THE EFFECT OF DIFFERENT INTRAVAGINAL PROGESTERONE DOSES ON THE CONCEPTION RATE OF BEEFMASTER COWS AND HEIFERS FOLLOWING FIXED TIME ARTIFICIAL INSEMINATION

GUILLAUME STEFANUS VAN NIEKERK

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by

Guillaume Stefanus van Niekerk

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Supervisor: Prof. J.P.C. Greyling

Co-Supervisor: Prof. C. van der Westhuizen

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DECLARATION AND COPYRIGHT

I declare that the dissertation hereby submitted in partial fulfilment for the requirements of the degree Magister of Technology: Agriculture at the Central University of Technology, Free State has not been submitted by me for any other degree at any other institution.

Name and Surname: Guillaume Stefanus van Niekerk____

Student Number: 212 087 525____________________

ID Number: 741212 5012 088________________

Signature: _______________________________

Date: _______________________________

DEDICATION

I dedicate my efforts, amongst others, to my late granddad Mr Guillaume Stefanus van Niekerk – I hope this takes us one step closer to our dreams…

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LIST OF ABBREVIATIONS

AI	-	Artificial insemination
BCS	-	Body condition score
CIDR	-	Controlled intravaginal drug release
CL	-	Corpus luteum
E2	-	Oestrogen (hormone)
EB	-	Oestradiol benzoate
FSH	-	Follicle stimulating hormone
FTAI	-	Fixed time artificial insemination
GnRH	-	Gonadotropin releasing hormone
ICP	-	Inter calving period
LH	-	Luteinizing hormone
LSD	-	Least significant difference
MGA	-	Melengestrol acetate
P4	-	Progesterone (hormone)
RIA	-	Radio-immuno-assay
RTS	-	Reproductive tract score

ABSTRACT

The effect of different intravaginal progesterone doses on the conception rate of Beefmaster cows and heifers following fixed time artificial insemination

The study was carried out to establish whether or not a decrease in the progesterone concentration of an intravaginal device used to synchronize oestrous in Beefmaster heifers and cows would lead to an increase in conception rates. The study evaluated the effect of different intravaginal progesterone device doses on the conception rates of Beefmaster cows and heifers following fixed time artificial insemination (FTAI). A total of 100 stud Beefmaster cows (Cow group) and 100 stud Beefmaster heifers (Heifer group) were used in the study. The animals were year round grazing residents of the farm Oribilaagte near the town of Vrede in the Free State province of South Africa. The Cow and Heifer groups were split into four experimental groups, respectively, and treated with either a 0.5 g, 1.0 g, 1.9 g or 1.9 g re-used intravaginal progesterone device during the spring synchronization program (P4 device insertion and 2 mg i.m. oestradiol benzoate treatment on d 0, 250 µg s.c. PGF2α treatment on d 7, P4 device removal on d 8, 1 mg i.m. oestradiol benzoate treatment on d 9, and FTAI on d 10). Blood samples were taken on the day of intravaginal insert removal (d 8), and on the day of FTAI (d 10), to monitor the blood serum P4 concentrations (solid phase RIA analysis) between the experimental groups. The experimental groups were examined for pregnancy 5 months post AI by means of rectal palpation. The Cow group recorded a pregnancy rate of 65%, 75%, 67% and 68% for the 0.5 g, 1.0 g, 1.9 g and 1.9 g re-used intravaginal progesterone device groups respectively. The Heifer group recorded a pregnancy rate of 19%, 39%, 41% and 47% for the 0.5 g, 1.0 g, 1.9 g and 1.9 g re-used intravaginal progesterone device groups respectively. The study concluded that a decrease in the progesterone concentration in the intravaginal device did not affect the serum progesterone concentrations in the Heifer and Cow groups on the day of the FTAI, and did not negatively affect the synchronization program. The study further concluded that, although a decrease in the progesterone concentration in the intravaginal device did not statistically influence the conception rates in the Heifer and Cow groups, the Cow group did record a higher numerical conception rate when the progesterone concentrations in the intravaginal devices were reduced.

Key words: serum progesterone, solid phase RIA, synchronization, CIDR, DIB, intravaginal device, Beefmaster cow, conception rate, fixed time artificial insemination.

Chapter 1

General Introduction

1.1 Background

Artificial insemination (AI) techniques are used to maximize the advancements gained by using, for instance top genetic bulls. Where a single bull could theoretically mate with 30 or 40 female animals during a 60 to 90 day breeding season, one ejaculate from the same bull could be diluted, packaged and used to inseminate in excess of 200 female animals in one day. The AI technique is performed by using a sterile, disposable pistolette, containing the semen which is inserted into the vagina and guided manually through the opening of the uterus (the cervix), via the rectum. The pistolette is passed through the cervix and the semen deposited directly into the uterus. The AI procedure gives varying results due to the magnitude of environmental and physiological factors that play a role. Some of these factors are discussed in the literature review section of this dissertation. The international consensus for an expectable conception result in cows following a CIDR based synchronization and FTAI program seems to be in the region of 40% to 45% on the 1st insemination. Heifers seem to do a bit worse with a conception rate of 30% to 40% after the 1st insemination (Webb, 2003).

Reproductive hormonal therapies are becoming increasingly common on modern day dairy and beef farms. The adoption of an ovulation based synchronization program is predominantly driven by their proven efficacy and ease of implementation. The incorporation of a CIDR device in the synchronization program significantly improves the synchronization of the oestrus cycles in heifers and cows and increases the probability of pregnancy after the 1st insemination (Martinez *et al*., 2012). The probability of achieving a pregnancy after the 1st insemination in a FTAI program is 5% to 20% higher in cows treated with a CIDR device and the accompanying hormonal therapy, than in cows not treated with a CIDR device and only treated with the accompanying hormonal treatments (Walsh *et al*., 2008).

The CIDR device is inserted into the vagina of either a heifer (a virgin cow) or a cow. The progesterone impregnated device releases progesterone into the vagina which is absorbed into the blood stream of the cow. This absorbed progesterone mimics the progesterone that is naturally released by the cow during the reproductive cycle (oestrous cycle) (Kirk, 2012).

Progesterone is the hormone secreted by the female reproductive system that functions mainly to regulate the condition of the inner lining (endometrium) of the uterus. Progesterone is produced by the ovaries, placenta and the adrenal glands. The term *progestin* is used to describe progesterone and synthetic steroid hormones with progesterone-like properties. Progesterone helps maintain the functions of the placenta; prevents the production of milk (lactation) until after birth; strengthens the mucus plug covering the entrance to the uterus to prevent infection; strengthens the pelvic walls in preparation to giving birth and stops the uterus from contracting during pregnancy up until giving birth (Hornby, 2005).

Enough progesterone is absorbed into the blood from the CIDR to keep the heifer or cow from coming into oestrus or heat. The CIDR has a “tail” or string that is left protruding from the vagina of the heifer or cow, which can be pulled to remove the CIDR from the vagina. When the CIDR is removed from the vagina, the blood progesterone levels fall. When the progesterone levels falls, the heifer or cow comes into oestrus and is ready to breed. The purpose of the CIDR is to synchronize the onset of oestrus in order to improve the timing and efficiency of a breeding program for dairy heifers. Some manufactures recommend the use of CIDRs in combination with other hormones for the most efficient results. By using the CIDRs in a group of heifers or cows, it is possible to have the majority of them ready to be breed within a 1-3 day period. Using the CIDR eliminates the need for heat detection in the heifers and cows, and can result in a higher pregnancy rate (Kirk, 2012).

1.2 Rationale and Motivation

It has long been speculated that the locally available 1.9 g progesterone (P4) controlled intravaginal drug release (CIDR) device results in lower conception results in heifers, than in cows.

The most cited reason for this result is that the high concentration of P4 overrides the normal endocrine functions in heifers shortly after removal, impairing the functions of oestrogens to produce a sufficient luteinizing hormone (LH) surge, thus reducing the occurrence of ovulation.

It has been recorded that a reduced level of progesterone (P4) in the intravaginal device leads to a decrease in the serum levels of P4 between days 1 and 8 of a fixed time artificial insemination (FTAI) program, and subsequently increases the dominant follicle diameter on day 9 of the FTAI program (Pegorer e*t al*., 2010). It has also been shown that a decrease in the P4 concentration of the intravaginal device increases the endogenous release of LH, resulting in an increased ovulation frequency in cows. High concentrations of P4 thus impair the ability of either endogenous or exogenous estradiol to induce a pre-ovulatory surge of LH and thus impede ovulation (Hatler *et al*., 2008). Some studies even claim to have used 1.9 g progesterone intravaginal devices four times in a row after having been inserted intravaginally for 9 days at a time, with no detrimental effects on fertility (Meneghetti *et al*., 2009).

There is thus a need to test these intravaginal devices with varying concentrations of P4 under South African conditions, to determine the effect of these devices on the conception rates in both heifers and cows, following synchronization and the FTAI program.

1.3 Problem statement

The South African market is currently being monopolised by a manufacturer of the only available intravaginal progesterone device registered for use in cattle in South Africa. The intravaginal device then comes in the form of a 1.9 g P4 controlled-internal-drug-release (CIDR®) device available from Pfizer, New Zealand (Registration No. G1916, Act 36 of 1947). The manufacturer offers no difference in the concentration of progesterone in the intravaginal device, and has a sole mandate when it comes to pricing the device. Other countries such as the United States, Australia and Europe offer a range of devices, ranging in the concentration of P4, which makes them more efficient when it comes to customizing synchronization programs according to the age, size, body weight, etc. of the target animals.

1.4 Main objective of the study

The study evaluated the effect of different intravaginal progesterone doses on the conception rates of Beefmaster cows and heifers following a fixed time artificial insemination protocol. The study was thus carried out to establish whether or not a decrease in the progesterone concentration of an intravaginal device used to synchronize oestrous in Beefmaster heifers and cows would lead to increases in the conception rates after artificial insemination (AI).

The **main objective** of this study was to determine which of the four intravaginal progesterone (P4) devices available in this study (0.5 g, 1.0 g, 1.9 g and 1.9 g re-used) would result in the highest conception rate after being used in a synchronization and fixed time artificial insemination (FTAI) program.

1.5 Specific objectives of the study

The study had the following specific objectives:

1.5.1 To determine if a decrease in the progesterone concentration of the intravaginal device would still induce an adequate serum progesterone concentration and allow the treated heifer and cow to maintain a normal luteal phase during the oestrous cycle.

1.5.2 To determine if a decrease in the progesterone concentration in the intravaginal device would result in a decreased serum progesterone concentration in the heifer and cow on the day of the fixed time artificial insemination.

1.5.3 To determine if a decrease in the progesterone concentration in the intravaginal device would result in a higher conception rate in the treated heifers and cows.

1.6 Hypothesis of the study

The hypothesis is that a decrease in the progesterone concentration in the intravaginal devices used to synchronize the oestrous cycles in both heifers and cows could improve the efficiency of the synchronization program and ultimately improve the conception rates currently obtained in similar cattle breeding programs, i.e. devices lower in P4 concentration (< 1.9g P4) will result in a more conducive RIA P4 profile and higher conception rate than the devices with a higher P4 concentration (> 1.0g P4).

Chapter 2

Literature review

2.1 Hormonal regulation of the reproductive cycle in the cow

The insemination of animals at a fixed time without recourse to oestrous detection to obtain repeatable high pregnancy rates is a practical, but not yet attainable goal in farm animals. A fixed time artificial insemination (FTAI) program then requires the regulation and synchronization of both the functional lifespan of the corpus luteum (CL) and the stage of follicular waves in all animals. A detailed knowledge of the endocrine system is important if one wishes to maximize any oestrous synchronicity (Roche *et al*., 1998).

2.1.1 Endocrinological review

The field of endocrinology generally refers to the study of the endocrine hormones, their receptors and the intracellular signalling pathways they utilise. Hormones are generally categorized into 4 structural groups (Ryke *et al.*, 1989; Beato *et al.*, 1996; Gordon, 1996, Hammes, 2003):

- Peptide and protein hormones e.g. LH and FSH
- Steroid hormones e.g. oestrogens and progesterone
- Fatty acid derivatives (eicosanoids) e.g. prostaglandin

By definition endocrine hormones are secreted into the blood and affect the target cells at distant sites in the body. Hormones in general can however also have an effect in close proximity (neighbouring cells), or even the cell responsible for its secretion. Three different hormonal pathways/signalling have been defined (Ryke *et al.*, 1989; Beato *et al.*, 1996; Gordon, 1996, Hammes, 2003):

- Endocrine pathway - the hormone is released into the blood and acts on distant target cells
- Paracrine pathway - the hormone acts locally by diffusing from its source cell to the neighbouring target cells
- Autocrine pathway - the hormone acts on the same cell that produced it (Figure 2.1).

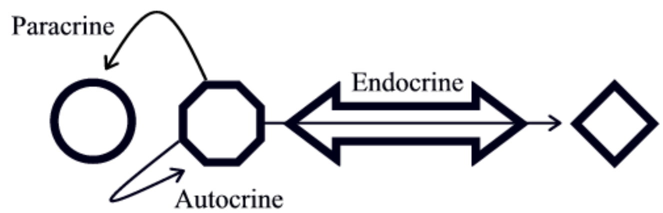

Figure 2.1: The different endocrine hormonal pathways in the body (Gordon, 1996)

In general, target cells recognize the hormones to which they respond to by having hormone specific receptors, either exposed on the cell membranes (cell surface receptors), or hidden within the cell (intracellular receptors) (Figure 2.2). Two important terms are used when describing the proteins that bind to these receptors:

- Agonists – molecules that bind to the receptors and lead to a biological effect
- Antagonist – molecules that binds to the receptors but do not lead to a biological affect

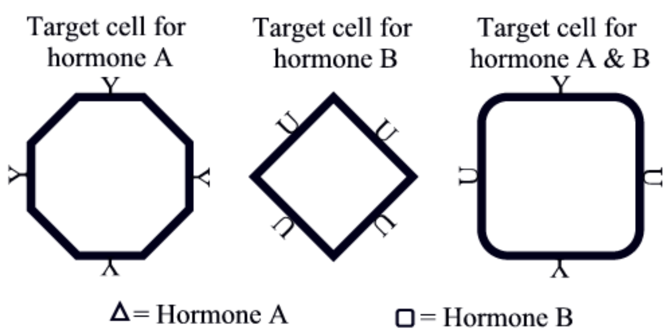

Figure 2.2: Illustration of target cell receptors to certain hormones (Gordon, 1996)

When target cell receptors bind to a specific hormone they generally undergo a conformational change, thus allowing the hormone to interact with the other components of that cell, ultimately leading to a change in the physiological state of the cell. Hormones generally have two principle mechanisms of action:

- The activation of enzymes and other dynamic molecules
- The modulation of gene expression (Ryke *et al.*, 1989; Beato *et al.*, 1996; Gordon, 1996, Hammes, 2003).

2.1.2 The gonadotropic and sex hormones

The sex hormones, most noticeably the gonadotropin releasing hormones (GnRH), follicle stimulating hormone (FSH), luteinizing hormone (LH), oestrogen (E2), progesterone (P4) and prostaglandin (PGF2α) are used to control and manipulate the reproductive cycle in cows. These hormones act on the gonads to initiate ovulation, regulate the oestrous cycle, allow fertilization, prepare the uterus for the accommodation of the fertilized ovum, maintain pregnancy, initiate parturition and ultimately control the lactation postpartum period. A complex interaction of positive and negative feedback systems thus exist between these interacting hormones, to maintain the different reproductive phases (Lewis *et al*., 1996) (Figure 2.3).

The gonadotropin releasing hormone (GnRH), for example, is secreted by the hypothalamus, which in turn regulates both the secretion of FSH and LH by the anterior pituitary gland. These two gonadotropins then stimulate the growth of the ovarian follicles and regulate ovulation and the formation of the corpus luteum (CL). As the ovarian follicle matures it secretes oestrogen, a steroid hormone which controls the LH function and induces oestrous behaviour. Oestrogen has a negative feedback effect on the blood FSH secretion and the levels of the FSH decreases as the dominant follicle on the ovary matures. Oestrogen however also has a positive feedback effect on LH secretion, and elevated oestrogen levels that lead to the induction of a LH surge, which in turn leads to the rupture of the mature follicle. Under the influence of LH, the ruptured follicle (ovulation) forms a CL, which then secretes progesterone, the steroid hormone responsible for maintaining a pregnancy compatible uterus. If fertilization does not occur the non-pregnant uterine wall secretes prostaglandin, a fatty acid derivative which causes the regression of the CL and initiates the start of the next oestrous cycle (Lewis *et al.*, 1996).

