APPLICATION OF TIMED ARTIFICIAL INSEMINATION IN SYNCHRONIZED DAIRY AND BEEF COWS USING SEXED AND NON-SEXED SEMEN

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by

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Declaration

I, **Thabang Luther Magopa** student number **63088622**, declare that this dissertation titled "**Application of timed artificial insemination in synchronized dairy and beef cows using sexed and non-sexed semen**" submitted for Master of Science degree in Agriculture at the University of South Africa:

It is my original research in design and execution.

It has never been submitted for any degree before, at this or any other institution of higher education by anyone else or myself in fulfilment of the prerequisites for obtaining this or any qualification.

It was under the supervision of Dr Masindi Lottus Mphaphathi and Ms Thendo Mulaudzi.

It does not comprise other person's writing, data, photos, figures, or tables, unless the source of the information is clearly acknowledged. Where citations of written sources have been made, however: (a) their words were rephrased and the precise information have been addressed. (b) Where their precise writing was used, then their words have been italicized and enclosed in proper citation.

It met the standards for originality after being submitted to originality-checking software.

Magopa T.L

SIGNATURE

31/01/2023

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Dedications

This dissertation is dedicated in honour and explicit affection:

To my mother Mapule Salome and my father Mosomane Peter, thank you for bringing me to this earth and thank you for the prayers, encouragement, endless support, and the love that you have always given me to further my career.

To the Magopa family, Mashilo family, and Mankgane family, thank you for your spiritual support, guidance, and understanding when I was not at home for holidays and family gatherings.

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List of abbreviations

Abbreviations	Description
ABS	American breeders service
ALH	Amplitude of lateral head displacement
AI	Artificial insemination
BCF (Hz)	Beat cross frequency
BCS	Body condition score
cm	Centimetre
CASA	Computer assisted sperm analysis
CIDR	Controlled intravaginal drug release
CL	Corpus luteum
ºC	Degrees Celsius
DNA	Deoxyribonucleic acid
EB	Estradiol benzoate
ECP	Estradiol cypionate
FC	Flow cytometry
FSH	Follicle-stimulating hormone
GnRH	Gonadotrophin-releasing hormone
g	Gram
HMD	Heat mount detector
Hz	Hertz
HOST	Hypo osmotic swelling test
Kg	Kilogram
Km	Kilometre
LIN (%)	Linearity
LN_2	Liquid nitrogen
IM (%)	Immotile
i.m.	Intramuscular
IVF	*In vitro* fertilization
LH	Luteinising hormone
MED (%)	Medium
µℓ	Microliter

µm/s	Micrometres per second
mℓ	Millilitre
mm	millimetre
NPM (%)	Non-progressive motility
E_2	Oestradiol
PAG	Pregnancy associated glycoproteins
P/AI	Pregnancy per artificial insemination
P_4	Progesterone
PM (%)	Progressive motility
$PGF_2\alpha$	Prostaglandin
RAP (%)	Rapid
SLW (%)	Slow
SCA®	Sperm Class Analyzer®
SD	Standard deviation
STR (%)	Straightness
TAI	Timed artificial insemination
TM (%)	Total motility
USDA	United States Department of Agriculture
UNISA	University of South Africa
VAP (µm/s)	Velocity average pathway
VCL (µm/s)	Velocity curvilinear
VSL (µm/s)	Velocity straight line
WOB (%)	Wobble

Conferences/symposiums proceedings and magazine article publications

Scientific paper

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Abstract

In beef and dairy cattle, using sexed semen improves genetic progress and increases the proportion of desired gender calves following artificial insemination (AI). Oestrus detection is a limiting factor for maximum reproductive efficiency in dairy and beef cows. Many intrinsic and extrinsic factors, however, can hinder oestrous synchronization. Advanced sperm analyses in cattle provide accurate results on sperm quality. The best measure of sperm quality, according to the computer assisted sperm analysis (CASA) system, is the highest sperm motility or velocity. Therefore, the present study aimed to assess the application of timed artificial insemination (TAI) in synchronized dairy and beef cows using sexed semen in emerging farmer's cattle herds of Gauteng province. A total of two hundred and thirty three cows (dairy; n = 136 and beef; n = 97) were selected from the emerging cattle herds. All cows were selected based on; age (3 to ≥ 7 years), body condition score (BCS) of ≤ 2.5, 3 and ≥ 3.5 (1 to 5 scale), not pregnant (excluding heifers), parity (1 to ≥ 5^{th}), negative to contagious abortion, 90 days postpartum and lactation status (lactating or dry) and assigned to a 9-day Ovsynch + controlled intravaginal drug release (CIDR) and TAI protocol. In brief, on any given day throughout the oestrous cycle (Day 0) the cows received an insertion of CIDR device into the vagina, with intramuscular (i.m.) administration of Estradiol benzoate® (EB). On Day 8, i.m. administration of prostaglandin ($PGF_2\alpha$), with adhesive tail-head heat mount detectors (HMD) and CIDR was removed. On Day 9, i.m. administration of EB. The TAI was performed by the same inseminator 55 hours following CIDR removal using frozen-thawed X-sexed or non-sexed semen from 8 bulls (4 Holstein Friesian and 4 Angus), 2 sexed and 2 non-sexed sperm from each cattle type (dairy and beef) were supplied by American breeders service (ABS) Global, South Africa. At AI, oestrus behaviour was assessed by activation of the HMD colour either as are red (oestrus/activated patch) or white (no oestrus/ not activated patch). The GameteTek Cryo-Mobile laboratory truck was used during thawing of semen straws and sperm quality parameters (sperm motility, velocity, morphology and membrane integrity) were immediately assessed before AI. Pregnancy diagnosis was performed on Days 35, 65 and 95 following TAI with the aid of a transrectal ultrasound scanner and transrectal palpation. The calving date and calf sex were recorded at calving. All data were analyzed by general linear model (GLM) procedure of Statistical Analysis System (SAS, 9.3.3). Analysis of variance (ANOVA) was tested to compare treatment means for semen quality (sexed vs non-sexed semen), bull (n = 8) and treatment × bull as a fixed effect. Chi-square test was used to determine significant differences for equal proportions. Differences between the variables were considered to be statistically significant at $P < 0.05$. The proportion of oestrus expression was greater in dairy (85.3%) than in beef (65.0%) cows ($P < 0.05$). The average sperm total motility on dairy (sexed; 66.8% and non-sexed; 70.7%) and

beef (sexed; 58.8% and non-sexed; 83.8%) bulls were recorded ($P < 0.05$). For morphology, high average live sperm percentages were recorded in both dairy (69.3%) and beef (71.2%) non-sexed sperm compared with dairy (57.2%) and beef (58.2%) sexed sperm, respectively ($P < 0.05$). However, sperm with tail abnormalities were recorded among dairy (sexed; 2.9% and non-sexed; 1.7%) and beef (sexed; 1.7% and non-sexed; 2.3%) bulls, ($P > 0.05$). The average sperm membrane integrity percentage was recorded among dairy (sexed; 51.0% and non-sexed; 64.1%) and beef (sexed; 52.3% and non-sexed; 71.2%) bulls. The proportion of pregnancy was high in dairy (sexed; 41.4% and non-sexed; 48.5%) compared with beef (sexed; 38.0% and non-sexed; 37.0 %) cows ($P < 0.05$). In conclusion, the acceptable oestrus synchronization expression and conception rates of dairy and beef cows were achieved. It is recommended that sexed semen can be successfully utilized through advanced reproductive biotechnologies in an organized emerging cattle farming system.

Keywords: Cattle, sexed semen, oestrous synchronization, thawed semen, timed artificial insemination, conception rate.

Chapter 1

General introduction

1.1 Background

It is worth reiterating from most recent information that cattle are the world's inextricable part of livestock farming. This is because they secure humanity with by-products such as milk, meat, leather, fertilizer, and draft power in various countries (Sheikh *et al*., 2017). Cattle production dominant livestock production in countries developing and most participants are small farmers trying to emerge. Cattle farming has great importance to the economy of South Africa as well as to independent farmers. The primary apprehension in livestock agriculture is maintaining and increasing livestock to produce sufficient by-products.

