------------------
```

```
Adjusted predictions                            Number of obs     =      1,499
Model VCE    : IID

Expression   : Linear prediction, predict()

1._at        : group           =           0

2._at        : group           =           1

------------------------------------------------------------------------------
             |            Delta-method
             |     Margin   Std. Err.      z    P>|z|     [95% Conf. Interval]
-------------+----------------------------------------------------------------
         _at |
          1  |   23.10119   .0990598   233.20   0.000     22.90704    23.29535
          2  |   25.33866   .3896532    65.03   0.000     24.57496    26.10237
------------------------------------------------------------------------------
```

```
Median regression                               Number of obs =      1,499
  Raw sum of deviations 1892.536 (about 25.874121)
  Min sum of deviations 1874.423                Pseudo R2     =     0.0096

------------------------------------------------------------------------------
          t2 |      Coef.   Std. Err.      t    P>|t|     [90% Conf. Interval]
-------------+----------------------------------------------------------------
       group |   2.282675   .4217911     5.41   0.000      1.58846    2.976889
       _cons |   25.80631   .0998473   258.46   0.000     25.64198    25.97065
------------------------------------------------------------------------------
```

```
Adjusted predictions                            Number of obs     =      1,499
Model VCE    : IID

Expression   : Linear prediction, predict()

1._at        : group           =           0

2._at        : group           =           1

------------------------------------------------------------------------------
```

```
             |            Delta-method
             |     Margin   Std, Err,      z    P>|z|     [95% Conf, Interval]
-------------+----------------------------------------------------------------
         _at |
          1  |   25,80631   ,0998473   258,46   0,000     25,61062    26,00201
          2  |   28,08899   ,4098027    68,54   0,000     27,28579    28,89219
------------------------------------------------------------------------------
```

```
Median regression                                 Number of obs =      1,491
  Raw sum of deviations 2349,581 (about 36,850527)
  Min sum of deviations 2346,823                  Pseudo R2     =     0,0012

------------------------------------------------------------------------------
          t3 |      Coef,   Std, Err,      t    P>|t|     [90% Conf, Interval]
-------------+----------------------------------------------------------------
       group |   1,746933   ,4958886     3,52   0,000     ,9307612    2,563105
       _cons |   36,83479   ,1134208   324,76   0,000     36,64811    37,02147
------------------------------------------------------------------------------

Adjusted predictions                            Number of obs     =      1,491
Model VCE    : IID

Expression   : Linear prediction, predict()

1,_at        : group           =           0

2,_at        : group           =           1

------------------------------------------------------------------------------
             |            Delta-method
             |     Margin   Std, Err,      z    P>|z|     [95% Conf, Interval]
-------------+----------------------------------------------------------------
         _at |
          1  |   36,83479   ,1134208   324,76   0,000     36,61249    37,05709
          2  |   38,58172   ,4827434    79,92   0,000     37,63556    39,52788
------------------------------------------------------------------------------
```

```
Median regression                                 Number of obs =      1,487
  Raw sum of deviations 2548,995 (about 37,759234)
  Min sum of deviations 2516,259                  Pseudo R2     =     0,0128

------------------------------------------------------------------------------
          t4 |      Coef,   Std, Err,      t    P>|t|     [90% Conf, Interval]
-------------+----------------------------------------------------------------
       group |   3,264669   ,5925153     5,51   0,000     2,289459    4,239878
       _cons |   37,65565   ,1330683   282,98   0,000     37,43664    37,87466
------------------------------------------------------------------------------

Adjusted predictions                            Number of obs     =      1,487
Model VCE    : IID

Expression   : Linear prediction, predict()

1,_at        : group           =           0

2,_at        : group           =           1

------------------------------------------------------------------------------
             |            Delta-method
             |     Margin   Std, Err,      z    P>|z|     [95% Conf, Interval]
-------------+----------------------------------------------------------------
         _at |
          1  |   37,65565   ,1330683   282,98   0,000     37,39484    37,91646
          2  |   40,92032   ,5773796    70,87   0,000     39,78868    42,05196
------------------------------------------------------------------------------
```

```
Median regression                                   Number of obs =      1,479
  Raw sum of deviations 3328,757 (about 49,669782)
  Min sum of deviations 3322,473                    Pseudo R2     =     0,0019

------------------------------------------------------------------------------
          t5 |      Coef,   Std, Err,      t    P>|t|     [90% Conf, Interval]
-------------+----------------------------------------------------------------
       group |   1,653201   ,7700868     2,15   0,032     ,3857259    2,920676
       _cons |   49,63217   ,1699111   292,11   0,000     49,35251    49,91182
------------------------------------------------------------------------------

Adjusted predictions                              Number of obs     =      1,479
Model VCE    : IID

Expression   : Linear prediction, predict()

1,_at        : group           =           0

2,_at        : group           =           1

------------------------------------------------------------------------------
             |            Delta-method
             |     Margin   Std, Err,      z    P>|z|     [95% Conf, Interval]
-------------+----------------------------------------------------------------
         _at |
          1  |   49,63217   ,1699111   292,11   0,000     49,29915    49,96519
          2  |   51,28537   ,7511085    68,28   0,000     49,81322    52,75752
------------------------------------------------------------------------------
```

```
Median regression                                   Number of obs =      1,483
  Raw sum of deviations 3459,949 (about 50,980027)
  Min sum of deviations 3437,633                    Pseudo R2     =     0,0064

------------------------------------------------------------------------------
          t6 |      Coef,   Std, Err,      t    P>|t|     [90% Conf, Interval]
-------------+----------------------------------------------------------------
       group |   2,948957   ,7083737     4,16   0,000     1,783056    4,114857
       _cons |   50,88729   ,1539008   330,65   0,000     50,63399    51,14059
------------------------------------------------------------------------------

Adjusted predictions                              Number of obs     =      1,483
Model VCE    : IID

Expression   : Linear prediction, predict()

1,_at        : group           =           0

2,_at        : group           =           1

------------------------------------------------------------------------------
             |            Delta-method
             |     Margin   Std, Err,      z    P>|z|     [95% Conf, Interval]
-------------+----------------------------------------------------------------
         _at |
          1  |   50,88729   ,1539008   330,65   0,000     50,58565    51,18893
          2  |   53,83625   ,6914534    77,86   0,000     52,48102    55,19147
------------------------------------------------------------------------------
```

```
Median regression                                   Number of obs =      1,475
  Raw sum of deviations 3886,869 (about 52,537271)
  Min sum of deviations 3867,587                    Pseudo R2     =     0,0050

------------------------------------------------------------------------------
          t7 |      Coef,   Std, Err,      t    P>|t|     [90% Conf, Interval]
-------------+----------------------------------------------------------------
       group |   3,015519   ,8467276     3,56   0,000       1,6219    4,409139
       _cons |   52,39317   ,1791099   292,52   0,000     52,09837    52,68796
------------------------------------------------------------------------------
```

