EFFECTS OF DIFFERENT ESTRUS SYNCHRONIZATION AND SUPEROVULATION TREATMENTS ON OVARIAN RESPONSE AND EMBRYO COLLECTION IN THE SOUTH AFRICAN BOER GOAT

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DECLARATION

I, the undersigned, hereby declare that the work contained in this thesis is my own original work and that I have not previously in its entirety or in part submitted it at any university for a degree.

Signature: Date:

ABSTRACT

Different synchronization and superovulation treatments were evaluated in the South African Boer goat (n = 367). Two progestagen implants, Synchro-Mate-B (SMB)/Crestar™ and Controlled Internal Drug Releases (CIDR), containing 3mg norgestomet and 0.33gm of natural progesterone, respectively, were used in the synchronization treatments. A luteolytic agent, Estrumate (Cloprostenol) 125µg, was administered 12h before progestagen withdrawal. Synchronization treatment groups were: 1) SMB x 1 (n = 123), one dose of SMB for 13 to 17 days; 2) SMB x 2 (n = 32), two doses of SMB implanted for 10 then 17 days; 3) CIDR x 1 (n = 187), one dose of CIDR; 4) CIDR x 2 (n = 25), two doses of CIDR, inserted for 9 to 17 days. On day 1 of the treatment, 0.5mg of estradiol cypionate (ECP) was administered to a group of randomly chosen goats (n = 112). Superovulation treatments consisted of Ovagen™ or Embryo-S™. An additional single dose (300 UI) of Pregnant Mare Serum Gonadotropin (PMSG) was administered to a group of randomly chosen does. Superovulation treatment groups were: 1) OV alone (n = 147), Ovagen 9 mg every 12h, 8 times starting 72h prior to progestagen removal; 2) OV + PMSG (n = 164), same treatment as 1 plus 300 IU of PMSG once 48h prior to progestagen removal; 3) E-S alone (n=16), Embryo-S 25 units twice a day, 8 times starting 72h before progestagen removal; 4) E-S + PMSG (n=40), same treatment as 3 plus 300 IU of PMSG once 48h prior to progestagen removal. Most does were naturally bred to bucks. Embryos were collected using the surgical laparascopic procedure on day 6 and corpora lutea counted. Data were not normally distributed and therefore analyzed using a nonparametric test (Wilcoxon, 1945 and Kruskal-Wallis, 1952) with outcome variable using the Mixed Procedure of SAS and the Tukey test. Differences were considered significant at $p<0.05$. Slightly more CL were on the left (52%) than on the right (48%) ovary. Superovulation treatment was significantly associated ($p<0.001$) while synchronization treatment was only marginally associated ($p=0.06$) with ovulation rate. Ovagen alone and Ovagen + PMSG were significantly more effective ($p<0.05$) than Embryo-S alone or Embryo-S + PMSG in influencing ovulation. Only synchronization treatment with 2 doses of CIDR was significantly more ($p=0.04$) effective in producing a high ovulation rate. Superovulation treatment was significantly ($p=0.02$) associated with the number of transferable embryos while synchronization treatment was not. Ovagen + PMSG was significantly ($p=0.02$) effective in producing more transferable embryos than Embryo-S + PMSG. Both superovulation and synchronization treatments were significantly ($p<0.05$) associated with producing unfertilized oocytes. Effectiveness of addition of ECP was shown in its association ($p=0.05$) with better quality embryos in univaraete analysis, though it did not have significant effect in the multivariate model. Though there was apparent advantage of CIDR over SMB, no significant difference in ovulation rate or embryo quality was associated with synchronization treatments. Effectiveness of Ovagen over Embryo-S was demonstrated and addition of PMSG improved embryo quality.

OPSOMMING

Verskillende sinkronisasie en multi-ovulasie behandelings is ge-evalueer in die Suid-Afrikaans Boerbok (n= 367). Twee progestagene, Synchro-Mate-B (SMB)/Crestar™ en Controlled Internal Drug Releases (CIDR), bevattende 3mg norgestomet en 0.33gm natuurlike progesteroon, respektiewelik, is gebruik tydens die sinkronisasiebehandelings. 'n Luteolitiese middel, Estrumate (Cloprostenol) 125µg, is toegedien 12 h voor progestageen verwydering. Sinkronisasie behandelings groepe was: 1) SMB x 1 (n = 123), een dosis SMB vir 13 tot 17 dae; 2) SMB x 2 (n = 32), twee dosisse SMB implante vir 10 tot 17 dae; 3) CIDR x 1 (n = 187), een CIDR vir die hele periode; 4) CIDR x 2 (n = 25), twee CIDRs, vir 9 tot 17 dae. Op dag 1 van die behandeling is 0.5mg estradiol cypionate (ECP) aan 'n willekeurige groep bokooie toegedien (n = 112). Multi-ovulasie behandelings het bestaan uit Ovagen™ of Embryo-S™. 'n Bykomstige dosis (300 UI) Dragtige Merrie Serum Gonadotrofien (PMSG) is toegedien aan 'n willekeurige groep ooie. Multi-ovulasie behandelingsgroepe was: 1) OV alleen (n = 147), Ovagen 9 mg elke 12h, 8 keer beginende 72 h voor progestageen verwydering; 2) OV + PMSG (n = 164), selfde behandeling as in (1) plus 300 IU PMSG eenmalig 48h voor progestageen verwydering; 3) E-S alleen (n=16), Embryo-S 25 eenhede tweemaal per dag, ag inspuitings beginende 72h voor progestageen verwydering; 4) E-S + PMSG (n=40), selfde behandeling as in (3) plus 300 IU PMSG eenmalig 48h voor progestageen verwydering. Die meerderheid ooie is natuurlik deur ramme gedek. Embrio's is gekollekteer deur gebruik te maak van die chirurgies-laparoskopiese metode op dag 6 en die aantal corpora lutea getel en aangeteken. Aangesien die data nie 'n eweredige verspreiding gehad het nie, is dit geanaliseer deur gebruik te maak van 'n nie-parametriese toets (Wilcoxon, 1945 en Kruskal-Wallis, 1952) met variërende uitkomste deur die Gemengde Prosedure Toets van SAS en die Tukey toets. Verskille is as beduidend aanvaar met 'n P-waarde van <0.05. Onbeduidend meer CLs is op die linker (52%) as op die regter (48%) ovarium opgemerk. Multi-ovuasie behandelings was beduidend geassosieer ($p<0.001$) met ovulasietempo, terwyl sinkronisasie behandelings net marginaal geassosieer was ($p=0.06$) met ovulasietempo. Ovagen alleen en Ovagen + PMSG was beduidend meer effektief ($p<0.05$) as Embryo-S alleen of Embryo-S + PMSG om ovulasie te beïnvloed. Slegs die sinkronisasie behandeling met 2 dosisse CIDR was beduidend meer ($p=0.04$) effektief om 'n hoër ovulasietempo te veroorsaak. Multi-ovulasie behandeling was beduidend geassosieer met die aantal oordraagbare embrio's, terwyl sinkronisasie nie dieselfde tendens gewys het nie. Ovagen + PMSG het beduidend meer ($p=0.02$) oordraagbare embrio's opgelewer as Embryo-S + PMSG. Beide multi-ovulasie en sinkronisasie behandelings was beduidend geassosieer ($p<0.05$) met onbevrugte oosiete. Die rol van die byvoeging van ECP is getoon in die assosiasie daarvan ($p=0.05$) met beter kwaliteit embrio's in 'n eenvariante analiese, alhoewel dit nie 'n beduidende effek op die multi-variante model gehad het nie. Alhoewel dit blyk dat CIDR 'n beter reaksie as SMB gee, kon geen beduidende verskil in die ovulasietempo of embriokwaliteit opgewys word nie. Die groter effektiwiteit van Ovagen oor Embryo-S is gedemonstreer, terwyl die byvoeging van PMSG embriokwaliteit verbeter het..

DEDICATION

To my father,

To my mother,

To my wife Carol,

To my son Fred and my young brothers Gad, Mulongo and Claude;

To my sisters Isa, Michou, Lily;

To my cousins, my nephews and nieces, including Freddy, Bob, Kyungu, Kabange and Mara, Laurette, Karine, Alain and Huguette;

To my grandfather Ilunga Ntshikala Epenetusa, who taught me the most wonderful lessons of life and love, among which I will always remember this amazing Luba proverb:

" Ella pa mema, bingi biya ku meso,"
Cast your bread upon the waters, for after many days you will get it back (Eccl. 11 :1)

With all my love, I dedicate this work to you.

"Kumeso kupite umo, amba lume lubapu." *Luba Katanga proverb (DR Congo)*
It is the early farm goer who absorbs all the morning dew on the footpath.

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CHAPTER 1

INTRODUCTION

1.1 PROBLEM STATEMENT AND MOTIVATION OF THE STUDY

According to the FAO 1978 report, the world population of goats was about 410 million while that of sheep was around 1028 million. The data of the year 1978 show that the goat population represented around 15% of the world population of grazing domestic animals, whereas the sheep to goat ratio was 2.5 during that year (Devendra & Coop., 1982). The goat number went over 470 million in 1982. The 1998 statistics of the world population of grazing domestic animals, according to the FAO 1998 annual report, was about 162 million buffaloes, 1318 million cattle, 1064 million sheep and 700 million goats. The FAO tables show that the ratio of sheep/goats has decreased to approximately 1.5 in 10 years. The same data of 1998 show also that, excluding pigs, compared to the world population of buffaloes, cattle and sheep, the goat population represents about 19% of the world population of grazing domestic animals. Recent data are putting the figure at more than 732 million (FAO 2000).

These figures clearly show how the world population of goats has increased steadily over the last decades, at an annual rate of 2.6%, during the last 20 years. Most of this increase in goat population has occurred, as we will notice, in those areas of the world where the goat is well established as an important domestic animal, namely in Africa, Asia and to a lesser extent in South America. It is most likely that the result of improved production systems and management, leading to better nutrition and herd health, is responsible for this increase in goat population number. The goat population in Africa, Asia and South America has grown from nearly 425 million in 1979, to reach over 667.57 million in 1998. That is about a 12 million increase per year. In Europe, North and Central America, and Oceania, the goat population grew from 25 to 32.4 million during the same period, representing an average growth of 0.4 million goats per year (FAO 1994, 1998).

To illustrate the importance of the goats in the tropics, where most developing countries are located, the following figures can be mentioned: over 60% of sheep are found in developed temperate zones (which mostly include industrialized countries) and less than 40% in tropical zones. The distribution of goats, however, is in sharp contrast to that of sheep. Nearly 80% of all goats are found within the band 0-40 degrees north, in the arid tropical and subtropical region

(Devendra & Coop, 1982). Strictly speaking, a big portion of South Africa is not part of the tropical region. However, with the wide variation of the climate, due to many geographical factors that occur in the tropical zone, some countries immediately outside the tropics, e.g. South Africa, are included (Devendra & Burns, 1970).

It should be borne in mind, however, that statistics used in goats, especially in developing countries, although official, may not be very reliable and that populations can vary considerably according to many factors. In my own experience in the Democratic Republic of Congo, small cattle breeders and goat-keepers were reluctant to give account of their livestock (goats and cows). They would underestimate the number because of the general tendency to undervalue property for fear of taxation or other causes. It should also be noticed that a great number of animals in the tropics are slaughtered outside official slaughterhouses and/or informally marketed, making the statistics found in the literature somehow biased.

The goat number relative to other livestock shows the importance of goats in the agriculture of several countries in the tropics. In the Democratic Republic of Congo, for example, there were four million goats in the 1978 FAO annual report, which is more than any other species of livestock. In Tanzania, according to the same report, the population of goats was about 2.5 times that of sheep. In India it was twice and in west Malaysia seven times the number of sheep, (Devendra & Burns, 1970). Paradoxically, South Africa has many times more sheep (30 million) than goats (7 million). In South Africa, the number of goats has fluctuated very little since 1946. About 70% of that number was owned by the African people, living in traditionally settled areas (Hugo, 1968). South African statistics (SA official Yearbook, 1998) show, however, that the indigenous meat producing Boer goats accounts for about 40% of all goats, and the Angora goats, used for mohair production, form the remaining 60%.

Goats are multi-purpose animals producing meat, milk, skin and hair. While in western countries goats are mainly kept as dairy animals, their worldwide importance is chiefly as producers of meat (Gall, 1981). In most Asian countries and in Africa (where dwarf breeds prevail), goats are considered almost exclusively as meat animals. One advantage of goats as suppliers of meat is that they produce small carcasses, which can be consumed by a family or a group of families without need of retailing or preserving. This is considered an advantage in the tropics in particular. On the other hand, the South African Boer goat (Fig. 1-1 and 1-2), whose primary function is meat production, is of excellent conformation according to Devendra & Burns (1970), and probably one of the breeds that exhibit the most pronounced meat production

qualities. For a growing world population, especially encountered in developing countries, the Boer goat may become both efficient and a more important supplier of high quality and quantity food, as well as a source of improved genetic material.

The Boer goats are fairly widespread over South Africa, although certain parts are more suitable for goat farming, e.g. where bushes grow higher and thicker, and rough mountain parts, to which others livestock species are not so well adapted. For this reason, the Western, Eastern and Northern Cape Provinces lodge more than half of the Boer goat population of the country (Greyling, 1988).

When the history of the development of the Boer goat is reviewed, it is apparent that almost every author has a different view on the subject. Some authors claim that the South African Boer goat breed was developed from goats imported from Nubia. According to Schreiner (1898), the Boer goat was developed from goats imported from Europe while Pegler (1886) stated that the South African Boer goat breed originated from goats brought to South Africa from India. Some others authors according to Hofmeyr & Meyer (1984), including Barrow, 1801; Epstein, 1971 and Mason, 1981 appear to have the most realistic view in their descriptions, ascribing the origin of the Boer goat to the goats of Hottentot tribes to which imported goats from India were crossed (Devendra & Burns, 1970). Whatever the origin of the Boer goat might be, what remains true is that the present improved Boer goat is the fruit of a human technological effort of genetic improvement and the demonstration of what can be achieved if careful genetic selection is practiced. As a result, several qualitative and quantitative traits such as excellent meat conformation, relative high average mass gains in kids, uniformity with regard to color (white hair with red head markings), hardiness and adaptability, and high fertility have been remarkably enhanced.

The motivation for this study resided in the fact that the world human population is growing, especially in developing countries. Large proportions of the world's livestock are kept in developing countries (95% of goats and 65% of cattle) under husbandry systems that need to be improved in order to meet the challenge of food security for the people. This also constitutes a tremendous gene pool that can contribute to the variation of these two species worldwide. To be able to achieve and maintain an efficient production of animal protein, a high level of reproductive performance is crucial. The impact of most recent scientific and technological advances on production in livestock breeding, especially in developed countries, is beyond doubt. As a result of implementation of research and improved management, South African

agricultural production has more than doubled during the last 30 years (SA Yearbook, 1993). The South Africans have also shown what is possible with respect to selective breeding of meat goats. Their improved Boer goat is a most impressive animal and quite unlike meat goats in any other countries (Mowlen, 1992). The use of artificial insemination in cattle for commercial purpose is an illustration of the application of the benefits of the technological advances. The techniques of multiple ovulation and embryo-transfer have been successfully practiced commercially for more than 20 years in the cattle industry in developed countries. Considering advances made in cattle embryo recovery and transfer techniques, during the last decades, can these technologies benefit goats in tropical countries?

1.2 OBJECTIVE OF THE STUDY

The principal objective of this study was to assess the results of different multi-ovulation programs used in Boer goats in our different experiments. Variability in ovarian response has been related to differences in superovulatory treatments such as gonadotropin preparation (FSH and PMSG), duration of treatment, and timing of treatment with respect to the estrous cycle, the total dose of gonadotropin, and the use of additional hormones in the superstimulatory scheme. As most of these above-mentioned factors have been reported in several publications in species like cows and sheep, the contribution of this study will be found in the application of some of these superovulatory and synchronization treatments in the Boer goat. This study was also to explore the impact of additional environmental factors such as season, age, location, and nutritional status as measured by the body condition score of animals. In exploring the different superovulatory and synchronization treatments in the South African Boer Goat, this thesis would contribute particularly to the study of this less reported and described species, the goat. The development of an embryo industry of goats in South Africa may play an important role in animal production within the country, in Africa and in many others tropical regions in the world. This technology may be part of or contribute to the development of the goat industry for production of the so much needed protein in this part of the world, since protein rather than energy is often the main deficiency in human nutrition.

Fig 1-1 Improved Boer goat male

Fig. 1-2 Improved Boer goat female

CHAPTER 2

LOCATIONS OF THE STUDY AND ANIMAL MANAGEMENT

2.1 DESCRIPTION OF THE STUDY AREAS

South Africa's location has had a great influence on many aspects of its geography and development. Thus its sunny, dry climate and the small variation between summer and winter daylight periods are attributable to its subtropical latitudinal position (SA Yearbook, 1994). The present study was carried out in two locations both situated in the former Province of the Cape, stretching latitudinally from 31 degrees 29 min. to 33 degrees 57 south, and longitudinally from 18 degrees 50 min. to 25 degrees east: the African Embryo & Semen (AES) station in Stellenbosch (Lat. 33 degrees 57 min, Long. 18 degrees 50 min.), in the Western Cape Province and the Grootfontein Agriculture College in Middelburg (Lat. 31 degrees 29 min., Long. 25 degrees 02 min.) in the Eastern Cape Province.

2.1.1 RELIEF FEATURES

The surface area of South Africa falls into two major physiographic provinces: the interior plateau, comprising extensive level surfaces with a mean altitude of 1200 m (Middelburg) and the marginal lands (Stellenbosch) between the plateau and the coast. Forming the boundary between these two areas is the Great Escarpment. Its height above sea level varies from 1500 m in the Namaqualand to a height of 3482 m in the Kwazulu Natal Drakensberg. Inland from the escarpment lies the interior plateau, which is the southern continuation of the great Africa plateau stretching north to the Sahara (SA Yearbook, 1998). The altitude of the locations of the study is 85 m for Stellenbosch and 1270 m for Middelburg.