The use of advanced reproductive biotechnologies in cattle allows for the suppression of major problems caused by poor reproductive function. Oestrous synchronization and AI are well-known adanced reproductive biotechnologies that dairy and beef industries continue to use for herd reproductive and genetic improvement, with high use of quality germplasm. Synchronization brings a group of selected cows/heifers into oestrus (heat) concurrently before insemination. According to Berg, (2020) AI is the most effective tool available in the cattle breeding industry other than natural mating which is the deliberate inseminating heifer or cow with semen into the reproductive tract (uterine body) to attain pregnancy by artificial means. The use of sexed semen with AI results in an accuracy of ≥ 90% calves with desired gender (Naniwa *et al*., 2019). The fluorescence-activated cell sorting is traditionally the most used type of flow cytometry (FC) (Garner *et al*., 2013), not long ago, the laser ablation of undesirable sperm with the X or Y chromosomes was developed (Faust *et al*., 2016a). Dr Lawrence Johnson established and modified the FC to be made into a specific sperm sorter with his colleagues at the Beltsville Agricultural Research Centre, United States Department of Agriculture (USDA) (Magopa *et al*., 2022). Thereafter, Dr George Seidel headed a team to develop a procedure for freezing sexed bull semen to be utilized in AI at the Colorado State University (CSU) in the late 1990s (Thomas, 2019). In cattle, there is variation in the Deoxyribonucleic Acid (DNA) content of sperm cells bearing X and Y chromosomes, with the X-bearing sperm dominating the Y-bearing sperm with 4% more genetic material (Naniwa *et al*., 2019). Naniwa *et al*. (2019) further reported differences among cattle breeds (4.24% in Jersey; 4.05% in Angus; 4.03% in Hereford; 3.98% in Holstein; 3.73% in Brahman).

South African cattle farming is distinguished into 3 subgroups of farmers based on the scale of production, availability of resources, herd size and breeding methods used, in a bottom-up approach which are communal farmers, emerging farmers and commercial farmers. Whereby, communal cattle farmers are those predominating the rural areas and they practice the old and indigenous knowledge farming system with indigenous cattle type grazing on communal land. Emerging cattle farmers only produce a few for the market (Pienaar & Traub, 2015), while others are trying to enter the market, they lack support and lease or own small farms with indigenous or exotic crossbred type cattle. Meanwhile, commercial cattle farmers produce to an enormous degree, as they depend more on technology and farm with crossbred and/or not genuine cattle breeds. An estimated 13.9 million cattle are found in South Africa (STATS SA, 2016), whereby 80% are beef cattle herds and 20% are dairy cattle herds, however, from the overall number 60% (8.3 million) are kept by commercial farmers although 40% (5.6 million) by emerging and communal farmers (DAFF, 2018). Therefore, the requisition to empower cattle emerging farmers to the level of being commercial farmers is still a quest to attain (Katikati, 2017). Livestock is an insurance and income source for almost every farmer during unpleasant incidences such as employment dismissal or dry periods (Sikhweni & Hassan, 2013).

There is a need for the transformation of the cattle production system in the communal and emerging livestock sectors, to improve the production system that has led to poor performance of cattle productivity (Odubote, 2022). Emerging cattle farmers have a long walk to be more productive compared to the commercial sector (Montshwe, 2006; Scholtz *et al*., 2008). The difference between commercial and emerging farmers is the level of production whereby management is the responsible influence (Nowers *et al*., 2013). Reproduction performance improvement of cows offers the possibility to enhance herd quality and rates of cattle consumed in a year or sold from emerging farms (Maqhashu *et al*., 2016). Therefore, a good breeding program benefits a breeding herd reproductive performance improvement that will enhance the turnover margin of a cattle enterprise (Katikati, 2017). However, another challenge encountered by emerging farmers is breeding management, whereby they raise crossbreed cattle types between *Bos taurus* and *Bos indicus* that exhibit behavioural variance and they are susceptible to unpleasant body conditions in a period of drought (Ciptadi *et al*., 2012).

Recently, livestock improvement has become imperative with the rise of interest from the South African government and agricultural sectors in food safety (Hanotte *et al*., 2002). The livestock industry is an important division within the agricultural sector, both in terms of food security and sustainable livelihoods. The application of artificial insemination (AI) following oestrus detection

using sexed semen has been practiced globally on cows/heifers in commercial dairy and beef operations (Sá Filho *et al*., 2014b). In livestock (dairy and beef cattle) the possibility of increasing the desired gender of calves has important economic benefits worldwide such as market variable (sexed semen straws are more affordable than breeding bulls) and technology variable (genetic improvement). Emerging cattle farming practices natural service methods commonly for breeding and allowing farmers to utilize any available bull, due to the high deficiency of breeding bulls (Muntswu *et al*., 2017).

1.2 Research problems

Low reproduction rates have been a challenge experienced in South Africa's communal and emerging cattle herds (Nengovhela & Nedambale, 2012). Moreover, there is a high demand for heifer calves (female) by dairy farmers to use as replacement heifers (Seidel Jr & Schenk, 2008); meanwhile, dairy male calves are susceptible to cause dystocia (difficult calving) unlike heifer calves (Holden & butler, 2018), whereas in beef the demand it is on both heifer and bull calves as replacers and for meat production. Moreover, heifer calves born as co-twins to bull calves (male) have a high rate of freemartinism (Kozubska-Sobocińska *et al*., 2016). Unfortunately, due to a lack of knowledge on modern breeding programs available to improve production currently, emerging cattle farmers are unable to produce the preferred gender of calves.

1.3 Justification

Production traits influencing productive performance in dairy and beef herds rely more on calf gender (Morotti *et al*., 2014). Therefore, the primary goal of using sexed semen in any dairy or beef production is to introduce a desired sex bias in the offspring.

It is generally known that dystocia increases the risk of retained membranes, uterine infection, delayed return of oestrus cyclicity, and sometimes mortality of both cow/heifer and calf. Therefore, considering that heifer calves are smaller and simpler to calve, sexed semen can reduce the incidence of dystocia by about 20% (Norman *et al*., 2010). According to Garcia-Ispierto *et al*. (2022), the incidence of freemartinism occurs in the cattle heterosexual twin whereby hormones from the male and female foetuses are shared in the uterus and resulting in an infertile heifer calf. However, the use of sexed semen was confirmed to prevent the condition of freemartinism since only co-twins of the same gender would be born (Kerby *et al*., 2021). Furthermore, the biosecurity risks associated with bringing in cattle from various herds may be improved and minimized by using sexed semen to produce extra heifers for herd expansion and herd replacements at a faster rate from inside the herd

(Holden & butler, 2018). The use of sexed semen with AI following oestrous synchronization enables cattle farmers to predetermine the sex (male or female) of calves from a specific dam and sire, resulting in production goals being met and improved genetic gain within herds. This technology reduces some undesirable calves that farmers/producers do not want in their herds, which they have to cull. The fertility of bull semen to be used for AI can be predicted by semen evaluation, which is an approved test (Kealey *et al*., 2006). Therefore, timing of AI should be observed precisely for successful breeding of cows. Furthermore, using proper management procedures along with advanced reproductive biotechnologies like oestrous synchronization and AI might help to raise the low reproduction rates in communal and emerging cattle farming systems. This study addresses the applications of sexed semen in both emerging dairy and beef herds and highlights the future benefits of sexed semen for optimal production.

1.4 Aim

This study aimed to assess the application of timed artificial insemination in synchronized dairy and beef cows using sexed and non-sexed semen.

1.5 Objectives

The objectives of the study were:

a) To compare the oestrus expression in dairy and beef cows following oestrous synchronization.
b) To evaluate the sperm quality in frozen-thawed sexed and non-sexed semen from dairy and beef bulls.
c) To compare conception rates in dairy and beef cows timed artificially inseminated with sexed and non-sexed semen.

1.6 Hypotheses

The study aimed to test the hypotheses accompanying the aforementioned objectives:

a) The dairy and beef cows will differ in their oestrous synchronization expression.
b) Frozen-thawed sexed and non-sexed sperm obtained from dairy and beef bulls will differ in semen quality.
c) Conception rates in dairy and beef cows timed artificially inseminated with sexed and non-sexed semen will be comparable.