```
Adjusted predictions                            Number of obs     =      1,475
Model VCE    : IID

Expression   : Linear prediction, predict()

1._at        : group           =           0

2._at        : group           =           1

------------------------------------------------------------------------------
             |            Delta-method
             |     Margin   Std, Err,      z    P>|z|     [95% Conf, Interval]
-------------+----------------------------------------------------------------
         _at |
          1  |   52,39317   ,1791099   292,52   0,000     52,04212    52,74422
          2  |   55,40869    ,827567    66,95   0,000     53,78669    57,03069
------------------------------------------------------------------------------
```

```
Median regression                                 Number of obs =      1,474
  Raw sum of deviations 5063,995 (about 54,612091)
  Min sum of deviations 5022,905                  Pseudo R2     =     0,0081

------------------------------------------------------------------------------
          t8 |      Coef,   Std, Err,      t    P>|t|     [90% Conf, Interval]
-------------+----------------------------------------------------------------
       group |   7,227214   1,219013     5,93   0,000     5,220853    9,233575
       _cons |   54,51545   ,2479845   219,83   0,000      54,1073    54,92361
------------------------------------------------------------------------------
```

```
Adjusted predictions                            Number of obs     =      1,474
Model VCE    : IID

Expression   : Linear prediction, predict()

1._at        : group           =           0

2._at        : group           =           1

------------------------------------------------------------------------------
             |            Delta-method
             |     Margin   Std, Err,      z    P>|z|     [95% Conf, Interval]
-------------+----------------------------------------------------------------
         _at |
          1  |   54,51545   ,2479845   219,83   0,000     54,02941    55,00149
          2  |   61,74266   1,193523    51,73   0,000      59,4034    64,08193
------------------------------------------------------------------------------
```

```
Median regression                                 Number of obs =      1,474
  Raw sum of deviations 5112,576 (about 68,660553)
  Min sum of deviations 5105,273                  Pseudo R2     =     0,0014

------------------------------------------------------------------------------
          t9 |      Coef,   Std, Err,      t    P>|t|     [90% Conf, Interval]
-------------+----------------------------------------------------------------
       group |   1,984464    1,19258     1,66   0,096     ,0216082    3,947319
       _cons |   68,52028   ,2426073   282,43   0,000     68,12098    68,91959
------------------------------------------------------------------------------
```

```
Adjusted predictions                            Number of obs     =      1,474
Model VCE    : IID

Expression   : Linear prediction, predict()

1._at        : group           =           0

2._at        : group           =           1
```

```
------------------------------------------------------------------------------
             |            Delta-method
             |     Margin   Std, Err,      z    P>|z|     [95% Conf, Interval]
-------------+----------------------------------------------------------------
         _at |
          1  |   68,52028   ,2426073   282,43   0,000     68,04478    68,99578
          2  |   70,50475   1,167643    60,38   0,000     68,21621    72,79328
------------------------------------------------------------------------------
```

```
Median regression                                   Number of obs =      1,466
  Raw sum of deviations  6181,41 (about 83,923056)
  Min sum of deviations 6117,587                    Pseudo R2     =     0,0103

------------------------------------------------------------------------------
         tsc |      Coef,   Std, Err,      t    P>|t|     [90% Conf, Interval]
-------------+----------------------------------------------------------------
       group |   8,140324    1,87342     4,35   0,000     5,056872    11,22378
       _cons |   83,49568   ,3562099   234,40   0,000      82,9094    84,08197
------------------------------------------------------------------------------

Adjusted predictions                            Number of obs     =      1,466
Model VCE    : IID

Expression   : Linear prediction, predict()

1,_at        : group           =           0

2,_at        : group           =           1

------------------------------------------------------------------------------
             |            Delta-method
             |     Margin   Std, Err,      z    P>|z|     [95% Conf, Interval]
-------------+----------------------------------------------------------------
         _at |
          1  |   83,49568   ,3562099   234,40   0,000     82,79753    84,19384
          2  |   91,63601   1,839243    49,82   0,000     88,03116    95,24086
------------------------------------------------------------------------------
```

```
Median regression                                   Number of obs =      1,441
  Raw sum of deviations 5195,889 (about 97,348355)
  Min sum of deviations 5144,219                    Pseudo R2     =     0,0099
```

```
------------------------------------------------------------------------------
         tsb |      Coef,   Std, Err,      t    P>|t|     [90% Conf, Interval]
-------------+----------------------------------------------------------------
       group |   7,212165    1,69683     4,25   0,000      4,41933      10,005
       _cons |   97,24725   ,3031694   320,77   0,000     96,74826    97,74624
------------------------------------------------------------------------------

Adjusted predictions                            Number of obs     =      1,441
Model VCE    : IID

Expression   : Linear prediction, predict()

1,_at        : group           =           0

2,_at        : group           =           1

------------------------------------------------------------------------------
             |            Delta-method
             |     Margin   Std, Err,      z    P>|z|     [95% Conf, Interval]
-------------+----------------------------------------------------------------
         _at |
          1  |   97,24725   ,3031694   320,77   0,000     96,65305    97,84145
          2  |   104,4594   1,669527    62,57   0,000     101,1872    107,7316
------------------------------------------------------------------------------
```

```
Median regression                                   Number of obs =      1,357
  Raw sum of deviations  5519,58 (about 105,56599)
  Min sum of deviations 5483,417                    Pseudo R2     =     0,0066

------------------------------------------------------------------------------
          tb |      Coef,   Std, Err,      t    P>|t|     [90% Conf, Interval]
-------------+----------------------------------------------------------------
       group |   7,137972   1,924016     3,71   0,000     3,971083    10,30486
       _cons |   105,3701   ,3303305   318,98   0,000     104,8264    105,9138
------------------------------------------------------------------------------

Adjusted predictions                              Number of obs     =      1,357
Model VCE    : IID

Expression   : Linear prediction, predict()

1,_at        : group           =           0

2,_at        : group           =           1

------------------------------------------------------------------------------
             |            Delta-method
             |     Margin   Std, Err,      z    P>|z|     [95% Conf, Interval]
-------------+----------------------------------------------------------------
         _at |
          1  |   105,3701   ,3303305   318,98   0,000     104,7227    106,0176
          2  |   112,5081   1,895447    59,36   0,000     108,7931    116,2231
------------------------------------------------------------------------------
```

```
Median regression                                   Number of obs =        989
  Raw sum of deviations  3858,13 (about 112,03987)
  Min sum of deviations 3837,527                    Pseudo R2     =     0,0053

------------------------------------------------------------------------------
         teb |      Coef,   Std, Err,      t    P>|t|     [90% Conf, Interval]
-------------+----------------------------------------------------------------
       group |   4,436407   1,785961     2,48   0,013     1,496002    7,376812
       _cons |   111,8455    ,305825   365,72   0,000      111,342     112,349
------------------------------------------------------------------------------