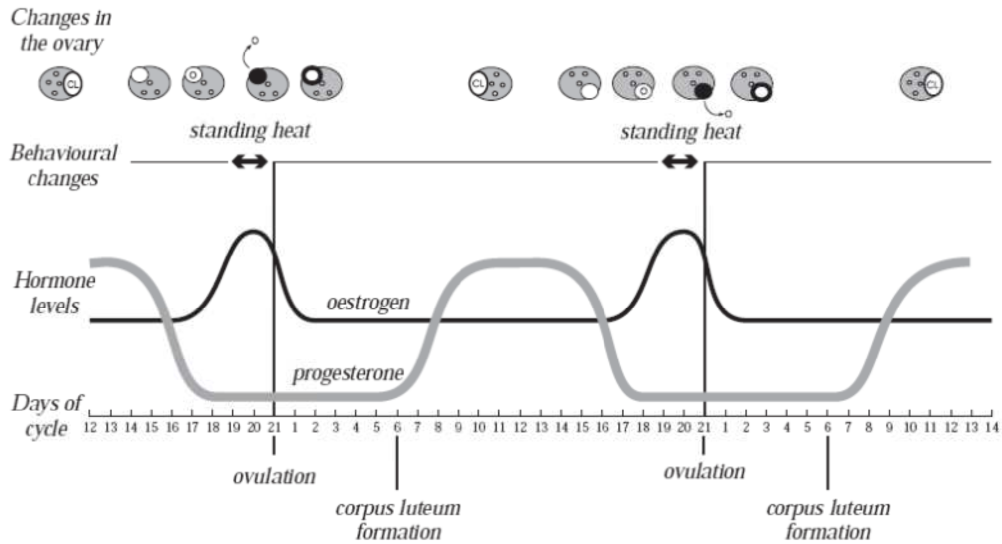

Figure 2.3: Changes in the ovary, behavioural changes and hormone levels during the oestrous cycle of the cow (Lewis *et al.*, 1996)

2.1.3 The bovine oestrous cycle

The bovine oestrous cycle is generally completed after 21 days in the mature cow and 20 days in the heifer. This cycle as such can be divided into 4 phases, namely the pro-oestrous phase, the oestrous phase, the met-oestrous phase and the di-oestrous phase (Lewis *et al.,* 1996, Roche *et al*., 1998, Hafez & Hafez, 2000) (Figure 2.4).

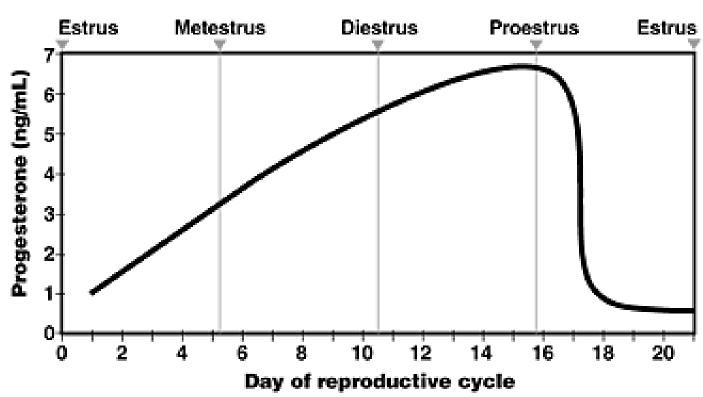

Figure 2.4: Blood progesterone levels in the natural oestrous cycle of the cow (Lewis *et al.*, 1996).

The pro-oestrous phase lasts for approximately 2 to 3 days and is characterized by the regression of the CL and the final growth phase of the ovulatory follicle. As the blood progesterone levels decrease, the serum LH and oestrogen levels increase, inducing a rapid increase in oestrogen production by the ovarian follicle (Hafez & Hafez, 2000).

The oestrous phase in the bovine lasts for approximately 6 to 30 h (average 20 h) and is characterised as the time period when the cow exhibits sexual desire and acceptance of the bull, by standing to be mounted and mated. No CL is present at this stage and therefore progesterone levels in the blood are very low. Circulating LH levels spike during this time and initiate ovulation, while oestrogen levels decrease from the high levels produced just prior to oestrous (Hafez & Hafez, 2000).

The met-oestrous phase lasts for approximately 3 to 5 days, during which ovulation may occur 10 to 15 h after the end of oestrous. During this period the CL starts to develop (luteal phase), but the blood progesterone levels are still relatively low (Hafez & Hafez, 2000).

The di-oestrous phase (luteal phase) lasts for approximately 12 days, and is characterized by the presence of a mature CL and increased blood progesterone levels (Lewis *et al.*, 1996, Hafez & Hafez, 2000). These different phases of the oestrous cycle in the cow are illustrated in Figure 2.5:

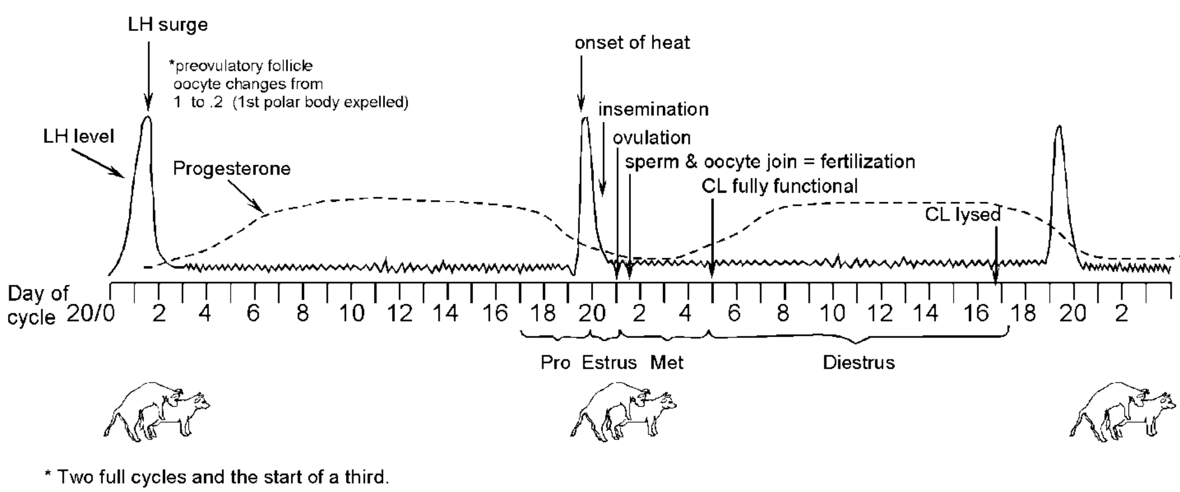

Figure 2.5: The different stages of the bovine oestrous cycle (Lewis *et al.,* 1996)

2.1.4 Oestrous synchronization in cattle

In general, 3 regimes can be used to synchronize the oestrous cycle of a cow, namely:

2.1.4.1 Prostaglandin (PGF2α) controlled breeding programs

Prostaglandin based protocols make use of either a one-injection or a two-injection regime. The one-injection protocol, which is based on the assumption that 65 – 70% of the cows are in the luteal phase of the oestrous cycle, and that prostaglandin therapy would thus terminate the luteal phase (luteolysis) and cause those cows to come into oestrous within 5 days following treatment. The two-injection protocol again is based on the assumption that 30 – 35% of the cows were not initially in the luteal phase of the oestrous cycle during the initial treatment, but have since progressed into the luteal phase of the oestrous cycle. Thus a second prostaglandin injection 11 days later would cause the remaining cows to return to oestrous, possibly during the following 5 days. Prostaglandin programs work well when combined with an oestradiol injection, 48 h after the last prostaglandin treatment (Deutscher, 2007).

2.1.4.2 Progesterone (P4) controlled breeding programs

Progesterone based programs generally make use of devices such as ear implants e.g. Crestar, or intravaginal devices e.g. CIDR®, to deliver the required concentration of progesterone. These devices are left intravaginally for 7 to 12 days, after which they are removed. Estrous observations are carried out for 5 days and animals showing signs of standing heat are inseminated according to the 12 h rule e.g. heat / estrus in the morning, fixed-time artificial insemination (FTAI) in the afternoon. The feed supplement MGA (melengestrol acetate), is also available to administer progesterone, but it is not always practical, as the quantity of progesterone taken in with the feed has to be monitored (Deutscher, 2007).

2.1.4.3 Combined breeding programs

The combination of endocrine hormones such as progesterone, prostaglandin, GnRH and oestradiol can be used very effectively. A typical combined synchronization program would work as follows:

Day 0	-	Progesterone (P4) device insertion
		Oestradiol treatment
Day 8	-	Progesterone (P4) device removal
		Prostaglandin treatment
		GnRH treatment
Day 9	-	Oestradiol treatment
Day 10	-	Fixed-time artificial insemination (FTAI)

Combined programs have the advantage of allowing the operator to perform the FTAI 48 to 72 h after P4 device removal (Whisnant *et al.*, 1999; Ferreira *et al.,* 2006; Deutscher, 2007; Stevenson, 2008; Leitman *et al.*, 2009; Sa Filho *et al.*, 2009; Starbuck & Mann*,* 2009). Generally 81% of heifers and cows treated with a progesterone device, combined with an oestradiol injection, show signs of oestrous and develop a functional CL (68%) within 2 days of the treatment (Rasby *et* al., 1998). Generally, 95.5% of heifers show signs of oestrous within 72 h, following this synchronization regime. Pregnancy rates in heifers are also generally higher when the synchronization program is initiated, with the animal in the di-oestrous phase (53.6%) of oestrous - compared to when the synchronization program is started, while the animal is in the pro-oestrous (44.4%) or met-oestrous (43.7%) phase of the oestrous cycle (Mathis *et al*., 2001).

2.1.5 The effect of pre-synchronization treatment on conception rate in the cow

It is important to establish the ovarian status, especially of early postpartum cows, in terms of luteal activity, before the onset of a synchronization program. A pre-synchronization treatment program can be conducted by administering two cloprostenol (PGF2α) injections 14 days apart, of which the second cloprostenol injection is given 7 days before the onset of the synchronization program. In cows with high blood progesterone concentrations at the onset of treatment, an Ovisynch program (d 0 = GnRH; d 7 = PGF2α; d 8 = GnRH; AI 16 – 20 h after d8 GnRH treatment) generally results in a significant improvement in conception rate, compared to a progesterone/GnRH based program. In cows with low blood progesterone concentrations at the onset of treatment, a progesterone (P4)/GnRH program (d 0 = P4 device insertion and GnRH; d 7 = PGF2α; d 9 = P4 device removal; d 10 = GnRH; AI 56h after d 9 P4 device removal) generally results in a significant improvement in conception rate over the Ovisynch program (Murugavel *et al*., 2003).

2.2 Factors that affect the fertility of beef heifers and cows

The fertility of beef cows and heifers can refer to aspects such as the conception and pregnancy rate, as well as the fertilization and embryonic mortality rates in female animals. The effect of breed (among others frame size), maternal heat stress, nutrition, suckling, and other factors on the fertility is also important. The following paragraphs will provide details in this regard.

2.2.1 Conception and pregnancy rate in the cow

Conception rate can be defined as the percentage of cows not returning to oestrous 19 – 22 days after being serviced (non-return rate). Pregnancy rate can then be defined as the percentage of cows conceiving and subsequently calving following a service or AI. The conception rate is generally used by AI stations and technicians to measure the efficiency of their AI service. Conception rates can be > 5% higher than the actual pregnancy rate and studies regularly report conception rates at e.g. 65%, but then report the actual pregnancy rate in the same group of animals at e.g. 60% (Colazo *et* al., 2006). Pregnancy rates are traditionally considered to be highest in the temperate regions. In the past, countries like the UK and parts of the USA regularly recorded pregnancy rates to first service of 60 to 65%, although this number has decreased to approximately 50% in recent years. In the tropical regions however, conception rates to first service are generally considered to be in the region of 40 to 50% (Gordon, 1996). Conception rates then generally increase as the lactation period progresses to up to 100 days in milk. Primiparous cows generally record higher conception rates than older multiparous cows. Cows that are anovular at the start of the synchronization program generally exhibit poor conception rates, while poor body condition and post-partum health disorders also affect the conception rates negatively (Tenhagen, 2005).

2.2.2 Fertilization and embryonic mortality rates in the cow

The biochemical recognition of pregnancy inhibits the endometrial secretion of prostaglandin and also reduces the receptiveness to oxytocin. The embryo maintains the corpus luteum (CL) by blocking the luteolytic action of prostaglandin from day 16 and later in gestation (Lewis *et al.,* 1996, Hafez & Hafez, 2000). The embryo either directly or indirectly decreases the follicular development on the ovary by increasing the speed of transition of immature follicles to mature follicles - but at the same time also increasing the rate of follicular atresia. With no dominant follicle to produce oestradiol and start the next cycle, the CL stays in an anti-luteolytic state and maintains the pregnancy by producing progesterone. Females with sustained ovarian follicular development (cystic follicles) on the adjacent ovary may be prone to higher embryonic mortality rates. Therapeutic treatments that prevent the luteolytic action of the CL before maternal recognition, which takes place at approximately day 16 of gestation, would theoretically be beneficial to the uterine environment and the developing embryo. The supplementation of exogenous progesterone by means of an intravaginal P4 releasing device such as e.g. a CIDR®, 1 to 2 days after breeding has been known to reduce conception rates. The supplementation of exogenous progesterone on day 5 to 12 after breeding however, has been found to benefit pregnancy in both fertile and less fertile cows (Fields & Sands, 1994).

Fertilization rates generally differ between heifers and cows, but generally range between 75 to 100%, when good quality tested frozen semen is used. On average, fertilization failure only account for about 10% of the overall reproductive failures, while embryonic mortality rates account for 30%, or more. On average 80% of the early embryonic mortalities occur between day 8 and 18 following fertilization. In some low fertility herds, the incidence of late embryonic mortalities occurring after 21 days of pregnancy can be as high as 25%. Genetic factors, stress factors, age of the animal, hormonal factors, season of the year, body condition, nutritional status and chromosomal abnormalities all contribute to the high occurrence of embryonic mortalities (Gordon, 1996; Grimard *et al*., 2006).

2.2.3 The effect of breed on the fertility in the cow

There are two different breeds or types of cattle, regarded as either closely related species, or sub-species of the same species. For example the *Bos taurus* type of cattle are the typical cattle of Europe, north-eastern Asia and certain parts of Africa and are generally more adapted to cooler climates. Generally speaking the *Bos taurus* type of cattle produce more milk than the *Bos indicus* cattle and are more fertile, when comparing AI conception rates, but they generally have a lower survival rate in the tropical areas. The *Bos taurus* type of cattle generally have a smaller cervix, but larger uterine horns than the *Bos indicus* type of cattle. The *Bos indicus* type of cattle (also referred to as Zebu cattle) are generally more adapted to the tropical areas, possessing a larger skin area, with folds on the neck and brisket, a larger prepuce and numerous sweat glands to facilitate heat loss. The ovaries are also smaller in the *Bos indicus* than in the *Bos taurus* types, while the *Bos indicus* cattle generally have a longer gestation period (282 d ± 3 to 25 d), compared to the *Bos taurus* cattle (Gordon, 1996). Embryos from the *Bos indicus* cattle are then generally also more resistant to heat shock than embryos from *Bos taurus* type of cattle, during the early stages of in vitro embryonic development. The embryos generally become more thermo-tolerant as their development progresses. The embryonic resistance to heat shock is as a result of the genetic contributions of both the oocytes and sperm, and the heat tolerance obtained from cross breeding e.g. *Bos indicus* oocytes and *Bos taurus* sperm differ from e.g. *Bos indicus* oocytes and *Bos indicus* sperm (Eberhardt *et al*., 2009).

2.2.4 The effect of maternal heat stress on the fertility of the cow

Factors such as high ambient temperatures and humidity are associated with a marked seasonal decline in the reproductive efficiency of cattle. In India for instance, pregnancy rates in high producing dairy cows generally decline from 52% in winter, to 24% in summer. In South Africa, the conception rates following the first service are generally lowest (33%), when the temperature-humidity index is highest compared to a time when the index is at its lowest (74%) (Du Preez *et al.,* 1991). Exposure of *Bos indicus* breeds to short term heat stress generally has no immediate effect on reproduction. However, long term heat stress can cause a delayed and adverse effect on the follicular growth, gonadotropin hormone production and oocyte competence. Cows that are experimentally housed at ambient temperatures of 38°C and 80% humidity during the day and 30°C and 80% humidity at night time for 28 days, generally develop an increase in the number of follicles with a diameter of > 9 mm, resulting in a co-dominance of these follicles. Cows exposed to prolonged periods of heat stress also exhibit longer periods of non-cyclic activity and shorter oestrous cycles, than cows housed at moderate ambient temperatures (Torres *et al*., 2008). Heat stress in the cow often results in lower levels of circulating progesterone, abnormal patterns of progesterone secretion, a shorter CL lifespan, higher oestrogen levels during the preovulatory phase, a higher incidence of ovulation without behavioural oestrous, smaller mammary glands, reduced calf birth weights and a decreased milk yield. Heat stress is partly caused by the body's tendency to redistribute blood flow from the body's warmer internal areas e.g. the reproductive tract, to the body's cooler periphery in an attempt to cool down the body temperature (Gordon, 1996). High ambient temperatures have also been shown to increase both the rectal and uterine temperatures in cows, resulting in a higher respiration rate and reduced conception rate, a longer postpartum anoestrous interval and a reduced duration of the oestrous cycle (Fields & Sands, 1994).

2.2.5 The effect of nutrition on the fertility of the cow

Nutrition, or more specifically the energy status of a cow, plays an important role in the reproductive performance of the animal. Cows that are losing body weight during the breeding season generally have a smaller chance of conceiving, compared to cows that are on an increasing plain of nutrition. Heifers that are fed a high energy diet, generally reach puberty at an earlier age than heifers fed a low energy diet. The size of the dominant ovarian follicle is also generally larger in heifers fed a high energy diet, than heifers on a low energy diet (Romano *et al*., 2007). Changes in the diet such as moving cows from silage to spring grass, generally cause a temporary period of reduced fertility, especially when these changes occur within the 3 weeks leading up to mating. Fertilization failure and embryonic mortality also occur when cows are fed excessive rumen degradable proteins e.g. spring grass - which results in a lowered pH and an acidification of the uterus. It is often argued that the apparent improvement in fertility, after mineral supplementation is purely coincidental. There is abundant evidence however to support the fact that the supplementation of protein rich minerals improve fertility (MacMillan *et al.,* 1993). The provision of high protein supplements to postpartum cows generally lead to an increase in forage intake, and thus total energy in the diet. Increasing the postpartum protein intake generally initiates oestrous activity and an increase in the pregnancy rate in cattle (Fields & Sands, 1994).