Chapter 2

Literature review

2.1 Introduction

Advanced reproductive biotechnologies are gaining popularity, particularly gender pre-selection technology, which assists with the production of gender-specific sperm for used during AI. These advanced reproductive biotechnologies' primary goal is to distribute germplasm worldwide and increase the number of offspring from genetic superior animals (Ferré *et al*., 2020). The technology brought an uprising to dairy and beef cattle industries by greatly enhancing the effectiveness and profitability of the livestock enterprise to fulfil the need for animal meat and dairy products (Naidu *et al*., 2022). There has been increasing interest in sexed sperm with reduced sperm number in each semen straw for AI, as expected to have economic returns (Macedo *et al*., 2013). Moreover, numerous field research have been successfully reported about the efficacy of AI using cryopreserved sexed bovine sperm (Reese *et al*., 2021). However, the most common and costly reason for AI protocol failure is incorrect oestrus detection, whereby cows are often misidentified as being in oestrus and are immediately inseminated (Roelofs *et al*., 2010). Automatic oestrus detection devices have been developed and one of the commercially available device detects mounting behaviour as part of oestrus behaviour, which is mounted to the cow's tailhead to detect the mounting activity (Röttgen *et al*., 2020). Furthermore, conception rates are a critical concern in the success of AI with sexed sperm, and one of the key variables influencing is the stress involved with the sexing process. It is considered that sexing process affects sperm viability, motility, and fertilization potential (Waheeb *et al*., 2020). However, the sexing effectiveness of ejaculates and the post-thaw quality of sexed sperm have both improved with advancement to the technology and processing method, which have reduced the fertility gap between non-sexed and sexed sperm (González-Marín *et al*., 2021). Several strategies were used to compensate the reduced fertility caused by sexed semen, some of which relied on increasing the amount of sperm used in each insemination (DeJarnette *et al*., 2011), others involved the use of sexed semen with fixed-TAI (Mallory *et al*., 2013). In cattle AI, semen is usually inserted directly into the body of uterus, by passing the cervix and allowing a far less amount of semen to be used (Pursley, 2021). However, the use of sexed semen is influenced by the effectiveness of AI and the accessibility of this technology by emerging farmers. (Khorshidi *et al*., 2017).

2.2 Success of sexed semen in cattle

In either dairy or beef, utilizing sexed semen to produce calves of the desired gender has been used progressively for genetic progress and profitability of the farmer. Previously the use of bovine sexed semen depended on the strategies used for management and geographical area in dairy and beef commercial operations (Sales *et al*., 2011). The primary goal for utilizing sexed semen in dairy or beef operations incorporate is to pre-select the gender of the impending calves (Holden & butler, 2018). Garner *et al.* (1983) were the first to report on sperm population to identify accurate X and Y chromosome bearing with the aid of FC. However later Johnson *et al*. (1989) used the technology to produce calves of the desired gender. The commercialization of sexed semen was proven by the success of the FC technology that is reliable in the separation of X and Y-chromosome-bearing sperm (Garner & Seidel Jr, 2008), however, other methods are available with different approaches to sex sorting semen for commercialization.

Tubman *et al*. (2004), further reported that the sorting of X-chromosome sperm was for heifer calves and resulted in 87.8% accuracy. However, a 37% increase was observed for heifer calves when comparing sexed semen and non-sexed semen. This will be an advantage for farmers to improve herd replacements. Razmkabir, (2018) observed a 1:1 bull to heifer calf sex ratio in non-sexed semen whereby 49.0% was found for heifer calves. The use of sexed sperm technology, which is now only used in more advanced cattle farming, is expected to expand globally in the future (Naniwa *et al*., 2019).

Impacts of using sexed semen in cattle are: (i) To produce calves of the desired gender in both dairy and beef cattle. (ii) Herd replacement and extension with genetically improved can be done at a faster rate (Seidel Jr, 2014; Holden & butler, 2018). (iii) To reduce a 28% rate of dystocia caused by bull calves in dairy (Norman *et al*., 2010). The main challenges with sperm sexing are (i) the high cost of the equipment used to sort sperm sex, and (ii) low sorting efficiency, which can lead to (iii) low conception rates (Singh *et al*., 2015). According to reports from Peippo *et al*. (2009) and Larson *et al*. (2010), fertility and embryo development can be affected negatively by the possible pre-capacitation. The primary contributing factors associated with fertility in sexed semen are the sexing procedures, a low sperm concentration per semen straw, and the deposition site of semen after AI

Sexed semen was proven to be effective in heifers and lactating or dry cows that were purposefully selected (Butler *et al*., 2014; Xu, 2014). Sperm sexing causes some damage to sperm quality, but not as much as cryopreservation, however, to date there is no evidence that those sexing procedures result in abnormal calves (Seidel Jr, 2002).

According to Seidel Jr (2003) and Weigel (2004), lightweight size calves as a result of more calves from female-sexed semen (X-sexed), minimize the incidence of dystocia, particularly in heifers. In addition, Tubman *et al*. (2004) observed calving ease in beef heifers and cows using the least squares means on a score of 1 (no assistance) to 4 (delivery by caesarean section) whereby heifer calves scored (1.1%) compared to bull calves (1.3%), however, no difference where observed between non-sexed (1.2%) and sexed semen (1.2%). Furthermore, they observed no differences in newborn death rates for sexed (3.5%) and non-sexed (4.0%) semen similarly for heifer (4.5%) and bull (3.0%) calves. According to a Danish study by Borchersen and Peacock (2009) in Holstein heifers, AI with sexed semen (heifer calves; 10% and bull calves; 14%) resulted in lower stillbirth rate compared with non-sexed semen (heifer calves; 12% and bull calves; 20%). Norman, *et al*. (2010) also reported that heifers had a higher rate of dystocia (non-sexed semen; 6.0% vs sexed semen; 4.3%) and stillbirth (non-sexed semen; 10.4% vs sexed semen; 11.3%) respectively, than cows (dystocia; non-sexed semen 2.5% vs sexed semen 0.9% and stillbirth; non-sexed semen 3.6% vs sexed semen 2.7%) respectively. Furthermore, DeJarnette *et al*. (2009) observed that bull calves produced from a population of sperm that was 90% X-sexed semen had a higher incidence of stillbirth (20%) compared with non-sexed semen (13%) and argued that this could be due to the inaccurate sexing process selecting Y-bearing sperm.

The birth of heavyweight calves from undesired gender (e.g. males) is a major problem in dairy production (Holden & Butler, 2018). Pre-determination of calf gender optimizes output and profitability in dairy herds in this aspect (Morotti *et al*., 2014). The most common use of sexed semen among reproductive biotechnologies is *in vitro* fertilization (IVF), which can yield high blastocyst rates (Matoba *et al*., 2014). Furthermore, when compared to AI, IVF requires considerably fewer sperm per egg to achieve satisfactory fertilization rates (Holden & Butler, 2018). However, blastocyst rates obtained using non-sexed sperm were reported to be lower (Seidel Jr, 2014). However, Cottle *et al*. (2018) observed that utilizing sexed semen in farms with herds that already had optimal fertility results was more advantageous financially than in farms with herds that had sub-optimal fertility. In any regard, the economic output of using sexed semen in dairy and beef operations is justified by production of desired gender calves. Sexed and non-sexed cattle semen characteristics are presented in Table 2.1.

Table 2.1 Characteristics of sexed and non-sexed semen in cattle.

	Non-sexed Semen	Sexed Semen
Number of sperm per straw	10 Million	2 Million
Proportion of female sperm	50%	≥ 90%
Semen wastage during processing	Minimal	> 50%

Source: (Adopted from: Razmkabir, 2018).

De Vries *et al*. (2008) reported that when sexed semen was applied a conception rate of 45% in heifers and more than 28% in cows. Moreover, heifers showed greater conception rate when using sexed cryopreserved semen where sperm per dose was 7-20 times more when inseminated in the uterus body (Gaur *et al*., 2020). However, heifers bred 12 hours after the start of standing oestrus seem to have the highest conception rates when sexed semen was applied (Rhinehart, 2015). Naniwa *et al.* (2019) reported that although conception rate of sexed semen after AI is low compared with conception rate of non-sexed semen after AI, >90% of sex selection accuracy was attained using sexed semen after AI, which is high compared with non-sexed semen. Razmkabir, (2018) also reported that insemination in Holstein heifers using sexed semen to attain either heifer calves or bull calves at birth respectively resulted in a sex ratio of 86.4% and 13.5%. However, significant variances between semen types (sexed and non-sexed semen) were observed concerning conception rate. Holstein heifer's conception rate and sex ratio for sexed and non-sexed semen are presented in Table 2.2.

Table 2.2 Conception rate and sex ratio for sexed and non-sexed semen in Holstein heifer.

Semen type	Calf sex	Calves born (*n*)	Conception rate (%)	Sex ratio[a] (%)
Sexed	Heifer	727	42.6	86.4
	Bull	114		
Non-sexed	Heifer	3223	54.8	49.0
	Bull	3348		

[a] Sex ratio was expressed as the number of heifer calves to total calves born.