Adjusted predictions                              Number of obs     =        989
Model VCE    : IID

Expression   : Linear prediction, predict()

1,_at        : group           =           0

2,_at        : group           =           1

------------------------------------------------------------------------------
             |            Delta-method
             |     Margin   Std, Err,      z    P>|z|     [95% Conf, Interval]
-------------+----------------------------------------------------------------
         _at |
          1  |   111,8455    ,305825   365,72   0,000     111,2461    112,4449
          2  |   116,2819   1,759582    66,08   0,000     112,8332    119,7306
------------------------------------------------------------------------------

Median regression                                   Number of obs =        143
  Raw sum of deviations 577,3394 (about 114,76014)
  Min sum of deviations 570,6745                    Pseudo R2     =     0,0115

------------------------------------------------------------------------------
         thb |      Coef,   Std, Err,      t    P>|t|     [90% Conf, Interval]
-------------+----------------------------------------------------------------
       group |   8,027658   2,902999     2,77   0,006     3,221069    12,83425
       _cons |      114,4   ,5946405   192,39   0,000     113,4154    115,3846
------------------------------------------------------------------------------
```

```
Adjusted predictions                            Number of obs     =        143
Model VCE    : IID

Expression   : Linear prediction, predict()

1._at        : group           =           0

2._at        : group           =           1

------------------------------------------------------------------------------
             |            Delta-method
             |     Margin   Std. Err.      z    P>|z|     [95% Conf. Interval]
-------------+----------------------------------------------------------------
         _at |
          1  |      114.4   .5946405   192.39   0.000     113.2345    115.5655
          2  |   122.4277   2.841444    43.09   0.000     116.8585    127.9968
------------------------------------------------------------------------------
```

Comparison of groups for ICSI used in both groups – no other method

```
, save "C:\Projekte\Dylan Ramsay\combined,dta"
file C:\Projekte\Dylan Ramsay\combined,dta saved

, tab fertilizationmethod group

Fertilization |         group
       Method |         0          1 |     Total
--------------+----------------------+----------
Both IVF/ICSI |       134          2 |       136
         ICSI |       848        166 |     1,014
         IMSI |       226          0 |       226
          IVF |       209          0 |       209
      Unknown |         0          8 |         8
--------------+----------------------+----------
        Total |     1,417        176 |     1,593,
```

Equivalence tested for green participants

```
,   foreach var of varlist pronucleitime tpb2 tpna tpnf t2  t3  t4  t5  t6 t7  t8  t9 tsc
tsb  t
> b  teb thb {
  2,   qreg `var' group if fertilizationmethod=="ICSI", quantile(50) level(90)
  3,   margins, at(group=(0 1))
  4,   }

Median regression                                       Number of obs =        996
  Raw sum of deviations 407,1218 (about 18,054461)
  Min sum of deviations 406,6169                        Pseudo R2     =     0,0012

------------------------------------------------------------------------------
pronucleit~e |      Coef,   Std, Err,      t    P>|t|     [90% Conf, Interval]
-------------+----------------------------------------------------------------
       group |   ,0814897   ,0191363     4,26   0,000     ,0499839    ,1129955
       _cons |   18,05011   ,0074015  2438,70   0,000     18,03792     18,0623
------------------------------------------------------------------------------

Adjusted predictions                              Number of obs     =        996
Model VCE    : IID

Expression   : Linear prediction, predict()

1,_at        : group           =           0

2,_at        : group           =           1

------------------------------------------------------------------------------
             |            Delta-method
             |     Margin   Std, Err,      z    P>|z|     [95% Conf, Interval]
-------------+----------------------------------------------------------------
         _at |
          1  |   18,05011   ,0074015  2438,70   0,000      18,0356    18,06462
          2  |    18,1316    ,017647  1027,46   0,000     18,09701    18,16619
------------------------------------------------------------------------------




Median regression                                       Number of obs =        927
  Raw sum of deviations 498,6355 (about 3,5937189)
  Min sum of deviations 494,7235                        Pseudo R2     =     0,0078

------------------------------------------------------------------------------
        tpb2 |      Coef,   Std, Err,      t    P>|t|     [90% Conf, Interval]
-------------+----------------------------------------------------------------
       group |   ,4413803   ,1240825     3,56   0,000     ,2370782    ,6456824
       _cons |   3,560619   ,0423528    84,07   0,000     3,490885    3,630353
------------------------------------------------------------------------------

Adjusted predictions                              Number of obs     =        927
Model VCE    : IID

Expression   : Linear prediction, predict()
```

```
1._at        : group           =           0

2._at        : group           =           1

------------------------------------------------------------------------------
             |            Delta-method
             |     Margin   Std. Err.      z    P>|z|     [95% Conf. Interval]
-------------+----------------------------------------------------------------
         _at |
          1  |   3.560619   .0423528    84.07   0.000     3.477609    3.643629
          2  |   4.001999   .1166306    34.31   0.000     3.773407    4.230591
------------------------------------------------------------------------------

Median regression                                 Number of obs =        945
  Raw sum of deviations 750.2846 (about 7.2830534)
  Min sum of deviations 740.7368                  Pseudo R2     =     0.0127

------------------------------------------------------------------------------
        tpna |      Coef.   Std. Err.      t    P>|t|     [90% Conf. Interval]
-------------+----------------------------------------------------------------
       group |   1.171087   .2077928     5.64   0.000     .8289617    1.513211
       _cons |   7.209845   .0708942   101.70   0.000      7.09312    7.326571
------------------------------------------------------------------------------

Adjusted predictions                              Number of obs     =        945
Model VCE    : IID

Expression   : Linear prediction, predict()

1._at        : group           =           0

2._at        : group           =           1

------------------------------------------------------------------------------
             |            Delta-method
             |     Margin   Std. Err.      z    P>|z|     [95% Conf. Interval]
-------------+----------------------------------------------------------------
         _at |
          1  |   7.209845   .0708942   101.70   0.000     7.070895    7.348795
          2  |   8.380932    .195325    42.91   0.000     7.998102    8.763762
------------------------------------------------------------------------------



Median regression                                 Number of obs =        936
  Raw sum of deviations 1166.938 (about 23.135415)
  Min sum of deviations 1150.082                  Pseudo R2     =     0.0144

------------------------------------------------------------------------------
        tpnf |      Coef.   Std. Err.      t    P>|t|     [90% Conf. Interval]
-------------+----------------------------------------------------------------
       group |   1.531166   .4112918     3.72   0.000     .8539796    2.208353
       _cons |   23.05097   .1268257   181.75   0.000     22.84216    23.25979
------------------------------------------------------------------------------

Adjusted predictions                              Number of obs     =        936
Model VCE    : IID

Expression   : Linear prediction, predict()