The body condition of a cow changes constantly during the lactation period. Daily milk production peaks at approximately 30 to 40 days after calving, but it may take 60 to 80 days before the cow starts consuming enough feed to make up for her increased energy needs. In the meantime the cow utilizes her body reserves and thus loses body condition. Excessive body condition loss has been associated with a delayed resumption of ovarian activity and reduced fertility. The technique of body condition scoring (BCS) can then be used to monitor an animal's nutritional requirements, estimate its nutritional status and manage the supplementation of feed, if needed. For optimum reproductive performance it has been reported that a cow needs to be on an increasing nutritional plane, and have a BCS of at least 2.5 (out of a score of 5) to achieve optimal reproductive results (Gordon, 1996). Cows should be managed to calve in a moderate BCS and maintain their body weight after parturition to stimulate the secretion of the anabolic hormones, promote fat deposition, decrease the interval to first oestrous, increase follicular development and to maximize the pregnancy rate at the first oestrous (Ciccioli *et al*., 2003; Lents *et al*., 2008). Beef producers may even benefit in meat production by delaying weaning dates in calves born to mature and well-nourished cows (age > 4yrs), with no detrimental effect on the cow's subsequent reproductive performance (Hudson *et al*., 2010).

2.2.6 The effect of suckling on the fertility of the cow

Suckling has also been identified as a major factor affecting the reproductive performance in postpartum beef cows. Reserve energy deposits are generally stored in the body as fat, which can be mobilized when the nutritional condition of the animal deteriorates. These reserves are important as they regulate the secretion of the endocrine hormones (GH, ACTH, TTH), via the hypothalamus and the pituitary gland. These endocrine hormones then directly control the ovarian function and thus reproduction. Suckling as such also induces the secretion of certain endocrine hormones e.g. oxytocin, which may affect the metabolic and reproductive activity. A cow's energy status and reproductive performance can thus be evaluated by means of body condition scoring, an indicator of body fat deposition (Fields & Sands, 1994). Most beef production systems are based on an annual calving season, which means that cows should be rebred within 82 days of calving (assuming a 283 day gestation prior to a 365 day inter-calving period (ICP)). Anoestrous can be seen as a condition or time that allows the cow time to recover after calving, and suppress the onset of a new oestrous cycle, commonly referred to as the postpartum anoestrous period. Postpartum anoestrous intervals in cattle typically range from 35 to 70 days, but it is possible to have intervals as short as 10 to 20 days in well nourished, non-suckled cows to over a 100 day period in suckled, nutritionally - stressed cows. The length of the postpartum anoestrous period can generally be affected by factors such as season, breed, parity, dystocia, presence of the bull, uterine palpation and consequences from previous pregnancies. Suckling and lactation also induce an anoestrous period in cows. During this anoestrous period there is a 2 to 3 week period after calving when the pituitary replenishes its stores of gonadotropins (sex hormones). During this time the pituitary and hypothalamus also regain their capacity to respond to the endocrine hormones. The hypothalamus is thus ready to release GnRH and the pituitary gland ready to release FSH and LH. The ovaries also become responsive at the end of the postpartum anoestrous period, but the inhibitory effect of the calves suckling prevents the onset of oestrous. The act of suckling creates a metabolic, neural and psychological message which prevents the hypothalamus from secreting sufficient levels of GnRH (Fields & Sands, 1994).

There are certain management alternatives that lessen the impact of suckling on induced anoestrous. Nutrition is the most common cause of a prolonged anoestrous period and the resumption of the oestrous cycle after calving seems to have a fairly low priority, compared to functions such as e.g. lactation, ovarian activity, growth and basic body maintenance. Suckling thus greatly exaggerates the effects of poor nutrition. Weaning, partial weaning (calf removed and allowed to suckle once or twice a day), or temporary weaning (calf removed for 2 to 4 days at the start of the breeding season) generally increase the number of cows cycling, but also increase the risk of lowering milk production and weaning weights. Oestrous synchronization and the use of progesterone or prostaglandin in cattle synchronization programs generally stimulate certain anoestrous cows into a renewed state of oestrous activity. The presence of a bull (male effect) has also been shown to decrease the postpartum interval to the first overt oestrous. Disease-free teaser bulls should thus be used to induce oestrous activity, especially if the aim is to AI the cows (Fields & Sands, 1994).

2.2.7 The effect of frame size on the fertility of the cow

Increasing the mature size of a cow, by selecting for hip height, the age at puberty generally increases and the maturity rate generally decreases. As a result the conception rates in lactating heifers generally decline. It is possible to correct this reduced fertility with nutrition. Large frame heifers will generally have to be fed more than smaller framed heifers, in order to achieve comparable levels of fertility at the same age (Fields & Sands, 1994).

2.2.8 Body condition score (BCS)

The body condition score (BCS) is an indicator of the amount of energy reserves in the body of the animal and is scored between 1 (emaciated) and 5 (obese) on a scale of 1 to 5. In general, adequate body reserves are essential to maintain health, production and the reproductive efficiency of an animal. Under-conditioned cows with a BCS of 1 to 2 are prone to reduced milk production and poor persistency in lactation. Over-conditioned cows with a BCS of 4 to 5 on the other hand, are prone to calving difficulties, fatty liver syndrome, impaired reproduction and metabolic disorders (Kellogg, 2010). The BCS at different stages of the female's reproductive cycle and particularly at the onset of the mating season show a significant correlation to the subsequent pregnancy rate achieved (Schwalbach, 2005). Kellogg (2010) suggested the use of the following criteria in farm animals, according to the BCS categories (Figure 2.6) of 1 (emaciated) to 5 (obese).

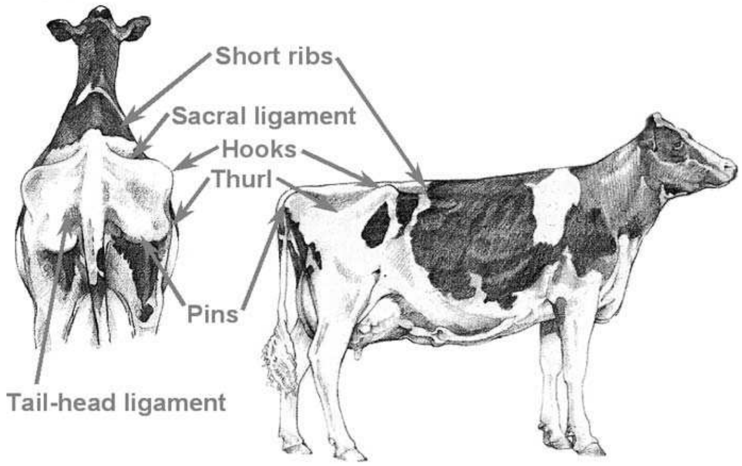

Figure 2.6: Sites of BCS evaluation in cattle

2.2.8.1 Body condition score (BCS) 1

For this score each vertebra of the animal is sharp and distinctly visible along the backbone. The short ribs are also clearly visible as individual bones (Figure 2.7). The ligaments connecting the sharp and well defined hook and pin bones to the backbone are also easily observed. The thurl is extremely concave and the area on either side of the tail head is sunken and hollow. There exist folds of skin in the depression between the tail bone and the pin bone (Kellogg, 2010).

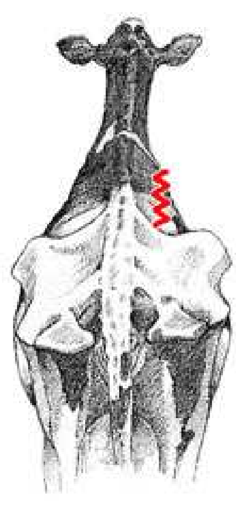

Figure 2.7: BCS 1 in an emaciated cow

2.2.8.2 Body condition score (BCS) 2

In animals with this score, the back bone is clearly visible, but does not stand out as individual vertebra. The short ribs are still prominent, with scalloping at the edges being very apparent. The thurl is generally very hollow, with prominent hook and pin bones (Figure 2.8). The ligaments connecting the bones to the back bone are very sharp and prominent. The area where the thigh bone meets the pelvis bone is evident, but unlike BCS 1, there is some muscle present. The area on either side of the tail head is concave, with folds of skin in the depression formed by the pelvis and tail (Kellogg, 2010).

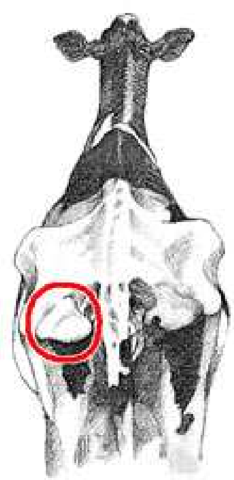

Figure 2.8: BCS 2 in a cow

2.2.8.3 Body condition score (BCS) 3

In this score, the vertebrae are rounded, but the back bone is still visible. There is 1 to 2 cm of tissue covering the short ribs. The edges of the ribs are rounded, but not as sharp as in the BCS 2. The hook and pin bones are clearly visible, but are rounded instead of angular (Figure 2.9). The ligaments connecting these bones to the backbone form clear boundaries between the cranial and caudal pelvic areas. The tissue covering makes it appear smooth and round. The thurl is concave, but not to the same extent as in the BCS 1 and 2. The area on either side of the tail head is hollow, but the folds of skin are not prominent (Kellogg, 2010).

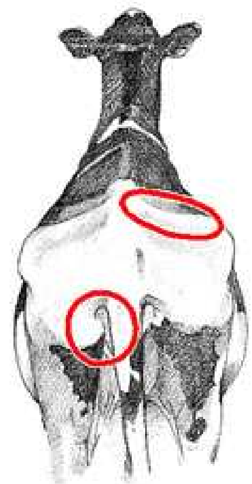

Figure 2.9: BCS 3 in a cow

2.2.8.4 Body condition score (BCS) 4

In this score the animal appears fleshy. The back appears flat like a table top. The short ribs still form a shelf, but cannot be identified as individual bones and only felt by strong palpation. The hook and pin bones are rounded and have prominent fat tissue padding (Figure 2.10). The area on either side of the tail head is not hollow, and there are no skin folds (Kellogg, 2010).

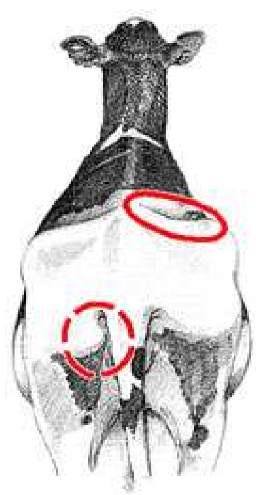

Figure 2.10: BCS 4 in a cow

2.2.8.5 Body condition score (BCS) 5

In this score the animals are obviously obese. The backbone and short ribs are not visible and only felt with difficulty. The shelf formed by the short ribs is well rounded and the thurl is filled. The hook bone appears like a ball and the pin bone is buried in the tissue (Figure 2.11). The fat deposition at the tail head gives a rippled appearance (Kellogg, 2010).

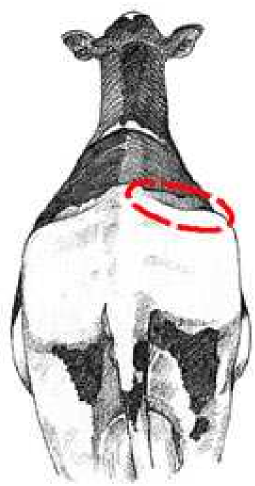

Figure 2.11: BCS 5 in a cow

2.2.9 Reproductive tract score (RTS)

The heritability of the reproductive tract score (RTS) is generally lower than expected, due to the fact that the reproductive track changes according to the oestrous status of the cow, and depends on the time and stage of the oestrous cycle. Cows that have completed uterine involution have not necessarily resumed ovarian activity, while cows that resume ovarian activity have not necessarily completed uterine involution. The following method of RTS evaluation for bovine has been suggested and described by Schwalbach *et al.* (2000):

Table 2.1: Reproductive track score for post-partum cows (Schwalbach *et al.,* 2000)

RTS score	Vulva & Vagina	Cervix	Uterus	Ovaries
1	Purulent discharge, recto-vaginal fistulae, pale mucosa	On the pelvic brim, not involuted, cervicitis, severe fibrosis	Not involuted, asymmetric, over the pelvic brim, irregular surface with content	Not active, no palpable structures, flat and small
2	Vaginitis or severe vulvae lesions with consequences to the shape and closure	Intrapelvic, not completely involuted, mild cervicitis, mild fibrosis	Not completely involuted, at the brim, distinct asymmetric, thick walled with content, no tone	Not active, no palpable structures but not flat
3	Vulvae lesions with consequences to the shape, but normal closure	Intrapelvic, involuted but with a small area of fibrosis	Not completely involuted, uterus intrapelvic, nearly symmetrical, thin walled with no content, no tone	Small developing follicles < 5mm, rounded ovaries
4	Normal, moist pink mucosa	Intrapelvic, normal	Involuted, intrapelvic, symmetrical, thin walled with no content, good tone	One ovary active with follicles > 10mm, possible CL
5	Normal, moist pink mucosa	Intrapelvic, normal	Involuted, intrapelvic, symmetrical, thin walled with no content, excellent tone	Both ovaries active with follicles > 10mm, CL present

2.3 Reproductive tract abnormalities affecting fertility in beef heifers and cows

The most common uterine abnormalities encountered in Zebu cattle are generally ovariobursal adhesions (5.5%), endometritis (3.9%) and cystic ovaries (3.5%). Other uterine abnormalities encountered are ovarian hypoplasia, vaginitis, cervicitis, tortuous cervical canal, mucometra, vaginal cysts, parovarian cysts, hypoplastic cervical rings, cervical cysts, freemartin, closed cervical os, uterine and oviduct adhesions, cystic uterine tubes, remnants of retained foetal membranes and cysts in the uterine wall. The prevalence of these abnormalities is generally higher in multiparous animals, compared to nulliparous animals and is presumably an important factor which may lead to possible infertility (Kumi-Diaka *et al*, 1981; Mickelsen *et al.*, 1986; Abalti *et al*., *2006*; Peretti *et al*, 2008).

2.3.1 The effect of congenital abnormalities on the fertility of the cow

Freemartin is a condition where a heifer is born as a twin to a bull. In general, 90% of Freemartins (heifer calves) are sterile. The sterility is caused by a chimeric condition where the haematitic cells intermingle in utero between the male and female foetus. This intermingling results in both XX and XY chromosomes being present in a Freemartin. The partial expression of the testicular-determining-factor from the Y male chromosome then inhibits the development of the female gonads (uterus and uterine horns).Ovarian aplasia which is a condition where either one or both of the ovaries are absent can result, and is caused by the abnormal development of the gonadal ridge during embryogenesis. Ovarian hypoplasia on the other hand, is a condition where either one or both of the ovaries are under-developed. It is a recessive trait that is caused by a single autosomal recessive gene (Kumi-Diaka *et al*, 1981; Mickelsen *et al.*, 1986; Abalti *et al*., 2006; Peretti *et al*, 2008).

2.3.2 The effect of pathological abnormalities on the fertility of the cow

Ovarian atrophy is a condition where either one or both of the ovaries regress and become inactive. This ovarian atrophy is generally caused by nutritional problems and is most commonly seen in high producing dairy cows. Silent heat is another condition where the cow will ovulate, but does not show any overt signs of oestrous. The first postpartum heat or oestrous is often an example of a silent heat and is caused by the low number of oestrogen receptors, subsequent to low circulating postpartum progesterone levels being present. Delayed ovulation is a condition that occurs when the cow ovulates later than 18h after the end of oestrous and generally occurs in less than 2% of the cows. Pyometra, again, is a condition where the uterus is filled with pus. The cervix is closed and thus blocks the pus from escaping from the uterus, which then prevents the normal luteolytic mechanisms from taking place. The fluid accumulation in the uterus could mimic a pregnancy, and the cow will also not return to oestrous. A prostaglandin treatment to lyse or degenerate the corpus luteum (luteolysis), generally return the cow to oestrous. A mummified foetus in the uterus will also mimic a pregnancy and prevent the normal luteolytic mechanism from taking place. A prostaglandin treatment to lyse the corpus luteum (decrease in progesterone levels), generally returns the cow to oestrous. Uterus unicornis is a condition where only one uterine horn is present, due to a Mullerian duct malformation. A persistent corpus luteum can also form on the contra lateral free-standing ovary - due to the lack of utero-ovarian counter current exchange from the uterine horn, ipsilateral to the persistent corpus luteum. Cystic ovarian disease is another condition where the cyst on the ovary may grow to more than 2.5cm in diameter. The ovarian cyst may persist for more than 10 days, or regress and be replaced by another cyst. There are two types of ovarian cysts e.g. follicular and luteal cysts (Kumi-Diaka *et al*, 1981; Mickelsen *et al.*, 1986; Abalti *et al*., 2006; Peretti *et al*, 2008).

2.4 AI related factors affecting fertility in the beef heifer and cow

There are many factors that affect the outcome of an AI program, some of which can be controlled and some of which cannot be controlled. An overview of the AI techniques available is useful in order to appreciate the difficulties that are faced in any AI program.

2.4.1 History of artificial insemination (AI)

According to Webb (2003), AI was documented in 1322 A.D. when an Arab Sheik wanted to mate his prize mare to an outstanding stallion belonging to his foe. He introduced a wand of cotton into the mare's reproductive tract and then used it to sexually excite the stallion causing the animal to ejaculate. The semen was then introduced into the desired mare, resulting in successful conception.