Source: (Adopted from: Razmkabir, 2018).

2.2.1 Sperm sexing techniques in the current market

- Flow-cytometry sperm sorting process

It is currently the available successful technology for sperm sorting (Naniwa *et al*., 2019), which involves the selection and segregation of X and Y chromosome-bearing sperm based on differences in their DNA content (Garner *et al*., 1983; Johnson *et al*., 1989).

The FC can sort 2500 sperm of each sex per second at nearly 60 miles per hour (Seidel Jr, 2002). This method is currently the only one that is dependable, repeatable, and verified for sorting live and healthy sexed sperm cells (Tubman *et al*., 2004). SexedULTRA and SexedULTRA 4M are trade names owned by Inguran LLC and represent sexed sperm prepared using a USDA-developed and registered method (flow-cytometry sperm sorter) that is now licensed under Sexing Technologies (Magopa *et al*., 2022). Over the past few years, the FC sperm sorter has undergone several advancements that have increased its effectiveness and viability. The sexed semen packaged using this method is currently marketed as SexedULTRA 4M for both dairy and beef breeds, and both Y- and X-bearing semen are available. The "4M" indicates that each straw contains 4×10^6 sperm cells, an increase in sperm concentration from previous standard of 2×10^6 sperm cells per straw (Thomas, 2021).

- Laser-based sperm ablation process

It is the latest method to produce sexed sperm but precisely not in a traditional sorting process, but rather the sperm cells are destroyed if they are of undesired gender. Sperm cells undergo a DNA content staining process to identify and destroy undesired gender sperm cells and conserve those sperm of the desired gender (Faust *et al*., 2016b). Sexcel semen products are available and presently marketed as X-bearing semen for a variety of breeds to produce heifer calves. Sexcel is a new sexing technique created by American Breeders Service (ABS) Global and based on their IntelliGen technology (Magopa *et al*., 2022). This sexing procedure also includes sperm cell labelling and DNA content-based differentiation of X and Y-chromosome-bearing sperm. However, a laser ablation method is employed to eliminate sperm cells bearing the undesired chromosome to produce a sexed result. (Thomas, 2021).

2.2.2 Impact of sexed semen on beef production

The acceptance and application of AI in beef production are far lower than it is in dairy production; as a result, there is also little usage of sexed sperm in the production of cattle (Holden & Butler, 2018). Sexed semen in beef cattle could be used in different production systems. Holden and Butler, (2018) further reported that sexed semen might be utilized in a single-sexed heifer breeding system to inseminate beef heifers with X-sexed sperm for production of replacements and then culled after first calving. However, in this system, age of the beef cow has no bearing on the price but increases its value. Sexed sperm in beef production would also be advantageous for the three-breed terminal crossbred system (Clasen *et al*., 2021).

2.2.3 Impact of sexed semen on dairy production

In dairy production, heifer calves are desired as replacements for herd expansion and surplus heifer calves may improve the market value of calves with desired gender (De Vries *et al*., 2008). There is an overabundance of undesirable bull calves in the dairy industry. In comparison to heifer calves, dairy bull calves have a higher risk of dystocia. In contrast to heifer calves, bull calves weigh more at birth. Dairy bull calves are an undesirable by-product of AI with non-sexed semen, due to their poor economic value for dairy production. In this regard, female-sexed sperm (X-sexed) could be used in dairy production to reduce the number of undesirable bull calves, which leads to dystocia.

2.3 Factors affecting cow reproductive efficiency

Herd reproductive efficiency has a significant impact on the economic efficiency of dairy or cow-calf production. Reproductive inefficiency caused by infertility not only limits herd expansion but also has a direct and considerable influence on farm profitability for dairy and beef farmers (Starbuck, 2005).

- Oestrous Cycle

The oestrous cycle in cattle lasts an average of 21 days (Pal & Dar, 2020), ranging from 18 to 24 days and regulated by gonadotrophin-releasing hormone (GnRH) (hypothalamus hormone), follicle-stimulating hormone (FSH) and luteinising hormone (LH) (anterior pituitary hormones), P_4, oestradiol (E_2) and inhibin (uterine hormone) (Murray & Orr, 2020). The oestrous cycle is a continuous ovarian process that enables cows/heifers to change from sexually non-receptive to receptive, enabling mating/service and successive pregnancy development. (Forde *et al*., 2011). The cycle is divided into 2 phases: follicular and luteal. The matured follicle releases an oocyte that is prepared for possible fertilization in the oviduct during the follicular phase (4-6 days) before ovulation (Figure 2.1).

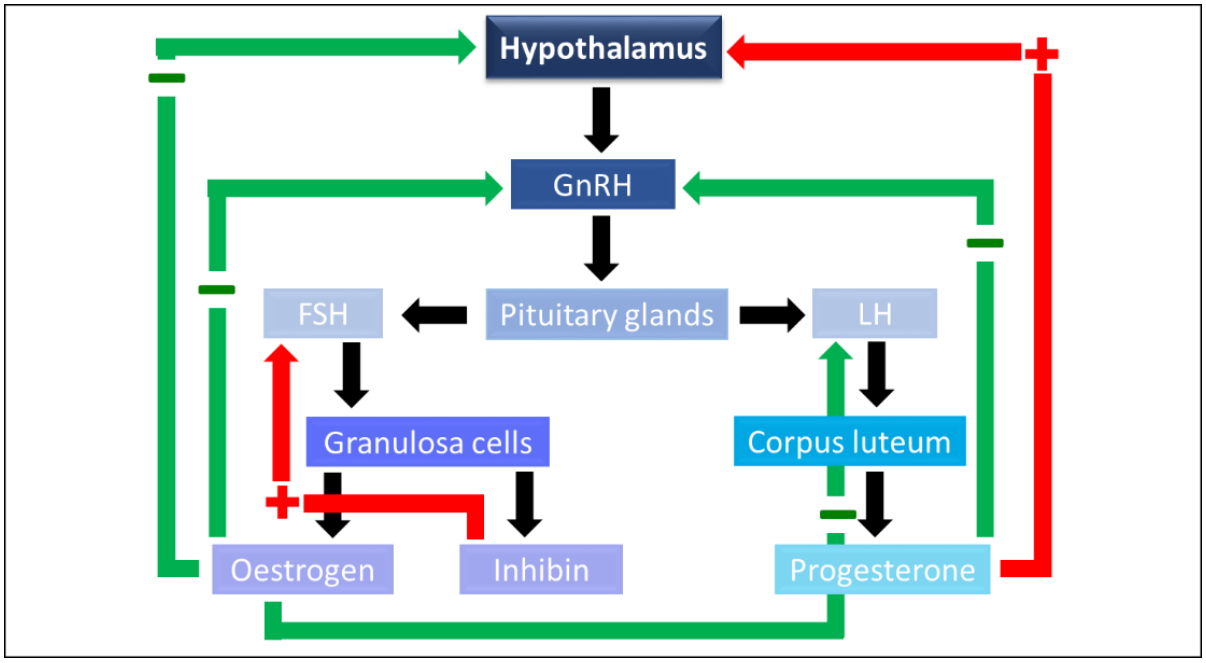

Figure 2.1 Schematic depiction of positive and negative hormonal feedback mechanisms of the hypothalamic-pituitary-gonadal axis in cattle. Source: (Modified and redrawn after: Scheun, 2018).

- Follicular Growth and Development

During the oestrous cycle, appropriate processes of ovarian follicle growth, development, and maturation are required for reproductive efficiency in cows. In dairy (2 waves) and beef (3 waves) cows, follicular growth waves occur differently, resulting in either ovulation or follicle atresia. According to Forde *et al*. (2011) in each wave, the process begins with the emergence of primordial follicles, followed by selection and dominance (Figure 2.2). Follicular growth occurs during early embryo development, near the time of embryonic genome activation (Wiltbank *et al*., 2016). However, follicle wave growth ceases in late embryo development (20-25 days of gestation) due to the suppression of FSH (necessary for follicle growth) by the P_4 and E_2 responding to the FSH (Crowe, 2008).