1._at        : group           =           0

2._at        : group           =           1

------------------------------------------------------------------------------
             |            Delta-method
             |     Margin   Std. Err.      z    P>|z|     [95% Conf. Interval]
-------------+----------------------------------------------------------------
         _at |
          1  |   23.05097   .1268257   181.75   0.000      22.8024    23.29955
          2  |   24.58214   .3912496    62.83   0.000      23.8153    25.34898
------------------------------------------------------------------------------



Median regression                                 Number of obs =        931
```

```
  Raw sum of deviations 1189,458 (about 25,851282)
  Min sum of deviations 1171,536                    Pseudo R2     =     0,0151

------------------------------------------------------------------------------
          t2 |      Coef,   Std, Err,      t    P>|t|     [90% Conf, Interval]
-------------+----------------------------------------------------------------
       group |   2,284657   ,4243891     5,38   0,000     1,585902    2,983412
       _cons |   25,69095   ,1267151   202,75   0,000     25,48231    25,89958
------------------------------------------------------------------------------

Adjusted predictions                              Number of obs     =        931
Model VCE    : IID

Expression   : Linear prediction, predict()

1,_at        : group           =           0

2,_at        : group           =           1

------------------------------------------------------------------------------
             |            Delta-method
             |     Margin   Std, Err,      z    P>|z|     [95% Conf, Interval]
-------------+----------------------------------------------------------------
         _at |
          1  |   25,69095   ,1267151   202,75   0,000     25,44259     25,9393
          2  |   27,97561   ,4050301    69,07   0,000     27,18176    28,76945
------------------------------------------------------------------------------



Median regression                                 Number of obs =        923
  Raw sum of deviations 1480,922 (about 36,801186)
  Min sum of deviations 1476,906                  Pseudo R2     =     0,0027

------------------------------------------------------------------------------
          t3 |      Coef,   Std, Err,      t    P>|t|     [90% Conf, Interval]
-------------+----------------------------------------------------------------
       group |    1,84142   ,5185335     3,55   0,000     ,9876498    2,695191
       _cons |    36,7403   ,1497688   245,31   0,000     36,49371     36,9869
------------------------------------------------------------------------------

Adjusted predictions                              Number of obs     =        923
Model VCE    : IID

Expression   : Linear prediction, predict()

1,_at        : group           =           0

2,_at        : group           =           1

------------------------------------------------------------------------------
             |            Delta-method
             |     Margin   Std, Err,      z    P>|z|     [95% Conf, Interval]
-------------+----------------------------------------------------------------
         _at |
          1  |    36,7403   ,1497688   245,31   0,000     36,44676    37,03384
          2  |   38,58172   ,4964336    77,72   0,000     37,60873    39,55471
------------------------------------------------------------------------------



Median regression                                 Number of obs =        917
  Raw sum of deviations 1604,934 (about 37,683111)
  Min sum of deviations 1573,869                  Pseudo R2     =     0,0194

------------------------------------------------------------------------------
          t4 |      Coef,   Std, Err,      t    P>|t|     [90% Conf, Interval]
-------------+----------------------------------------------------------------
       group |   3,268878   ,5796396     5,64   0,000      2,31449    4,223267
       _cons |   37,51578   ,1635441   229,39   0,000      37,2465    37,78506
------------------------------------------------------------------------------

Adjusted predictions                              Number of obs     =        917
Model VCE    : IID
```

```
Expression   : Linear prediction, predict()

1,_at        : group           =           0

2,_at        : group           =           1

------------------------------------------------------------------------------
             |            Delta-method
             |     Margin   Std, Err,      z    P>|z|     [95% Conf, Interval]
-------------+----------------------------------------------------------------
         _at |
          1  |   37,51578   ,1635441   229,39   0,000     37,19524    37,83632
          2  |   40,78466   ,5560894    73,34   0,000     39,69475    41,87458
------------------------------------------------------------------------------
```

```
Median regression                                 Number of obs =        915
  Raw sum of deviations 2098,802 (about 49,685473)
  Min sum of deviations 2093,385                  Pseudo R2     =     0,0026

------------------------------------------------------------------------------
          t5 |      Coef,   Std, Err,      t    P>|t|     [90% Conf, Interval]
-------------+----------------------------------------------------------------
       group |   1,446404   ,7968138     1,82   0,070     ,1344311    2,758378
       _cons |   49,67274   ,2219604   223,79   0,000     49,30728     50,0382
------------------------------------------------------------------------------

Adjusted predictions                            Number of obs     =        915
Model VCE    : IID

Expression   : Linear prediction, predict()

1,_at        : group           =           0

2,_at        : group           =           1

------------------------------------------------------------------------------
             |            Delta-method
             |     Margin   Std, Err,      z    P>|z|     [95% Conf, Interval]
-------------+----------------------------------------------------------------
         _at |
          1  |   49,67274   ,2219604   223,79   0,000     49,23771    50,10777
          2  |   51,11914    ,765275    66,80   0,000     49,61923    52,61906
------------------------------------------------------------------------------
```

```
Median regression                                 Number of obs =        914
  Raw sum of deviations 2144,224 (about 51,086308)
  Min sum of deviations 2124,216                  Pseudo R2     =     0,0093

------------------------------------------------------------------------------
          t6 |      Coef,   Std, Err,      t    P>|t|     [90% Conf, Interval]
-------------+----------------------------------------------------------------
       group |   2,869014   ,7325623     3,92   0,000     1,662831    4,075197
       _cons |    50,9251   ,2012779   253,01   0,000     50,59369    51,25651
------------------------------------------------------------------------------

Adjusted predictions                            Number of obs     =        914
Model VCE    : IID

Expression   : Linear prediction, predict()

1,_at        : group           =           0

2,_at        : group           =           1

------------------------------------------------------------------------------
             |            Delta-method
             |     Margin   Std, Err,      z    P>|z|     [95% Conf, Interval]
-------------+----------------------------------------------------------------
```

```
         _at |
          1  |    50,9251   ,2012779   253,01   0,000     50,53061     51,3196
          2  |   53,79412   ,7043684    76,37   0,000     52,41358    55,17465
-------------------------------------------------------------------------------



Median regression                                 Number of obs =        910
  Raw sum of deviations 2433,861 (about 52,632555)
  Min sum of deviations 2415,741                  Pseudo R2     =     0,0074

------------------------------------------------------------------------------
          t7 |      Coef,   Std, Err,      t    P>|t|     [90% Conf, Interval]
-------------+----------------------------------------------------------------
       group |   2,981352   ,8773213     3,40   0,001     1,536813    4,425891
       _cons |   52,42734   ,2362707   221,90   0,000     52,03831    52,81636
------------------------------------------------------------------------------

Adjusted predictions                              Number of obs     =        910
Model VCE    : IID

Expression   : Linear prediction, predict()