2.4.2 Artificial insemination (AI) techniques in cattle

Webb (2003) further identified the following different AI techniques used following controlled breeding in cattle:

i) The deposition of semen directly into the vagina. This method is however not very effective, as conception rates are generally low and large numbers of sperm are required for fertilization

ii) AI performed with the aid of a speculum. This technique is easily learnt, but proper hygiene and sterilization of the equipment is essential - making it less practical to inseminate large groups of animals. Semen is then deposited at the mouth of the cervix, also resulting in limited success.

iii) The recto-vaginal technique using a sterile, disposable pistolette, containing the semen which is inserted into the vagina and guided manually through the cervix, via the rectum. The pistolette is passed through the cervix and the semen deposited directly into the uterus. This technique gives acceptable fertility results.

2.4.3 The effect of AI timing relative to oestrous on the conception rates in cows

True standing heat or oestrous occurs when a cow is receptive to the male and stands to be served by the bull or allows other cows to mount her. This can last for between 4 to 27h from the time that the cow stands to be mounted, with the average duration of oestrous being 18h. The event of ovulation then involves the release of an oocyte from the mature ovarian follicle and this usually occurs 24 to 30h after the onset of oestrous, or 10 to 12h after the end of oestrous in the cow (Lewis *et al.*, 1996) (Figure 2.12).

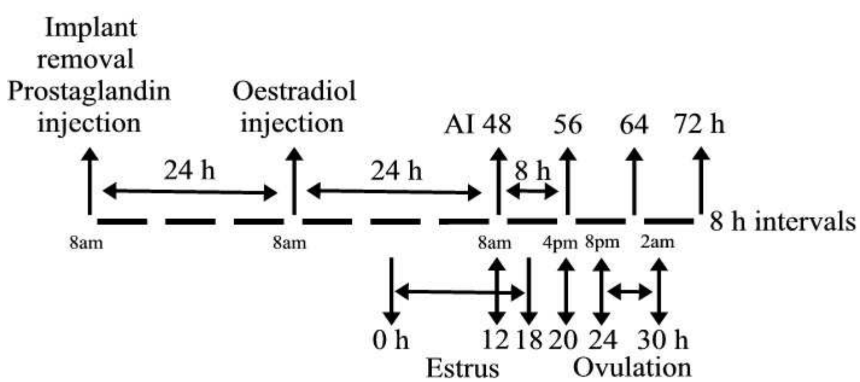

Figure 2.12: Timing of oestrous and ovulation following controlled breeding in cows (Lewis *et al.*, 1996)

It has been demonstrated that heifers generally achieve a higher conception rate when inseminated 56h after P4 device removal, than when FTAI is implemented at 48, 64 or 72h. After device withdrawal, although cows generally have similar conception rates when FTAI is performed at 48, 56, 64 or 72h after P4 device removal. Embryonic mortalities are however higher between days 32 to 63, when FTAI is performed at 48 and 72h after P4 device removal (Dobbins *et al.*, 2009). Similarly postpartum beef cows generally recorded a higher conception rate with FTAI performed at 66h after P4 device withdrawal (67%), compared to when FTAI was performed 54h (61%) after P4 device removal (Busch *et al.*, 2008).

In embryo flushing programs the time of AI in relation to the onset of oestrous plays an important role in the total number of recovered viable, degenerate or unfertilized embryos/oocytes. FTAI (fixed time AI) in cows at either 0 and 12h or 12 and 24h after the onset of oestrous has resulted in comparable embryo yields. However heifers generally respond better when FTAI is performed at 12 and 24h after the onset of oestrous (Sales & Souza, 2005). AI performed 12h after the onset of oestrous provides the best compromise between the lower fertilization rate of AI obtained at 0h after the onset of oestrous, and the lowered embryo quality (although higher fertilization rate), due to the increased occurrence of degenerate embryos following AI 24 h after the onset of oestrous (Dalton *et al.*, 2001).

2.4.4 The effect of AI timing relative to oestrous on the gender ratio of the offspring in the cow

Certain studies have reported the possibility that cows that are inseminated at approximately 10h before ovulation predominantly produce bull calves, while cows inseminated at approximately 20h before ovulation, predominantly produce heifer calves. The rationale being that the lighter XY chromosome carrying the male sperm travel faster and reach the oocyte before the heavier XX chromosome carrying female sperm. However, the male sperm generally have a shorter life span and die off earlier than the female sperm. These results have however been difficult to reproduce in practice. The site of semen deposition could also play a role in the gender ratio of the offspring. So for example, semen deposition into the uterine body generally result in more bull calves being (21%) born. While semen deposited deeper into the uterine horn, ipsilateral to the ovary with the dominant follicle, generally result in more heifer calves (20%) being born. Semen deposition into the uterine body then generally also has a higher conception rate (> 7%), than when the semen is deposited into the uterine horn (Pursley, 2004; Rorie *et al.*, 1999; Zobel *et al*., 2010).

2.5 Semen related factors affecting the fertility of beef heifers and cows

Synchronization programs generally facilitate FTAI, by altering the length of the oestrous cycle by manipulating ovarian follicular growth. Thereby making the occurrence of oestrous more predictable and allowing for FTAI, without the need for oestrous detection. Although protocol compliance e.g. accurate cow identification, appropriate drug dosage and route of administration, the correct time and day of treatment, etc., are very important factors contributing to the success of an oestrous or ovulation synchronization program, many other factors may however also play a role (Dalton *et al.,* 2010).

2.5.1 The effect of semen thawing on artificial insemination conception rates in the cow

Bovine semen frozen in liquid nitrogen, is traditionally thawed in water at 32 to 37°C for ± 40 seconds. There is generally no significant difference in the overall post-thaw sperm motility or acrosome integrity in sperm thawed at 37°C for 40s, 50°C for 15s or 70°C for 5s. A higher proportion of the sperm usually still exhibit progressive motility after a 2h post-thawing incubation period compared to bovine semen thawing performed at a faster rate (37°C/40s = 8.3%; 50°C/15s = 18.1%; 70°C/5s = 16.5%) (Muino *et al*., 2008). In semen processed to allow for flexible thawing, thawing by either pocket thawing or warm-water thawing methods generally does not significantly affect the conception rate of the semen when used under commercial conditions (Kaproth *et al*., 2005). The simultaneous thawing of straws generally has no effect on the conception rate, provided that the straws are used within 15 minutes of thawing (Dalton *et al.,* 2004).

2.5.2 The effect of sperm dosage on the artificial insemination conception rate in cows

Fertility generally increases with an increase in the number of viable sperm inseminated, up to a threshold level. After this threshold level has been reached, the female population becomes the limiting factor and an increase in the number of sperm per insemination does not result in any further increase in fertility or fertilization. Generally the minimum number of motile sperm required for maximum fertility differs between bulls, but the fertility increases with increasing numbers of structurally intact and motile sperm. On average, 5 to 7 x 10^6 viable sperm per inseminate has been suggested as being optimal for fertilization. Low fertile bulls require more sperm per inseminate than higher fertile bulls, in order to obtain maximum conception (Dalton *et al.,* 2010). Generally, there is no significant difference in the conception rate when using semen packaged in either 0.25 or 0.5 ml French straws (Johnson *et al*., 1995).

2.5.3 The effect of sperm morphology on artificial insemination conception rates in the cow

Fertilization failure and abnormalities in embryogenesis are both considered to be of seminal origin. Bulls generally differ in the number of sperm required to achieve maximum conception. Males requiring more sperm are considered to have morphologically compensable seminal deficiencies. These compensable traits in semen quality relate to the ability of inseminated sperm to not only reach the ovum, but also bind to and penetrate the zona pellucida and initiate the block to polyspermia. The effect of compensable seminal deficiencies can be lessened by increasing the number of available sperm and include e.g. dag defects, mid-piece reflex, broken tails, loose heads, etc. Differences in fertility between bulls or inseminates, independent of sperm dosage, are generally considered to be due to morphologically uncompensable seminal deficiencies. Uncompensable traits in semen quality on the other hand, relate to the competence of the fertilizing sperm to complete the fertilization process and sustain early embryonic development. The effect of uncompensable seminal traits cannot be lessened by increasing the number of available sperm and include sperm with e.g. nuclear vacuoles, pyriform heads, deteriorating acrosomes etc. Uncompensable seminal traits have been reported to have a detrimental affect on the overall embryo cleavage rates. (Miller *et al.*, 1982; DeJarnette *et al.*, 1992; Saacke *et al.*, 2000; Acevedo *et al.*, 2002; Fernandes *et* al., 2008; Saacke, 2008).

2.5.4 The effect of the semen deposition site on artificial insemination conception in cows

Many bovine studies have compared semen deposition near the greater curvature of the uterine horns, to the conventional deposition of semen into the uterine body. Some claim an increased conception rate when the semen is deposited in the uterine horns, rather than the uterine body (Pursley, 2004). Others however have found no difference between the two insemination deposition sites (Diskin *et al.*, 2004). In general, 20% of AI's aimed at depositing the semen within the uterine body, are actually wrongly deposited within the cervix, which generally results in a 10% reduction in conception rate (Peters *et al.*, 1984).

2.5.5 The effect of fresh vs. frozen semen on artificial insemination conception rates in cows

The use of quality tested fresh semen at a concentration of 3×10^6 sperm/straw, generally achieve more acceptable fertilization results, compared to quality tested frozen semen at a concentration of 20×10^6 sperm/straw. Fresh semen generally provides an alternative to the use of frozen semen for the more efficient utilization of superior genetics (Bucher *et al.*, 2009). The use of either fresh or frozen semen does however not affect the total embryo yield during embryo flushing (MOET) programs, but frozen semen generally result in fewer grade 1 and 2 embryos and more grade 4 and 5 embryos, than fresh semen (Goulding *et al.*, 1994).

In New Zealand, 95% of the semen used for AI in cattle is processed as fresh semen. Fresh semen can be stored for up to 3 days in Caprogen® diluents, without a significant reduction in fertility (Verberckmoes *et al.*, 2005). The lifespan of sperm in the cow's oviduct is believed to be 24 to 48 h, when freshly ejaculated semen is used, and 12 to 24 h if frozen-thawed semen is used (Gordon, 1996). A fertilization failure rate of 10 to 12 % seems to be common when both fresh and frozen semen is utilised (Diskin *et al*, 2004).

2.5.6 The correlation between accessory sperm numbers and embryo quality

"Accessory sperm" is the term generally given to sperm that have been trapped within the zona pellucida during the zona reaction. Accessory sperm are able to penetrate the oviduct, undergo capacitation and the true acrosome reaction, recognize and bind to the zona pellucida, and then at least partially penetrate the zona pellucida. These accessory sperm are then trapped in the zona pellucida by the zona reaction, a functional block to polyspermia that occurs immediately after fertilization, by the fertilizing sperm cell. The accessory sperm trapped within the zona are thought to be an indirect indicator of the sperm transport, and a quantitative measure of the available sperm, competing for fertilization. Good or excellent embryos possess more associated accessory sperm, compared to fair, poor or degenerated embryos or unfertilized ova (DeJarnette *et al.*, 1992). There is however a large variation in the associated accessory sperm numbers within the embryo quality categories, and this precludes the use of accessory sperm numbers as a reliable predictor of bull fertility (Howard *et al.*, 1993).

2.6 Considerations when breeding heifers at twelve months of age

Heifers are generally selected for breeding either as replacement cows, to further expand the size of the herd or to be sold as pregnant heifers. In recent years a mind shift has been made from breeding heifers at 2 years of age, to breeding yearling heifers to calve at 2 years of age (Fields & Sands, 1994).

2.6.1 The endocrine regulation at puberty in the heifer

From as early as 1 month of age, heifers are capable of producing fertile ova when administered exogenous gonadotropins - demonstrating that the reproductive organs are functional well before the physical onset of puberty (the stage at which a functional CL is present). Furthermore, the exogenous administration of oestradiol has been found to induce pre-ovulatory LH surges in pre-pubertal heifers, again showing that the endocrine system responsible for these hormonal surges are functional long before the onset of puberty. Puberty as such is generally delayed by the fact that the pre-pubertal ovaries secrets low levels of oestradiol, too low to elicit a worthy LH / FSH response. As time progresses the ovaries mature and more oestradiol is secreted, which finally induces puberty to occur. All too often an increase in dietary energy intake is sufficient to help tip this balance (Hafez & Hafez, 2000).

2.6.2 Advantages and disadvantages of breeding yearling heifers

Advantages of breeding yearling heifers:

- Shorter interval to return on capital investment
- Increased lifetime production of the cow

- Increased calving rate (output) per herd per year
- Less pressure on pastures to separate heifers and cows during the breeding season

Disadvantages of breeding yearling heifers:

- Increased feed costs associated with breeding heifers as yearlings
- Increased calf losses due to dystocia and associated managerial problems
- Lower conception rates in second-calf heifers, compared to older cows
- Fewer and smaller calves weaned, compared to the older heifers which were mated later (Fields & Sands, 1994).

2.6.3 Managerial factors affecting age at puberty in the heifer

The genotypes of different breeds demonstrate different maturing rates and therefore reach puberty at different ages. Individual sires within the same breed may however also differ in their maturing rate. Sires known for producing early maturing heifers should preferably thus be selected. The season of the year also affects the pasture quality through the available photoperiod and rains, and has an effect on the age at puberty. Heifers born during increasing periods of day light length generally reach puberty 2 months earlier than those born during periods of decreasing day length. The growth rate and nutritional intake also have an effect on the age at puberty and faster growing individuals generally reach puberty sooner, but only until a certain threshold body weight limit is reached. Heifers born late in the calving season are also generally lighter and younger at puberty than their counterparts. Both pre- and post-weaning weight gain (ADG) has an effect on the body weight at which puberty occurs (Fields & Sands, 1994).

2.6.4 The effect of age at puberty on the reproductive performance of heifers

Age at puberty of heifers is generally regarded as 50% heritable and closely related to the scrotal circumference in yearling bulls. The degree of puberty can thus be measured using a reproductive tract score (RTS) of 1 to 5, where RTS 1 represents a small, immature tract with no palpable corpus luteum (definitely not cycling), and a RTS of 5, a tract containing a palpable CL (definitely cycling). An examination performed on the reproductive tract of the heifer approximately 30 to 60 days prior to the breeding season can serve as an indication of the nutritional and sexual status of the animal. From a genetic viewpoint, faster growing heifers generally mature earlier. A positive correlation exists between the age of the heifer and the body weight at puberty. A seasonal effect also exists, whereby heifers born later in the calving season reach puberty at an earlier age, although heifers born earlier in the breeding season reach puberty at an earlier age (Fields & Sands*,* 1994). Some heifers show signs of behavioural oestrous, without the subsequent development of a functional CL, termed non-pubertal oestrous (NPE). There is a higher tendency in heifers that weigh below the group body weight average, to exhibit NPE later than in heifers weighing on or above the group average. The heifers that show pubertal oestrous (behavioural oestrous with the subsequent development of a functional CL) are also generally older than heifers exhibiting NPE (Rutter & Randel, 1986). Heifers tend to be more fertile from their 3rd pubertal oestrous onwards, than during the pubertal oestrous (1st ovulation), probably due to the maturational changes associated with the cycling activity (Byerley *et al*., 1987).

2.6.5 The effect of postpartum anoestrous on the conception rate in heifers

Heifers calving at 2 years of age still weigh approximately 80% of their mature body weight, and thus still require energy for growth. This partitioning of nutrients is in direct competition with the energy needed for lactation and other reproductive activities. Consequently, the 2 year-old heifers generally wean lighter calves (80% of the mature cows body weight), have a longer interval between calving and the subsequent first post-partum oestrous, and thus also record a lower second calving pregnancy rate. This problem can be partially addressed by strategic feed management, to improve the body condition and satisfy the nutritional needs of the second calf heifers (Fields & Sands*,* 1994). Cows generally resume their oestrous cycles within approximately 41 days after calving. Incidences of reproductive problems are generally low once oestrous cycling has commenced (6.5%). Cows that do develop reproductive problems exhibit a delayed interval to first service, lower conception and calving rates, increased services per pregnancy and a higher barren rate, compared to cows that start cycling normally (Mann *et al*., 2005).

2.6.6 The correlation between scrotal circumference and age at puberty

As approximately 90% of the genetic change in a herd over time can be ascribed to the contribution of the bull, it would make sense to identify the hereditable reproductive traits in bulls and use them to optimally improve the reproductive performance of the female offspring. Scrotal circumference in yearling bulls has been shown to be a moderate to highly hereditable trait (approximately 50%), and selection for this trait could be very effective in improving the scrotal circumference in bull calves. Scrotal circumference is generally accepted as a better indicator of puberty, compared to age or body weight. Bulls generally reach puberty when they produce 50×10^6 sperm/ml, with a minimum of 10% sperm motility - normally at an average scrotal circumference of 27.9cm. In general, as the scrotal circumference of the bull increases, so does the sperm motility, the percentage morphologically normal sperm, ejaculate volume, sperm concentration and total sperm output. Age at puberty and scrotal circumference are thus essentially the same trait, or closely related. Scrotal circumference is thus favourably related to several female reproductive traits, most notably the age at puberty (Fields & Sands, 1994).