2.1.2 CLIMATIC FEATURES

The climate at any place on earth is mainly controlled by latitude, position relative to distribution of land and sea, height above sea level, general circulation of the atmosphere and its local effects, influence of ocean currents, and position relative to hills and mountains (Anderson, 1969). The subtropical location, south of 30 degrees south, accounts for the warm temperature conditions so typical of South Africa. The country also falls squarely within the subtropical belt of high pressures, making it dry, and with an abundance of sunshine. The wide expanses of ocean on three sides of South Africa have a certain moderating influence on its climate. More apparent, however, are the effects of the warm Agulhas current (south-flowing stream skirting

the east and south coast as far as Cape Point) and the cold Benguela current (flowing north along the west coast as far as southern Angola). While Durban (east coast) and Port Nolloth (west coast) lie more or less in the same latitude, there is a difference of no less than 6°C in their mean annual temperatures. Even more significant is the effect on rainfall. Because east coast air masses are warmer, they tend to be less stable and more likely to cause abundant rain. Indeed moist, warm air masses from the Indian Ocean are in fact the chief source of rainfall for most of the country. The cold water of the Benguela current, by contrast, chills from beneath west coast air masses. Coupled with others conditions, rain-forming processes are therefore severely discouraged, hence Port Nolloth's meager annual total rainfall of only 63 mm. Durban (east coast), on the other hand, records 1018 mm of rain annually (SA Yearbook, 1998.)

South Africa has an average annual rainfall of only 464 mm, against a world mean of 857 mm. Sixty-five percent of the country has less than 500 mm annually, usually regarded as the absolute minimum for successful dryland farming. The distribution of annual precipitation has two main features. Firstly, there is a fairly regular decrease over the plateau from east to west. The moist Indian Ocean air masses gradually lose their moisture as they move towards the western interior. The very lowest rainfall - less than 50 mm in places - occurs on the west coast in Namaqualand (Northern Cape). The second main feature of the rainfall distribution pattern is the strong orographic influence. In the Wemmershoek and Jonkershoek mountains (near the Stellenbosch embryo station) of the south-western Cape, an annual rainfall figure as high as 3200 mm have been recorded. Using periods of maximum rainfall as a measure, three precipitation sub-regions can be distinguished. The winter rainfall region is a comparatively small area along the Cape west and southwest coasts and has a Mediterranean rainfall pattern, with a prominent winter maximum. In this study, Stellenbosch annual rainfall of 633 mm belongs to the winter rainfall region. The summer rainfall region covers most of the rest of the country. Middelberg (Grootfontein) with an annual rainfall of 360 mm is located in this region. Between the winter and the summer rainfall regions lies a transitional area where rain falls in all seasons (SA Yearbook, 1998).

South Africa's rainfall is unreliable and unpredictable. Large fluctuations in the average annual figure are the rule rather than the exception in most areas of the country. Years with a below-average figure are more common than years with an above-average total. As is the case of other countries in similar latitudes, South Africa is periodically afflicted by severe and prolonged droughts, which often end in severe floods (SA Yearbook, 1998).

Three main features characterize temperature conditions. Firstly, the temperature tends to be lower than in other countries in a similar latitude, for example North Africa and Australia. This is primarily due to the greater elevation above sea level of the subcontinent. Secondly, despite a latitudinal span of 13 degrees, average annual temperatures are remarkably uniform throughout the country. The third feature is the striking contrast between temperatures on the east and west coasts. Mean annual temperature figures, however, conceal almost as much as they reveal. This is shown by an examination of extreme temperatures, and seasonal and daily ranges. The mean annual temperature recorded in Stellenbosch (1994) was 18°C, and in Middelburg (1920-1962) was 14.5°C, (SA Yearbook, 1994).

2.2 ANIMAL MANAGEMENT

Experiments were conducted on two farms where goats were kept in intensive management systems. Each of these farms housed an embryo-collection unit, the African Embryos and Semen (AES) Station in Stellenbosch and the Grootfontein Agricultural College Embryo Station (GACES) in Middelburg.

2.2.1 AES STELLENBOSCH

The animals were all housed in well sheltered and ventilated woody (females) and brick worked (males) barns under roof by night with a provision of pellets and lucern *ad libitum*. During the day the animals were permitted to browse on Kikuyu grass (*Penicetum clandestinum*) while pellets was given *ad libitum* as well. The approximate daily quantity of pellets taken per animal was 0.5 kg. The commercial pellet given was Budget Pride 12 (Table 2-1). The bucks were sheltered in a separated barn from the does during the night, and kept in open-air camp next to that of the does during the day. Water was always freely available. Four weeks prior to the starting of the experiments, all animals were given Zn, Mn, Se and vitamin A injections, vaccinated against *Pasteurella* and pulpy kidney disease, and treated against worms with Avermectin (Ivomec, Logos Agvet). Oxytetracycline was injected in those animals showing disease symptoms of coughing.

Table 2-1 Composition of the AES pellets ration (Budget Pride 12)

Ingredient	Quantity	Unit	Max/min
Protein	120	g/kg	Min
Fat	25	g/kg	Min
Fibre	90	g/kg	Max
Moisture	120	g/kg	Max
Calcium	15	g/kg	Max
Phosphorus	6	g/kg	Min
Urea	10	g/kg	Max

Body conditions of animals were assessed and dentition used to estimate the ages. Average body condition of the herd was about three in a scale ranging from one for poor to five for excellent condition. The experimental animals were randomly chosen upon the base of body condition.

2.2.2 GACES MIDDELBURG

The animals were kept in well-ventilated pens under roof by night and in a well-fenced open-air camp during the day. Pellets (Table 2-2) and lucerne hay were given *ad libitum*. The approximate daily ration per animal was 1.75 kg of pellets, and 750 g of lucerne hay. Males were separated from females and water was freely available to both sexes.

Table 2-2 Composition of the GACES pellets ration

Ingredients	Percentages
Crude protein	12.9%
T.D.N.	68.3%
Crude fiber	11.3%
Metabolizable energy	10.3 Mj /kg
Calcium	1.18%
Phosphorus	0.29%

By the time of the arrival of the goats at the embryo station farm, one month before the beginning of the experiment, the general condition of the animals was very bad, especially because of their previous poor nutritional status consisting of an unimproved pasture diet without any supplementation. The average weight of the donors then was about 42 kg. Three months later, the average weight of donor animals was 47 kg and 52 kg six months later.

Fig. 2-1 Location of the study in South Africa

CHAPTER 3

SUPEROVULATION, SYNCHRONIZATION AND FERTILIZATION

3.1 SUPEROVULATION

Superovulation is defined as the treatment of a female with hormones to allow more ova to be ovulated than the usual (one or two). In this process, ovaries are stimulated to produce more than the normal number of ovulatory follicles and thus release more ova. In order to exploit the superior genetic potential of an outstanding female to the full, using embryo transfer, it is obviously essential to obtain as many eggs as possible from that female (Peters & Ball, 1987). With current procedures, superovulation increases the yield of normal embryos about fivefold in the cow, goat, sheep, and rabbit (Hafez, 1993).

In cattle, superovulation typically results in the ovulation of about 10 ova, six of which are recovered as normal embryos (Seidel & Elsden, 1989.) In goats and sheep about 10 to 12 normal embryos should be recovered (Table 3-1). In all species, there are tremendous individual variations in response and wide ranges in superovulatory response and embryo yield have been revealed in several reviews of commercial embryo transfer. The variability in response to current superovulatory regimes is one of the problems limiting the success of multiple ovulation and embryo transfer (MOET) programs (Wallace, 1992; Mapletoft & Bo, 1994). Therefore, much research effort has been directed toward improving superovulation regimes. This variability in ovarian response has been related to differences in superovulatory treatments such as gonadotropin preparation, the batch of gonadotropin, the duration of treatment, the timing of treatment with respect to the estrous cycle, the total dose of gonadotropin, the use of additional hormones in the superstimulatory scheme and other additional factors.

To date, the gonadotropin preparations most frequently used include pregnant mare serum gonadotropin (PMSG), porcine follicle stimulating hormone (FSH-p), horse anterior pituitary extracts (HAP) and human menopausal gonadotropin (hMG) (Wallace, 1992). Discovered in 1930 by proff. Cole and Hart at the university of California at Davis, according to Peters & Ball (1987), in the serum of pregnant mares, PMSG has been found to have both FSH-like and LH-like proprieties. It differs from the endogenous pituitary gonadotropin in cattle (LH and

FSH) in that it has a much longer half-life in the body (in excess of 50 h) as compared with approximately 0.5 - 1 h for LH and FSH (Peters & Ball, 1987).

Traditionally, a single injection of 1500 to 3000 IU of PMSG has been used to superovulate cows. However, more ovulations, embryos recovered and pregnancies have been reported after a superovulatory regimen with FSH and LH in a 5:1 ratio, administered twice a day in decreasing doses for 5 days (Mapletoft, 1986). Another treatment commonly used, especially because of its simplicity in on-farm work, is 5 mg FSH daily for 5 days (Mapletoft, 1986). It has been demonstrated that an improved response could be obtained when LH was not added in decreasing doses for 5 days. FSH and PMSG may be given either subcutaneously or intramuscularly (Mapletoft, 1986). The majority of donor cows will respond most predictably and optimally if superovulation treatments are instituted between days 8 and 14 of the cycle (Mapletoft, 1986).

Table 3-1 Distribution of ovulation rate following various superovulatory hormone regimes (Wallace, 1992)

Superovulation Program	**No of ewes treated**	**No of ewes with ovulation rates**		**Mean ovulation rate ± Sem [1]**	
		#4	∃5	All ewes	Ewes with CL∃5
hMG [2]	23	3	20	11.6± 1.31	13.1 ±1.21
HMG	10	5	5	7.7± 2.30	13.2 ± 2.94
PMSG [3] + GnRH [4]	73	24	49	8.4 ± 0.75	11.0 ± 0.90
PMSG + GnRH	48	26	22	5.2 ± 0.64	7.8 ± 1.17
PMSG + GnRH	38	11	27	8.9 ± 1.11	11.5 ± 1.27
porcine FSH [5]	43	4	39	14.2 ± 1.24	15.3 ± 1.24
porcine FSH	46	17	29	7.6 ±0.82	10.5 ± 0.95

[1] Sem: Standard error of mean

[2] hMG: Human Menopausal Gonadotropin

[3] PMSG: Pregnant Mare Serum Gonadotropin

[4] GnRH: Gonadotropin Releasing Hormone

[5] FSH: Follicle Stimulating Hormone

Boer goats can be superovulated during the breeding and anestrous seasons. Some reports suggest a slightly lower response during the anestrous period, particularly for those breeds with strict seasonal limitations on cyclic behavior. The use of PMSG and FSH both have now been used successfully for over a decade to superovulate donor does. Over the years, however, FSH stimulation has been reported to produce more normal corpora lutea and better quality embryos

for transfer in both breeding season and non breeding season does than PMSG (Armstrong *et al*, 1982 a, 1982 b), (Pendleton *et al.*, 1992). Table 3-2 lists the timing and the doses for PMSG and FSH in goats. In the cyclic donor, gonadotropins are administered near the end of the luteal phase of the cycle (days 17 to 18) so that follicles are recruited before lysis of the existing CL (Bondurant, 1986).

Table 3-2 Superovulation in goats (Bondurant, 1986)

Drug	**Total dose/route**	**Day of estrous cycle**	**Comments**
PMSG	1000 to 2000 IU IM	14 to 18	Single injection
FSH	18 to 24 mg IM or SC. (begin at 5 mg)	Begin days 13 to 16	Twice daily decreasing dose for 3 to 4 days

Because effective functional estrous detection requires much time, labor, skill and expense, it has been cited as a major factor limiting the widespread use of artificial insemination in cattle (and goats). Therefore, the elimination of estrous detection from artificial breeding programs was the principal stimulus that led to research in the development of prostaglandin and progestational compounds that have the ability to control estrus without affecting fertility (Wenkoff, 1986). Estrous control offers several applications in addition to synchronized breeding programs in farm animals. Estrous synchronization in embryo transfer programs is one thereof.

3.2 ESTROUS SYNCHRONIZATION

The control of the estrous cycle is dependent on manipulation of the hormonal events occurring during the normal ovarian/estrous cycle. The overriding event controlling the development of an ovarian follicle to the point of ovulation in the cyclic cow (or doe) is believed to be the process of luteolysis or decrease in progesterone secretion occurring at the end of the luteal phase of the cycle (Peters & Ball, 1987). The fall in peripheral progesterone concentrations may be manipulated artificially in two ways, by: 1) Artificial induction of premature luteolysis using luteolytic agents, for example, prostaglandin $F_{2\alpha}$; 2) Simulation of CL function by administration of progesterone (or one of its synthetic derivatives) for a number of days, followed by abrupt withdrawal (Peters & Ball, 1987).

3.2.1 INDUCTION OF LUTEOLYSIS

The single greatest advance in superovulation methodology in the last decade has been the use of prostaglandin $F_{2\alpha}$ or $PGF_{2\alpha}$ analogues, such as cloprostenol, because superovulatory treatment can be initiated anytime between day 6 of the estrous cycle and natural CL regression. The optimal time for treatment however is between days 8 and 12 of the cycle in cattle (Betteridge, 1977), because the luteolytic rate is low in young corpora lutea (days 5 to 6) and does not approach 90% until the CL is over 7 to 9 days old (Wenkoff, 1986). Injection of exogenous $PGF_{2\alpha}$ or one of its analogues during the mid-luteal phase of the cycle, results in a premature luteolysis and consequential fall in peripheral progesterone concentrations. This is followed by a rise in secretion of gonadotropins and estradio-17β culminating in the pre-ovulatory (LH and FSH) surges and eventual ovulation (Peters & Ball, 1987).

Normally, a luteolytic dose of prostaglandin $F_{2\alpha}$ is administered 48 to 72 hours after initiation of superovulatory treatment. Estrus is expected to occur around 48 hours after the administration of $PGF_{2\alpha}$. Cows that are late coming into heat by one or more days usually yield poor results. Occasionally, estrus will occur 12 to 24 hours early. These cows usually have a good superovulatory response with good embryo quality. Once heat is expressed, treatments cease and inseminations (or natural service) begin. If ovulation problems are suspected in a given donor cow (based on previous experience), 2500 IU of hCG or 200 μg of GnRH may be administered at the onset of heat. Donor cows can be superovulated every 50 to 60 days rather indefinitely. However, it is often useful to allow a donor cow to have a calf to rejuvenate the system and to milk some fat off her back (Mapletoft, 1986). Prostaglandins have been used to control the estrous cycle in several different ways. One possible method, the so-called "two plus two" or "double-injection" technique, was designed to synchronize groups of animals cycling at random without prior knowledge of their precise ovarian status. All cattle are injected on day 0 of treatment and the injection repeated 10 days later. AI is then carried out usually three and four days after the second $PGF_{2\alpha}$ injection (Peters & Ball, 1987).

Prostaglandin $F_{2\alpha}$ manipulates the reproductive events of goats very effectively. $PGF_{2\alpha}$ has been used successfully in dairy goats for the induction of estrous and synchronization of estrus among others applications (Ott, 1986). During the breeding season, synchronization may be achieved by inducing resorption of the CL with $PGF_{2\alpha}$ and, therefore, shortening the cycle. Prostaglandin $F_{2\alpha}$ injection has been shown to be effective on any doe that is between days 4 to 17 of the estrous cycle. Does which are on day 18 or later will soon come into estrus anyway. $PGF_{2\alpha}$ will

not cause does on days 1, 2, or 3 to come into estrus early (Ott, 1986). Does in this category are unresponsive to $PGF_{2\alpha}$ receptors for the simple reason that the corpus lutea present have very few $PGF_{2\alpha}$ receptors at that stage. However, seven days later, these does will now be between days 8 and 10, in mid-luteal phase, and therefore responsive to prostaglandin. Successful induction of estrus with $PGF_{2\alpha}$ in goats treated as early as day 4 of the estrous cycle indicates that $PGF_{2\alpha}$ is luteolytic at an earlier stage of the estrous cycle in goats than sheep or cattle (Bretzlaff *et al*, 1983). In sheep, prostaglandin will not induce luteolysis prior to days 4 to 5 of the cycle.

It is often necessary to control the time of estrus and ovulation in donors so that gonadotropin treatment can be administered properly timed to the recruitment of follicles and subsequent estrus. When information is available about the stage of the donor's estrous cycle, a single injection of $PGF_{2\alpha}$ given two days after initiation of gonadotropin treatment (middle to late cycle) will induce estrus in one to four days. In goats for which no such information is available, two injections of $PGF_{2\alpha}$ may be given 10 to 12 days apart. Gonadotropin treatment is begun 2 days before the second injection of $PGF_{2\alpha}$ (Bondurant, 1986). Prostaglandins are not effective when administered to anestrous does outside the breeding season, because the ovary can only respond to prostaglandin if there is a functional corpus luteum. Therefore, does with no ovarian activity during the anestrous period, will not respond to prostaglandine $F_{2\alpha}$

3.2.2 USE OF PROGESTAGENS

The second method of controlling the cycle is to simulate the function of the CL by the administration of progesterone or one of its derivatives. In this method, gonadotropin release, and hence follicular maturation, is suppressed until progesterone withdrawal.

If a group of cows is treated with progesterone and the progesterone is then withdrawn from all cows simultaneously, this will theoretically synchronize ovulation in the group. In order to synchronize a group of randomly cycling cows effectively, it is necessary to treat them with progesterone for a period equivalent to the length of the natural luteal phase i.e. at least 16 days. This is due to the fact that exogenous progesterone has little or no effect on the life span of the natural CL and therefore in some cases the natural CL might outlive a short-term progesterone treatment, resulting in a failure of synchrony. However, it has been shown that long-term progesterone treatments (18-21 days) result in poor pregnancy rates. Shorter-term progesterone treatments generally result in a more acceptable pregnancy rate. Unfortunately short-term progesterone treatment does not control the cycle adequately. If treatment is started early in the

cycle, the natural CL may outlast the progesterone treatment. Therefore, it is necessary to incorporate a luteolytic agent with short-term progesterone treatments in order to eliminate any natural corpus luteum (Table 3-3). Prostaglandin is usually injected on the day before progestagen withdrawal (Peters & Ball, 1987).