1,_at        : group           =           0

2,_at        : group           =           1

------------------------------------------------------------------------------
             |            Delta-method
             |     Margin   Std, Err,      z    P>|z|     [95% Conf, Interval]
-------------+----------------------------------------------------------------
         _at |
          1  |   52,42734   ,2362707   221,90   0,000     51,96425    52,89042
          2  |   55,40869   ,8449075    65,58   0,000      53,7527    57,06468
------------------------------------------------------------------------------

Median regression                                 Number of obs =        907
  Raw sum of deviations  3098,44 (about 54,929337)
  Min sum of deviations 3059,987                  Pseudo R2     =     0,0124

------------------------------------------------------------------------------
          t8 |      Coef,   Std, Err,      t    P>|t|     [90% Conf, Interval]
-------------+----------------------------------------------------------------
       group |   7,146366   1,303658     5,48   0,000     4,999842    9,292891
       _cons |    54,5963   ,3380844   161,49   0,000     54,03963    55,15297
------------------------------------------------------------------------------

Adjusted predictions                              Number of obs     =        907
Model VCE    : IID

Expression   : Linear prediction, predict()

1,_at        : group           =           0

2,_at        : group           =           1

------------------------------------------------------------------------------
             |            Delta-method
             |     Margin   Std, Err,      z    P>|z|     [95% Conf, Interval]
-------------+----------------------------------------------------------------
         _at |
          1  |    54,5963   ,3380844   161,49   0,000     53,93366    55,25893
          2  |   61,74266   1,259057    49,04   0,000     59,27496    64,21037
------------------------------------------------------------------------------



Median regression                                 Number of obs =        907
  Raw sum of deviations 3116,058 (about 68,644957)
  Min sum of deviations  3108,21                  Pseudo R2     =     0,0025

------------------------------------------------------------------------------
          t9 |      Coef,   Std, Err,      t    P>|t|     [90% Conf, Interval]
-------------+----------------------------------------------------------------
       group |   2,064437    1,27895     1,61   0,107    -,0414045    4,170279
```

```
       _cons |   68,44031   ,3316767   206,35   0,000     67,89419    68,98643
------------------------------------------------------------------------------

Adjusted predictions                            Number of obs     =        907
Model VCE    : IID

Expression   : Linear prediction, predict()

1._at        : group           =           0

2._at        : group           =           1

------------------------------------------------------------------------------
             |            Delta-method
             |     Margin   Std, Err,      z    P>|z|     [95% Conf, Interval]
-------------+----------------------------------------------------------------
         _at |
          1  |   68,44031   ,3316767   206,35   0,000     67,79023    69,09038
          2  |   70,50475   1,235194    57,08   0,000     68,08381    72,92568
------------------------------------------------------------------------------
```

```
Median regression                                   Number of obs =        900
  Raw sum of deviations 3777,648 (about 83,358787)
  Min sum of deviations 3706,177                    Pseudo R2     =     0,0189

------------------------------------------------------------------------------
         tsc |      Coef,   Std, Err,      t    P>|t|     [90% Conf, Interval]
-------------+----------------------------------------------------------------
       group |   8,505921   1,890018     4,50   0,000     5,393907    11,61793
       _cons |   83,13009   ,4586514   181,25   0,000     82,37489    83,88528
------------------------------------------------------------------------------

Adjusted predictions                            Number of obs     =        900
Model VCE    : IID

Expression   : Linear prediction, predict()

1._at        : group           =           0

2._at        : group           =           1

------------------------------------------------------------------------------
             |            Delta-method
             |     Margin   Std, Err,      z    P>|z|     [95% Conf, Interval]
-------------+----------------------------------------------------------------
         _at |
          1  |   83,13009   ,4586514   181,25   0,000     82,23115    84,02903
          2  |   91,63601   1,833523    49,98   0,000     88,04237    95,22965
------------------------------------------------------------------------------
```

```
Median regression                                   Number of obs =        884
  Raw sum of deviations 3183,368 (about 97,549109)
  Min sum of deviations 3134,061                    Pseudo R2     =     0,0155

------------------------------------------------------------------------------
         tsb |      Coef,   Std, Err,      t    P>|t|     [90% Conf, Interval]
-------------+----------------------------------------------------------------
       group |   7,137105   1,695046     4,21   0,000     4,346071     9,92814
       _cons |   97,32231   ,3866646   251,70   0,000     96,68563    97,95898
------------------------------------------------------------------------------

Adjusted predictions                            Number of obs     =        884
Model VCE    : IID

Expression   : Linear prediction, predict()

1._at        : group           =           0
```

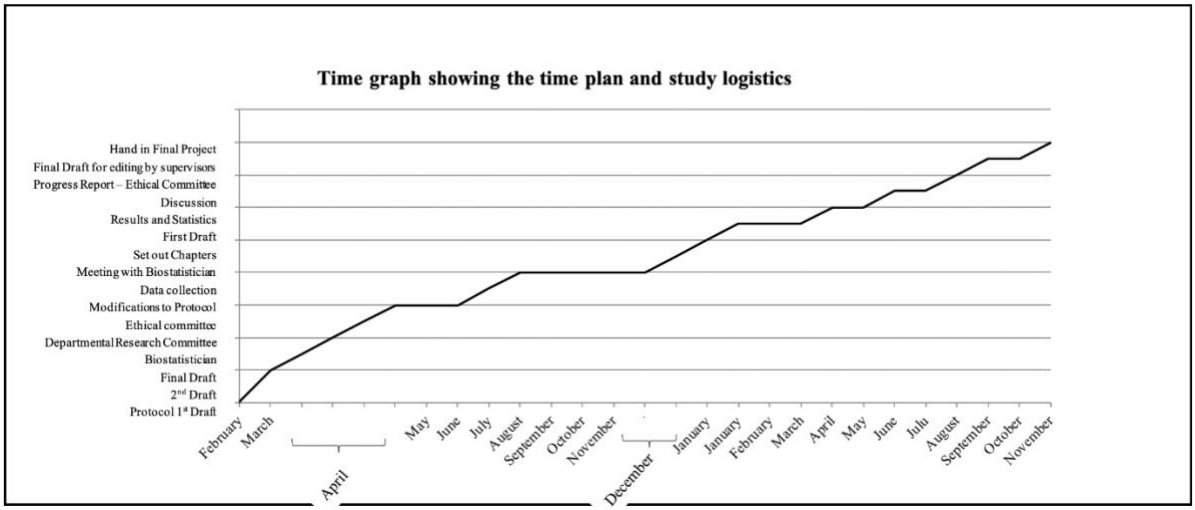
```
2,_at        : group           =           1

------------------------------------------------------------------------------
             |            Delta-method
             |     Margin   Std, Err,       z    P>|z|     [95% Conf, Interval]
-------------+----------------------------------------------------------------
         _at |
          1  |   97,32231   ,3866646   251,70   0,000     96,56446    98,08015
          2  |   104,4594   1,650355    63,30   0,000     101,2248     107,694
------------------------------------------------------------------------------
```