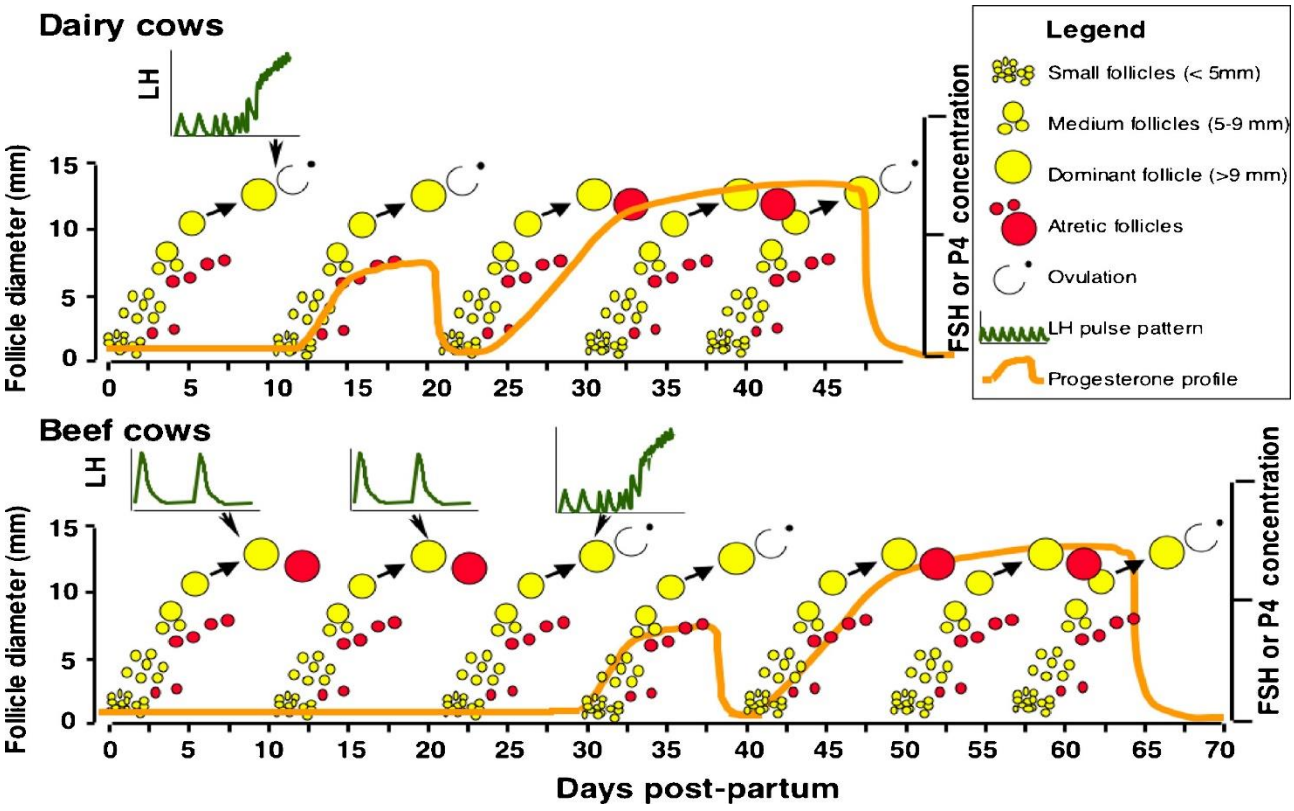

Figure 2.2 Graphic depiction of oestrous cycle of dairy and beef cows in postpartum period with secretion of hormones and ovarian follicular growth pattern. Source: (Adopted from: Crowe, 2008).

2.4 Fertility in cattle

Any breeding plan's purpose (AI or natural breeding; synchronized or non-synchronized) is to increase the number of pregnant heifers/cows. As a result, fertility is critical to the success of any breeding plan. Profitable dairy and beef production systems require optimal reproductive efficiency in both bulls and cows/heifers (Berry *et al*., 2014). According to Perry *et al*. (2010), fertility is controlled by various variables; however, the "Equation of Reproduction" is one of the greatest ways to look at components that affect fertility. Perry *et al*. (2010) further clarified that the equation considers four major topics: the percentage of cows/heifers discovered in standing oestrus and inseminated, inseminator efficiency, herd fertility rate, and sperm quality level.

According to Kubkomawa, (2018), fertility decreases whenever breeding of cows occurs a few hours before the targeted 12 hours and evidently when bred more hours away from the targeted 12 hours from the end of standing oestrus. However, Nafarnda *et al*. (2005) also observed that little decrease in fertility could occur when AI is performed once in cows/heifers observed in the following 24 hours after standing oestrus. The use of female-sexed semen (X-sexed) in dairy commercial operations has targeted heifers, as their fertility levels are superior to cows **(**DeJarnette *et al*., 2011). Lower fertility following timed artificial insemination (TAI) is usually related to insufficient synchronized oestrus and ovulation, which would have allowed for the optimal TAI similarly to ovulation (Cardoso *et al*., 2021). However, even a small decrease in sexed semen fertility compared with non-sexed semen can offset most of the economic benefits (Holden & Butler, 2018).

According to Laven (2009), poor fertility has a direct impact on decreased production, culling rate, genetic makeup of the herd, and profitability. Laven (2009) further explained that, for cows to become pregnant as soon as they are ready for service while using a minimal number of inseminations, reproductive management must be efficient regardless of the farm system implemented. The first step in managing reproduction for optimum quality is to provide the best care for newborn heifer calves. According to Laven (2009), to enhance heifer fertility for calving-mating-pregnancy cycle first must begin at 13 months, pregnancy at 15 months, and calving at 24 months. To optimize fertility, an efficient management program (fertility cycle) is necessary at every phase of a cow's life (Figure 2.3).

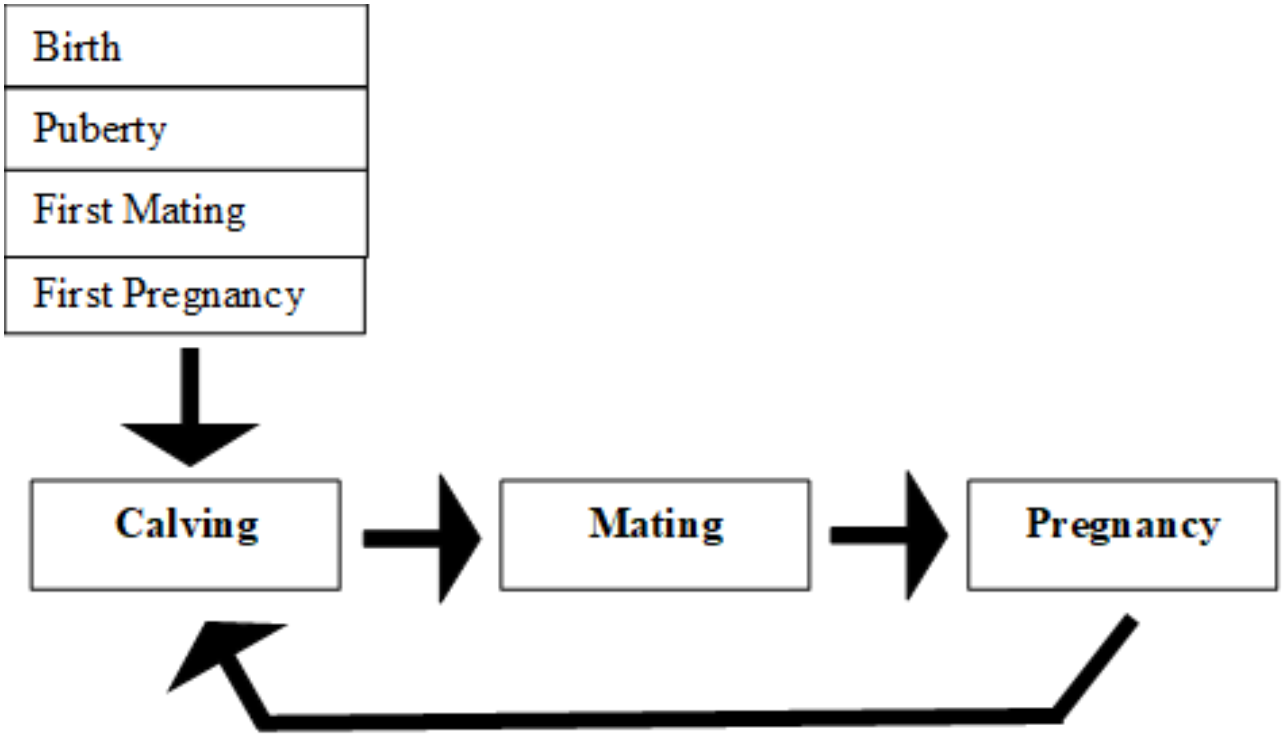

Figure 2.3 The fertility cycle of a cow. Source (Adopted from: Laven, 2009).