2.6.7 The use of pelvic measurements as a predictor of the age at puberty in the heifer

The pelvic measurement of offspring are considered to be moderately to highly hereditary (h = 0.29 and h = 0.23 for width and h = 0.86 and h = 0.93 for height and area (H x W) in cows and heifers respectively), and it would appear that relying on pelvic measurements, instead of body weight and skeletal size, is justified. Generally most genetic progress is made by sire selection, and the pelvic measurement of yearling bulls could predict the performance of the female offspring. The pelvic dimension in bulls appears to be highly correlated to female pelvic height, width and area. The selection for male pelvic height should thus result in an increased pelvic dimension in female offspring, decreasing the frequency of dystocia (Fields & Sands, 1994).

2.7 Pregnancy diagnosis in cows

The success of any AI program is ultimately measured in the conception, and later pregnancy rate achieved by the AI program. It is therefore necessary to have a basic knowledge of the different techniques uses to measure the outcome of the AI program.

2.7.1 Rectal palpation

Rectal palpation of the uterus is one of the most accurate and extensively used techniques (95%) for determining the pregnancy status of a cow. Rectal palpation at 35 days after AI can be performed by an experienced operator with 99% accuracy, while real time ultrasound scanning of the uterus at 23 to 31 days after AI is approximately 70% accurate (Badtram *et al.,* 1991).

The following occurrences can generally be detected by rectal palpation:

- The presence of a mature CL, approximately 3 weeks after oestrous, after the cow had been served or inseminated, is suggestive of pregnancy
- Disparity in the uterine horn size from about 30 to 35 days gestation due to the distension of the allantochorion by the allantoic fluid is generally an indicator of pregnancy. The uterine wall appears thinner to the touch and fluctuates due to the presence of the allantoic fluid
- Poor postpartum uterine involution and pyometra can also be felt manually at this stage, but is distinguishable from early pregnancy by a thickened uterine wall
- The membrane slip can be felt from 35 to 40 days of gestation. The allantochorion can be identified as a delicate strand of tissue which is felt to slip away from the grasp of the fingers, just before the wall of the uterine horn during palpation
- The foetus becomes palpable from 65 days gestation onward and generally remains within the pelvic brim for the first 3 months
- From 4 months of gestation and onward, the uterine horns reach over the pelvic brim and into the posterior abdominal cavity
- Placentomes are palpable from 70 to 80 days gestation and considered to be a reliable sign of pregnancy (Gordon, 1996).

2.7.2 Real time ultrasound scanning

Real time ultrasound scanning involves the use of a rectal probe which transmits harmless ultrasound waves through the body tissue. These waves are reflected to the transducer when they reach the foetus and are converted to produce an image on a screen. By using a 5 MHz transducer, amniotic fluid can be detected from 24 days of gestation and the foetal membranes from 28 days of gestation, with 98% accuracy (Herak *et al.,* 1993).

2.7.3 Milk and blood progesterone radio-immuno-assay (RIA)

Milk and blood progesterone radio-immuno-assay (RIA) can be used as an early alternative to rectal palpation when diagnosing pregnancies, oestrous confirmations and reproductive disorders. Blood or milk samples are collected on day 0, day 10 to 12 and day 22 to 24 after AI. The blood sample is centrifuged to separate the serum, which is then aspirated and analysed using a direct solid phase RIA, marked with an I^{125} labelled progesterone tracer. The serum progesterone concentrations are interpreted as low (<1nmol/l), intermediate (1 to 3nmol/l) or high (>3nmol/l). The RIA serum progesterone profiles are then used to determine the ovarian activity and pregnancy status of the cow and will indicate if an animal was e.g. inseminated at the wrong time. A low-high-low serum progesterone profile is indicative of a non-pregnant animal, a low-high-high serum progesterone profile is indicative of a pregnant animal, a high-high-high serum progesterone profile is indicative of an animal pregnant at the time of AI or of an animal with a persistent CL, while a high-low-high serum progesterone profile can indicate an animal inseminated at the wrong time during the oestrous cycle, etc. (International Atomic Energy Agency, 2000; Toleng *et al*., 2000; Zhenghua *et al*., 2000).

The low accuracy of oestrous detection, coupled with the poor response to the synchronization hormones, the fertilisation rate of oocytes, a high embryonic mortality rate and functional hormonal disturbances are factors that could determine the outcome of the FTAI programs (Pedroso *et al.,* 2000; Busch *et al*., 2008).

Chapter 3

Material and Methods

3.1 Trial location

This trial was carried out to coincide with the annual spring breeding program of WO Beefmasters on the farm Oribilaagte, near the town of Vrede in the Free State Province. The stud of Beefmaster heifers and cows have been FTAI'd according to an established synchronization program for many years.

WO Beefmasters is a family livestock business run by the Odendaal family. The Free State Province falls within a summer rainfall area; known for abundant *Themeda triandra* (red grass) pastures during the summer months, followed by cold winter months where the quality of the grazing deteriorates to protein values of less than 2%. The Odendaal family started farming with predominantly Sanga x Tauricus crossbred cattle in the early 1970's. The owner Mr. Willem Odendaal visited Beefmaster breeders in the USA during the 1980's and decided to implement the 6 principles described by Tom Lasater, a Beefmaster breeder, who today is regarded by many as the founder of the modern day Beefmaster breed. The 6 principles include (i) fertility, (ii) adaptability, (iii) durability, (iv) milk production, (v) growth and (vi) temperament - which today form the basis of the WO Beefmasters breeding strategy.

Artificial insemination has become a vital tool to the WO Beefmaster enterprise and semen from mainly the USA and Zimbabwe were initially used. The *Bos indicus* type of animals were inseminated with *Bos taurus* breeds, and vice versa, to produce an animal that is adaptable and durable, yet still early maturing and fertile. The Odendaal family believes that a heifer should calve at 2 years of age and produce a calf annually thereafter. Currently 90% of the breeding herd is inseminated with the top 2% of the previous year's progeny bulls, while the remaining 10% of the breeding herd is selectively inseminated to improve calving ease, colour, hair type, size, conformation, etc. The progeny bulls are generally selected on birth weight, a weaning weight of at least 350 kg, a yearling scrotal circumference of more than 40 cm and cow : calf weight ratio of 60%. Calves born from these bulls boast some of the best cow : calf weight ratios in the breed, with many calves weaning in excess of 300 kg at 7 months of age.

The Odendaal family attribute their livestock breeding success to the high selection criteria that they have implemented through the selective use of artificial insemination.

3.2 Research design

Two groups of cattle were used in the trial, namely a group of cows (n=100) and a group of heifers (n=100). Each group was then sub-divided into four progestagen treatment groups (n=25/treatment group). Following synchronisation with the aid of CIDR's and Ciderol treatment, fixed-time AI was performed 50h after CIDR withdrawal. Thus the experiment was performed using a 4 x 2 factorial design (i.e. treatment vs. age group).

3.3 Experimental animals

The experimental animals in this study included 100 stud Beefmaster heifers (mean age of 14 ± 2 months; body weight 320 ± 20kg; BCS 3 ± 0.5) and 100 stud Beefmaster first-calf cows (mean age of 3 years; body weight 450 ± 50kg; BCS 3 ± 0.5; parity 1). The cattle were all kept on natural grazing and maintained year round under similar managerial conditions (environment, level of nutrition, facilities, etc.), and all originated from the farm Oribilaagte and were thus adapted to the environment.

3.4 Treatment groups

Both the Beefmaster heifer and cow groups (n=100 per group) were randomly allotted to the following four treatment groups:

1. Group 1: 0.5g P4 DIB® intravaginal device treatment group (n=25)
2. Group 2: 1g P4 DIB® intravaginal device treatment group (n=25)

3. Group 3: 1.9g P4 CIDR® re-used intravaginal device treatment group (n=25)
4. Group 4: 1.9g P4 CIDR® intravaginal device treatment group (n= 25)

3.5 Trial time-frame

The Beefmaster heifer group was treated with the P4 intravaginal devices on the 22nd of November 2010 (d 0), at which time the body condition score (BCS) and reproductive tract score (RTS) evaluations were also performed. The cloprostenol (PGF2α) treatment was administered on the 29th of November 2010 (d 7). The P4 intravaginal devices were removed on the 30th of November 2010 (d 8), followed by an estradiol benzoate treatment on the 1st of December 2010 (d 9). The fixed time artificial insemination (FTAI) was then performed on the 2nd of December 2010 (d 10). The Beefmaster cow group was treated with the P4 intravaginal devices on the 23rd of November 2010 (d 0), at which time the BCS and RTS evaluations were also performed. The cloprostenol (PGF2α) treatment was administered on the 30th of December 2010 (d 7). The P4 intravaginal device was removed on the 1st of December 2010 (d 8), followed by an estradiol benzoate treatment on the 2nd of December 2010 (d 9). The FTAI was performed on the 3rd of December 2010 (d 10). The conception status (pregnancy) of each animal was determined on the 4th and 5th of May 2011.

3.6 Body condition score (BCS) and reproductive tract score (RTS) evaluations

The BCS and RTS evaluations were performed as described by Kellogg (2010) and Schwalbach *et al.* (2000), respectively. The cow and heifer treatment groups were very uniform and the variation in both BCS and RTS were found to be negligible.

3.7 Oestrous synchronization protocol

Apart from the different P4 concentrations contained in the DIB® and CIDR® intravaginal devices, the different treatment groups were treated and managed as one group. The synchronization program applied was as follows (Pfizer, 2010):

Day 0: i) Body condition scoring (BCS)

ii) Reproductive tract scoring (RTS)

iii) P4 intravaginal device (DUB® or CIDR®) insertion

iv) 1mg i.m. treatment with oestradiol benzoate (1ml Ciderol) for heifers or 2mg i.m. treatment with oestradiol benzoate (2ml Ciderol) for cows

Day 7: i) 250µg s.c. treatment with PGF2α cloprostenol (1ml Estrumate)

Day 8: i) P4 intravaginal device (DIB® or CIDR®) withdrawal

ii) Collection of blood samples (7ml) from the coccygeal tail vein of all animals

iv) 50ml F10 disinfectant solution introduced into the vagina

Day 9: i) 1mg i.m. treatment of oestradiol benzoate (1ml Ciderol)

Day 10: i) Fixed time artificial insemination (FTAI) 50 ± 1 h after P4 intravaginal device withdrawal

ii) Collection of blood samples (7ml) from the coccygeal tail vein of all animals

Month 5: i) Rectal pregnancy diagnoses of all animals

3.8 Artificial Insemination technique

The general recto-vaginal AI technique was used to inseminate all the cattle, with the semen being deposited directly into the uterine body (Webb, 2003). All the animals within the same treatment group (heifer or cow groups) were FTAI'd with quality tested (80% individual sperm motility, 70% normal sperm morphology) fresh semen collected from a 20 month old Beefmaster bull directly before commencing with the AI program. The semen was collected on the farm and extended with Triladyl® to a final sperm dose concentration of > 10 x 10^6 sperm / dose. The extended semen was microscopically evaluated pre- and post- FTAI to avoid any sperm deterioration bias.

3.9 Serum Radio-Immuno-Assay progesterone analyses

The first blood sample was taken on the day of progesterone (P4) device withdrawal in order to demonstrate a high serum P4 level, indicative of an animal in the luteal phase of the oestrous cycle. The second blood sample was taken on the day of the fixed time artificial insemination (FTAI) to demonstrate a low serum P4 level indicative of an animal in the non-luteal phase of the oestrous cycle. The blood samples were centrifuged and the serum aspirated and frozen at -20 °C, where after it was delivered to the Onderstepoort Veterinary Institute as one batch for comparative solid-phase Radio-Immuno-Assay (RIA) serum P4 analyses.

3.10 Pregnancy diagnosis

Rectal palpation for the identification of pregnancy was performed in May 2011 (end of autumn); approximately 60 days after the follow-up bulls have been removed from the breeding group. Individual animals that were found to be 5 months pregnant, were recorded as pregnant as a result of the FTAI performed in December 2010 (onset of summer). Animals that were found to be less than 5 months pregnant were recorded as been serviced by the follow-up bulls.

3.11 Statistical analysis

A one way analysis of variance (ANOVA) was performed on the serum concentration of P4 on day 8 and day 10, and the conception rate for the different levels of P4 treatments recorded. Factorial analysis of variance (ANOVA) was performed on the P4 concentrations with the day and the conception rate as factors. Factorial analysis of variance (ANOVA) was performed to compare conception rate of cows and heifers, with overall group (cows/heifers), and treatment as factors. The LSD test was used to compare the P-value means at a 5% level of probability, using STATISTICA version 8.0 (StatSoft Inc., 2004).

Chapter 4

Results

This chapter reports on the results recorded for the first parity 2008 cow group (Group A) and the 2009 heifer group (Group B), respectively. In line with the objectives of the study, par. 4.1 and 4.2 will provide an overview of the progesterone concentrations at different days after application and the conception rates of the cow- and heifer treatment groups respectively. Paragraph 4.3 will provide detailed information on each of the these aspects, including the LSD test results indicating the meaningful differences ($p < 0.05$) that is used in par. 4.1 and 4.2.

4.1 Group A – 2008 Cow Group

The 2008 cow group was treated with different concentrations (g) of the P4 DIB® intravaginal device and recorded different results as can be seen in Table 4.1.

Table 4.1: D8 and d10 serum P4 concentrations and conception rates of the cow P4 treatment groups

P4 (nmol/l)	*0.5g*	*1.0g*	*1.9g*	*1.9g/r*
P4 (d8)	3.70 [a]	3.58 [a]	4.95 [b]	4.00 [a]
P4 (d10)	1.78 [a]	1.72 [a]	1.76 [a]	2.46 [a]
Conception rate	65% [a]	75% [a]	67% [a]	68% [a]

[a b] values in a row with different superscripts differ statistically significantly ($p < 0.05$)

4.1.1 Cows treated with the 0.5 g intravaginal progesterone device

The cow group treated with the 0.5g P4 DIB® intravaginal devices, recorded a serum P4 profile of high (d8) – intermediate (d10), instead of a high (d8) – low (d10) serum P4 profiles, and was only partially comparable to the theoretical IAEA model for a serum P4 profile recorded, conducive of conception rates in cattle (IAEA, 2000). The high d8 serum P4 concentration of 3.70nmol/l was indicative of cows that were in the luteal phase of the oestrous cycle on the day that the intravaginal P4 devices were withdrawn. The intermediate d10 serum P4 concentration of 1.78nmol/l again was indicative of cows that still retained some circulatory serum P4 on the day of fixed-time AI. Even though this group did not follow the theoretical IAEA model for P4 serum profiles, the average serum P4 concentration between d8 and d10 decreased by 1.92nmol/l, and the group managed to record an overall conception rate of 65% (Table 4.1).

4.1.2 Cows treated with the 1.0 g intravaginal progesterone device

The cow group treated with the 1.0g P4 DIB® intravaginal device also recorded a serum P4 profile of high (d8) – intermediate (d10) - again only partially comparable to the theoretical IAEA model for a serum P4 profile, conducive to conception (IAEA, 2000). The high d8 serum P4 concentration of 3.58nmol/l recorded for this group was indicative of cows that were in a luteal phase of the oestrous cycle on the day that the intravaginal P4 devices were withdrawn. The intermediate d10 serum P4 concentration of 1.72nmol/l was again indicative of cows that still retained some circulatory serum P4 on the day of fixed-time AI. Even though this group did not fall into the low serum P4 category, as would be expected when compared to the theoretical IAEA model, the average serum P4 concentration level between d8 and d10 decreased by 1.86nmol/l and the group managed to record an overall conception rate of 75% (Table 4.1).

4.1.3 Cows treated with the 1.9 g intravaginal progesterone device

The cow group treated with 1.9g P4 CIDR® intravaginal devices recorded a serum P4 profile of high (d8) – intermediate (d10), again only partially comparable to the theoretical IAEA model, as set out for a serum P4 profile, conducive to conception (IAEA, 2000). The high d8 serum P4 concentration of 4.95nmol/l was indicative of cows that were in the luteal phase of their oestrous cycle, on the day that the intravaginal P4 devices were withdrawn. Here the intermediate d10 serum P4 concentration of 1.76nmol/l was indicative of cows that still retained some circulatory serum P4 on the day of the FTAI. Even though this group did not fall into the low serum P4 concentration category as would be expected, when compared to the theoretical IAEA model, the serum P4 concentration between d8 and d10 decreased by 3.19nmol/l and the group managed to record an overall conception rate of 67% (Table 4.1).

4.1.4 Cows treated with the 1.9 g re-used intravaginal progesterone device

The cow group treated with the 1.9g re-used P4 CIDR® intravaginal devices recorded a serum P4 profile of high (d8) – intermediate (d10), again only partly comparable to the theoretical IAEA model, set out for a serum P4 profile conducive to conception (IAEA, 2000). The high d8 serum P4 concentration of 4.0nmol/l was indicative of cows that were in the luteal phase of their oestrous cycle on the day that the intravaginal P4 devices were withdrawn. The intermediate d10 serum P4 concentration of 2.46nmol/l was again indicative of cows that still retained some circulatory serum P4 on the day of FTAI. Even though this group did not fall into the low serum P4 concentration category, as would be expected when compared to the theoretical IAEA model, the average serum P4 concentration between d8 and d10 decreased by 1.54nmol/l and the group managed to record an overall conception rate of 68% (Table 4.1). An important aspect recorded here was the fact that the re-used device offered a cheaper alternative, without having to compromise on fertility.

4.2 Group B – 2009 Heifer Group

The 2009 heifer group was also treated with different concentrations (g) of the P4 DIB® intravaginal device and recorded different results as can be seen in Table 4.2.