```
Median regression                                 Number of obs =        832
  Raw sum of deviations  3451,31 (about 105,63158)
  Min sum of deviations 3414,903                  Pseudo R2     =     0,0105

------------------------------------------------------------------------------
          tb |      Coef,   Std, Err,      t    P>|t|     [90% Conf, Interval]
-------------+----------------------------------------------------------------
       group |   7,163575   2,139589     3,35   0,001     3,640331    10,68682
       _cons |   105,3445    ,469136   224,55   0,000      104,572     106,117
------------------------------------------------------------------------------

Adjusted predictions                            Number of obs     =        832
Model VCE    : IID

Expression   : Linear prediction, predict()

1,_at        : group           =           0

2,_at        : group           =           1

------------------------------------------------------------------------------
             |            Delta-method
             |     Margin   Std, Err,       z    P>|z|     [95% Conf, Interval]
-------------+----------------------------------------------------------------
         _at |
          1  |   105,3445    ,469136   224,55   0,000      104,425     106,264
          2  |   112,5081   2,087523    53,90   0,000     108,4166    116,5996
------------------------------------------------------------------------------
```

```
Median regression                                 Number of obs =        622
  Raw sum of deviations  2509,82 (about 111,99442)
  Min sum of deviations 2487,975                  Pseudo R2     =     0,0087

------------------------------------------------------------------------------
         teb |      Coef,   Std, Err,      t    P>|t|     [90% Conf, Interval]
-------------+----------------------------------------------------------------
       group |   4,574492   1,812987     2,52   0,012     1,587931    7,561053
       _cons |   111,7074     ,39147   285,35   0,000     111,0625    112,3523
------------------------------------------------------------------------------

Adjusted predictions                            Number of obs     =        622
Model VCE    : IID

Expression   : Linear prediction, predict()

1,_at        : group           =           0

2,_at        : group           =           1

------------------------------------------------------------------------------
             |            Delta-method
             |     Margin   Std, Err,       z    P>|z|     [95% Conf, Interval]
-------------+----------------------------------------------------------------
         _at |
          1  |   111,7074     ,39147   285,35   0,000     110,9401    112,4746
          2  |   116,2819   1,770218    65,69   0,000     112,8123    119,7514
```

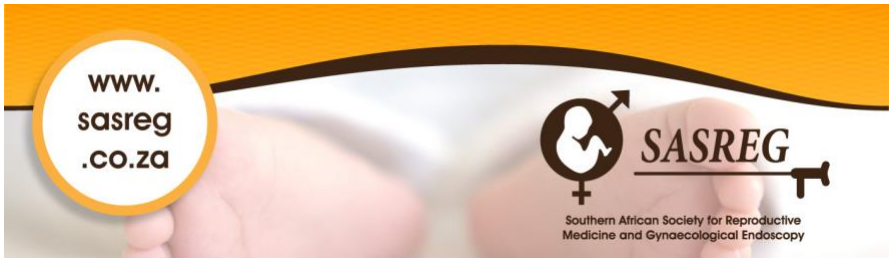
```
------------------------------------------------------------------------------

Median regression                                   Number of obs =         99
  Raw sum of deviations 372,1796 (about 114,13766)
  Min sum of deviations 364,1368                    Pseudo R2     =     0,0216

------------------------------------------------------------------------------
         thb |      Coef,   Std, Err,      t    P>|t|     [90% Conf, Interval]
-------------+----------------------------------------------------------------
       group |   8,532569    3,27981     2,60   0,011      3,08574     13,9794
       _cons |   113,8951   ,8074335   141,06   0,000     112,5542     115,236
------------------------------------------------------------------------------

Adjusted predictions                            Number of obs     =         99
Model VCE    : IID

Expression   : Linear prediction, predict()

1,_at        : group           =           0

2,_at        : group           =           1

------------------------------------------------------------------------------
             |            Delta-method
             |     Margin   Std, Err,      z    P>|z|     [95% Conf, Interval]
-------------+----------------------------------------------------------------
         _at |
          1  |   113,8951   ,8074335   141,06   0,000     112,3125    115,4776
          2  |   122,4277   3,178869    38,51   0,000     116,1972    128,6581
------------------------------------------------------------------------------