2.5 Evaluation of cryopreserved sperm and semen

Semen evaluation is performed im methods which can be either conventional or advanced. The conventional methodology of analysing semen involves the use of a light microscope, warmed stage and slides to estimate cryopreserved semen quality and fertility subjectively (Partyka *et al*., 2012). Advanced methodology for analysing semen involves the use of FC (Hossain *et al*., 2011), and CASA (Lu *et al*., 2014; Cenariu *et al*., 2018) to purposely estimate cryopreserved semen quality and fertility objectively. The key goal for semen evaluation is to rate the semen concerning its volume, good progressive motility and sperm morphology, which further estimates the fertility of a bull. According to Mahmoud *et al.* (2013), the major distress in breeding bulls to be considered are semen quality and its potential fertility. Mahmoud *et al*. (2013) further reported the importance of semen evaluation in forecasting the fertility of bull semen when AI with cryopreserved semen is utilized. Semen quality characteristics such as sperm motility, morphology, membrane integrity, and concentration have all been evaluated using light microscopy on a regular basis (Ugur *et al*., 2019). Moreover, before semen evaluation, practical measures need to adhere, therefore dilution of semen with a medium solution or an appropriate extender aid in increasing the individual sperm cells observation (Lekola, 2015).

2.5.1 Sperm motility evaluation

The CASA is one of the powerful tools utilized for sperm motility evaluation for fertility in males, numerous researchers evaluated semen with the aid of CASA on male livestock species such as bull (Veznik *et al*., 2001), buck (Sundararaman & Edwin., 2008), boar (Vyt *et al*., 2008), ram (Spalekov *et al*., 2011) and cock (Mphaphathi *et al*., 2012). According to Lu *et al*. (2014), the same CASA system is a routinely used tool worldwide to examine human semen in clinic laboratories. However, the CASA system was simply regarded as a tool to rely on when coming to sperm motility and velocity parameters analyses (Alessandra *et al*., 2010). One of the most important parameters of fertile sperm cells is motility. It was the first and is still the most widely used sperm function indicator. The percentage of total motile or progressively motile sperm cells is used to calculate sperm motility (Partyka *et al*., 2012). Partyka *et al*. (2012) further explained that the main advantage of this technique is that it ensures objective semen evaluation, whereas the main disadvantage of traditional semen evaluation is the variability of the results. However, immediate evaluation of sperm concentration, the total number of sperms in the ejaculate, and automated calculation of the number of insemination units that could be prepared from one ejaculate are all significant advantages of CASA. Table 2.3 presents several sperm motility and velocity parameters, which describe the movement of individual sperm cells evaluated using CASA-sperm class analyzer® (SPA®).

Table 2.3 Detailed description of sperm motility and velocity parameters for the CASA-SCA® system.

Parameters	Units	Descriptions	References
Total motility rate	%	The percentage of motile sperm relative to the total sperm concentration.	Kathiravan *et al*., 2008
Non-progressive motility	%	The percentage of sperm not progressing in a straight line.	Vyt *et al*., 2008
Progressive motility	%	The percentage progressively moving sperm.	Vyt *et al*., 2008
Rapid	%	The percentage rapidly moving sperm.	Vyt *et al*., 2008
Medium	%	The percentage of sperm moving at 11-25 µm/second.	Vyt *et al*., 2008
Slow	%	The percentage of sperm moving at 1-10 µm/second.	Vyt *et al*., 2008
Static	%	The proportion of immobile sperm (inactive during the analysis)	Vyt *et al*., 2008
Curvilinear velocity	µm/s	The rate at which the sperm's trajectory progressively recorded in real time expressed in units of time.	Somi *et al*., 2006
Straight line velocity	µm/s	This is the sperm's straight trajectory over time (= the distance travelled in a straight line from start to finish of the track divided by the time taken).	Somi *et al*., 2006
Average path velocity	µm/s	The mean trajectory of the sperm per unit of time.	Somi *et al*., 2006
Linearity	%	This is expressed as (VSL/VCL) × 100 and indicates the proportion of straight displacement to the total of the displacements at the time of measurement.	Somi *et al*., 2006
Straightness	%	This is expressed as (VSL/VAP) × 100 and the linearity of the mean trajectory of the sperm.	Somi *et al*., 2006
Wobble	%	This is expressed as (VAP/VCL) × 100 and indicates the oscillation of curvilinear sperm trajectory onto the mean trajectory.	Somi *et al*., 2006
Amplitude of lateral head displacement	µm	This is the oscillation of the sperm head mean width	Somi *et al*., 2006
Beat cross frequency	Hz	This is the number of times the sperm head oscillates laterally around its mean trajectory.	Somi *et al*., 2006

Source: (Adapted from: Mphaphathi, 2017).

2.5.2 Sperm morphology evaluation

Sperm morphology is of ought most importance since infertility is related to a high percentage of sperm with defects (Umesiobi, 2010). Sperm defects are categorized into 3 types (Primary /secondary, major/minor and compensable/uncompensable). The primary and secondary sperm defects categories are intended to aid in the mechanics of checking totals, monitoring sperm development and prediction. Meanwhile, the major and minor sperm defects categories are intended to differentiate sperm that are proven to cause less harm from those that are related to infertility. However, the compensable and uncompensable sperm defects categories are intended to show great ability, though an increased dose is required for further enhancement and analysing before utilization takes place. Kubkomawa, (2018) reported that an uncompensable defect persists to cause low fertility irrespective of the number of sperm per insemination. Furthermore, Kubkomawa, (2018) reported that with an oocyte fertilized by a sperm with an uncompensable defect, pregnancy may not be detected due to lacking ability to accomplish the fertilization results or produce an embryo, and as such, is associated with chromosomal abnormalities, perhaps abnormalities of messenger ribonucleic acid (mRNA), and protamine status. DNA fragmentation was anticipated to be signified by sperm morphological defects (Enciso *et al*., 2011). Effortlessly observed live, dead and morphological sperm defects can be attained with the aid of staining solutions under a microscope. According to (Attia *et al*., 2016), the necessity for successful and profitable cattle AI is associated with the use of good quality semen. Beef breeders considered the >70% live normal sperm morphology as the normal acceptable morphology (Menon *et al*., 2011).

2.5.3 Sperm hypo-osmotic swelling test

The hypoosmotic swelling (HOS) test was developed as an assay for determining the plasma membrane's functional integrity and osmoregulatory capability of mammalian sperm plasma (Nur *et al*., 2004, 2005). The test is based on the observation that fluid transfer occurs through an intact cell membrane under hypoosmotic circumstances up until the cell's interior and outside are in equilibrium. The cell expands as a result of the influx of fluid, causing the plasma membrane to bulge (Nateq*et al*., 2020). This assay is based on the intact cell membrane's semi-permeability, which causes sperm to enlarge under hypoosmotic environment. When sperm cells with intact plasma membranes are exposed to hypo-osmotic solution, their tails swell (Nateq *et al*., 2020), indicating that water transport across the membranes is normal and the membrane's functional integrity has been preserved (Belala *et al*., 2019).

2.6 Body condition score in cattle

A cow's body condition is an indicator of its fat reserves (Roche *et al*., 2009) and energy status (Nazhat *et al*., 2021) which is controlled by diet type (McCarthy *et al*., 2007), feed efficiency (Rathbun *et al*., 2017), and farm stocking rate (Coffey *et al*., 2017). Body condition is usually quantified by a BCS, which is a subjective method of distinguishing the nutritional status and the amount of metabolic energy contained in fats and muscles for both cows and heifers using a predefined numerical scoring protocol. The procedures required animals to be under restraint while scoring and rely on observing and palpating specific areas of the animal's anatomy before assigning a score (Nazhat *et al*., 2021). The condition scoring ranges from 1 to 5 (whereby a score of 1 characterises being emaciated, 2 being thin, 3 being moderate, 4 being fat and 5 being obese). The BCS can be determined by looking or/and feeling the level of muscle and fat around the back, tailhead, pins, hooks, ribs, and brisket region. Moreover, cows that need immediate care are those in BCS 1, which are in a life-threatening situation, therefore those that are in BCS 4-5 are the costliest to maintain, as they are over-conditioned. However, due to the excessive fat in the pelvic region, cows with BCS of 4-5 may encounter dystocia since it is commonly overestimated as a result of ideal body condition (Schatz, 2011). According to Nazhat *et al*. (2021), a body condition management system has been adopted as a basic, reliable, and economically effective tool to predict cow fertility during the postpartum period. Body weight alone is not a reliable indicator of a cow's condition considering that weight is closely correlated with cow genetic makeup (Zieltjens, 2020). However, body weight is mistakenly utilized to determine level of fat reserves and body condition in cows. Moreover, poor BCS at calving was associated with decreased postpartum body condition loss but still resulted in poor fertility (Ayres *et al*., 2014). According to Mullins *et al*. (2019) body condition monitoring with the BCS system is an important farm management tool when assessing production efficiency. In Figure 2.4, different areas for visual determination of BCS in beef cattle are presented.