Table 3-3. The effect of a nine-day progestagen treatment without a luteolytic agent on the estrous cycle in cow (Peters & Ball, 1987)

Stage of cycle at implant	Will the cows synchronize?	Mechanism
Days 9-17	Yes	Treatment coincides with or outlasts corpus luteum.
Days 18-1	Yes	Progesterone blocks ovulation.
Days 2-8	No	Corpus luteum outlasts progesterone treatment.

Progestagens (progesterone-like compounds) can be administered in the feed, by injection or by implant. Treatment by food requires that the compound is "orally active," i.e. that it is absorbed into the systemic circulation unchanged. However, this route of administration presents problems of controlled dosing. Progestagens can be given by injection, but repeated treatments may be necessary and the rate of absorption may be too imprecise to allow synchronized withdrawal of the compound. Implants are the most suitable method of administration of progestagens since withdrawal can then be precisely controlled by implant removal (Peters & Ball, 1987).

Because progesterone itself is relatively inactive orally, some synthetic analogues such as medroxyprogesterone acetate (MPA) and melengestrol acetate (MGA) were developed in the mid 60's as possible synchronization compounds. The level of synchrony was adequate, but conception rates were low because of delayed embryo cleavage. The recently developed synthetic progesterone, norgestomet, on the other hand, can be used to synchronize estrus without lowering fertility. Norgestomet (also called Crestar in RSA or Synchro-Mate B in the USA) consists of an impregnated silastic subcutaneous implant (Wenkoff, 1986; Peters & Ball 1987).

Used in the cow, Synchro-Mate B (SMB)/Crestar treatment consists of an ear implant that contains 3 mg of norgestomet and an intramuscular injection of 5 mg estradiol valerate and 3 mg norgestomet mixed together in a single 2 mL dose (Fig. 3-2). The norgestomet acts as "artificial CL" and therefore prevents the LH surge and ovulation, CL formation, and CL maintenance.

When the implant is removed 9 days later, the pituitary gland is released from the inhibitory effects of norgestomet, and the animal returns to estrus 24 to 36 hours later (Wenkoff, 1986), (Miksch *et al*, 1987). In either cyclic or anestrous donors does, estrus and ovulation may be controlled with progesterone or synthetic progestagins (Table 3-4).

Table 3-4 Estrous control in goats (Bondurant, 1986)

Drug	**Dose**	**Route**	**Duration of treatment (days)**	**Comment**
Progesterone	10 to 12 mg	IM	14-18	In oil
Fluorogestone acetate (FGA) = Cronolone	30 to 45 mg	Vaginal pessaries	14-18	Immature animals may require higher dose
Methyl-acetoxyprogesterone (MAP)*	60 mg	Vaginal pessaries	14-18	
$PGF_{2\alpha}$	4 to 10 mg	IM	1	At mid-cycle
Cloprostenol	100 to 250 mg	IM	1	At mid-cycle

*MAP: also referred to as Medroxyacetate progesterone (Hafez, 1993) or Medroxyprogesterone acetate (Peters & Ball, 1987).

During the breeding season, synchronization may be achieved by overriding the goat's system with exogenous progesterone to prolong a cycle. Progesterone used to prolong the estrous cycle can be supplied in several forms. When fluorogestone acetate (FGA, 45 mg) impregnated vaginal pessaries are implanted for 16 to 21 days (preferably with administration of 400 to 500 IU of PMSG at the time of removal), ovulation has been successfully induced with 55 to 90 per cent fertility. Estrus occurs 20 to 40 hours after sponge removal. To avoid the difficulties sometimes associated with pessaries (the evident mucopurulent vaginal discharge at the sponge removal, possible extraction of sponges by others goats in the pen), removable subcutaneous implants of 375 mg progesterone or 60 to 70 mg MAP in silastic tubing have been used instead (Smith, 1986).

An intra-vaginal device consisting of 9% (w/v 0.38 g) progesterone (Fig. 3-1), moulded over a nylon core called Controlled Internal Drug Release dispensers (CIDRs) have been claimed to have a much less foul-smelling discharge (mucus) on removal. CIDRs also has a lower rate of loss during treatment and a quicker reaction time, i.e. earlier and more compact synchronization, when compared to the intra-vaginal progestagen sponge in sheep (Harvey *et al.*, 1984; Welch *et*

al., 1984). A portion of a bovine norgestomet implants (3 mg/goat) described above is another alternative.

Outside the breeding season, progestagen vaginal pessaries have been used with variable results. FGA sponge implanted for 16 to 21 days can be used successfully (40 per cent conception with AI) and administration of 400 to 600 IU of PMSG 48 hours before sponge removal improves fertility up to 65 per cent conception rate (Smith, 1986).

3.3 FERTILIZATION

3.3.1 NATURAL BREEDING

In wild animals, there is a well-defined breeding season when both sexes show sexual activity. Many wild species of *bovidae* are seasonal breeders. The change in the daily photoperiod is the cue for the onset or termination of ovarian activity. Spring and summer in these species are the most suitable times of the year for calving. During the course of domestication, both dairy and beef cattle were selected against seasonality, facilitating them to ovulate and conceive throughout the year. However, beef cows might remain sensitive to photoperiodicity. A seasonal variation of fertility in temperate climates only becomes evident after studying a large number of herds over a period of several years (Hafez, 1993).

Some goat breeds are seasonally polyestrous so that the young are born during the most favorable time of the year, namely the spring. The length of the sexual season varies with day length, breed and nutrition. This seasonality is governed by photoperiodicity with estrous activity commencing during a period of decreasing day length. In temperate zone latitudes, most breeds of goats are anovulatory and in anoestrus during spring and summer, but start cycling as the length of daylight decreases during fall. In the tropical zones, where there is less variation in day length, indigenous goats tend to breed throughout the year. High environmental temperatures and lack of feed may restrict sexual activity during some months of the year in the tropics. In sheep and goats there are important breed differences in the duration of the sexual season (Hafez, 1993).

Although periods of complete anestrus were never observed within the Boer goat herd, it would appear that the Boer goat doe is seasonally polyestrous, with an extended breeding season. In the southern hemisphere, the seasonal pattern displays the peak of sexual activity during April

and May (autumn) and the period of lowest sexual activity from October through January (late spring to mid-summer), (Hofmeyr *et al*, 1966; Greyling, 1988).

In farm animals (cows, goats), mating is limited during estrus, coinciding with the time of ovulation. The duration of estrus is species-dependent (32 to 40 hours in goats and 18 to 19 hours in cows) and varies slightly from one female to another within the same species (Table 3-5). This is also true in respect to the time of ovulation, which occurs 32 to 36 hours from the beginning of estrus in the goat, 10-11 hours after the end of estrus in the cow. The length of estrus and time of ovulation also vary in relation to internal and external factors. In ewes the interval between the onset of estrus and LH ovulatory surge (and therefore the interval between estrus and ovulation) lengthens as the number of ovulation increases (Hafez, 1993).

In the mature Boer goat, the mean duration of a natural estrous period was recorded as 37.4 hours, with a variation from 24 to 56 hours between animals. The mean time of ovulation in Boer goats (from the onset of the estrous period) is 36.8 hours (86.7% of does ovulating 38 hours after the onset of estrus). The mean time interval between the onset of estrus to the LH peak is 11.6 hours (Greyling, 1988).

Goats have fairly high fertilization rates after superovulation. The general rate of ovum fertilization for goats tends to be higher than for sheep, and natural mating of superovulated does is generally successful. Fertilization rates after artificial insemination of superovulated does have not been as high (Bondurant, 1986).

Table 3-5 Estrous cycle, estrus, and ovulation in farm animals (Hafez, 1993)

	Length of estrus cycle (days)	**Duration of estrus (hours)**	**Time of ovulation**
Sheep	16-17	24-36	30-36 hours from beginning of estrus
Goat	21 (Also short cycle)	32-40	
Cow	21-22	18-19	10-11 hours after end of estrus

3.3.2 ARTIFICIAL INSEMINATION

Artificial insemination (AI) is the most important single technique devised for the genetic improvement of animals. This is possible because a few highly selected males produce enough spermatozoa to inseminate thousands of females per year, whereas only relatively few progeny per selected female can be produced per year even by embryo transfer. AI has many advantages to offer the dairy farmer but the problem of estrus detection limits its value in beef herds (Peters

& Ball, 1987). Major advantages of AI as opposed to natural service include genetic improvement, venereal disease control, cost effectiveness, and safety through elimination of dangerous males on the farm, and in cows, flexibility (Peters & Ball, 1987). In spite of these advantages, AI is used in probably only 5 per cent of beef cows in the UK and in the USA, whereas most dairy herds use an AI service. Most of the world's population of sheep and goats is managed under free ranging conditions where natural mating is widely practised. Unlike with cattle, AI of sheep and goats has been generally limited, owing to the high cost of labor, difficulty of accurately identifying superior sires, and low conception rates, especially with frozen semen (Hafez, 1993).

CHAPTER 4

EMBRYO RECOVERY AND EVALUATION

4.1 EMBRYO RECOVERY

Embryos can be collected from the oviducts or uteri after the slaughter of the animal or the excision of the reproductive tract, or they can be removed either surgically or non-surgically from the intact animal (Hafez, 1993). Prior to 1976, most bovine embryos were collected via mid-line laparotomy or, less commonly, via a flank incision. In that year, several groups published efficacious methods for non-surgical (transcervical) recovery of embryos, and the industry changed to these procedures rather abruptly (Betteridge, 1977). Ova representing 40 to 80% of the corpora lutea can usually be recovered from superovulated, intact animals. However, recovery rates are slightly higher from excised tracts (Hafez, 1993). For many applications, non-surgical techniques for the collection of ova are desirable, because all surgical techniques may lead to the formation of adhesions to the reproductive organs. There is also less risk to the life and the health of the donor with the non-surgical methods (Hafez, 1993).

In goats and sheep, the majority of embryo recoveries in multiple ovulation and embryo transfer schemes are carried out via mid-ventral laparotomy under general anaesthesia. The limitations of current superovulation treatments require that females on genetic improvement programs be flushed repeatedly. The formation of adhesions following surgical embryo recoveries severely limits the number of times an animal can be flushed, and often compromises its ability to breed naturally. It was for this reason that non-surgical laparoscopic procedures to repeatedly recover embryos were developed at the Rowett Research Institute (McKelvey et *al.*, 1986).

4.2 EVALUATION OF EMBRYOS

4.2.1 EMBRYOLOGICAL TERMINOLOGY

The terminology of embryo transfer can be confusing, even to the scientists. One of the reasons for confusion is that different terms are used in different countries. Following are some terms used commonly in the field of embryo transfer as explained by G E & S M Seidel and R P Elsdein in their books Embryo Transfer in Diary Cattle (1989) and Training Manual for Embryo Transfer in Cattle (1991).

In mammals, the female gamete ovulated is called an **egg** or **ovum** (Latin word for egg); the correct technical term for the newly female gamete is an **oocyte**. Upon fertilization, the oocyte matured into ovum becomes a one-cell embryo, sometimes referred to as a zygote. The embryo then divides into two cells, four-cells, eight-cells. At the 16-cells stage, the embryo becomes a **morula** (Latin for mulberry). When a cavity (blastocoele) forms between the cells of the embryo, it is termed a **blastocyst.** To add further confusion, all of these stages are frequently called eggs or ova. During the morula stage, cells of embryos change from spherical to polygonal in shape. This phenomenon is termed compaction (compacted morula). Frequently, **compacted morulae** are termed tight morulae. Compacted morulae are smaller than pre-compacted embryos. Compaction is an excellent sign that the embryo is developing normally; lack of compaction by the sixth day after estrus in cattle and goats indicates retarded development. As a morula develops into a blastocyst, it forms a cavity (the blastocoele) by expending energy to pump fluid between the cells. Thus blastocyst formation also is indicative of continued normal embryonic development. Conversely, lack of blastocoele formation by seven days after estrus in cattle and goats signifies retarded development.

The zona pellucida is a gelatin-like capsule that surrounds the oocyte and early embryo. It has receptors for sperm that are inactivated after fertilization; it keeps the cells of the pre-compaction embryo together, and protects these young cells from the immune system and from pathogens. If the zona pellucida is removed from pre-compaction embryos, the cells come apart upon embryo transfer and then degenerate. When the bovine blastocoele becomes very large, the embryo expands (normally 8 to 9 days after estrus), which thins the zona pellucida. This is the expanded blastocyst stage. After one to one days more, the expansion is so great that the embryo hatches out of the zona pellucida, perhaps aided by enzymes. A **hatched blastocyst** become ellipsoid in shape 11-13 days after estrus and then elongates markedly by 14-16 days post-estrous.

4.2.2 EVALUATION

One problem is terminology for evaluation of ova recovered from donors. They can be classified based on appearance as **unfertilized** or **fertilized**, and as ranging in quality from degenerated or very poor to excellent. The exact terminology varies considerably among technicians who evaluate embryos. Frequently embryos are classified on a scale of one to three, one to four or one to five, but for some systems one is best and for other systems one is worst. In the end, however ova always are placed into two groups, those not worth transferring and those worth transferring; the later are said to be **"transferable"** (Seidel & Elsden, 1989).

Morphological microscopic appraisal is the most practical criterion for evaluating embryos. The microscopic inspection of ova and embryos at 70X magnification permits classification of uncleared, degenerated, delayed, or regular embryos (Baril *et al.*, 1989). In summary, they are considered normal if blastomeres are uniform in size and spaced regularly within the zona pellucida. Quality is assessed by the stage of development, integrity of the zona pellucida, nature of the shell surrounding the embryo, color and texture of the cytoplasm of the embryo.

There are three elements to successful evaluation of embryos: training, experience, and proper equipment. Training includes learning the correct morphology of embryos at different times post-estrous (Table 4-1). One must also learn how to manipulate and examine embryos. Experience is gained by examining many embryos at different stages of development. Ideally hundreds of embryos should be studied under the guidance of someone experienced in this area. Photographs, drawings or slide of different kinds of embryos are useful. However, they can only substitute partially for real embryos. As for the equipment, with a good stereomicroscope at X30 to X40 magnification or less, an experienced technician can evaluate more than ninety percent of embryos accurately. However, a small percentage of embryos require a compound microscope (at least a X10 objective with X8 to X20 eyepieces) for accurate evaluation. For learning purposes, a compound microscope is especially useful.

It is important to be able to recognize the various stages of development and to compare these with the developmental stage that the embryo should be at, based on the occurrence of heat (Table 4-1).

Table 4-1 Stages of embryonic development at various times after estrus

Stage of embryonic development	**Days after ovulation, by species**	
	Cow	**Goat**
1 Cell	0-1	0-1
2 Cell	0-2	0-1
4 Cell	1-2	1-2
8 Cell	2-4	2-3
Early morula (EM)	3-5	2-4
Compacted morula (CM)	4-6	4-5
Early blastocyst (EB)	6-7	5-6
Blastocyst (B)	6-8	6-7
Expanded blastocyst (EB)	7-9	7-8
Hatching blastocyst (HB)	8-10	7-9

CHAPTER 5

MATERIAL AND METHODS

5.1 ESTROUS SYNCHRONIZATION

5.1.1 SYNCHRONIZATION PRODUCTS

Synchronization was performed by the administration of progestagen alone or in combination with estradiol depending on the superovulation program used. Four products were used: Synchro-Mate-B (SMB)/Crestar and Controlled Internal Drug Release (CIDR), as progestagen implants; Estradiol Cypionate (ECP) and Estrumate (Cloprostenol), a prostaglandin analogue, were given as luteolytic agents.

Synchro-Mate-B (SMB)/**Crestar** (Intervet SA. 86/2 86/1) consists of a subcutaneous ear implant impregnated with 3-mg norgestomet, inserted by means of a special syringe on day 0 (Fig. 5-1). In this study, a subcutaneous ear implant containing 3 mg (SMB x 1) or 6 mg (SMB x 2) of norgestomet, depending on the superovulation program used, was inserted for 13 to 17 days. This norgestomet treatment was in combination with either ECP on day one or no ECP administration. The principle of the insertion and the removal of the implant of the goat are similar to that shown in cows (Fig. 5-1). The Crestar implant was removed on day 13 by making a small scalpel incision in the skin of the ear at the implant entry point, and pulling the implant out of there by means of haemostat forceps. In some cases the spot of the needle entrance at the time of implant was slightly swollen and infected, easing the pulling out of the implant removal without the need of any incision. To avoid such infection, the spot of implant behind the ear was carefully disinfected with 70% alcohol prior to the implant.

Controlled Internal Drug Releases (CIDR) (EAZI-breed CIDR G, Solvay A.H) device consists of a silicone rubber elastomer moulded over a nylon spine, containing 0.33 grams of natural progesterone. The device was administered intravaginally using the specifically designed applicator. Placed in the applicator rod hole through its tip, the wings of the device folded inward and were held in the folded position by the applicator. Squeezing of the plunger handle expelled the device in the anterior part of the vagina. When in place, the nylon string attached to the device protruded from the vulva. Between each insertion the applicator was plunged in an antiseptic solution Rocal II (quantenary ammonium compound, QAC), and then rinsed in clean water to prevent any vaginal infection spreading from one animal to the next one. The applicator

was coated with lubricant when needed (e.g. for a doe with a restricted vaginal opening) to ease the applicator penetration. The removal at the end of the required time, according to the program used, was achieved by gently but firmly pulling on the nylon string of the device. CIDR G was used in a single dose (CIDR x 1) or in a double (CIDR x 2), either in combination with estradiol at the device insertion or not.

Estrumate (Cloprostenol, Hoechst Ag-Vet) is a synthetic prostaglandin $F_2\alpha$ which is a potent luteolytic agent consisting of 250 μg per mL of the solution. In this study 0.5 mL of Estrumate (125 μg) was administered intramuscularly 12 hours before progestagen implant (SMB or CIDR) withdrawal.

Estradiol Cypionate (ECP, Upjohn) consists of 2 mg per mL of estradiol cypionate. In this study 0.25 mL (0.5 mg) was administered intramuscularly on day one and used for two reasons: 1) to destroy the CL within its first 4 days of life (during metestrus stage); 2) to stop follicular wave development to allow the stimulation of a new wave of follicular development before the dominant follicle has been identified that will suppress the development of inferior follicles.