,
end of do-file

,

end of do-file

,
```

Appendix Q: Time plan and logistics



Appendix R: Budget and Funding 2018/2019

DRAMSAY SU 18170560						
HREC ETHICS APPLICATION						
ESTIMATED BUDGET						
SELF FUNDED / DEGREE PUROSE BUDGETT FOR MASTERS THROUGH STELLENBOSCH UNIVERSITY						
Reason	Distance (km)	Fuel Cost (R/km)	Maintenance (R/km)	Occurance (days)	Years of Study	Cost
Petrol from Cape Town to Wijnland, Stellebosch	49	R [illegible],00	R [illegible],50	62	2	R [illegible],00
Petrol from Cape Town to Aevitas, Pinelands	10	R [illegible],00	R [illegible],50	180	2	R [illegible],00
Petrol from Cape Town to TBH, Belville	25	R [illegible],00	R [illegible],50	120	2	R [illegible],00
					TOTAL BUDGETT FOR 2018 AND 2019	R [illegible]



Date: 01 March 2019

Dear Dylan Ramsay

CONGRATULATIONS! It is a pleasure to inform you that you have been selected to be a recipient of the **Merck Serono Scholarship as a student in Reproductive Biology – 2019/2020**

Grant Information:
R40 000 per year for 2 years to be utilized as supervised.

- **R30 000 for personal use**
- **R10 000 to be used for any research needs throughout the year – these purchases will be determined by your supervisor – DR ML de Beer**
  - **Any funds not used at end of year will be paid over to you in December of the year.**

Merck Serono would also like to congratulate you on your success and is committed towards continued education in the field of reproductive health.

Important:

- In order for the scholarship grant to be paid out, you must return a signed copy of this letter as proof that you have accepted the scholarship.
- To be eligible for the scholarship, you must be considered a student in good standing by your institution.
- **Please supply SASREG with your banking details if you have not yet done so for the transfer of funds.**

☐ I do not accept the scholarship because _____

☒ I accept the scholarship.

_____ 05/03/2019

Signature of Recipient Date

Yours sincerely

Dr Sulaiman Heylen
SASREG President

EXECUTIVE COMMITTEE

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Dr Sulaiman Heylen
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Email: info@sasreg.co.za

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Dr Sulaiman Heylen

Appendix S: HREC approval letter

UNIVERSITEIT
STELLENBOSCH
UNIVERSITY

Amendment Approval Letter

27/11/2018

Project Reference #: 7454

Ethics Reference #: S18/06/120

Title: TIME-LAPSE ANALYSIS AND MORPHOKINETIC EVALUATION OF FRESH VS VITRIFIED OOCYTES, INCLUDING DONOR AND SIBLING OOCYTE CYCLES

Dear Mr Dylan Ramsay ,

Your amendment request # 1 dated 17- Oct-2018 refers.

The Health Research Ethics Committee (HREC) reviewed and approved the amended documentation through an expedited review process.

The following amendment was reviewed and **approved:**

- To include ONLY the morphogenetic data from time lapse incubated embryo development at the Wijnland Fertility clinic.

Correspondingly the protocol version 2 dated 17 October, 2018 had been **approved.**

Where to submit any documentation

Kindly note that the HREC uses an electronic ethics review management system, *Infonetica*, to manage ethics applications and ethics review process. To submit any documentation to HREC, please click on the following link: https://applyethics.sun.ac.za.

Please remember to use your **Project ID** [7454] and ethics reference number S18/06/120 on any documents or correspondence with the HREC concerning your research protocol.

The Health Research Ethics Committee complies with the SA National Health Act No. 61 of 2003 as it pertains to health research and the United States Code of Federal Regulations Title 45 Part 46. This committee abides by the ethical norms and principles for research, established by the Declaration of Helsinki and the South African Medical Research Council Guidelines as well as the Guidelines for Ethical Research: Principles, Structures and Processes 2015 (Department of Health).