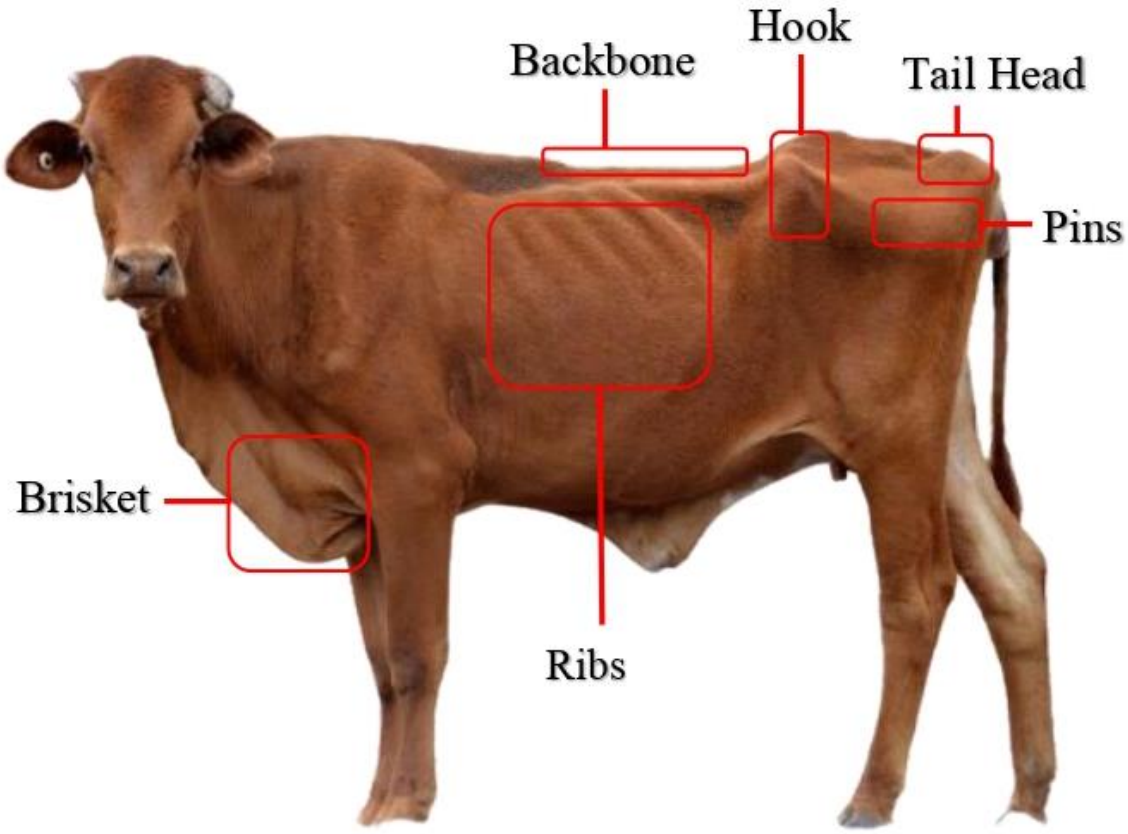

Figure 2.4 Depiction of areas useful for visually determining BCS in beef cows. Source: (Modified from: Body condition score for beef cattle, 2012)

2.7 Applied reproductive biotechnologies in dairy and beef cattle

2.7.1 Oestrous synchronization in cattle

The cost and difficulty of implementing AI, as well as the labour required to handle cattle and observe oestrus, have hampered the widespread adoption of oestrous synchronization programs and AI in beef herds (Epperson, 2019). To make oestrous synchronization more accessible to beef producers, protocols must limit costs, time, and labour, which can be accomplished by reducing the number of times and frequency at which cows are handled during the process. Cattle are polyestrous, which means they experience oestrus at regular intervals. The inability to recognize oestrus, along with miss-detection of oestrus, can result in major economic consequences (Perry *et al*., 2010). Therefore, controlling the timing and season of ovulation would make planning easier.

Studies have revealed that the proper detection of cows/heifers in oestrus is the greatest preventive factor in AI programs (Kubkomawa, 2018). Oestrous synchronization shortens the number of days that oestrus detection must be done as it brings all cows/heifers in oestrus at the same time. The

exogenous hormones are used to imitate bovine female (cows and heifers) endogenous hormones that are produced naturally for oestrus timing (Mabry, 2013). Oestrous synchronization is a valued reproductive technique for both dairy and beef producers and could be used to help producers gain the importance of AI as an ideal method now available. However, herdsman needs to know and understand oestrus signals in dairy and beef cattle industries where cows/heifers are bred artificially. Furthermore, oestrous synchronization also allows for natural breeding, breeding synchronized cows with bulls.

Naturally, oestrus in sexually matured heifers/cows takes place after every 18 to 24 days (Perry *et al*., 2010) for a short period (15 to 18 hours), with oestrus signs behaviour such as standing to be mounted or seeking to mount other cows followed by occurrence of ovulation 10 to 14 hours later (Rae *et al.*, 2004). There are 3 methods currently available for synchronizing oestrous: intravaginal insertion of CIDR, feeding melengestrol acetate (MGA), and ear implanting Syncro-Mate-B® (Pal & Dar, 2020). The control of the oestrous cycle's follicular and luteal phases determines the oestrous synchronization's major success (Mukkun *et al*., 2021). Alphonsus *et al*. (2014) and Mai *et al*. (2014) reported that a cow/heifer that remains standing to allow other cattle to ride on its back, shows finest signal of a fertile time of oestrus. Therefore, the greatest times to spot cattle for oestrus detection are early dawn and late dusk (Kubkomawa, 2018). The use of heat-mount detector device is an effective way aid to determine cows/heifers on the oestrus. However, cows doubted to be in oestrus soon they are applied with the device that is agglutinated to the tail head. Eventually, the original white colour of the detector after prolonged mounting pressure will turn red within 3 seconds (Kubkomawa, 2018). However, appropriate techniques for oestrus detection are necessary to increase the conception rate following AI.

Appropriate reproduction functioning in cows is vital, with the aid of current protocols for oestrous synchronization used; cows can be pregnant at the preferred period of the breeding season. Prior selection of an oestrous synchronization protocol, producers have to identify resources available and all cows and heifers selected ought to be evaluated (Johnson *et al*., 2013). Selection of an oestrous synchronization protocol requires certain aspects to consider for effectiveness. According to Smith *et al*. (2012), aspects to consider involves labour requirement, protocol cost, inseminating on a TAI or inseminating in an advance determined time, and whether the AM/PM rule of insemination is implemented. Smith *et al*. (2012) further reported that an effective oestrous synchronization protocol stimulates fertility level that has to be acceptable when utilizing AI or anoestrus ovulation and cycling of heifers and cows.

To date the utmost used protocol selected by AI professional industries it is the standard 7-day Ovsynch + CIDR protocol. Bringing cows out of anoestrus is advantageously attained by CIDR inclusion, which contains P_4 hormone. Beuchat *et al*. (2013) confirmed the benefits of CIDR insertion in lactating cows to improve fertility in low serum P_4 before $PGF_2\alpha$ administration throughout the Ovsynch protocol. The regression of CL and eradication of P_4 concentration is attained by administration of $PGF_2\alpha$, following seven days of GnRH administration (Colazo & Ambrose, 2013). The Ovsynch protocol (Figure 2.5) is similar, except that administration of the second GnRH occurs 48 hours after $PGF_2\alpha$ and the TAI occurs 12 to 24 hours later. In addition, higher levels of circulating P_4 during the period of follicle growth before AI has been associated with enhanced embryo quality (Cerri *et al*., 2009; Rivera *et al*., 2011), and fertility in lactating dairy cows (Bisinotto *et al*., 2010).