5.1.2 SYNCHRONIZATION TREATMENTS

Goats (n=367) were assigned to different synchronization treatments using 2 different products SMB (Crestar) and CIDR as shown in the table below.

Table 5-1 Summary of different synchronization (Synchro) treatments used during the experiment

Synchro groups	Synchro drug	Dosage & administration	Other treatment	Symbol used	Number of goats
1	SMB	3 mg norgestomet inserted from 13 to 17 days.	No farther treatment	SMB x 1	123
2	SMB	3 mg norgestomet inserted for 10 days.	One more implant of 3-mg norgestomet inserted from day 10 to17.	SMB x 2	32
3	CIDR	0.33 g of natural progesterone inserted from 13 to 17 days.	No farther treatment	CIDR x 1	187
4	CIDR	0.33 g of natural progesterone inserted for 9 days.	One more implant of 0.33 g of natural progesterone inserted from day 9 to 17.	CIDR x 2	25

Estradiol Cypionate treatments were given to ECP group 1 goats (n=112) while ECP group 2 goats (n=255) did not get ECP treatments as shown in the table below.

Table 5-2. Summary on the use of Estradiol cypionate treatments during the experiment

	ECP use groups	Dosage & administration	Symbol used	Number of goats
1	Estradiol Cypionate (ECP) was used	0.5 mg of ECP injected intra-muscularly on day one after implant insertion	ECP used	112
2	Estradiol Cypionate (ECP) was not used	-	No ECP	255

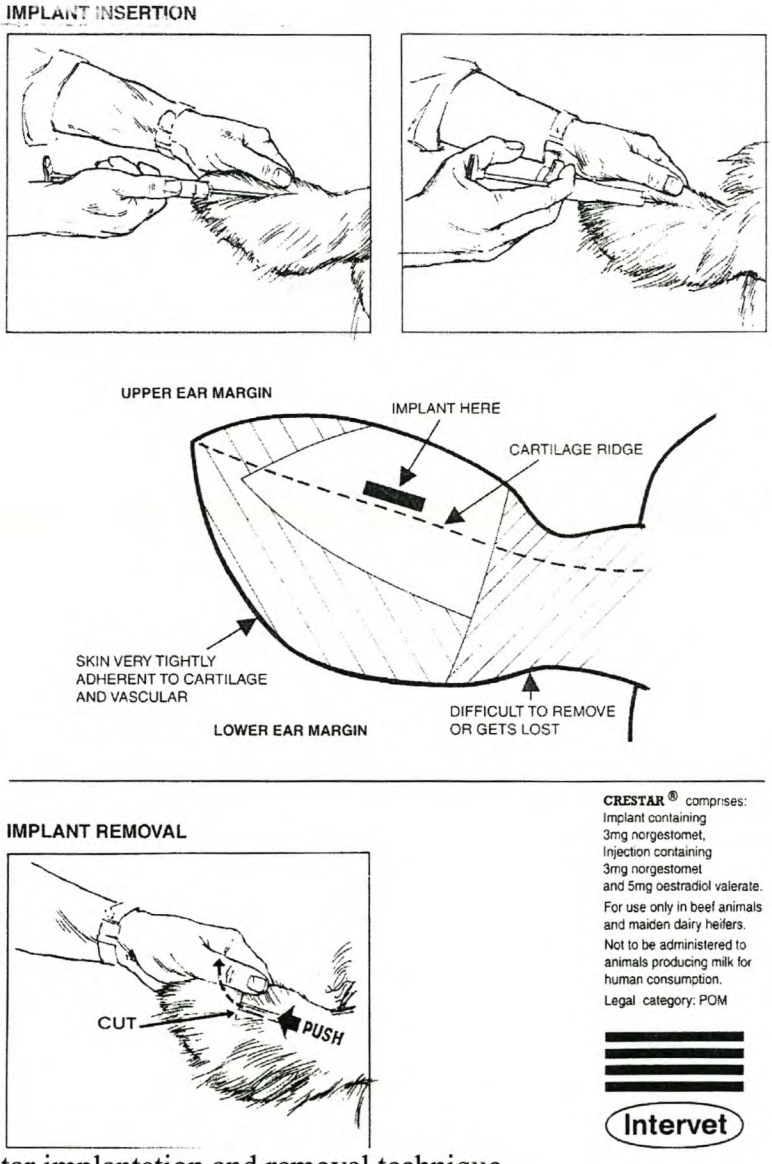


Fig. 5-1 Crestar implantation and removal technique

5.2 SUPEROVULATION

5.2.1 SUPEROVULATION PRODUCTS

Two ovine FSH preparations, Embryo-S (E-S) and Ovagen (Ov) were used. These products were either used alone or combined with PMSG, depending on the superovulation program used.

Pregnant Mare Serum Gonadotropin (PMSG) (Fostim, Upjohn) was administered intramuscularly as a single dose of 300 IU, 48 to 72 hours prior to the implant removal. Supplied as a white-dried crystalline powder of serum gonadotropin, the product was protected from light and was only reconstituted with distilled, sterile water immediately prior to use.

Ovagen ™ (ICP, NZ Pat 220169) is a follicle stimulating hormone preparation produced from ovine pituitary glands and freeze-dried immediately following purification to protect its potency. Each vial contains the equivalent of 18 mg *NIADDK-oFSH-17 ovine pituitary FSH (1 mg NIADDK-oFSH-17 is 20 units **NIH-FSH-S1). Each vial of Ovagen ™ was reconstituted with 20 mL of sterile isotonic saline diluent immediately prior to use. A total of 10 mL of Ovagen™ alone or in combination with a single PMSG dose was injected intramuscularly (8 or 10 mL), in a four-day period, injected twice daily. Protected from sunlight, the PMSG reconstituted solution was used once, and any partially used vials were destroyed.

* National Institute of Arthritis, Diabetes and Digestive and Kidney Diseases, USA Ovine Pituitary FSH Standards.

**National Institute of Health

Embryo-S (Jurox, Aus. Pat. 601421) is a purified ovine FSH, lyophilized to maintain its potency under normal storage conditions (below 25 degrees). Embryo-S was available in two sizes: one size contained 25 units NIH-FSH-S1 with 10 mL of sterile diluent each, and the second contained 50 units NIH-FSH-S1 with 20 mL of sterile diluent. The sterile isotonic saline diluent contains sodium chloride solution. Once reconstituted, the solution was immediately used or stored at 2 - 8°C and any unused solution was discarded after four days. Embryo-S was used either as 1) 8 to10 mL (25 units) by intramuscular injection given in a single dose or fractioned in 8 doses of 1.25 mL every 12 hours during 4 days, in combination with 300 or 400 IU of PMSG, 48 or 72 hours prior to the progestagen removal or 2) 10 mL fractioned in 8 doses were given without PMSG.

5.2.2 SUPEROVULATION TREATMENTS

Goats (n=367) were assigned to four different superovulation treatments (SUPOV-T) using 2 different products, Ovagen and Embryo-S. Each of these products was either used alone (E-S alone or Ov alone) or in combination with PMSG (E-S + PMSG or Ov + PMSG) as shown in Table 5-3.

Table 5-3 Summary of different Superovulation (Supov) treatments used during the experiment

Supov* group	Supov* drug	Dosage & administration	Associated with	Symbol used	No of goats
1	Ovagen	9 mg i.m. every 12 h, 8 times, starting 72 h prior to implant removal.	None	OV alone	147
2	Ovagen	9 mg i.m. every 12 h, 8 times starting 72 h prior to implant removal.	PMSG 300 IU i.m., once 48 to 72 h prior implant removal.	OV + PMSG	164
3	Embryo-S	25 units i.m. twice a day, 1 or 8 times, 72 h prior implant removal.	None	E-S alone	16
4	Embryo-S	25 units i.m. twice a day, 1 or 8 times, 72 h prior to implant removal.	PMSG 300 IU i.m., once 48 h to 72 h prior to implant removal.	E-S + PMSG	40

* Supov = Superovulation

5.3 FERTILIZATION

5.3.1 NATURAL SERVICE

Most of the does were naturally bred during this study. Because does mated more than once are more likely to conceive than those mated only once, they were introduced in the buck pen twice a day (morning and evening) for three days. The first attempt at mating was performed 12 hours after the progestagen withdrawal. Only one buck was ascribed to a particular doe during the 3 days of mating. In some cases, the does were left in the buck pen overnight from the second to the third day and removed the following morning.

5.3.2 ARTIFICIAL INSEMINATION

5.3.2.1 Semen collection

The collection of semen by artificial vagina (AV) was the method of choice, since the technique was successful in most of the trained bucks and representative ejaculates were obtained. Alternatively, the electro-ejaculator was sometimes used in untrained males or those that refused

to serve the artificial vagina. The AV consists of a stiff plastic tube casing about 15 cm long and 7 cm in diameter, with an air/water-filling valve in its wall. A cylindrical synthetic latex sleeve of about 20 cm long and about 5 cm diameter was located inside the tube and both ends of the rubber sleeve folded over the ends of the tube. The space between the tube and the rubber sleeve was then filled with water at about 55°C until the space was almost full. The inner wall of the rubber sleeve was lubricated on one side with a non-toxic sterile gel. In order to create appropriate pressure during the simulated copulation, the space between the tube and the synthetic rubber sleeve was further filled with water and air through the valve until the inner lumen of the artificial vagina was occluded, allowing only one finger to be inserted. The inner lumen temperature was monitored to an optimal temperature of 40 to 45°C. A synthetic rubber cone with an attached glass tube was placed over the non-lubricated end to channel the semen into the tube for collection. In order to prevent the spermatozoa from cold temperature shock, the semen collection tube was covered with the palm of the hand.

The semen collection with the AV was made during attempts by a given buck to breed a superovulated doe in which estrous behaviour was observed after progestagen withdrawal. An assistant was needed to hold the doe in position while the semen was being collected. The collection site had enough space for freedom of movement for the buck, the assistant, and semen collector. Prior to the collection, dirt and debris were gently removed from the buck's prepucial orifice by means of a sterile paper towel. The collector then knelt laterally to the doe's hindquarters, on the same side of the doe as the hand holding the artificial vagina. The buck was led to the doe's hindquarters where, in first response, he sniffed the perineal region and began to protrude his penis, then mounted by grasping the doe's hindquarters and began short pelvic thrusts in attempts to bring the extended penis into contact with the vulva. During these attempts, the collector gently grasped the prepuce and diverted the tip of the penis into contact with the artificial vagina. Ejaculation occurred with pronounced pelvic thrusts, while the collector still held the AV at the prepucial orifice as the buck dismounted. The AV was held at an angle downwards towards the glass tube to allow the ejaculated semen to flow into the tube. After semen collection, the buck and doe were separated in order to avoid any reinitiated breeding attempts.

An artificial vagina is preferable to the others methods of collection if time can be afforded for the initial training period, which may occupy several weeks. This was not the case with bucks in this study as they were accustomed only to free natural service. Consequently, the inability or unwillingness of certain bucks to use the AV forced us to use the electro-ejaculation methods, in

order to save time. The electro-ejaculator apparatus used consisted of a stimulator powered by batteries connected to a probe via conductor wires. An assistant restrained the buck either in standing or in lateral recumbence. The probe was then introduced in the buck's rectum with the electrodes positioned toward the pelvic floor by a second assistant while the semen collector was manually holding the shaft of the penis (cleaned with a paper towel) inserted in a dry, clean plastic collection tube. A rhythmic and increasingly intensified application of electric current was administered, with the current on for 2 to 3 seconds and off for 2 to 3 seconds. The extension of the penis followed by ejaculation was the expected reaction.

5.3.2.2 Semen evaluation

Visual assessment of the motility of spermatozoa is one of the most rapid and widely used tests of semen quality. It is the one that was used during this study. The semen sample was kept warm (35 to 38°C) in order to avoid any temperature shock, and motility was assessed immediately after collection. A drop of semen was placed on a warmed slide and examined under a microscope at low magnification (X10 objective). The wave motion was assessed in a system where the absence of wave indicated bad semen with motionless spermatozoa, and dense, rapidly moving waves signified good semen with 90% or more spermatozoa being alive and active.

5.3.2.3 Semen dilution

Semen diluents provide some nutrient for the metabolic processes of the sperm, creates pH stability, protect against the cold shock, and also make possible the extension of the volume of semen to allow many females to be inseminated. A variety of extenders have been utilized to dilute ram and buck semen for use in the short or long term. Most semen diluents utilize egg yolk or milk as the basic ingredient, which protects the spermatozoa against the cold shock by the presence of the lipoprotein and lecithin in the yolk and the casein in the milk. While both egg yolk and milk can be used in sheep semen, only skimmed and ultra heat-treated fat-free milk should be used to extend goat semen. Indeed, the presence of phosphotidase in the seminal plasma of goats catalyses the hydrolysis of lecithin in egg yolk into fatty acid and lysolecithins, which are toxic to the spermatozoa. Otherwise, for goats, diluents with a low concentration of egg yolk (≤1.5%) can be used, or the seminal plasma, containing phosphotidase, should be removed by centrifugation and washing (Wallace, 1992)

In this experiment, however, the dilution of the semen was made with fat free ultra high temperature treated (UHT) milk at the rate of 1:4. Therefore with about 1 mL semen collected

per ejaculate, we would add 4 mL of UHT milk to make 5 mL of the extended semen that would serve up to 20 does at the dose of 0.25 mL per doe. The refrigerated milk was warmed in hot water to 38-40°C before diluting the collected semen. The extended semen was kept in warm water (around 35°C) contained in a polystyrene box, and the temperature was closely monitored with a thermometer, during the insemination process.

5.3.2.4 Insemination procedure

Artificial insemination was only used in one site (at the AES station) during the experiment. Cervical insemination was the method used. A pre-warmed insemination pipette, or a catheter attached to the syringe was loaded with about 0.25 mL of semen and made ready for introduction into the cervix. The assistant restrained the doe with her hind legs lifted over a 60 to 80 cm high bar. When the doe was in position, the vagina was wiped clean using tissue paper. A lubricated speculum was then inserted into the vagina and the cervix brought into view with the aid of a headlamp. The catheter or pipette, protected from the cold shock, in a sterile plastic sleeve covered by a clean cloth, was released from there and the tip was introduced gently with one hand about 1 cm, and the plunger was slowly depressed to release 0.25 mL of semen into the cervix (see Fig. 5-3).

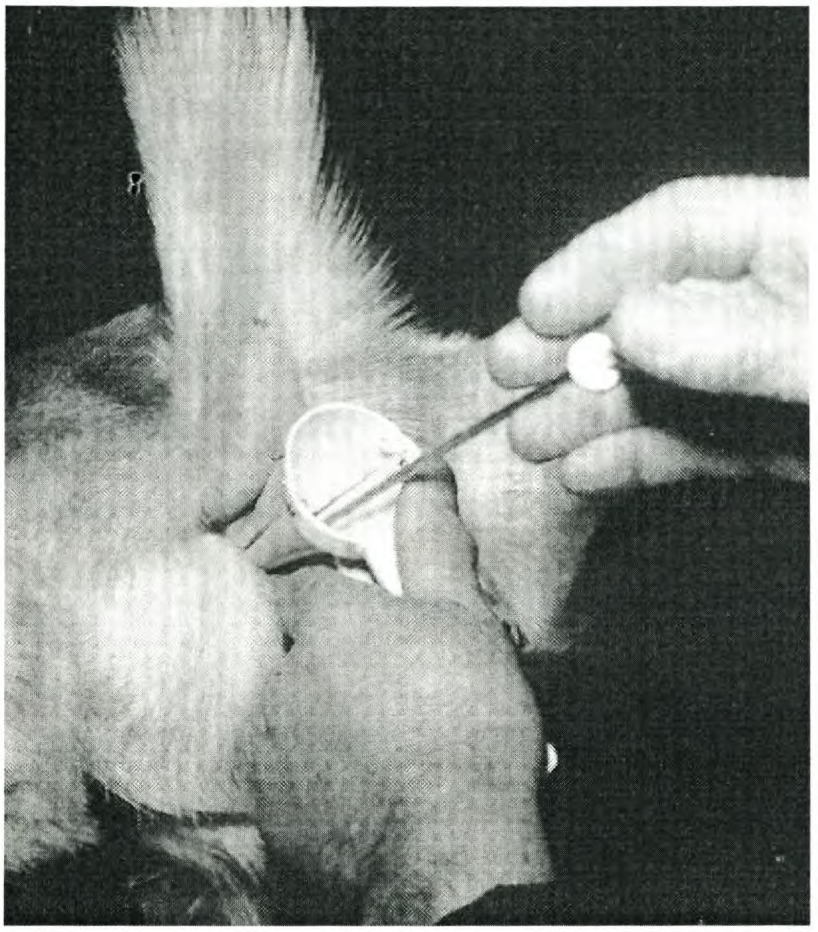


Fig 5-2 Cervical insemination in goats

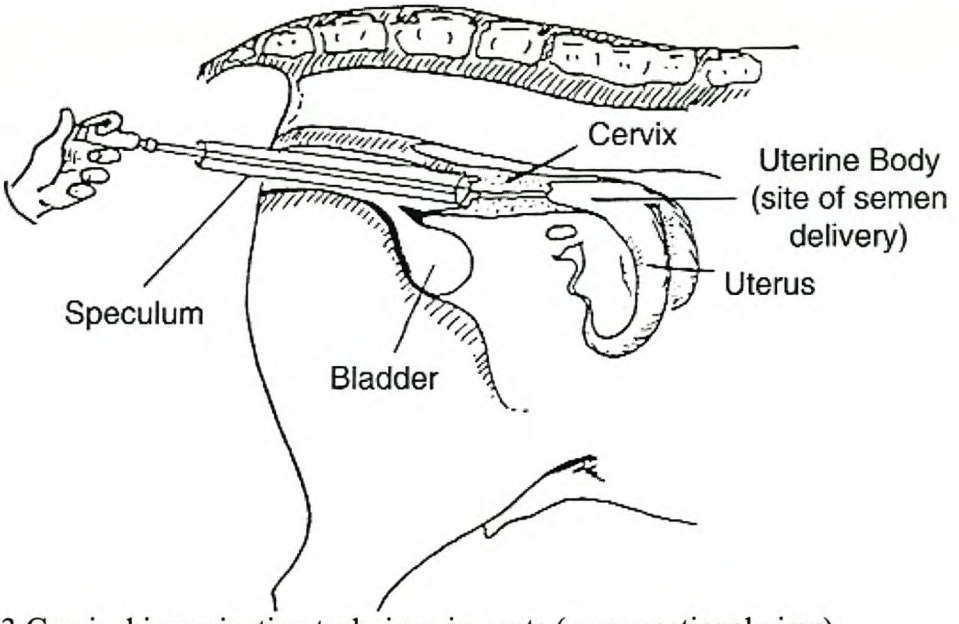


Fig 5-3 Cervical insemination technique in goats (cross sectional view)

5.4 EMBRYO RECOVERY

5.4.1 MEDIA USED FOR EMBRYO COLLECTION

Medium type and quality is a critical factor in an ET program. The medium must be free of any contamination and should be sufficient to maintain embryo viability. Dulbeccos Phosphate buffered saline (D-PBS) was the medium used during this study in the embryo collection, storage, micromanipulation, and freezing process.