Table 4.2: D8 and d10 serum P4 concentrations and conception results in the heifer P4 treatment groups

P4 (nmol/l)	*0.5g*	*1.0g*	*1.9g*	*1.9g/r*
P4 (d8)	1.61 [b]	1.84 [b]	1.44 [a]	1.66 [b]
P4 (d10)	1.06 [a]	1.04 [a]	1.28 [a]	1.35 [a]
Conception rate	19% [a]	39% [a]	41% [a]	47% [a]

[a b] values in a row with different superscripts differ statistically significantly ($p < 0.05$)

4.2.1 Heifers treated with the 0.5 g intravaginal progesterone device

The heifer group treated with the 0.5g P4 DIB® intravaginal devices recorded a serum P4 profile of intermediate (d8) – intermediate (d10), instead of a high (d8) – low (d10) serum P4 profile. This was not comparable to the theoretical IAEA model for a serum P4 profile, conducive to successful conception in cattle (IAEA, 2000). The intermediate d8 serum P4 concentration of 1.61nmol/l was indicative of heifers with a suppressed oestrous period on the day that the intravaginal P4 device was withdrawn. The intermediate d10 serum P4 concentration of 1.06nmol/l showed that the heifers still retained some circulatory serum P4 on the day of fixed-time AI, with resultant depressed gonadotropin secretion. The average serum P4 concentration decreased by 0.55nmol/l between d8 and d10 and this group recorded an overall low conception rate of 19% (Table 4.2). This decrease in the serum P4 level could have induced this low conception rate in the heifers.

4.2.2 Heifers treated with the 1.0 g intravaginal progesterone device

The heifer group treated with the 1.0g P4 DIB® intravaginal devices recorded a serum P4 profile of intermediate (d8) – intermediate (d10), once again not comparable with the theoretical IAEA model for a serum P4 profile conducive to conception in cattle (IAEA, 2000). The intermediate d8 serum P4 concentration of 1.84nmol/l was indicative of heifers with a suppressed oestrous period (oestrogen secretion) on the day that the intravaginal P4 devices were withdrawn. The intermediate d10 serum P4 concentration of 1.04nmol/l showed that the heifers still retained relatively high levels of circulatory serum P4 on the day of the fixed-time AI, which was detrimental to fertility. The average serum P4 concentration decreased by 0.80nmol/l between d8 and d10 and this group only recorded an overall conception rate of 39%, which was unacceptably low. The decrease in P4 levels during oestrous is a prerequisite for high oestrogen and hence LH production during this period (Table 4.2).

4.2.3 Heifers treated with the 1.9 g intravaginal progesterone device

The heifer group treated with 1.9g P4 CIDR® intravaginal devices recorded a serum P4 profile of intermediate (d8) – intermediate (d10), again not in line with the theoretical IAEA model for a serum P4 profile conducive to conception in cattle (IAEA, 2000). The intermediate d8 serum P4 concentration of 1.44nmol/l was indicative of heifers with suppressed oestrous activity (too high progesterone levels) on the day that the intravaginal P4 devices were withdrawn. The intermediate d10 serum P4 concentration of 1.28nmol/l was also indicative of heifers that still retained some circulatory serum P4 in their systems on the day of fixed-time AI – suppressing the blood oestrogen and LH levels. The average serum P4 concentration had decreased by 0.16nmol/l between d8 and d10 and this group recorded an overall conception rate of 41% (Table 4.2).

4.2.4 Heifers treated with the 1.9 g re-used intravaginal device

The heifer group treated with the 1.9g re-used P4 CIDR® intravaginal devices recorded a serum P4 profile of intermediate (d8) – intermediate (d10), levels again not in line with the theoretical IAEA model for a serum P4 profile recommended to be conducive for acceptable conception in cattle (IAEA, 2000). The intermediate d8 serum P4 concentration of 1.66nmol/l (relatively high) was indicative of heifers with a suppressed oestrous cycle on the day that the intravaginal P4 devices were withdrawn. The intermediate d10 serum P4 concentration of 1.35nmol/l was indicative of heifers that still retained some circulatory serum P4 on the day of fixed-time AI. The average serum P4 concentration had decreased by 0.31nmol/l between d8 and d10 and this group recorded an overall conception rate of 47% - ironically even better than an unused CIDR® device (Table 4.2).

4.3 Comparative analyses as hypothesised in the original protocol

This paragraph provides detailed information on the serum progesterone concentration (at certain intervals after treatment) and the conception rate of cows and heifers respectively with selected P4 device concentrations, and also providing the LSD test results indicating the meaningful differences ($p < 0.05$) between treatments.

4.3.1 Intravaginal P4 device concentration vs. the conception rate in cows

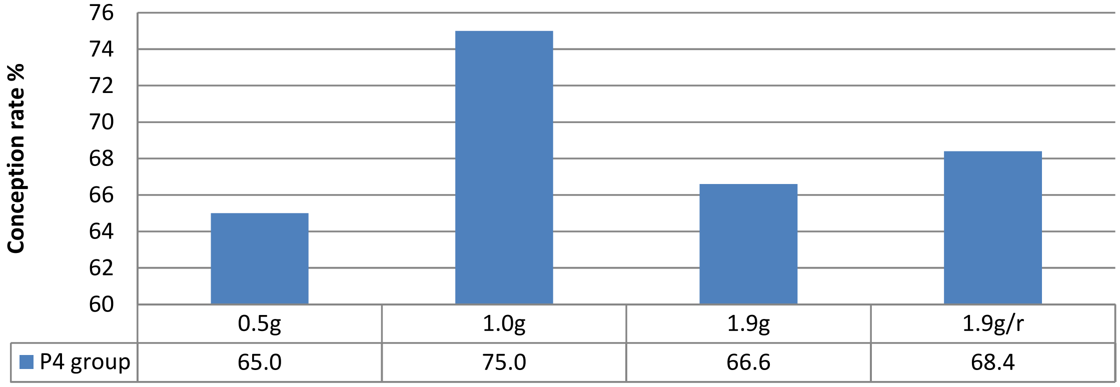

Figure 4.1: Intravaginal P4 device concentration vs. the conception rate in cows

The 1.0g DIB® intravaginal P4 devices recorded the highest conception rate (75%) of the four cow treatment groups - with the 1.9g re-used CIDR® recording the second highest conception rate (68.4%), the 1.9g CIDR® being third (66.6%) and the 0.5g DIB® recording the lowest conception rate (65%). There was however no significant statistical difference between the P4 treatment groups and the pregnancy data recorded for the groups ($p > 0.1$) (Table 4.3).

Table 4.3: LSD Test – the relationship between P4 treatment and conception rate

P4 Treatment	***0.5***	***1.0***	***1.9***	***1.9r***
0.5		0.533172 ^{ns}	0.911106 ^{ns}	0.823171 ^{ns}
1.0	0.533172 ^{ns}		0.599514 ^{ns}	0.685047 ^{ns}
1.9	0.911106 ^{ns}	0.599514 ^{ns}		0.907679 ^{ns}
1.9r	0.823171 ^{ns}	0.685047 ^{ns}	0.907679 ^{ns}	

^{ns} No significant statistical differences

The results seem to suggest that a reduction in the concentration of P4 in the intravaginal device from 1.9g to 1.0g could lead to improved conception rates in similar cows. The fact that the re-used 1.9g CIDR® recorded the second highest conception rate of the four treatment groups supports this finding. Although the exact concentration of P4 in the re-used 1.9g CIDR® was unknown, it can be speculated that the concentration of the P4 intravaginal device lies somewhere between 0.5g and the original 1.9g CIDR®. The results further suggest that a P4 concentration of 1.0g in the intravaginal device is optimal for improving the conception rate in similar cows, and that either an increase or decrease in the P4 concentration would lead to a reduced conception rate.

4.3.2 Intravaginal P4 device concentration vs. the d8 serum P4 concentration in cows

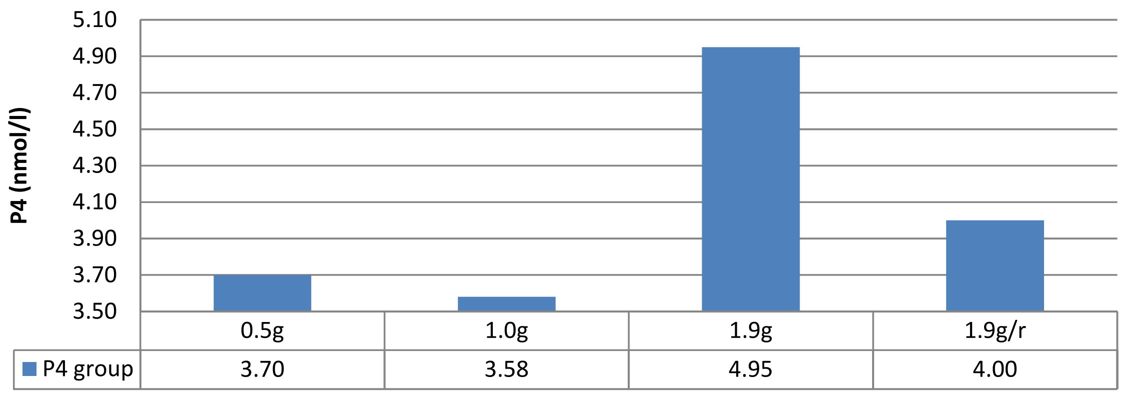

Figure 4.2: Intravaginal P4 device concentration vs. the d8 average serum P4 concentration in cows

The 1.9g CIDR® intravaginal P4 devices recorded the highest d8 serum P4 concentration (4.95nmol/l) of the four cow treatment groups recorded. The 1.9g re-used CIDR® recorded the second highest P4 concentration (4.00nmol/l), the 0.5g DIB® third highest (3.70nmol/l) and the 1.0g DIB® the lowest serum P4 concentration (3.58nmol/l). There was a significant statistical difference in the d8 P4 serum concentrations of the 1.9g CIDR® and the other three treatment groups ($p < 0.05$). No significant statistical difference was recorded between the 0.5g DIB®, the 1.0g DIB® and the 1,9g re-used CIDR® treatment groups ($p > 0.1$) (Table 4.4).

Table 4.4: LSD Test – the relationship between P4 treatment and the serum P4 concentration on d8 in cows

P4 Treatment	*0.5*	*1.0*	*1.9*	*1.9r*
0.5		0.763349 [a]	0.000887 [b]	0.411737 [a]
1.0	0.763349 [a]		0.000638 [b]	0.284507 [a]
1.9	0.000887 [a]	0.000638 [a]		0.011686 [a]
1.9r	0.411737 [a]	0.284507 [a]	0.011686 [b]	

[a b] values in a row with different superscripts differ statistically significantly ($p < 0.05$)

The results seem to suggest that an increase in the P4 concentration of the intravaginal device would lead to an increase in the d8 serum P4 levels in cows under similar conditions, but a higher d8 P4 concentration does not necessarily equate to a higher conception rate. In fact, the lowest d8 P4 serum concentration, as recorded for the 1.0g DIB® treatment group, resulted in the highest conception rate of the four cow treatment groups.

4.3.3 Intravaginal P4 device concentration vs. the d10 serum P4 concentration in cows

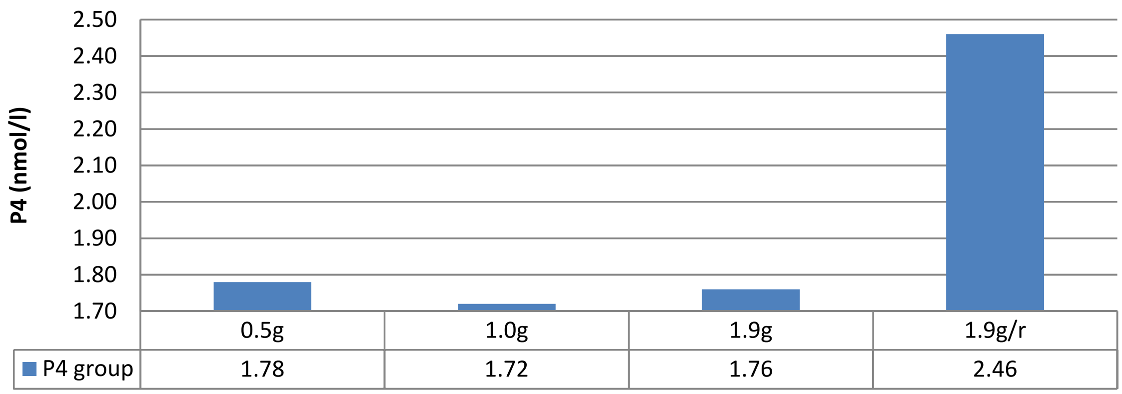

Figure 4.3: Intravaginal P4 device concentration vs. the d10 average serum P4 concentration in cows

The 1.0g DIB® intravaginal P4 devices recorded the lowest d10 serum P4 concentration (1.72nmol/l) of the four treatment groups, with the 1.9g CIDR® recording the second lowest serum

P4 concentration (1.76nmol/l), the 0.5g DIB® third lowest (1.78nmol/l) and the 1.9g re-used CIDR® the highest serum P4 concentration (2.46nmol/l). There was no significant statistical difference in the d10 P4 serum concentrations recorded between any of the four treatment groups ($p > 0.5$) (Table 4.5).

Table 4.5: LSD Test – the relationship between P4 treatment and the serum P4 concentration on d10 in cows

P4 Treatment	*0.5*	*1.0*	*1.9*	*1.9r*
0.5		0.882610 ^{ns}	0.968410 ^{ns}	0.099623 ^{ns}
1.0	0.882610 ^{ns}		0.910848 ^{ns}	0.089453 ^{ns}
1.9	0.968410 ^{ns}	0.910848 ^{ns}		0.088439 ^{ns}
1.9r	0.099623 ^{ns}	0.089453 ^{ns}	0.088439 ^{ns}	

^{ns} No significant statistical differences

The results seem to suggest that an increase in the P4 concentration in the intravaginal device does not affect the serum P4 concentration on d10, and that similar cows are possibly able to metabolise the circulatory P4 with no residual effect within 48hr after the intravaginal device is withdrawn.

4.3.4 Intravaginal P4 device concentration vs. the conception rate in heifers

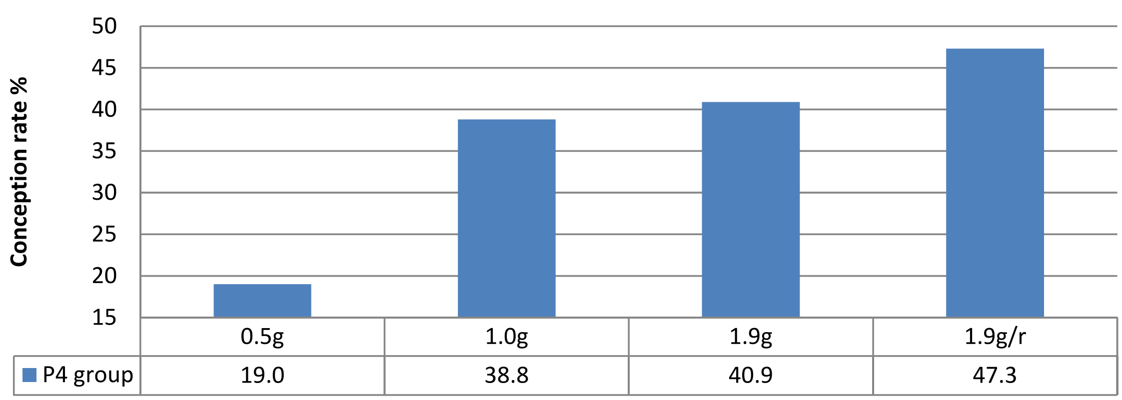

Figure 4.4: Intravaginal P4 device concentration vs. the conception rate in heifers

The 1.9g re-used CIDR® intravaginal P4 devices recorded the highest conception rate (47.3%) of the four heifer treatment groups - with 1.9g CIDR® being the second highest (40.9%), the 1.0g DIB® the third highest (38.8%) and 0.5g DIB® the lowest conception rate (19%). There was no significant statistical difference in the conception rate between the four P4 heifer treatment groups ($p > 0.1$) (Table 4.6).

Table 4.6: LSD Test – the relationship between P4 treatment and the conception rate in heifers

P4 Treatment	***0.5***	***1.0***	***1.9***	***1.9r***
0.5		0.169383 ^{ns}	0.402422 ^{ns}	0.527475 ^{ns}
1.0	0.169383 ^{ns}		0.554341 ^{ns}	0.459294 ^{ns}
1.9	0.402422 ^{ns}	0.554341 ^{ns}		0.858833 ^{ns}
1.9r	0.527475 ^{ns}	0.459294 ^{ns}	0.858833 ^{ns}	

^{ns} No significant statistical differences

The results seem to suggest that a reduction in the concentration of P4 in the intravaginal device from 1.9g to somewhere between 1.9g and 1.0g would lead to an improved conception rate in similar animals. The conception rate recorded by the re-used 1.9g CIDR® further suggests that the P4 concentration of the re-used 1.9g CIDR® now lies somewhere between 1.0g and 1.9g. This would support the earlier finding that an intravaginal device P4 concentration of between 1.0g and 1.9g would improve conception rates in heifers under similar conditions. It is interesting to note that there was no significant statistical difference in the conception rate between the 1.0g DIB® and the 1.9g CIDR®.