Yours sincerely,

Mrs. Melody Shana,

coordinator,

HREC1.

National Health Research Ethics Council (NHREC) Registration Numbers: REC-130408-012 for HREC1 and REC-230208-010 for HREC2

Federal Wide Assurance Number: 00001372

Institutional Review Board (IRB) Number: IRB0005240 for HREC1

Institutional Review Board (IRB) Number: IRB0005239 for HREC2

Appendix T: HREC progress report

UNIVERSITEIT
STELLENBOSCH
UNIVERSITY

Approval Letter
Progress Report

23/07/2019

Project ID: 7454

Ethics Reference No: S18/06/120

Title: Time-lapse analysis and morphokinetic evaluation of fresh vs. vitrified oocytes, including donor and sibling oocyte cycles.

Dear Mr Dylan Ramsay

Your request for extension/annual renewal of ethics approval dated 17/07/2019 12:03 refers.

The Health Research Ethics Committee reviewed and approved the annual progress report you submitted through an expedited review process.

The approval of this project is extended for a further year.

Approval date: 23 July 2019

Expiry date: 22 July 2020

Kindly be reminded to submit progress reports two (2) months before expiry date.

Where to submit any documentation

Kindly note that the HREC uses an electronic ethics review management system, *Infonetica*, to manage ethics applications and ethics review process. To submit any documentation to HREC, please click on the following link: https://applyethics.sun.ac.za.

Please remember to use your Project Id 7454 and ethics reference number S18/06/120 on any documents or correspondence with the HREC concerning your research protocol.

Yours sincerely,

Mrs. Ashleen Fortuin
Health Research Ethics Committee 1 (HREC1)

National Health Research Ethics Council (NHREC) Registration Number:
REC-130408-012 (HREC1)•REC-230208-010 (HREC2)

Federal Wide Assurance Number: 00001372
Office of Human Research Protections (OHRP) Institutional Review Board (IRB) Number:
IRB0005240 (HREC1)•IRB0005239 (HREC2)

The Health Research Ethics Committee (HREC) complies with the SA National Health Act No. 61 of 2003 as it pertains to health research. The HREC abides by the ethical norms and principles for research, established by the World Medical Association (2013). Declaration of Helsinki: Ethical Principles for Medical Research Involving Human Subjects; the South African Department of Health (2006).Guidelines for Good Practice in the Conduct of Clinical Trials with Human Participants in South Africa (2nd edition); as well as the Department of Health (2015). Ethics in Health Research: Principles, Processes and Structures (2nd edition).

The Health Research Ethics Committee reviews research involving human subjects conducted or supported by the Department of Health and Human Services, or other federal departments or agencies that apply the Federal Policy for the Protection of Human Subjects to such research (United States Code of Federal Regulations Title 45 Part 46); and/or clinical investigations regulated by the Food and Drug Administration (FDA) of the Department of Health and Human Services.

Appendix U: Normative value infographics

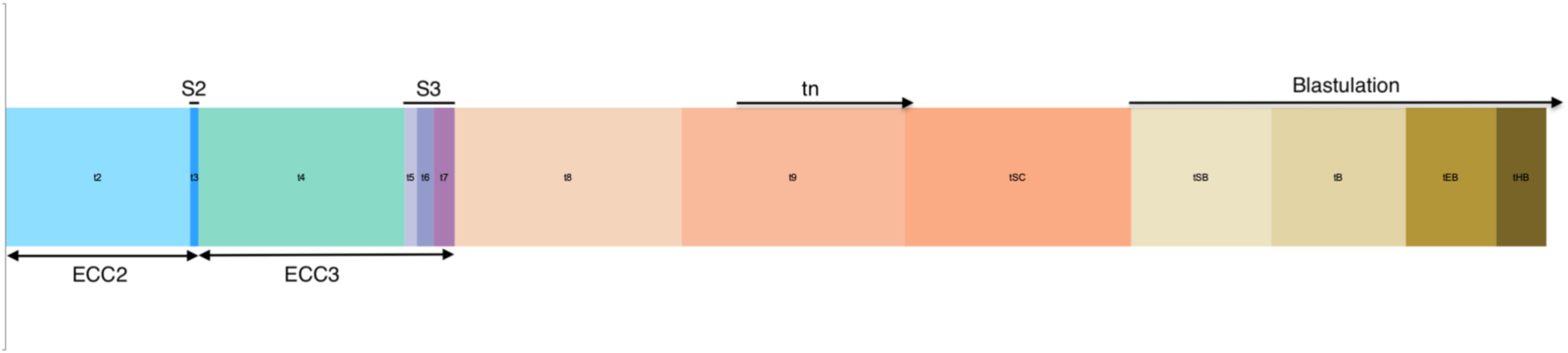


Figure 12: Normative population timeline

Figure 13: Normative population cell cycle timeline

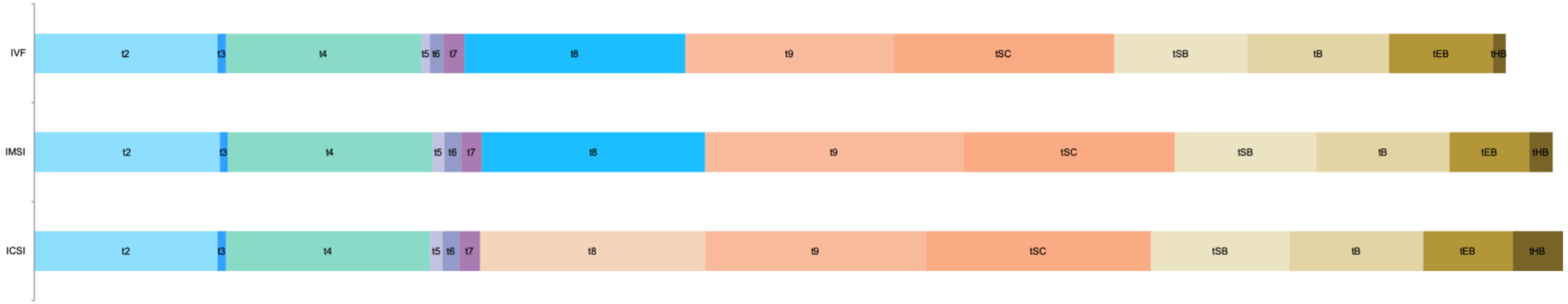


Figure 15: Cell stage durations for normative population by fertilization method

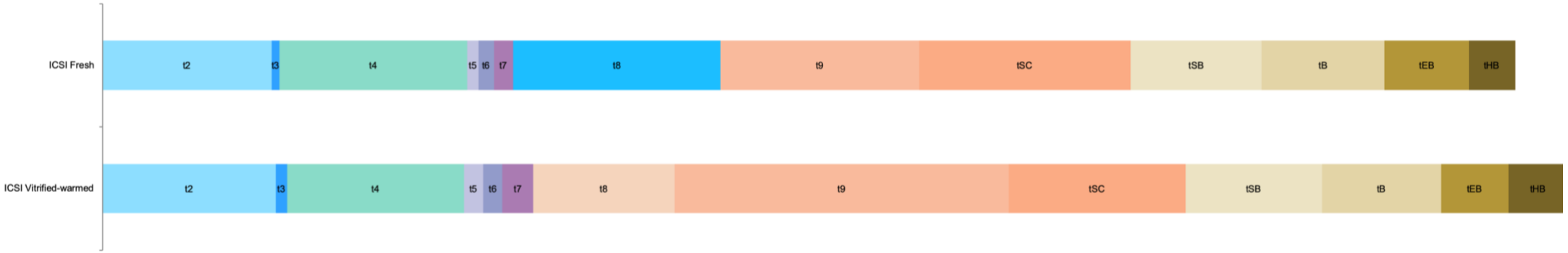
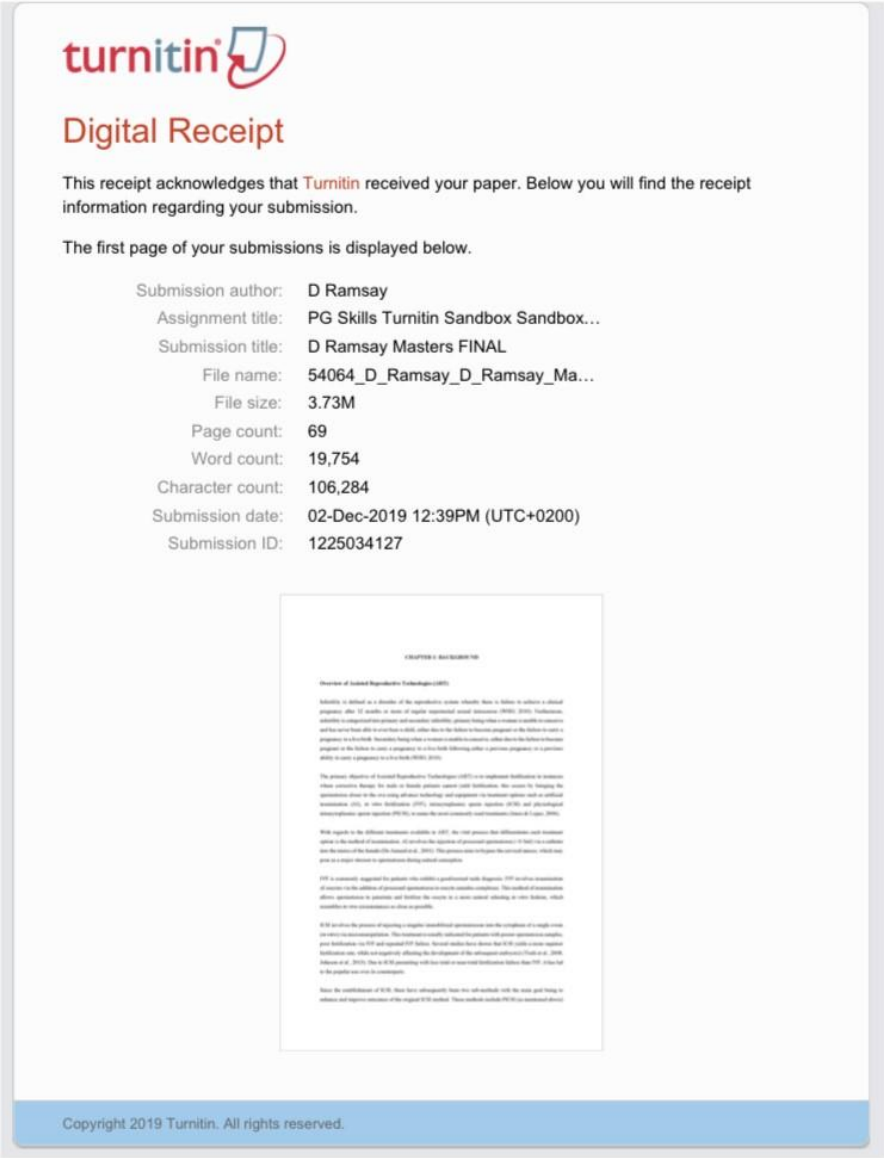


Figure 19: Durations of fresh vs vitrified/warmed oocyte populations

Appendix V: Turnitin report



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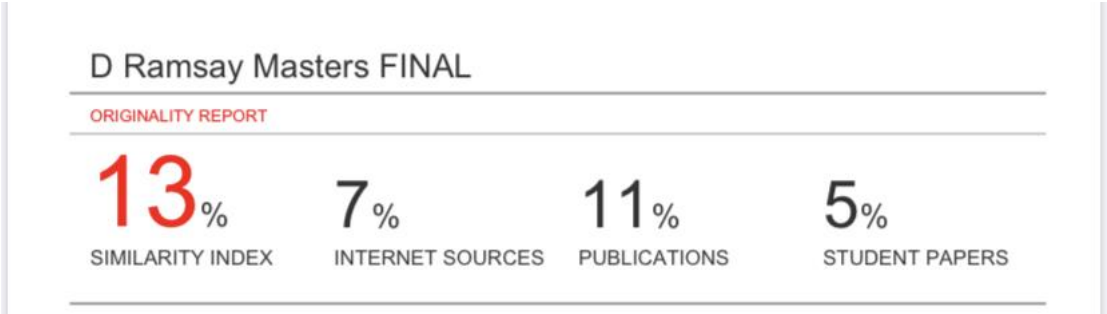

D Ramsay Masters FINAL

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