5.4.1.1 Media mixing

PBS used was purchased as a pre-weighed powder. The calcium chloride for powdered D-PBS was supplied in a separate packet. Powdered D-PBS was first dissolved in double-distilled water contained in a flask. Then the calcium chloride, dissolved separately in double distilled water, was added to the former solution after the contents of the first packet were completely dissolved. Failure to completely dissolve the powder from the first packet prior to adding the calcium chloride would cause the formation of a precipitate. After all the powder was dissolved, the medium was generally used at once. One packet of D-PBS was used in every 1000 mL of double-distilled water.

5.4.1.2 Macromolecules and antibiotics

Lyophilized newborn calf serum to which antibiotics (100 iu of penicillin and 100µg of streptomycin per mL of mixed solution) were added, was used in collection and holding media as a macromolecule. The main function of the calf serum was to coat plastic and glassware to keep the embryos from sticking to them due to surface tension and electrical polarity. After any protein (serum or bovine serum albumin) is added to D-PBS, it becomes an excellent growth medium for bacteria and other microorganisms. For that reason, protein combined with antibiotic was added just prior to use in a proportion of 1% (10 mL of protein for 1000 mL of the medium) for the collection medium. Unused medium containing protein was not stored longer than 24 hours in the refrigerator.

In summary, the following were used for the collection medium:

1) 1 litre phosphate buffered saline (PBS)
2) 100,000 IU penicillin
3) 100,000 IU streptomycin
4) 10 mL calf serum (1%)

5.4.2 EMBRYO COLLECTION TECHNIQUE

The surgical laparoscopic method was used during this experiment. The procedure was essentially equivalent to a surgical uterine flush, except that all manipulations of the reproductive tract, during the checking of CL numbers, were carried out with the aid of grasping forceps, and fine trochars were used. However, exteriorization of the tract at laparotomy, for the purpose of embryo collection, inevitably involved some degree of surgical trauma, and often leads to the formation of post-operative adhesions involving the uterus and ovaries. The following steps were taken:

5.4.2.1 Anaesthesia

Food and water were withheld 24 to 36 hours prior to the embryo collection of the animals. To facilitate manipulation of the animal, general anaesthesia was used. Anaesthesia was induced by injecting about 15 to 25 mg of thiopentone sodium (Intraval Sodium, Rhone Poulenc) per kg of body mass in the vena jugularis in the neck region (1 g Intraval Sodium dissolved in 20 mL water would anaesthetized a 70-kg doe). Thiopentone sodium is a short-acting barbiturate employed as a general anaesthetic for short surgical procedures, or in this case as a basal anaesthetic before the use of inhalation.

An endotracheal tube was placed through the pharynx and larynx into the trachea with the aid of a curved stainless steel rod. The rod was removed, the cuff inflated with about 20 mL of air, and the air inlet valve closed. This was done to prevent any pharyngeal contents that could possibly be vomited during anaesthesia from entering the lungs and to keep the tracheal tube in position. The tube was taped to the mouth with masking tape to secure the tube position. The doe was then placed in a specially designed cradle on its back with legs securely tied to the cradle bars over the Achilles tendons. The cradle was made so that it could be inclined at an angle of about 30 to 45°C to the horizontal. Then the end of the Y-piece of a fluothane anaesthetic machine was connected to the endotracheal tube, and the gas (fluothane) setting put at around 2%. The fluothane (Halotane, ICI) gas was then increase to 3% or 4% depending on the need of the animal. Oxygen flowed through the system at a rate of about 0.5 litre per minute. After

restraining the animal on a surgical table in dorsal recumbency, the head was held down at a 45% angle to the horizontal so that the hindquarters were above the head. The abdominal area anterior to the udder was shaved, washed and disinfected.

5.4.2.2 Surgery

Three stab incisions were made approximately 3 to 4 cm on either side of the mid-line. The first was 25 cm anterior to the udder for the laparoscope, the second was about 15 cm anterior to the udder for the insertion of the uterine grasping forceps. Prior to the insertion of the laparoscope the peritoneal cavity was insufflated with approximately 4-5 litres of air. After establishment of the pneumoperitoneum (insufflation of the abdominal cavity), the laparoscope was inserted, the uterus and ovaries located, and ovarian response was recorded by counting the number of corpora lutea. While viewing the uterus through the laparoscope, the tip of the horn of the uterus was grasped with Allis tissue forceps and exteriorized through a mid-ventral line incision (5-10 cm). The incision was made cranial to the udder attachment to allow elevation of each side of the uterus body and horn. The forefingers of the right hand were inserted into the abdominal cavity and directed downwards and backward to locate the uterus, which was recognized by its firm horns and pink color. The uterus was withdrawn from the abdominal cavity. From then on, the uterus was kept wet with a saline-heparin-antibiotics solution. The reproductive tract was flushed with D-PBS as already described.

5.4.2.3 Flushing

Uterine flushing was the technique used for embryo-collection (Fig. 5-4 and 5-5). A puncture wound was made into one uterine horn at the uterine bifurcation (1), using a pair of closed artery forceps. The hole made was slightly widened by opening the end of the forceps in the hole. A special glass cannula, 25-30 cm long with a double bend, was inserted into the uterine lumen through the puncture to a depth of between 2 to 5 cm and tightly held in that position by means of a grasping modified bowl clamp. A 20-mL syringe (4) fitted with a 20 to 22 G plastic IV catheter was inserted at the utero-tubal junction (3). The needle supporting the catheter during the insertion was removed. Twenty millimeters of flushing media was injected through the IV catheter, while the assistant was massaging the tip of the uterine horn, and out through the glass cannula. The flushings (flushed media) were collected into a small bowl (glass beaker) (5-5). Occasionally, the fluid did not flow readily and checks were then made to ensure that the catheter and glass cannula were in good position. The syringe was withdrawn and the fluid pressed gently towards the uterine body so that as much flushed medium as possible was collected into the bowl. The glass cannula was then removed from the uterine horn

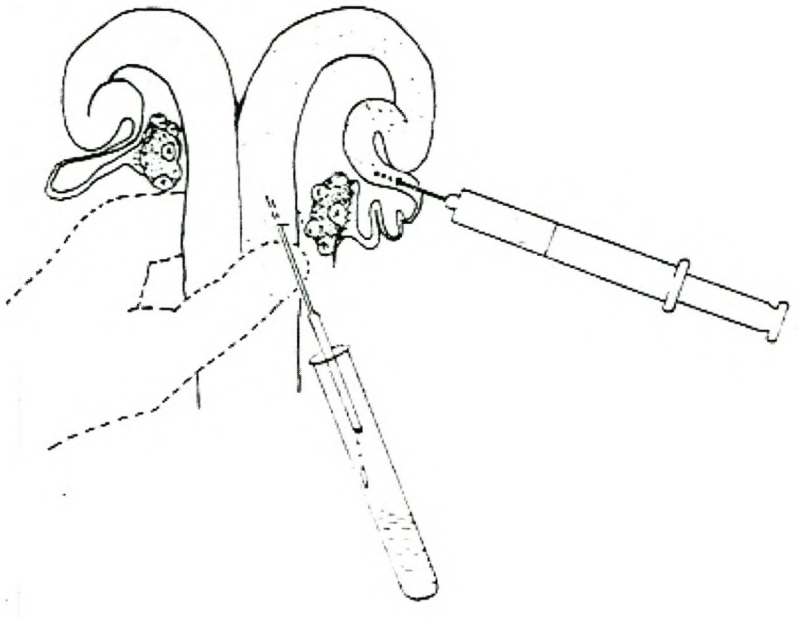
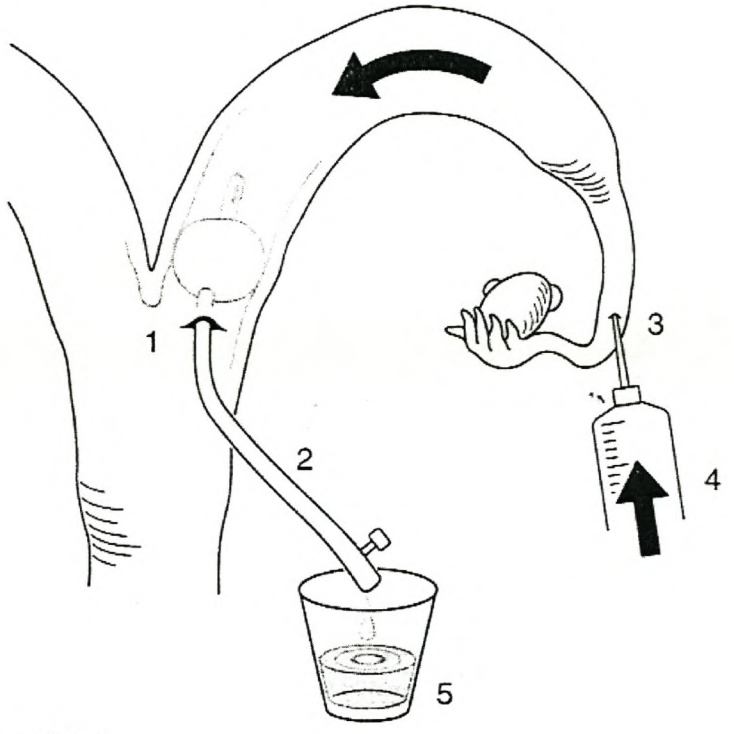


Fig. 5-4 Embryo flushing technique using glass catheter



1 – Insertion point to Foley catheter
2 – Foley catheter
3 – Insertion point for flushing needle
4 – Syringe with flushing medium
5 – Embryo collection vessel

Fig. 5-5 Embryo flushing technique using Foley catheter

and the fluid in it allowed to run into the bowl or into sterile petri dishes for immediate examination and counting under a dissecting microscope by another technician. Then the other uterine horn was treated in a similar manner. As a rosette of endometrium protruded from the puncture wound after catheter removal, this was always closed with an inverted horistal mattress suture, using atraumatic needles and fine catgut. The reproductive tract was irrigated with warm saline, and returned to the abdominal cavity.

Care was taken throughout the surgery to minimize postoperative development of abdominal adhesions. To prevent this problem, 50-100 mL of a solution of 5% dextrose with heparine and gentomycin was dripped into the peritoneal cavity before closure. The peritoneum (and the body wall sutured together) and muscles layers were closed by continuous polyglycolic acid suture.

5.4.2.4 Post-surgery

The skin incision was closed with interrupted sutures. The wound was sprayed with wound aerosol. The doe received an injection of 5 mL long acting penicillin. The anaesthetic machine was removed from the endotracheal tube. The doe was placed upright on its sternum, the cuff of the tube deflated and the endotracheal tube removed from the trachea. The doe was allowed to recover quietly from the anaesthetic in a well-bedded room.

5.4.3 ISOLATION OF EMBRYOS

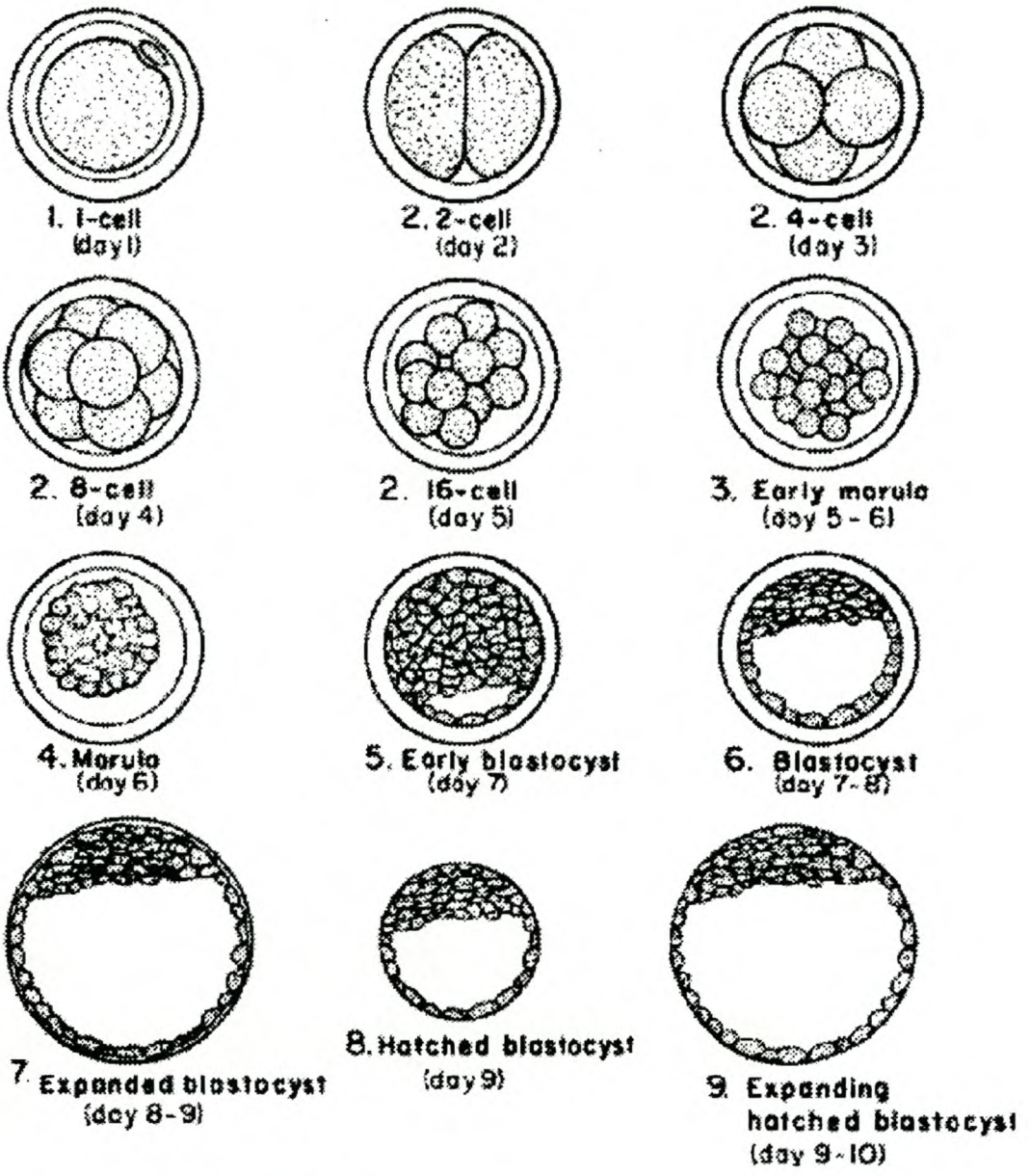
In both cows and goats the method used for isolation of embryos is essentially similar. The filtration method of isolating embryos was used in this study. Flush fluid collected in a glass beaker was poured through the filter unit (Emcon filter) and was allowed to escape through a short length of tubing. The outflow was controlled by means of a clamp. To prevent dehydration of the embryos at least 1 cm of the medium was retained in the filter to cover the filter grid on which the embryos rested. To recover the embryos from the filter, the filter was swirled and the content was poured into a searching dish. Immediately after, the filter was quickly rinsed in concentric circles while holding it partly inverted, moving from the outer rim of the grid to the centre, using a 22 G needle mounted on a 50 mL syringe containing flushing medium without serum or bovine serum albumin (BSA). The omission of protein from the medium for this step was important to prevent foaming when it was ejected from the needle, since embryos are easily lost among the bubbles, which can persist for hours. The sides of the filter and the grid were rinsed several times until all vestiges of mucus and cellular debris was gone. This took considerable rinsing at high pressure.

Medium containing 10 % calf serum was added to the searching dish after the filter was thoroughly rinsed to keep embryos from sticking to the dish or pipette and also serving as a nutrient medium. The flush fluid was examined systematically at about X10 to X15 magnifications to locate embryos and isolate them. A fine pipette was used to move them into fresh medium. The pipette was made from Pyrex glass tubing, with a 4 mm outside diameter. A 15 cm length glass of tubing was heated in the center with a Bunsen burner, and pulled to make an outside diameter of less than 1 mm. This was then scored with a diamond pencil and broken to make two pipettes. All ends were fire-polished. After pipettes had been washed and rinsed thoroughly, they were placed in clean glass test tubes with screw tops and sterilized (and dried) by dry heat. For use, pipettes were connected to a 0.5 or 1 mL syringe or plastic mouthpiece with rubber tubing. Embryos recovered were transferred to fresh holding medium (PBS containing 10 % calf serum) when they were found. As far as labor was available, two people examined each dish twice. Dishes that were not being examined were covered and stored where they would not be exposed to excessive light. As soon as all embryos were located, they were evaluated and immediately prepared for cryopreservation.

5.5 EVALUATION OF EMBRYOS

The procedure for classifying the embryos consisted of isolating them; removing debris (which occurred in the process of washing them); and then separating them into 2 main groups of 1) **Transferable** (or **freezable**), and 2) **Non-transferable** (**Unfertilized Oocyte** or **UFO**, severely degenerated, and early stage embryos).