4.3.5 Intravaginal P4 device concentration vs. the d8 serum P4 concentration in heifers

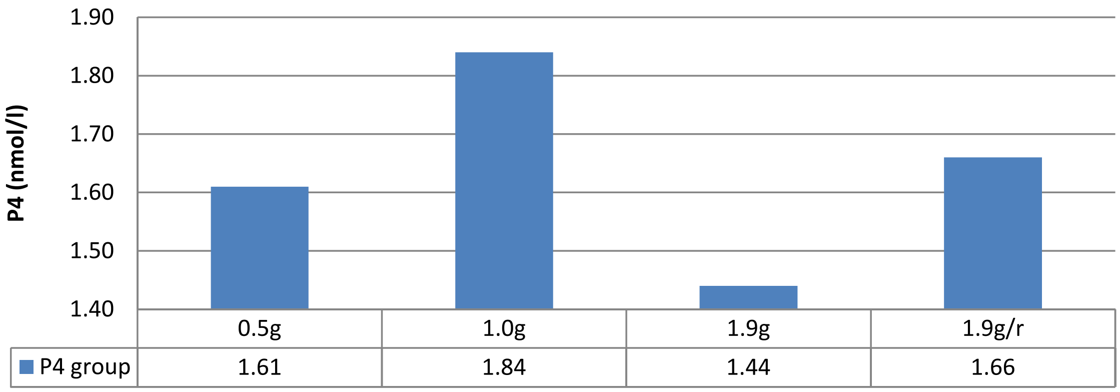

Figure 4.5: Intravaginal P4 device concentration vs. the d8 serum P4 concentration in heifers

The 1.0g DIB® intravaginal P4 devices recorded the highest d8 serum P4 concentration (1.84nmol/l) of the four heifer treatment groups - with the 1.9g re-used CIDR® being the second highest (1.66nmol/l), the 0.5g DIB® the third highest (1.61nmol/l) and the 1.9g CIDR® recording the lowest serum P4 concentration (1.44nmol/l). There was a significant statistical difference in the d8 serum P4 concentration between the 0.5g DIB® and the 1.0g DIB® ($p < 0.5$); and between the 0.5g DIB® and the 1.9g re-used CIDR® ($p < 0.5$) (Table 4.7).

Table 4.7: LSD Test – the relationship between P4 device treatment and the serum P4 concentration on d8

P4 Treatment	***0.5***	***1.0***	***1.9***	***1.9r***
0.5		0.010328^{b}	0.184119^{a}	0.041713^{b}
1.0	0.010328^{b}		0.174249^{a}	0.567349^{a}
1.9	0.184119^{a}	0.174249^{a}		0.432864^{a}
1.9r	0.041713^{b}	0.567349^{a}	0.432864^{a}	

$^{a\,b}$ values in a row with different superscripts differ statistically significantly ($p < 0.05$)

The results seem to suggest that there is no significant correlation between the P4 concentration of the intravaginal device and the d8 serum P4 concentration in heifers. The results also recorded no significant correlation between the d8 serum P4 concentration and the conception rate in heifers under similar conditions. It is important to point out at this time, that the d8 serum P4 concentration recorded in the heifer treatment group was lower than what would be expected for heifers in the luteal phase of the oestrous cycle. The low serum P4 concentration is indicative of a functional corpus luteum (CL), but the activity is low and may have influenced the conception results in the heifer group in general.

4.3.6 Intravaginal P4 device concentration vs. the d10 serum P4 concentration in heifers

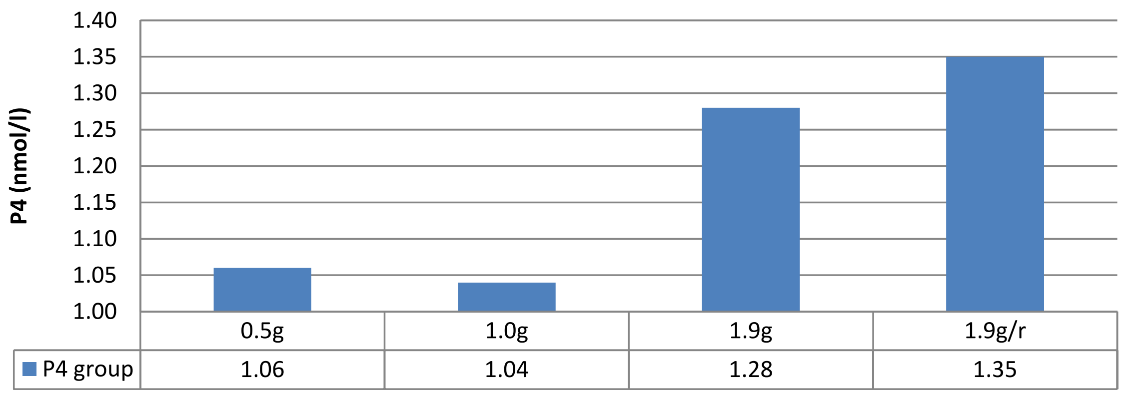

Figure 4.6: Intravaginal P4 device concentration vs. the d10 serum P4 concentration in heifers

The 1.0g DIB® intravaginal P4 devices recorded the lowest d10 serum P4 concentration (1.04nmol/l) of the four heifer treatment groups, with the 0.5g DIB® recording the second lowest (1.06nmol/l) P4 concentration. The 1.9g CIDR® recorded the third lowest P4 concentration (1.28nmol/l) and the 1.9g re-used CIDR® recorded the highest P4 concentration (1.35nmol/l). There was however no significant statistical difference recorded in the d10 serum P4 concentrations between the four treatment groups ($p > 0.1$) (Table 4.8).

Table 4.8: LSD Test – the relationship between P4 treatment and the serum P4 concentration on d10 in heifers

P4 Treatment	***0.5***	***1.0***	***1.9***	***1.9r***
0.5		0.169383 ^{ns}	0.402422 ^{ns}	0.527475 ^{ns}
1.0	0.169383 ^{ns}		0.554341 ^{ns}	0.459294 ^{ns}
1.9	0.402422 ^{ns}	0.554341 ^{ns}		0.858833 ^{ns}
1.9r	0.527475 ^{ns}	0.459294 ^{ns}	0.858833 ^{ns}	

^{ns} No significant statistical differences

It is interesting to note that the 1.9g re-used CIDR® intravaginal devices recorded both the highest d10 serum concentration and the highest conception rate in heifers.

4.3.7 Overall serum P4 concentration vs. the conception rate in cows

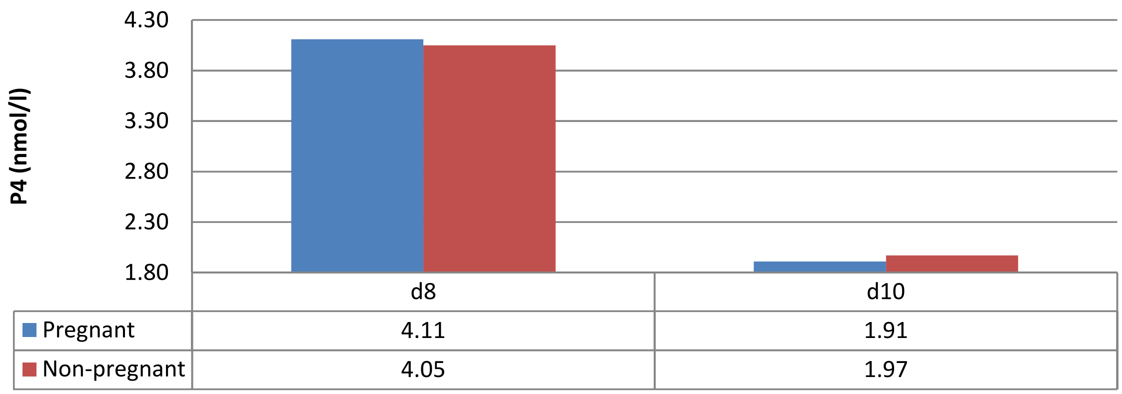

Figure 4.7: Overall serum P4 concentration vs. the conception rate in cows

The cow treatment group recorded a combined overall conception rate of 68.4% over the four P4 treatment groups, with an average d8 serum P4 concentration of 4.11nmol/l and an average d10 serum P4 concentration of 1.91nmol/l. The pregnant population in the cow treatment group recorded a 2.20nmol/l decrease in serum P4 concentration between d8 and d10. The non-pregnant population of the cow treatment group (31.6%) recorded an average d8 serum P4 concentration of 4.05nmol/l and a d10 serum P4 concentration of 1.97nmol/l - a 2.08nmol/l decrease in serum P4 concenration between d8 and d10. A significant statistical difference was recorded between the d8 and d10 serum P4 concentrations, both in the pregnant and non-pregnant treatment groups ($p < 0.05$). There was however no significant statistical difference between the d8 serum P4 concentrations of the pregnant and non-pregnant treatment groups ($p > 0.1$); with no significant statistical difference between the d10 serum P4 concentrations of the pregnant and non-pregnant treatment groups ($p > 0.1$) (Table 4.9).

Table 4.9: LSD Test – the relationship between serum P4 concentrations on d8 and d10 and the conception rate in heifers

	D8 PD(-)	***D8 PD(+)***	***D10 PD(-)***	***D10 PD(+)***
D8 PD(-)		0.847273 [a]	0.00000 [b]	0.00000 [b]
D8 PD(+)	0.847273 [a]		0.00000 [b]	0.00000 [b]
D10 (PD(-)	0.00000 [b]	0.00000 [b]		0.844409 [a]
D10 PD(+)	0.00000 [b]	0.00000 [b]	0.844409 [a]	

[a b] values in a row with different superscripts differ statistically significantly ($p < 0.05$)

The results seem to suggest that there was no significant difference in the oestrous cycle activity of the pregnant and non-pregnant populations in the cow treatment groups - based on the serum P4 profiles it could be that the non-pregnancies may have been caused by other external factors. The cow treatment group was homogenous as far as body condition score (BCS), parity, days post-partum and general management was concerned.

4.3.8 Overall serum P4 concentration vs. the conception rate in heifers

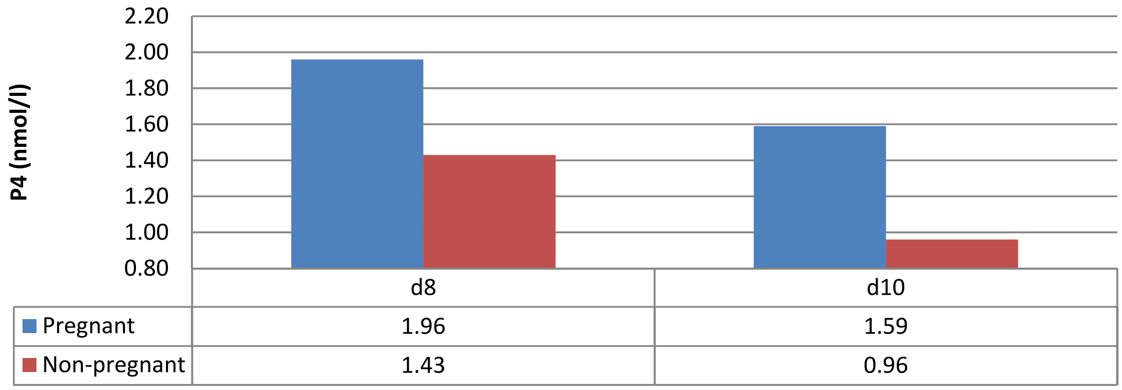

Figure 4.8: Overall serum P4 concentration vs. the conception rate in heifers

The heifer treatment group recorded an overall conception rate of 36.3% over the four heifer treatment groups, with an average d8 serum P4 concentration of 1.96nmol/l and a d10 serum P4 concentration of 1.59nmol/l. The pregnant heifer population in the treatment groups recorded a 0.37nmol/l decrease in serum P4 concentration between d8 and d10. The non-pregnant heifer population of the treatment groups (63.8%) again recorded a d8 serum P4 concentration of 1.43nmol/l and a d10 serum P4 concentration of 0.96nmol/l - a 0.47nmol/l decrease in serum P4 concenration between d8 and d10. There was no significant statistical difference between the d8 serum P4 concentrations of the pregnant and non-pregnant treatment groups ($p > 0.1$). There was however a significant statistical difference between the d8 serum P4 concentrations of the pregnant treatment groups and the d10 serum P4 concentrations of the non-pregnant group ($p < 0.05$), as well as between the d10 serum P4 concentrations of the pregnant and non-pregnant treatment group ($p < 0.05$) (Table 4.10).

Table 4.10: LSD Test – the relationship between serum P4 concentrations on d8 and d10 and the conception rate in heifers

	D8 PD(-)	*D8 PD(+)*	*D10 PD(-)*	*D10 PD(+)*
D8 PD(-)		0.159521 [a]	0.147261 [a]	0.052583 [a]
D8 PD(+)	0.159521 [a]		0.008788 [b]	0.633291 [a]
D10 (PD(-)	0.147261 [a]	0.008788 [b]		0.001701 [b]
D10 PD(+)	0.052583 [a]	0.633291 [a]	0.001701 [b]	

[a b] values in a row with different superscripts differ statistically significantly ($p < 0.05$)

The results seem to suggest that there was a significant difference in the oestrous cycle activity of the pregnant and non-pregnant populations of the device treatment groups. It would seem that a large portion of the population was sexually inactive at the time when the breeding program was initiated, and that this influenced the conception rate to a large extent. The heifer treatment group was homogenous as far as body condition score (BCS) and general management was concerned. There may however have been a genetic predisposition that favoured the age at puberty in a certain population of heifers, enabling them to conceive at an earlier age.

4.3.9 Day 8 and d10 serum P4 concentration variation vs. the conception rate in cows

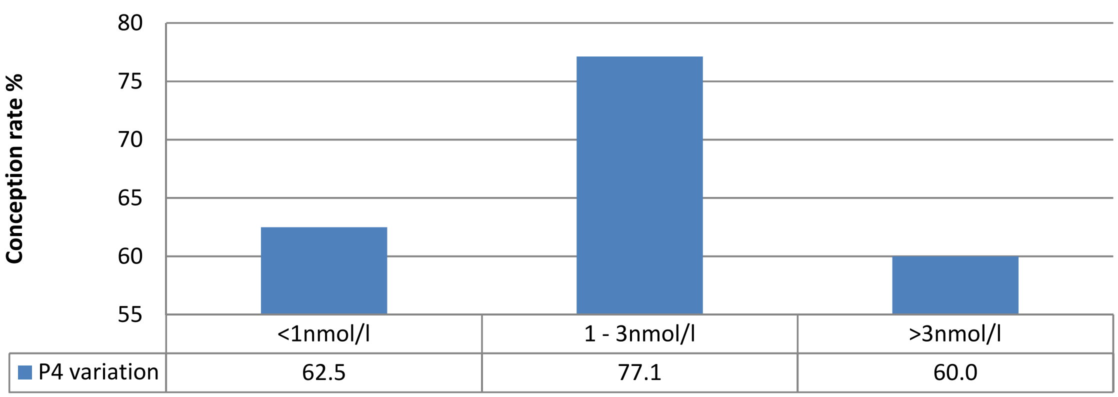

Figure 4.9: Day 8 and d10 serum P4 concentration variation vs. the conception rate in cows

The cow treatment group that recorded a decrease in serum P4 concentration between d8 and d10 of < 1nmol/l (low; 21%), recorded a conception rate of 62.5%. The treatment group that recorded a decrease in serum P4 concentration between d8 and d10 of between 1 to 3nmol/l (intermediate; 46%) recorded a conception rate of 77.1%. The group that recorded a decrease in serum P4 concentration between d8 to d10 of > 3nmol/l (high; 32.9%) recorded a conception rate of 60%. There was however no significant statistical difference between the conception rates of the three different P4 device groups ($p > 0.1$) (Table 4.11).

Table 4.11: LSD Test – the relationship between the variation in the d8/d10 P4 device groups and the conception rate in cows

P4 variation (d8/d10)	*<1nmol/l*	*1 - 3nmol/l*	*>3nmol/l*
<1nmol/l		0.211734 [ns]	0.658142 [ns]
1 - 3nmol/l	0.211734 [ns]		0.247772 [ns]
>3nmol/l	0.658142 [ns]	0.247772 [ns]	

[ns] No significant statistical differences

The results seem to suggest that a decrease in the serum P4 concentrations between d8 and d10 of 1 to 3nmol/l may be conducive to the highest conception rates in similar animals. A decrease in the serum P4 concentration between d8 and d10 of either <1nmol/l or >3nmol/l seemed to have a similar effect on the conception rates in cows under similar conditions.

4.3.10 Day 8 and d10 serum P4 concentration variation vs. the conception rate in heifers

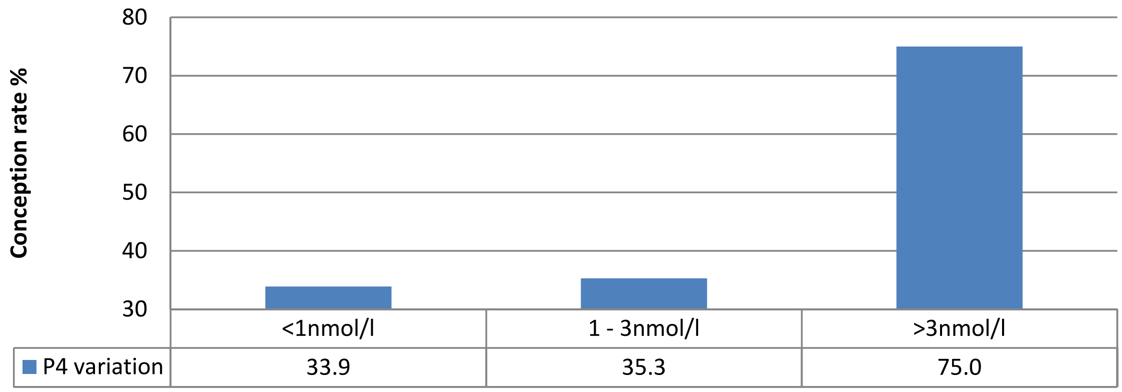

Figure 4.10: Day 8 and d10 serum P4 concentration variation vs. the conception rate in heifers

The heifer treatment group that recorded a decrease in the serum P4 concentration between d8 and d10 of < 1nmol/l (low; 73.8%) recorded a conception rate of 33.9%, while the treatment group that recorded a decrease in serum P4 concentration from d8 to d10 of between 1 to 3nmol/l (intermediate; 21.3%) recorded a conception rate of 35.3%. Finally, the group that recorded a reduction in serum P4 concentration from d8 to d10 of > 3nmol/l (high; 5%), recorded a conception rate of 75%. A significant statistical difference was recorded in the conception rate between the < 1nmol/l group and the > 3nmol/l P4 device variation groups ($p < 0.05$). There was however no significant statistical difference in the conception rates between the 1 to 3nmol/l and the < 1nmol/l and the > 3nmol/l P4 device variation group ($p > 0.1$) (Table 4.12).