Evaluation of embryos was done as described by Seidel & Seidel (1991) (Fig. 5-6) at X50 to X100 magnification under a stereoscopic microscope, with the embryos in the 4 well culture dish. Each embryo or ovum was then carefully examined individually by focusing up and down and in certain cases rolling the embryo with a pipette or by swirling the dish. Embryos that were of doubtful quality were discarded. Retarded embryos at the time of collection (4, 8 cell-embryos) were equally discarded. In our laboratory, two criteria were taken in account for the classification of embryos including the stage of its development (as adapted from Table 4-1) and its quality or health status.



1 – Unfertilized oocyte and one cell embryo
2 – Embryo with 2 – 16 cells
3 – Precompacted morula
4 – Compacted Morula
5 – 9 Blastocyst

Fig. 5-6 Stage of embryo development (*Seidel & Seidel, 1991*)

5.5.1 EMBRYO STAGE

In term of this study, we called

- **Morula (M)** a mass of at least 16 cells. Individual blastomeres are difficult to discern from one another after compaction. The cellular mass of the embryo occupies most of the space within the zona pellucida.
- The expression **compact morula (CM)** was individual blastomeres that have joined together, forming a compact mass. The embryo occupies 60 to 70 % space within the zona pellucida.
- **Early blastocyst (EB)** was used to describe an embryo that has formed a fluid-filled cavity or blastocoele and gives a general appearance of a signet ring. The embryo occupies 70 to 80 per cent of space within the zona pellucida. Early in this stage the embryo may appear of very questionable quality.
- **Blastocyst (B)** was characterized by a pronounced and evident differentiation of the outer trophoblast layer and the darker, more compact inner cell mass. The blastocoele is highly prominent, with the embryo occupying most of the perivitelline space. Visual differentiation between the trophoblast and the inner cell mass is possible at this stage of development.
- In the **expanded blastocyst (XB)**, the overall diameter of the embryo dramatically increases, with a concurrent thinning of the zona pellucida to approximately a third of its original thickness.
- **Hatched blastocyst (HB)** was an embryo recovered at the stage in which it undergoes the process of hatching or may have completely shed the zona pellucida. Hatched blastocysts may be spherical with a well-defined blastocoele or may be collapsed. Identification of embryos at this stage can be difficult for the inexperienced operator. Embryos collected for transfer purposes in this study ranged from morula through expanded blastocysts.

5.5.2 EMBRYO QUALITY

- **Grade 1** or excellent was an ideal embryo, spherical, symmetrical and with cells of uniform size, color and texture. Less than 5 % of the blastomeres are visually abnormal.
- **Grade 2** or good was characterized by small imperfections such as a few extruded blastomeres, irregular shape and a few vesicles. Between 5 and 25 % of the blastomeres are visually abnormal.

- **Grade 3** or fair were embryos with more definite problems including presence of numerous extruded blastomeres, vesiculation and a few vesicles. More then 25 % of the blastomeres are visually abnormal.
- **Grade 4** or Poor was embryos with severe problems, numerous extruded blastomeres, degenerated cells, cells of varying sizes, large numerous vesicles but no apparently viable embryo mass. Also included in this category were all the unfertilized oocytes.

In this study, Grade 1 and 2 were considered as transferable embryos, and Grade 3 and Grade 4 were rejected as non-transferable.

5.6 STATISTICAL METHODS AND CONSIDERATIONS

5.6.1 STATISTICAL DESIGN OF THE STUDY

Table 5-4 Summary of the design of the superovulation and synchronization program of this study

Synchronization Products (Synch-P)		**Synchronization Treatments (Synch-T)**		**Total CL (Means)**	**Total Eggs (Means)**	***Transf Embryos (Means)**	**UFOs (Means)**
1	SMB	1	SMB one dose				
		2	SMB two doses				
2	CIDR	3	CIDR one dose				
		4	CIDR two doses				
Superovulation-Products (SupOv-P)		**Superovulation-Treatments (SupOv-T)**		**Total CL (Means)**	**Total Eggs (Means)**	***Transf Embryos (Means)**	**UFOs (Means)**
1	Ovagen	1	Ovagen alone				
		2	Ovagen + PMSG				
2	Embryo-S	3	Embryo-S alone				
		4	Embryo-S + PMSG				

As shown in Table 5-4, the design of this study, in accordance with the objective stated in the introduction, was to assess different superovulation and synchronization treatments in the Boer goat. In exploring the different treatments used, this study attempted to determine the efficiency of the different products used during these experiments namely two superovulation products (Supov-P), Ovagen and Embryo-S; and two synchronization products (Synch-P), SMB and CIDRs, used during the experiments, through their effects on ovulation rate, and fertilization rate of the South African Boer goat (See first column of Table 5-4).

In summary, the study has evaluated and compared four different synchronization treatments (Synch-T) consisting of:

1) One dose of SMB (SMB x 1)
2) Two doses of SMB (SMB x 2)
3) One dose of CIDR (CIDR x 1)
4) Two doses of CIDR (CIDR x 2) (see second column of Table 5-4.) and;

Four superovulation treatments (Supov-T) consisting of

1) Ovagen alone
2) Ovagen associated with PMSG
3) Embryo-S alone
4) Embryo-S associated with PMSG

Because these different treatments took place in diverse locations, environmental conditions shaping the body condition, seasons, and ages of animals, as well as the use of ECP (Estradiol Cypionate), we investigated to what extent these additional factors can impact the different results obtained.

Independent variables (factors) analyzed in this study were:

1) Superovulation treatments (four groups as shown in Table 5-3)
2) Synchronization treatments (four groups as indicated in Table 5-1)
3) Use or no of ECP (two groups as described in Table 5-2)
4) Age was divided in four groups (1 = one year, 2 = 2 years, 3 = 3 years, 4 = 4 years and up)
5) Body condition was divided in four groups (1 = poor, 2 = fair, 3 = good, and 4 = excellent)
6) Season was divided in two groups (1 for low fertility in spring and summer and 2 for high fertility in fall and winter.)

Dependent variables in this study included the:

1) Total number (left and right ovary) of corpora lutea (TOT_CL),
2) Total number (embryos plus ova) of eggs (TOT_EGGS),
3) Number of transferable embryos (TransEmb),
4) Number of unfertilized oocytes (UFOs.)

The data from seven does were excluded from the data analysis. Two of them did not stand during the service, suggesting they have not come into estrus. Two more were sick and in very bad physical condition. Two had early pregnancies, and one of them had massive uterine adhesions.

5.6.2 STATISTICAL METHODS

The data were analyzed using the SAS system (SAS Institute, Cary, NC, USA). Descriptive statistics were obtained for each treatment group (superovulation and synchronization treatments) and other covariates (location, season, use of estradiol, age and body condition). Because the data were not normally distributed and log- or square root transformations were not effective in achieving normality, the data were analyzed as follows. First, the statistical significance of the relationship between each of the dependent variables (total number of corpora lutea, total number of eggs, number of transferable embryos and number of unfertilized oocytes) and the independent variables (superovulation and synchronization treatments, location, season, use of estradiol, age and body condition score) were assessed using nonparametric tests i.e., Wilcoxon test for binary variables and Kruskal-Wallis test for polytomous variables (Siegel & Castellan, 1988). Each outcome variable was then modeled using the Mixed Procedure of SAS to determine the significance of the differences between treatment groups in models adjusted for other covariates. Because of lack of normality in the dependent variables and because of some variation due to location and season, the models were fit using the Empirical option in proc mixed and treating location and season as random effects in the models. This approach uses the sandwich estimator for variances of fixed effects and is robust where the data are not normally distributed. The significance of the differences in the least squares means for the main effects were tested using the Tukey test. Differences were considered significant at $p<0.05$.

CHAPTER 6

RESULTS, DISCUSSION AND CONCLUSION

6.1 OVERALL RESULTS

Basic descriptive statistics (Table 6-1) show a mean ovulation rate (average number of eggs shed per superovulation = total eggs shed / number of goats superovulated) of 10.35 ± 0.39 per donor. The proportion of animal that produced a number of CL equal or higher than one by the total of animal treated was 87.46 %. Four or less CL were counted in 22.62 % of superovlated does while 77.38 % of them produced above or equal to five CL. The mean ovulation in this latter group was 13 CL, with a higher mean (15.4) from the superovulation treatment group using Ovagen alone (Table 6-2). On the other hand, the group that produced CL ≤ 4, had mean ovulation of 1.1 with the lowest mean (0.9) in the Ovagen alone group.

Table 6-1 Ovulation response and egg/embryo collection

	Number of does	Min	Max	Mean	Standard Error of Mean	Standard of Deviation
TOT_CL	367	0	34	10.35	0.39	7.43
TOT_EGGS	367	0	39	7.92	0.43	8.40
TRANSEMB	367	0	38	4.64	0.36	6.99
UFOS	367	0	31	2.86	0.28	5.34

TOT_CL= Total number of corpura lutea; Tot-Eggs = Total number of eggs; TransEmb = Number of transferable embryos; UFOs = Number of unfertilized oocytes.

The recovery rate, obtained by dividing the total number of eggs (ova and embryos recovered) by the total number of CL, performed was 76.5%. The conception rate is the proportion of animals mated that conceived. In this study, the conception rate has been expressed as the number of does with more than one transferable embryo divided by the total number of does mated and was 50 %.

This study revealed in general a slightly higher number of CL on the left ovary (52%) than on the right ovary (48%). This was consistent for most variables measured (age, body condition, season, synchronization treatment, superovulation group), except in animals of poor body

condition (BCS = 1), those where Embryo-S + PMSG were used, and those where two doses of SMB were used.

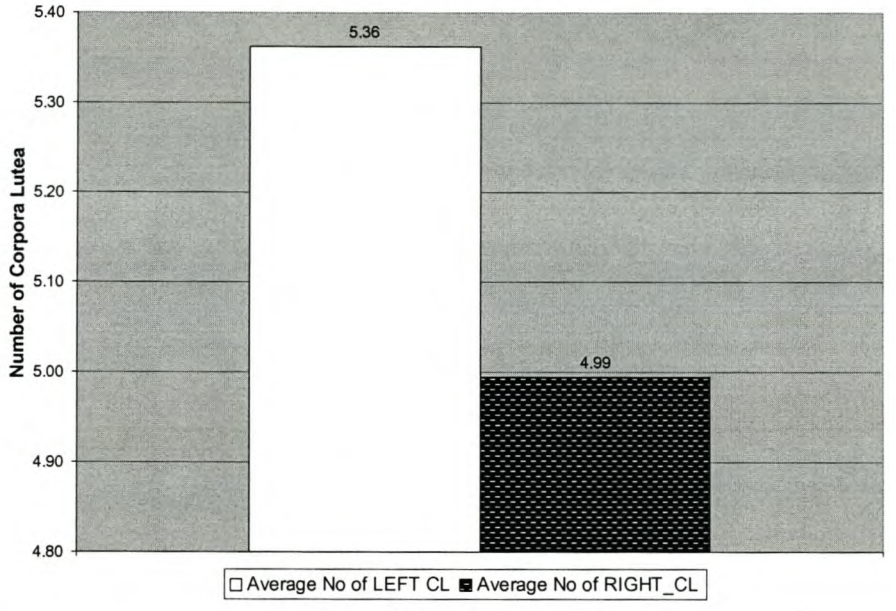


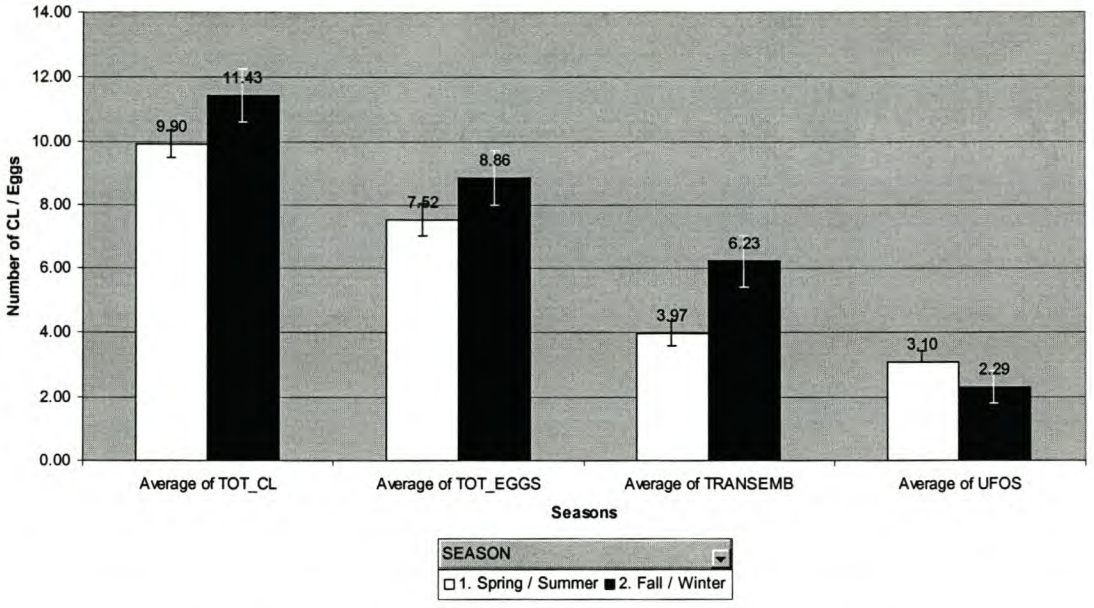
Fig. 6-1 Corpora Lutea produced on the left and right side of the Boer goat ovary

Table 6-2 Distribution of ovulation rate following different superovulation treatments

	Superovulation Treatment	Number of Doe treated	Mean ovulation per doe	% of all treated animals
CL equal to or below 4	Ovagen alone	28	0.9	
	Ovagen + PMSG	35	0.9	
	Embryo-S alone	5	3.0	
	Embryo-S + PMSG	15	1.6	
Total CL ≤ 4		83	1.1	22.62 %
CL equal to or above 5	Ovagen alone	119	15.4	
	Ovagen + PMSG	129	11.8	
	Embryo-S alone	11	10.0	
	Embryo-S + PMSG	25	9.6	
Total CL ≥ 5		284	13.0	77.38 %
Total CL all does		367	10.4	100 %

6.2 EFFECTS OF DIFFERENT FACTORS ON OVULATION AND EGG/EMBRYO COLLECTION

6.2.1 Effects of season on ovulation and egg/embryo recovery



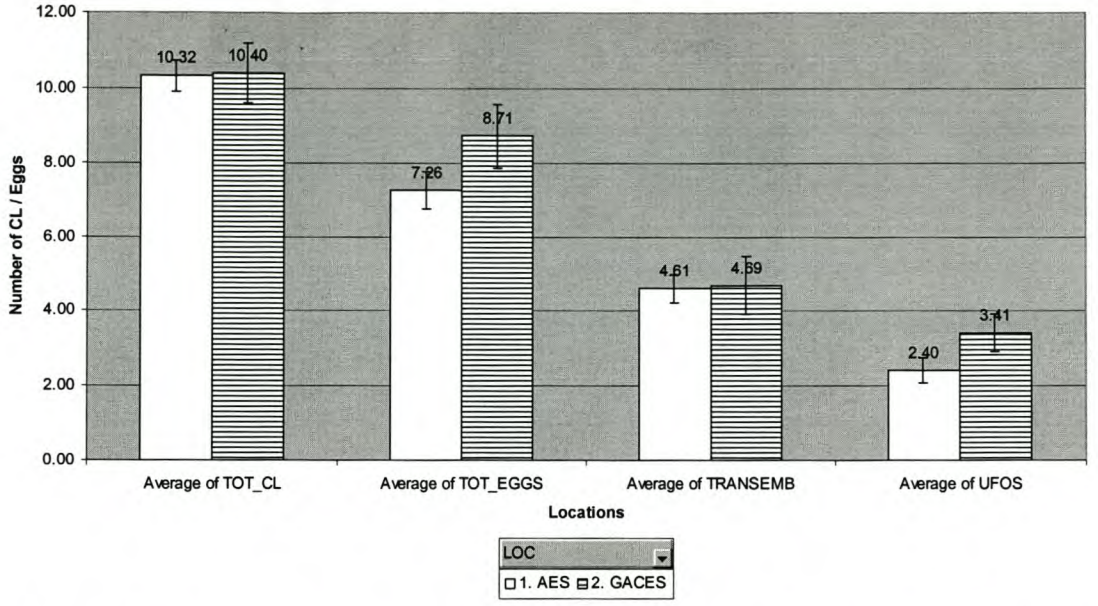
TOT_CL= Total number of corpura lutea; Tot-Eggs = Total number of eggs; Trans-Emb = Number of transferable embryos; UFOs = unfertilized oocytes.

Fig. 6-2 Effects of season based on observed means

As shown in Fig. 6-2, the means of total number of CL, total number of eggs and transferable embryos were higher in fall and winter than in spring and summer except for the number of unfertilized oocytes. However, the univariate analyses demonstrated significant difference ($p<0.05$) only for the number of transferable embryos and the number unfertilized oocytes.

6.2.2 Effects of location on ovulation and egg /embryo recovery

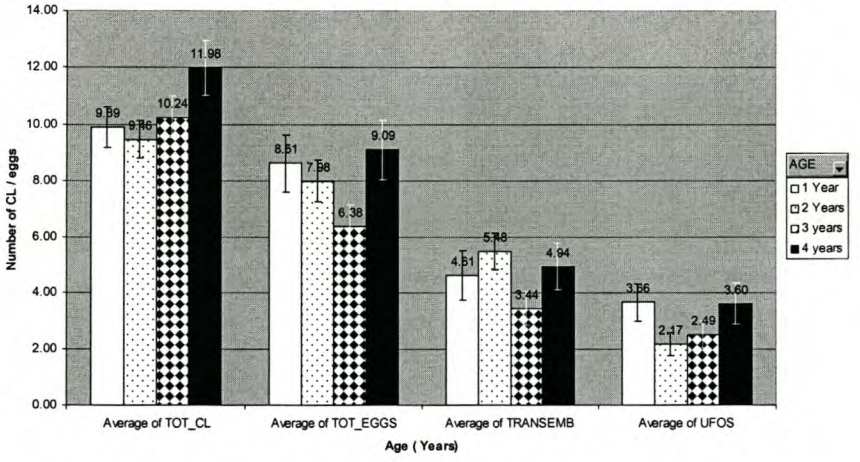
The average number of total eggs (ova and embryos), as shown on Fig. 6-3, has been demonstrated by the non-parametric one-way test (Wilcoxon) to be significantly different in regards to the location. There was significantly higher number of eggs ($p<0.05$) recovered in the Grootfontein Agricultural College Embryo Station (GACES) in Middleburg than in the African Embryos and Semen location (AES) in Stellenbosch. In addition, the number of unfertilized oocytes collected was more in the GACES location than AES location, although not significantly so ($p>0.05$).



TOT_CL= Total number of corpura lutea; Tot-Eggs = Total number of eggs; Trans-Emb = Number of transferable embryos; UFOs = unfertilized oocytes.

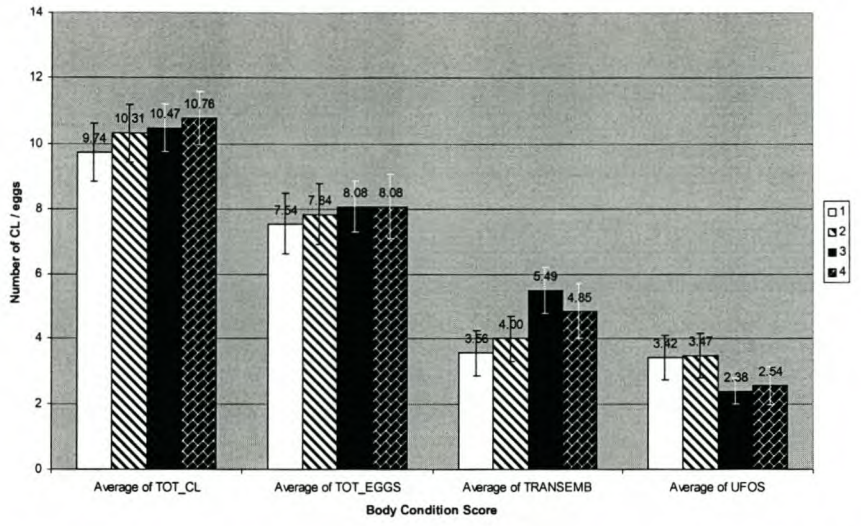
Fig. 6-3 Effects of location based on observed means

6.2.3 Effects of age and body condition on ovulation and egg/embryo recovery



TOT_CL= Total number of corpora lutea; Tot-Eggs = Total number of eggs; Trans-Emb = Number of transferable embryos; UFOs = unfertilized oocytes.

Fig. 6-4 Effects of age based on observed means



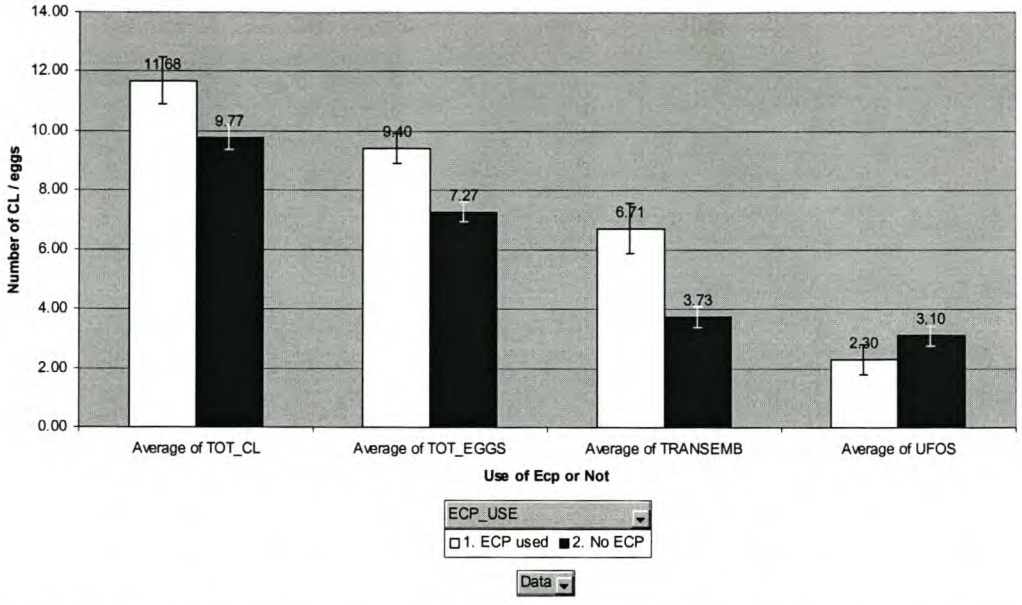
TOT_CL= Total number of corpura lutea; Tot-Eggs = Total number of eggs; Trans-Emb = Number of transferable embryos; UFOs = unfertilized oocytes.

Fig. 6-5 Effects of body condition based on observed means

Figures 6-4 and 6-5 show the effects of age and body condition on ovulation and egg/embryo recovery based on observed means. No significant univariate association ($p>0.05$) was detected for age and body condition. Likewise, in the multivariate model adjusted for age and body condition, among other main effects, these two factors didn't show any significant differences ($p>0.05$).

6.2.4 Effects of the use of estradiol cypionate (ECP) on ovulation and egg/embryo recovery

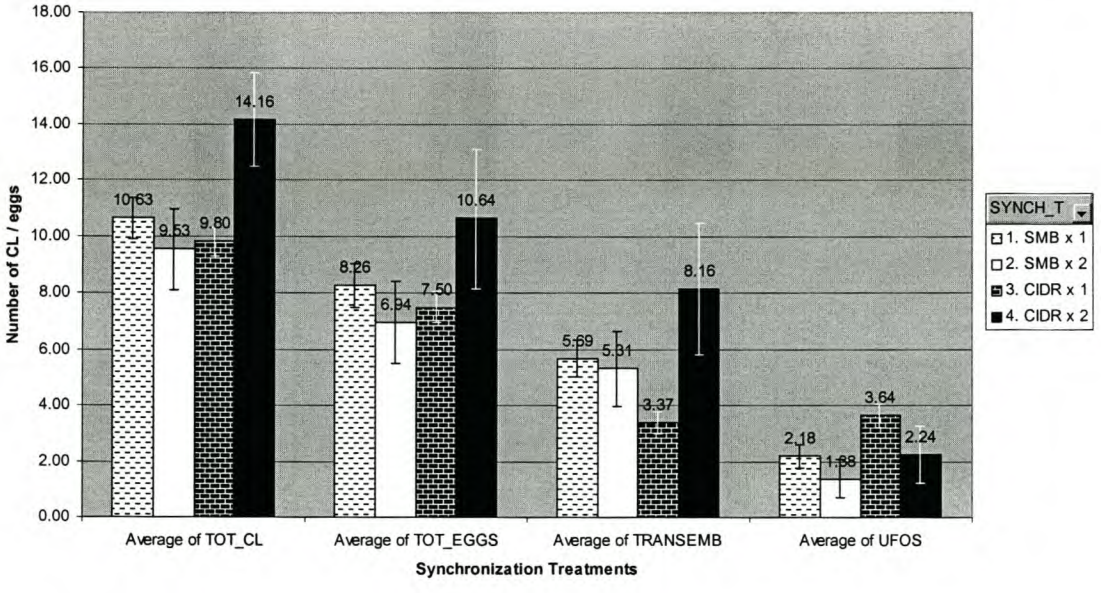
Figure 6-6 represents the effects of using ECP in superovulation treatment on ovulation and egg/embryo recovery based on observed means. Generally, more eggs/embryos were recovered from ECP-used than no ECP group except for UFOs. There was a significant univariate association ($p<0.05$) between the use of estradiol cypionate and the number of transferable embryos, and a marginal association ($p=0.08$) between the ECP use and the number of unfertilized oocytes. The association between ECP use and the number of total CL and eggs recovered were not significant ($p>0.05$). On the other hand, the multivariate analyses in the models in which ECP use was adjusted as main effects, no significant association ($p>0.05$) was shown.



TOT_CL= Total number of corpura lutea; Tot-Eggs = Total number of eggs; Trans-Emb = Number of transferable embryos; UFOs = unfertilized oocytes.

Fig. 6-6 Effects of the use of ECP or not based on observed means

6.2.5 Effects of synchronization treatment on ovulation and egg/embryo recovery

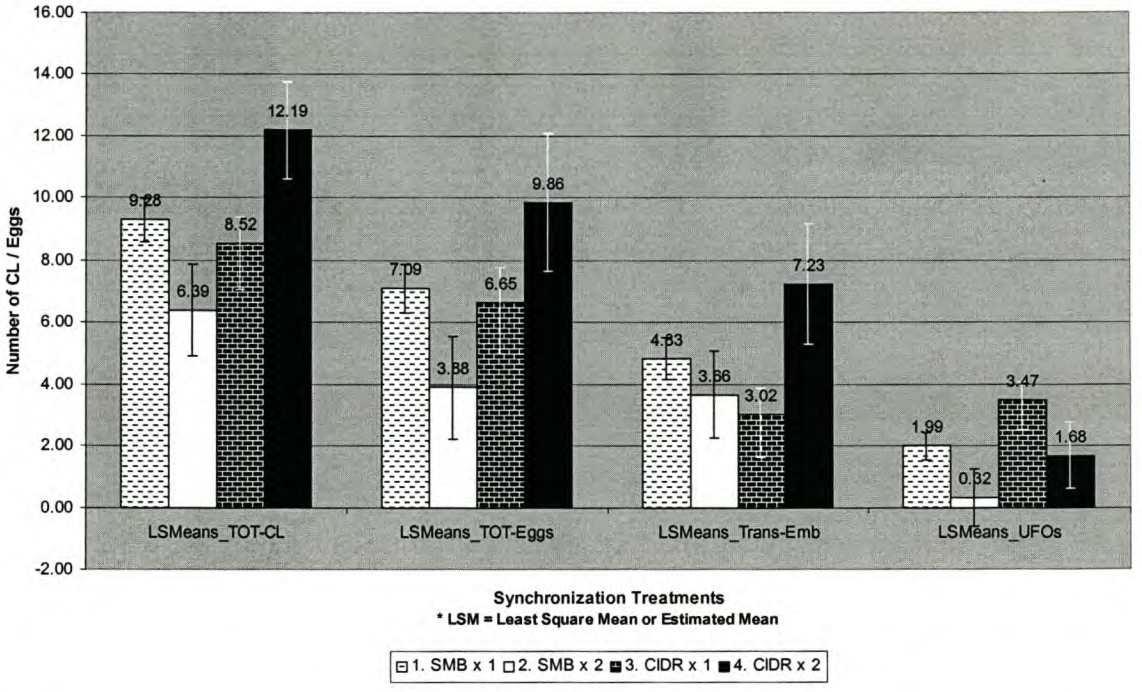


TOT_CL= Total number of corpura lutea; Tot-Eggs = Total number of eggs; Trans-Emb = Number of transferable embryos; UFOs = unfertilized oocytes.

Fig. 6-7 Effects of synchronization based on observed means

Figure 6-7 shows the effects of synchronization treatment on ovulation and egg/embryo recovery based on observed means. Transferable embryos and unfertilized oocytes were significantly

associated ($p<0.05$) with the estrous synchonization treatment in univariate analysis. The multivariate analysis showed a marginal association between synchronization and the number of CL, transferable embryos and unfertilized oocyte.



TOT_CL= Total number of corpura lutea; Tot-Eggs = Total number of eggs; Trans-Emb = Number of transferable embryos; UFOs = unfertilized oocytes.

Fig. 6-8 Effects of synchronization treatments on ovulation and egg/embryo recovery based on the Estimated Means (LSM*)

Figure 6-8 and Table 6-3 portray the results obtained in the multivariate model adjusted for synchronization, superovulation, age, and body condition score as main effects and location and season as random effects. In this model, with the total number of CL as a dependant variable, synchronization treatment was marginally significant ($p=0.06$) as main effect. In a post-ANOVA analysis using Tukey multiple comparison test, only the use of two doses of CIDR (CIDR x 2) was significantly ($p=0.001$) different from the use of two doses of SMB (SMB x 2), while the others treatments were not significant difference ($p>0.05$).

Table 6-3 Ovulatory response and egg/embryo recovery following different synchronization treatments in Boer goats (LSM* ± SEM**)

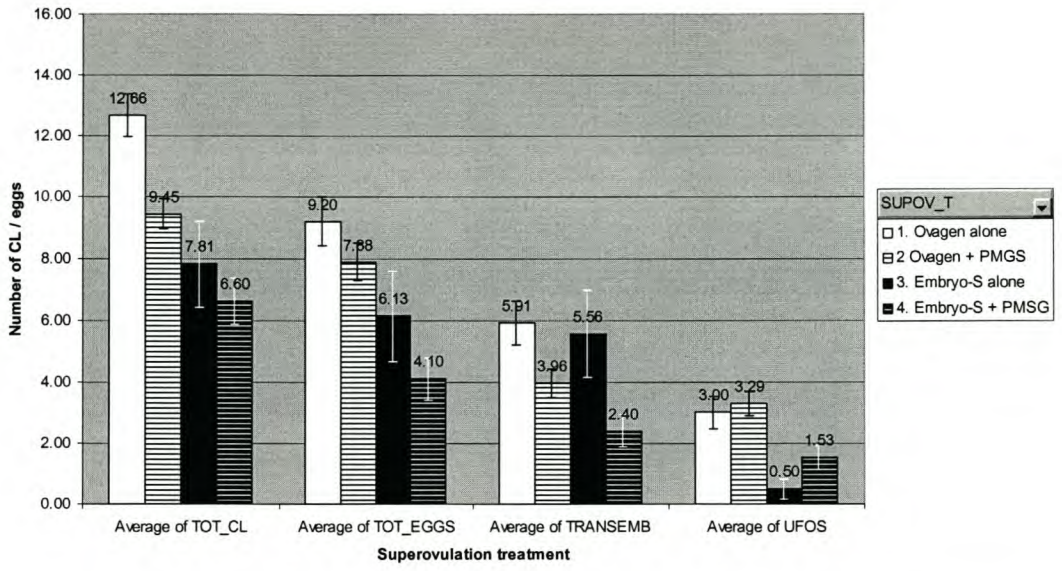
Synchronization treatment	No of Animals	No of CL	No of Eggs	No of Transferable embryos	No of UFOs
1. SMB x 1	123	9.28 ± 0.71 [ab]	7.09 ± 0.79 [a]	4.83 ± 0.67 [a]	1.99 ± 0.43 [a]
2. SMB x 2	32	6.39 ± 1.46 [b]	3.88 ± 1.66 [a]	3.66 ± 1.41 [a]	0.32 ± 0.94 [a]
3. CIDR x 1	187	8.52 ± 0.88 [ab]	6.65 ± 1.13 [a]	3.02 ± 0.86 [a]	3.47 ± 0.58 [a]
4. CIDR x 2	25	12.19 ± 1.59 [a]	9.86 ± 2.22 [a]	7.23 ± 1.96 [a]	1.68 ± 1.07 [a]

Values in columns with different superscripts are significantly different. $P \leq 0.05$
* LSM = Least square means or estimated means
**SEM = Standard error of means

In a similar model, there was no significant multivariate association between synchronization and the total number of eggs recovered and the number of transferable embryos. However, in the model for unfertilized oocytes, synchronization treatment was significant ($p>0.05$) as a main effect but no significant differences were detected between different treatment groups in the post ANOVA analysis.

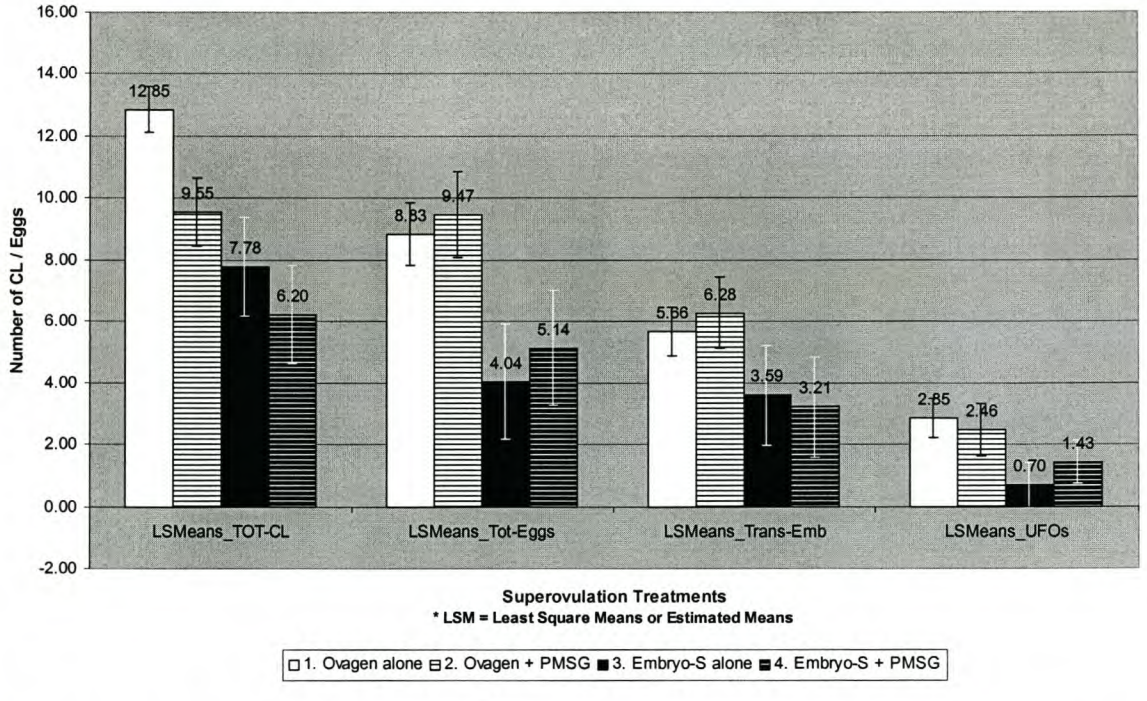
6.2.6 Effects of superovulation treatment on ovulation and egg/embryo recovery

Figure 6-9 below shows the effects of superovulation on ovulation and egg/embryo recovery based on observed means. Overall, there was a strong univariate association ($p<0.05$) between superovulation treatment and the total number of CL, and the total number of unfertilized oocytes. The multivariate model (Fig. 6-9) strongly corroborates this association.