Table 4.12: LSD Test – the relationship between the variation in the d8/d10 P4 device groups and the conception rates in heifers

P4 variation (d8/d10)	*<1nmol/l*	*1 - 3nmol/l*	*>3nmol/l*
<1nmol/l		0.034380 ^{b}	0.005591 ^{b}
1 - 3nmol/l	0.034380 ^{b}		0.075778 ^{a}
>3nmol/l	0.005591 ^{b}	0.075778 ^{a}	

$^{a\ b}$ values in a row with different superscripts differ statistically significantly ($p < 0.05$)

The results seem to suggest that a decrease in the serum P4 concentration between d8 and d10 of >3nmol/l is conducive to the highest conception rate in heifers. A decrease in the serum P4 concentration between d8 and d10 of <1nmol/l or 1 – 3nmol/l seem to have a similar effect on the conception rates in heifers under similar conditions. The fairly low percentage of the heifer population (5%) that managed to record a >3nmol/l decrease in the serum P4 concentration between d8 and d10 is evident of the fact that a large population of the heifers were sexually inactive during the breeding program. In fact, only 32.25% of the heifer population manage to record a decrease in the serum P4 concentration between d8 and d10 of >1nmol/l, which could explain the low overall heifer conception rate of only 36.25%.

Chapter 5

Discussion

5.1 The relationship between the progesterone concentration in the intravaginal device and the conception rate in heifers and cows

The results of this study agree with that of other studies in that the conception rates of the heifers and cows did not significantly differ when using intravaginal implants ranging in progesterone concentration between 0.5 g and 1.9 g. The conception rate to fixed-time AI did not generally differ between cows synchronized with a new or once re-used CIDR®. Cows treated with a twice re-used CIDR® however, have recorded a lower conception rate and the addition of a second twice re-used CIDR® did not significantly improve the conception rate to fixed-time AI. The conception rate of heifers treated with intravaginal devices with different concentrations of progesterone ranging from 0.5 g, 0.78 g, 1.0 g and 1.56 g (CueMate® and DIB®), did not statistically differ from that of heifers treated with an intravaginal CIDR® device containing 1.9 g of progesterone. Despite these findings, it has been well documented that although the difference in conception rate is statistically insignificant, heifers treated with intravaginal devices with less than 1.9 g of progesterone (CueMate® and DIB®), record consistently higher numerical conception rates than heifers treated with the 1.9 g CIDR® implants (Cutaia *et al*., 2006; Pincinato *et al.*, 2007; Phillips e*t al*., 2010). These findings agree with the current study, in that a higher numerical number of heifers conceived when treated with the 1.9 g re-used CIDR® (47.3%), compared to the 1.9 g CIDR® (40.9%).

There was generally also no significant difference recorded in the conception rates of cross-bred *Bos indicus* heifers treated with the 0.5 g or a 1.0 g DIB® intravaginal progesterone device, suggesting that the progesterone concentration in the device does not affect the conception rate in cross-bred *Bos indicus* heifers (Cutaia e*t al.*, 2006; Pincinato *et al.*, 2007). The use of low level intravaginal progesterone devices may thus shorten the interval to oestrous, an important point when one considers the timing of AI in cattle (Martinez *et al*., 2011).

The amount of progesterone released by an intravaginal progesterone device over a 15 day period is reported to be highly repeatable and a CIDR® can be used at least twice in a 7 or 8 day synchronization program (Macmillan *et al*., 1996; Macmillan and Peterson, 1993), resulting in a serum progesterone concentration of above 1ng/ml - the minimum concentration expected to be sufficient to suppress the endogenous LH surge (Savio *et al*., 1993). This finding seems to agree with the current findings in that there was no significant difference in the d 8 serum progesterone level of pregnant and non-pregnant cows and heifers treated with either a 0.5 g DIB®, a 1.0 g DIB®, a 1.9 g CIDR® or a 1.9 g re-used CIDR®. The lower conception rates recorded in cattle treated with a twice re-used CIDR® suggests that the minimum serum progesterone concentration of 1 ng/ml may not be maintained in all cattle throughout the 7 or 8 day protocol. It is suspected that the ability of estradiol to consistently induce follicular regression (and synchronize follicular wave emergence) at any stage of the oestrous cycle is dependent on elevated serum progesterone concentrations (Colazo e*t al.,* 2006). Inadequate serum progesterone levels may desensitise the hypothalamus to the estradiol surge, which in turn inhibits the pre-ovulatory surge of GnRH and LH to occur. This could then lead to the occurrence of metabolic disorders such as cystic ovarian follicles (Amer and Mahdi, 2008). When ovariectomized cows received a once re-used CIDR®, a concurrent injection of 100 mg of progesterone was needed to achieve serum progesterone concentrations 24 h after CIDR® insertion - that is similar to those of cows treated with a new CIDR® (Martinez *et al*., 2003).

An interesting observation made was that heifers and cows fitted with re-used CIDR®s, tend to lose more CIDR®s than heifers and cows treated with new CIDR®s. A possible explanation could be that the re-used CIDR® may be relatively worn out and does not press as tightly against the vaginal wall, as do a new CIDR®. The loss of a CIDR® may be associated with a reduction in the conception rate and could diminish or even nullify the economic benefit of using re-used CIDR®s (Colazo e*t al*., 2004). Another factor to consider when using re-used CIDR®s is the transmission of disease. Re-used CIDR®s can be soaked in a povidone-iodine-based detergent, followed by scrubbing with a brush and rinsing in clean water to remove the detergent and debris. Re-used CIDR®s can also be autoclaved. It is important to thoroughly dry the CIDR® before long term storage to reduce the potential for microbial growth (Colazo *et al*., 2003).

5.2 The relationship between the progesterone concentration in the intravaginal device and the serum progesterone concentration, measured during the synchronization program in heifers and cows

The results of this study showed a significant difference between the day 8 serum progesterone levels recorded for the 1.9 g CIDR® cow treatment group and the other cow treatment groups; and between the 0.5 g DIB® and 1.0 g DIB®, and the 0.5 g DIB® and the 1.9 g re-used CIDR® heifer treatment groups. There was however no significant difference between the serum progesterone levels in any of the treatment groups on the day of the AI. Other studies also report no significant difference in the serum progesterone concentrations between day 0 and 10 of the synchronized cows treated with either the 0.5 g and 1.0 g DIB® device, the 0.78 g and 1.56 g CueMate® device or the 1.9 g CIDR® device (Rogan *et al*., 2007; Phillips *et al*., 2010). In ovariectomized cows the serum progesterone profiles were generally characterised by a sudden increase in serum progesterone within 12 h of intravaginal device insertion, followed by a gradual decrease in serum progesterone concentration during the subsequent days. A recent study analysed and compared the serum progesterone levels in ovariectomized beef cows treated with a new CIDR® for 7 days, a once re-used CIDR® for 7 days or a twice re-used CIDR® for 7 days; and recorded serum progesterone levels 24 h after device insertion of 4.0 ± 0.1 ng/ml, 2.4 ± 0.2 ng/ml and 1.8 ± 0.2 ng/ml, respectively. The same cows recorded serum progesterone levels of 1.4 ± 0.1 ng/ml, 1.0 ± 0.1 ng/ml and 0.9 ± 0.1 ng/ml on the day of CIDR® removal (day 7) for the new CIDR®, once re-used CIDR® and twice re-used CIDR® treatment groups, respectively. The results again suggest that a once re-used CIDR® is still effective and can produce adequate levels of serum progesterone (Long *et al*., 2009).

The fixed-time AI conception rate for heifers with a serum progesterone concentration of > 1 ng/ml at day 16 (6 days post AI) of the synchronization program was on average 60%, compared to the

20% conception rate for heifers that recorded a progesterone concentration of < 1 ng/ml - indicating that a pregnancy is more likely to be sustained in the presence of a CL at 6 days post AI, that is detectable and produces adequate levels of progesterone. Heifers are more likely to conceive if the dominant follicle is > 10mm in diameter. The size of the ovulatory follicle is then related to the size of the subsequent CL, and the size of the CL critically affects progesterone secretion during the early embryonic period (Vasconcelos *et al*., 1999; Bo *et al*., 2003). The induction of luteolysis in cows with a pre-ovulatory follicle of <10mm generally results in a longer follicular phase, the ovulation of a smaller pre-ovulatory follicle and a delayed post-ovulatory increase in serum progesterone concentration (Robinson *et al*., 2006). Where ovulation is induced for fixed-time AI, the smaller pre-ovulatory follicle may be forced to ovulate prematurely, which will then result in a decreased number of granulosa cells being present in the ovulated follicle. A reduction in granulosa cells in the ovulated follicle in turn will result in a decreased number of large steriodogenic luteal cells in the subsequently formed CL - which then results in a reduction in the concentration of progesterone produced and could then lead to an increased risk of early embryonic mortality (Perry *et al*., 2005; Perry & Perry, 2009). It is possible for as many as 34% of the synchronized heifers to fail to ovulate, because of the inability of the immature dominant follicle to respond to the LH surge induced by the oestradiol benzoate treatment (Cavalieri e*t al*., 2002). In infrequently handled *Bos indicus* heifers, a proportion of the heifers generally experienced acute stress during handling, that may also reduce the secretion of GnRH and LH, resulting in reduced follicular growth, which then leads to a disturbance in the ovarian function. It is possible for heifers to suffer from disturbances in the ovarian function after oestrous synchronization with an intravaginal device, irrespective of the concentration of progesterone in that device. Generally, up to 25% of the heifers that ovulate during the initial synchronized oestrous do not ovulate at the return cycle, and up to 20% of the heifers develop a persistent CL after the synchronization program (Butler *et al*., 2008; Butler *et al*., 2009).

Cycling cows generally recorded a serum progesterone concentration of > 1 ng/ml, whereas anovular cows generally recorded a serum progesterone concentration of < 1 ng/ml. Pre-synchronization of the cows by means of two injections of prostaglandin F2α, 14 days apart, can therefore be used to accurately manipulate the cow's oestrous cycle, and so be able to start the subsequent synchronization program somewhere in the mid-luteal phase of the cow's oestrous cycle. Pre-synchronization has been shown to increase the proportion of cows with a high serum progesterone concentration (> 1 ng/ml), indicative of luteal activity during the second PGF2α injection (Silva *et al*., 2007).

The onset of the first luteal activity post calving is a highly heritable trait, when compared with the heritability of traditional measures of fertility (Martin *et al*., 2010). The onset of ovarian activity in first-parity cows was compared with older multiparious cows, possibly due to a greater negative energy balance in first-parity cows, compared to older cows. First-parity cows generally recorded a higher incidence of delayed cyclisity, compared to older cows, but older cows, especially cows in their 6^{th} to 11^{th} lactation, demonstrating an increased risk of atypical progesterone profiles (Petersson *et al*., 2006).

5.3 The relationship between the serum progesterone concentration and the conception rate in heifers and cows

The serum progesterone concentration measured on the day of the artificial insemination (day 10) and on the day when the increase in the post-ovulatory serum progesterone concentration begins (day 16), affects the conception rate and embryonic mortality rate in repeat-breeder cows. The cows with a serum progesterone concentration of more than 0.5 ng/ml at AI generally show a significantly higher incidence of late embryonic deaths, compared to those recording a serum progesterone concentration of less than 0.5 ng/ml at AI ($p<0.01$). As the serum progesterone level at insemination increased, so the conception rate declined (Ghanem *et al*., 2006). This finding seems to support the findings in the current study in that the pregnant cow group recorded a lower serum progesterone level, when compared to the non-pregnant cow group – although this was only a numerical finding and not statistically significant. A negative correlation existed between the conception rate and the serum progesterone level in the cow on the day of AI. Recent studies have shown that cows that record an increase in circulatory progesterone of 1 ng/ml or more by the 6th day post AI, generally record a 50% conception rate, as opposed to the 20% conception rate in cows that fail to record an increase in circulatory progesterone concentrations of at least 1 ng/ml by the 6th day post AI. Thus, an increase in the serum progesterone concentrations at the time of the AI and a delayed increase in the concentration of serum progesterone by the 6th day post AI generally result in a decrease in the fertility of repeat breeder cows (Ghanem *et al*., 2006).

It is quite possible for repeat breeder cows to suffer from different types of luteal dysfunctions. A recent study reported that only 38% of repeat breeder cows to have a normal milk progesterone profile, and that the milk progesterone levels in these cows increased to 1 ng/ml or higher by the 5th day post AI and thereafter steadily increased to 2 ng/ml or higher during the mid-luteal phase of the oestrous cycle. The same study reported that, on the other hand, 62% of the repeat breeder cows to have an abnormal milk progesterone profile, which indicates some sort of luteal phase defect after AI. Although the concentration of progesterone is slightly higher when measured in milk, compared to serum, these levels are still closely correlated (Rodes, [s.a.]). The study further reported that 54% of the repeat breeders show a delayed rise in the milk progesterone level between day 6 - 11 post AI and that 15% of the cows recorded a comparatively low level of milk progesterone levels of below 2 ng/ml, through most of the luteal phase. In total, 31% of the repeat breeder cows had a combined progesterone profile with both a delayed increase in the milk progesterone concentration and a low level of progesterone during the luteal phase. In comparison, 63% of the cows with a normal progesterone profile conceived, while none of the cows that recorded insufficient luteal function conceived. This suggests that the delayed formation of the corpus luteum either combined or not combined, with the lowered secretion of progesterone during the luteal phase, being one of the causes of repeat breeding in heifers and cows (Kimura *et al*., 1987). This finding seems to support the findings of the current study in that 75% of the heifers that recorded a three-fold decrease in serum progesterone concentration between the day of intravaginal device withdrawal and the day of the fixed-time AI conceived, regardless of the progesterone concentration in the intravaginal devices used.

It is important to realize, however, that the use of serum progesterone concentrations to confirm pregnancy in cows should be done cautiously. A recent study compared the accuracy of diagnosing pregnancy in cows by interpreting either the ISG15 mRNA or the serum progesterone concentrations in cows 17 to 25 days post AI. ISG15 mRNA is produced by a foetus-derived interferon in the endometrium of the pregnant cow on day 15 to 45 post AI. The study concluded that low levels of both ISG15 mRNA and serum progesterone, 17 to 25 days post AI are 100% accurate in confirming non-pregnancy in cows. However, high levels of ISG15 mRNA is only 78% accurate and high serum progesterone levels only 58% accurate in confirming pregnancy in cows during the same time (Han e*t al*., 2006).

Chapter 6

General conclusions and recommendations

This study set out to determine whether or not a decrease in the progesterone concentration in the intravaginal devices used to synchronize the oestrous cycles in both heifers and cows could improve the efficiency of the synchronization program and ultimately improve the conception rates currently obtained in similar cattle breeding programs. The hypothesis was based on three assumptions – the first assumption being that a decrease in the progesterone concentration in the intravaginal device from 1.9 g to 0.5 g would still be adequate to induce high serum progesterone concentrations in the heifer and cow to maintain a normal oestrous cycle. The second assumption was that a decrease in the progesterone concentration in the intravaginal device would result in a decreased serum progesterone concentration in the heifer and cow on the day of the fixed-time AI. Finally, the third assumption was that a decrease in the progesterone concentration in the intravaginal device would result in higher conception rates in the treated heifers and cows.

This study concluded that a decrease in the progesterone concentration in the different intravaginal devices that were studied did not decrease the serum progesterone concentrations in first parity cows to a level below the minimum critical limit of 1 ng/ml (Colazo *et al*., 2004) during the luteal phase of the synchronization program, and thus did not negatively affect the synchronization program. The study further concluded that a decrease in the progesterone concentration in the intravaginal device did not affect the serum progesterone concentrations in the heifer and cow groups on the day of the fixed-time AI, and again did not negatively affect the synchronization program. In summary, the study concluded that although a decrease in the progesterone concentration in the intravaginal device did not statistically influence the conception rate in the heifer and cow group, the cow group did record a higher numerical conception rate when the progesterone concentrations in the intravaginal devices were lowered. This finding has been supported by numerous researchers (Cutaia *et al*., 2006; Pincinato *et al.*, 2007; Phillips e*t al*., 2010). Based on the results of the study, the hypothesis (par. 1.6) must thus be accepted.

Based on the conclusions of this study it is recommended that the cattle industry further investigates the importation and registration of intravaginal devices containing a range of different progesterone concentrations. In addition to the 1.9 g CIDR® intravaginal device, the international community have the option of choosing between different concentrations in the intravaginal devices, ranging from 0.5 g in the DIB® intravaginal device to 1.56 g in the CueMate® intravaginal device. The 1.9 g CIDR® intravaginal device is currently the only available intravaginal progesterone device registered for use in South Africa. Further in-depth investigations are warranted.

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