TOT_CL= Total number of corpura lutea; Tot-Eggs = Total number of eggs; Trans-Emb = Number of transferable embryos; UFOs = unfertilized oocytes.

Fig. 6-9 Effects of superovulation treatments based on observed means



TOT_CL= Total number of corpura lutea; Tot-Eggs = Total number of eggs; Trans-Emb = Number of transferable embryos; UFOs = unfertilized oocytes.

Fig. 6-10 Effects of superovulation treatments on ovulation and egg/embryo recovery based on the Estimated Means (LSM*)

Figure 6-10 and Table 6-4 depict the results obtained in multivariate analysis adjusted for superovulation, synchronization, ECP use, body condition score and age as main effects, and location and season as random effects. In this model, with the total number of CL as a dependant variable, superovulation treatment was significant (p=0.0001) as main effect. In a post-ANOVA analysis using Tukey multiple comparison test, treatment 1 consisting of the use of Ovagen alone was significantly (p<0.05) different from treatment 3 (Embryo-S alone) and treatment 4 (Embryo-S + PMSG). In other words, higher number of corpora lutea were detected in the treatment group were Ovagen alone was used than in the treatment groups which used Embryo-S alone and Embryo-S + PMSG. Also, Ovagen + PMSG treatment group produced significantly higher number of CL (p<0.05) than Embryo-S + PMSG, while the number of corpora lutea in Ovagen alone group were marginally higher (p=0.06) than in Ovagen + PMSG group.

Table 6-4 Ovulatory response and egg/embryo recovery following different superovulation treatments in Boergoat (LSM* ± SEM**)

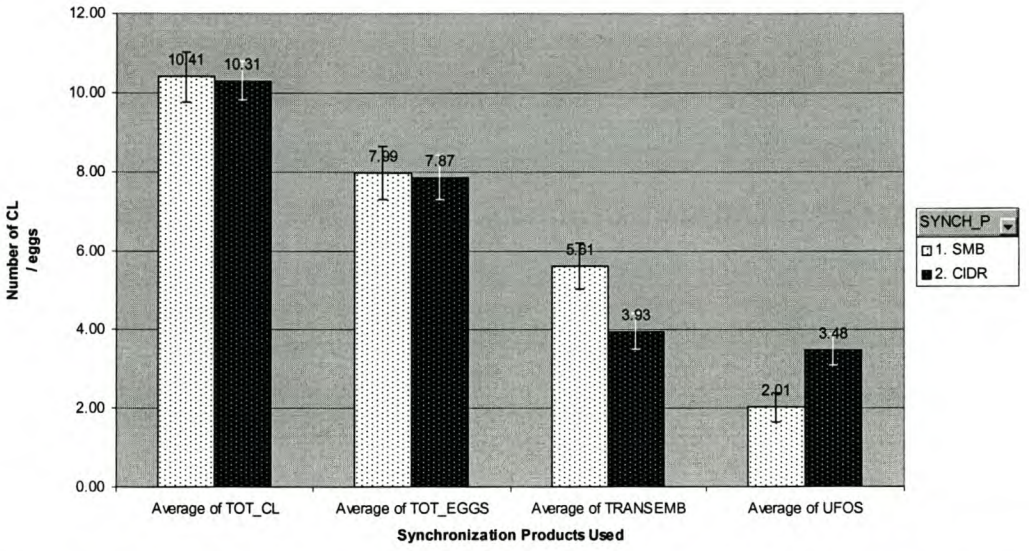
SuperovulationTreatments	No of Animals	No of CL	No of Eggs	No of Transferable embryos	No of UFOs
1. Ovagen alone	147	12.85 ± 0.74 ^{a}	8.83 ± 0.99 ^{ab}	5.66 ± 0.80 ^{ab}	2.85 ± 0.63 ^{a}
2. Ovagen + PMSG	164	9.55 ± 1.11 ^{ab}	9.47 ± 0.41 ^{a}	6.28 ± 1.15 ^{a}	2.46 ± 0.84 ^{ab}
3. Embryo-S alone	16	7.78 ± 1.61 ^{b}	4.04 ± 1.87 ^{ab}	3.59 ± 1.61 ^{ab}	0.70 ± 0.71 ^{b}
4. Embryo-S + PMSG	40	6.20 ± 1.29 ^{b}	5.14 ± 1.47 ^{b}	3.21 ± 1.29 ^{b}	1.43 ± 0.78 ^{ab}

Values in columns with different superscripts (except between treatments 2 and 3) are significantly different. P ≤ 0.05
*LSM = Least square means or estimated means
**SEM = Standard error of means

In a similar model using the total number of eggs as dependant variable, only superovulation treatment was significant as main effect. However, in the post-ANOVA analysis, only Ovagen + PMSG treatment group produced significantly higher total number of eggs (p=0.01) than Embryo-S + PMSG group. For transferable embryos, superovulation was significant (p=0.02) as main effect. In the post-ANOVA analysis, only superovulation treatment using Ovagen + PMSG was significantly different (p=0.02) from treatment using Embryo-S + PMSG. In the model for unfertilized oocytes superovulation treatment was again significant (p<0.05) as a main effect. Only Ovagen alone treatment group was found to be significantly different from Embryo-S alone in the post-ANOVA analysis.

6.2.7 Comparison between the two synchronization products (SMB and CIDR)

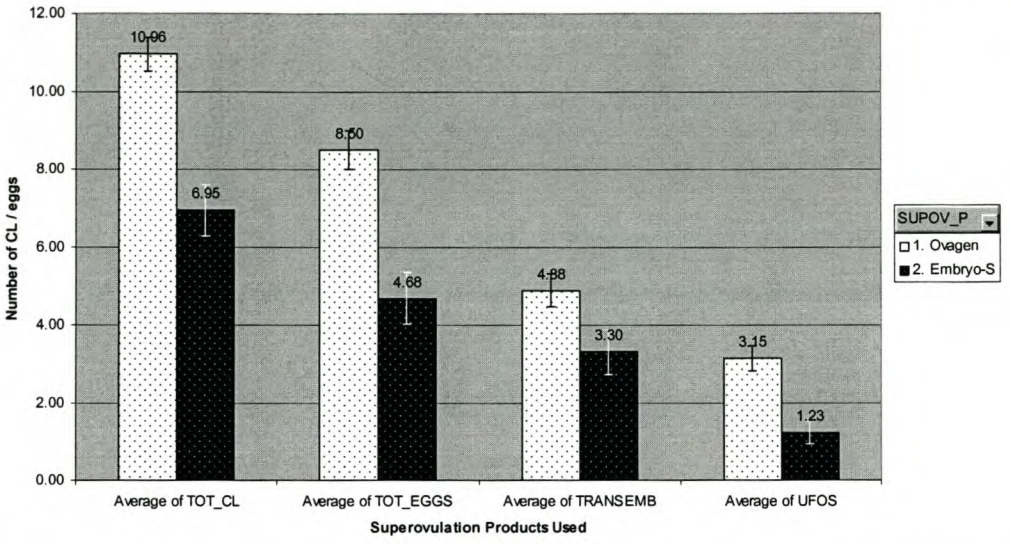
When the two synchronization products (SMB and CIDR) used during the experiment were evaluated, there was a significant univariate association ($p<0.05$) between synchronization and the number of transferable embryos. There were also significantly higher ($p<0.05$) transferable embryos and less UFOs produced in the overall SMB group than there was in the CIDR group (Fig. 6-11). However, the multivariate analysis of the synchronization products used could not back up that assertion while it demonstrated an evident significant difference between treatment using two doses of CIDR over that using two doses of SMB on corpura lutea production with the CIDR leading.



TOT_CL= Total number of corpura lutea; Tot-Eggs = Total number of eggs; Trans-Emb = Number of transferable embryos; UFOs = unfertilized oocytes.

Fig. 6-11 Synchronization using SMB vs CIDR based on observed means

6.2.8 Comparison between the two superovulation products (Ovagen and Embryo-S)



TOT_CL= Total number of corpura lutea; Tot-Eggs = Total number of eggs; Trans-Emb = Number of transferable embryos; UFOs = unfertilized oocytes.

Fig. 6-12 Superovulation using Ovagen vs Embryo-S based on observed means

Generally Ovagen alone or in combination with PMSG gave higher number of eggs than the Embryo-S alone or in combination with PMSG (Fig. 6-12). In assessing the two superovulation products used, Ovagen and Embryo-S, there was significant association ($p<0.05$) between the superovulation products used and the production of corpora luteum in univariate analysis.

6.3 DISCUSSION AND CONCLUSION

The primary goal of superovulation is to obtain consistently high numbers of transferable embryos. Successful techniques of estrous synchronization in a superovulation programme must not only establish tight synchrony, but also provide an acceptable level of fertility upon AI and natural breeding (Wideus, 1999). This study assessed synchronization and superovulation treatments that could enhance ovulation rates, improve embryos quality (transferable embryos), establish optimum doses of synchronization agents, and explore the possible benefits resulting from a superovulation co-treatment with an additional agent.

The multivariate analysis of the effect of synchronization treatments on ovulation rate in this study showed only a marginal association ($p=0.06$). In post ANOVA analysis, however, only two doses of CIDR (CIDR x 2) were significantly associated ($p<0.05$) with a high ovulation rate as compared to two doses of SMB (SMB x 2). This was demonstrated by a higher number of CL in CIDR x 2 than SMB x 2.

Related studies comparing the two synchronization agents, CIDR and SMB, in a design similar to our investigation in Boer goats have, to our knowledge, not been done. However, several comparisons among other synchronization agents are found in the literature, as is analysis of the effect of synchronization treatments on ovulation rate. Our findings agree with the study by Thompson *et al.* (1990) reported by Cognie (1999), which found that both ovulation rate and the number of embryos of transferable quality (assessed on day 6) increased when ewes were primed for 12 days with two consecutive (CIDR x 2) versus a single (CIDR x 1) controlled internal drug release device (CIDR, containing 9 % progesterone.). But our study disagrees with that same study in that the difference was not significant in our analysis. This might be due to the different species between the two studies (does vs. ewes) or the nature of the data commending different statistical analysis. However, the univariate analysis of our study demonstrates a significant association between synchronization and the number of transferable embryos and the number of UFOs, results consistent with a couple of studies reported by Wallace (1992) in her report on recent progress in sheep and goat research, suggesting that embryo quality was linked to progesterone priming at the time of superovulatory hormone administration. One such study by Scudamore *et al.* (1991) - based on ewes primed and synchronized to ovulate with the intravaginal pessary containing 30 or 40 m of synthetic progestagen (fluorogestone acetate), superovulated with PMSG and GnRH, and then inseminated at 48 h after pessary withdrawal - established that ovulation rate was not influenced

by the level of progestagen priming, a result consistent with our univariate analysis. These other reported studies suggest that the level of progesterone / progestagen priming prior to superovulation may be influencing follicle growth and development and/or the oviduct-uterine environment by mechanisms as yet unknown (Wallace, 1992). Our study, however, shows no significant difference in ovulation rate associated with the use of different doses for any synchronization treatment used (CIDR or SMB), findings consistent with the Scudamore *et al.* (1991) study reported above.

The findings of our study may suggest the benefit of two doses of CIDR over two doses of SMB, though the study found no statistical evidence to substantiate that claim. Apart from a significant difference between two doses of CIDR and two doses of SMB on ovulation rate, our study comparing CIDR to SMB found no other significantly different (embryo quality) advantage between the two synchronization products.

Other studies attempting to establish a connection between different synchronization dosages and ovulation rate, embryo quality and pregnancy rate resulted in different outcomes. In a study conducted in Mexico (Mellado & Valdez, 1997) trying to determined the minimal dose of norgestomet (SMB) necessary to induce estrus in goats, and to examine the effect of new or recycled norgestomet implants on goats, concluded that the effect of norgestomet dosage and status of the implant (new or previously used in goats) had no significant effect on reproductive performance (shown in the study by the absence of relationship between pregnancy rate and the dose level of the norgestomet used). The same study concluded that one-fifth of the SMB implant (1.2 mg of norgestomet) used for cattle was sufficient to control estrus in the crossbreed Criollo goats. The lowest dose effective for estrous synchronization in the above Mexican study (1.2 mg of norgestomet) was less than half the dose reported successful for induction of estrus in dairy goats (Bretzlaff & Madrid, 1985.) That latter dose was the minimum dose used in our study in Boer goats (3 mg for 1 dose of SMB). Another study from Brazil trying to determine the efficiency of different protocols in inducing and synchronizing the estrous cycle of Saneen goats by using new or reused SMB and CIDR in combination with either PMSG or cloprostenol concluded that auricular implants or intravaginal devices may be reused, at least one more time, because they are efficient for inducing and synchronizing the estrus in the cyclic dairy goats (Oliviera *et al.* 2001).

As for studies comparing the efficiency of different synchronization agents in the Boer goat, one South African study conducted by Motlomelo *et al.* (2002) comparing the CIDR devices

medroxyprogesterone acetate (MAP) and fluorogestone acetate (FGA) for synchronization found no significant difference in estrous response and conception rate, concluding that the use of CIDR, MAP and FGA treatments are equally efficient in synchronizing estrus in goats.

The multivariate model of this study found a strong association between superovulation treatments and ovulation rates ($p<0.0001$), the total number of embryos collected ($p<0.001$), the number of embryos of transferable quality ($p=0.02$), and the number of UFOs ($p=0.05$). The post-ANOVA analysis found that the use of Ovagen alone or in association with PMSG induced the production of significantly higher corpora lutea, eggs and transferable embryos than did the use of Embryo-S, whether alone or in association with PMSG. Our study also interestingly found that the addition of PMSG to Ovagen significantly increased the number of transferable embryos and showed a non-significant decrease of UFOs (Table 6-4).

Similar comparative studies between the two superovulation agents (Ovagen vs. Embryo-S) from the same source (ovine pituitary) have not been conducted, as far as we know, showing the uniqueness of our study. However, many other studies consulted in the literature were comparing the efficiency of superovulation agents from an ovine source with those from other sources; porcine (FSH-P), equine (eCG), or human (hMG). One such investigation conducted in India by Kumar *et al.* (2003) had the objective of comparing the efficacy of FSH-O (an ovine FSH preparation, Ovagen) with FSH-P and eCG on superovulatory response in Tellicherry goats. The result of that study demonstrated the effectiveness of FSH-O over the other agents in regard to the ovulation rate, total embryo recovery and number of transferable embryos. The higher ovulation rate seen in the FSH-O group compared with the FSH-P and eCG groups may be due to the lower LH:FSH ratio in the FSH-O preparation than in the FSH-P and eCG preparations (McNatty *et al.*, 1989; Henderson *et al.*, 1990). The study therefore recommended FSH-O (Ovagen) as a gonadotropin of choice for superovulation of Tellicherry goats when compared to FSH-P and eCG. We might also imply in our study comparing Ovagen and Embryo-S that the advantage observed of Ovagen over Embryo-S in producing corpora lutea and transferable embryos may be due to the high proportion of LH contained in the Embryo-S preparation.

One other study, performed in Spain on the Murciano-Granadina goat by Gonzales-Bulnes *et al.* (2003), found the mean ovulation rate in goats treated with purified ovine FSH (Ovagen) to be higher than reported in the previous studies on the same breed using a single superovulatory

dose of eCG or using several doses of porcine FSH or FSH in combination with eCG (Pintado *et al.*, 1998). The same Spanish study resulted in a higher number of recovered and viable embryos, compared to the previous one, confirming the advantages of using highly purified gonadotropins (Cognie, 1999).

The benefit of the addition of PMSG to Ovagen, found in this study to significantly increase the number of transferable embryos with a non-significant decrease of UFOs, might be explained in Cognie's report on the state of the art in sheep-goat embryo transfer (Cognie, 1999). He reported that even though highly purified FSH-O (Ovagen) and FSH-P (Folltropin) are efficient, a minimum amount of LH is essential after the removal of progestagen (FSH/LH: 0.3-0.4 in the last two injections, respectively) to increase the superovulatory responses and the number of transferable embryos recovered (Cognie, 1999). LH contained in the PMSG added to the highly purified Ovagen may have caused the increase found in our study.

Apart from synchronization (marginal effect) and superovulation treatments (significant effect), the multivariate analysis did not show any significant effect by other factors (the use of ECP, body condition, age) as a main effect. The effectiveness of the addition of estradiol cypionate (ECP) was shown in its association ($p=0.05$) with better quality embryos in the univariate analysis, although it did not have significance as a main effect in the multivariate model. These results agree with many findings in cows attributing to the estrogen treatment the ability to cause regression of ovarian follicles with emergence of new follicular wave (Mapletoft & Bo, 1994). One using a synchronization program with SMB and estradiol valerate concluded that estradiol valerate induces atresia of the antral follicles in cows with SMB implant. It thus seems possible to synchronize, by inducing atresia of existing antral follicles, a new wave of follicular development that would be responsive to the super-stimulatory treatments and therefore result in more ovulations and higher ova/embryo quality (Mapletoft *et al.*, 1994). There were no significant effects by age and body condition in this design. A difference of season was significant ($p<0.05$) in the univariate analysis on the number of transferable embryos and UFOs. The number of transferable embryos was higher in the fall/winter season than spring/summer. This is consistent with seasonality in the South African Boer does, which have a peak of sexual activity during April and May (Fall / Autumn) and lowest sexual activity from October to January (late Spring to mid-Summer) (Greyling, 1988). Our study also found the mean ovulation rate of 10 in all does and 13 in does with CL $\exists$ 5. Compared to several findings reported by Wallace, 1992 (Table 3-1), this rate is acceptable. Our recovery rate was 76.5%. This study

also shows a generally higher number of CL on the left ovary (52%) than on the right ovary (48%).

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