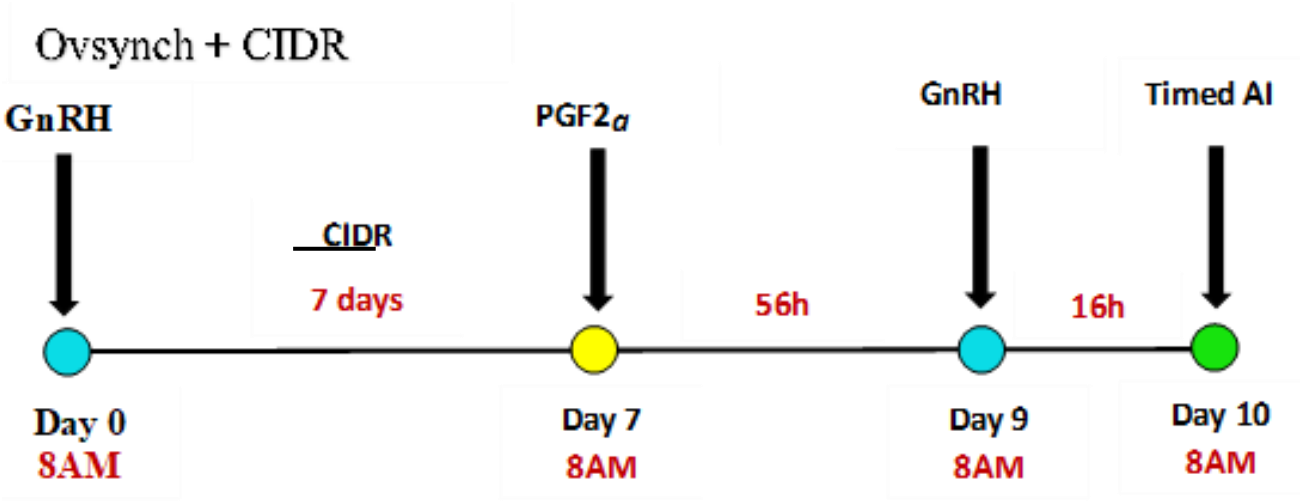

Figure 2.5 Pfizer cattle 9-day Ovsynch + CIDR protocol. Source (Adopted from: Moradi-Kor *et al*., 2012).

2.7.1.1 Oestrous synchronization protocols in cattle

Hormonal agents that synchronize oestrus for natural mating or AI are used in synchronization protocols. Follicular dynamics, CL regression, and ovulation synchronization protocols have been established and are now accessible to cattle farmers. The overall response of a protocol is known to be affected by the stage of the oestrous cycle (Dirandeh *et al*., 2018). Ovulation synchronization (Ovsynch), Pre-synchronization (Presynch), Re-synchronization (Resynch) and Heat-synchronization (Heatsynch) are the main categories in which these protocols fall.

- Ovsynch protocol

The Ovsynch was designed as an ovulation synchronization protocol to allow for TAI at an ideal time with ovulation (Fricke & Wiltbank 2022). This procedure has drawn more attention and is used to synchronize the oestrous/ovarian cycles of several farm animals, like sheep (Hashem *et al*., 2015), dairy cows (Borchardt *et al*., 2018) and goats (Panjaitan *et al*., 2020). According to Chaikol *et al*. (2022), the program stimulates the ovarian follicle to ovulate by the first gonadotropin-releasing hormone (GnRH) injection, which results in the formation of the corpus luteum. A GnRH was administrated at a random time in the oestrous cycle, and a new follicular wave with a dominant follicle appeared following the initial dose. The CL was regressed 7 days later with administration of $PGF_2\alpha$. The second administration of GnRH given 48 hours after $PGF_2\alpha$ synchronized ovulation thereafter, cows were artificially inseminated 16 hours following second GnRH administration, with no need for oestrus detection. Other bovines, such as buffalos (Paul & Prakash, 2005) and yaks, have been effectively treated with Ovsynch (Sarkar & Prakash, 2005).

- Presynch protocol

As compared with Ovsynch, a Presynch protocol allow the use of double $PGF_2\alpha$ administration 14 days apart starting 26 days before Ovsynch to enhance conception rate per artificial insemination (P/AI) in lactating dairy cows following TAI (Moreira *et al*., 2001; El-Zarkouny *et al*., 2002). Within seven days, this program should synchronize 90 to 95% of all cycling cows to exhibit oestrus. Therefore, administration of $PGF_2\alpha$ was used on Presynch cows to start TAI protocol at a desired early luteal phase (Moreira *et al*., 2001). According to Moreira *et al*., (2001) it is important to Presynch cows before administering the first dose of GnRH. Furthermore, Presynch protocols have been used to enhance the percentage of cows that can begin the Ovsynch with the largest follicles responding to the first GnRH within the ideal interval (Silva *et al*., 2018).

- Resynchronization protocol

The Resynch protocol occurs when an Ovsynch or Ovsynch modification is used for the second time following TAI (Bartolome *et al*., 2005; Sterry *et al*., 2006). Resynch 32 days after TAI is a standard strategy to improve conception rates at the start of the breeding season, and it is seen as a significant tool to increase the herd's reproductive effectiveness and financial success (Sá Filho *et al*., 2014a). Moreover, Ovsynch or Ovsynch +CIDR are commonly used to treat non-pregnant cows, which had not displayed behavioural oestrus symptoms to resynchronize the expression of oestrus (Abdalla *et*

al., 2019). According to Giordano *et al*. (2012), these protocols are often started at the time or 7 days before diagnosis of non-pregnancy. Despite higher overall insemination rates and shorter interbreeding periods, P/AI for resynchronized insemination are usually lower than for first insemination (Silva *et al*., 2009; Thompson *et al*., 2010). One cause of the low fertility in Resynch insemination is that 15 to 26% of the cows lack a CL or have low P_4 when the protocol start (Sterry *et al*., 2006; Silva *et al*., 2009). This is essential since resynchronizing cows in low P_4 decreases fertility in cows that are ovulating (Fricke *et al*., 2003; Silva *et al*., 2007). To increase the fertility of Resynch protocols, several ways have been utilized. Silva *et al*. (2007) found that cows Presynch 12 days with a single $PGF_2\alpha$ dose before starting Resynch had improved fertility. Although no differences in the percentage of cows with high versus low P_4 at the first GnRH administration of resynchronization were observed between treatments, an important finding was that cows with high P_4 concentration (1 ng /mℓ) at the first GnRH administration of resynchronization had more P/AI than cows with low P_4, regardless of treatment (Silva *et al*., 2007).

- Heatsynch protocol

Heatsynch is a version of Ovsynch (Pancarci *et al*., 2002), that uses estradiol cypionate (ECP) instead of the second GnRH administration (Lopes *et al*., 2000). Between the $PGF_2\alpha$ administration and the TAI moment, this administration of ECP promotes LH surge, ovulation, and CL development, as well as oestrus behaviour. Heatsynch has a significant cost advantage over GnRH since ECP is less costly (Sarkar *et al*., 2008). However, for Heatsynch protocol, in replacement of the second administration of GnRH, an administration of E_2 (ECP or EB) is given 48 hours following $PGF_2\alpha$ administration (Masoumi *et al*., 2017). Heatsynch conception rate was said to be comparable to Ovsynch results in dairy cows (Lima *et al*., 2015).

2.7.1.2 Oestrus synchronization in natural cattle breeding programs

When cows are synchronized and bred naturally, management measures for the bull's breeding ability should be considered. Some farmers will benefit by breeding synchronized cows with natural-breeding bulls, while others will not (Perry *et al*., 2010). Bull: cow ratios after synchronization are currently recommended at 1:25 bulls per cow. (Timlin *et al*., 2021). In comparison to natural service without oestrus induction or synchronization, natural service applied after hormonal stimulation can increase the reproductive effects of the herds (Baruselli *et al*., 2018). Furthermore, the method used most to get non-pregnant cows pregnant after synchronization is the introduction of clean-up bulls up until the end of the breeding season (Epperson *et al*., 2020). However, the major advantage of oestrus

synchronization with natural breeding is the ability to increase the number of cows that become pregnant within the first 5 to 7 days of the mating season (Perry *et al*., 2010). Perry *et al*. (2010) further explained that a cow's oestrous cycle lasts an average of 21 days (range 18 to 23 days), giving her one chance to conceive every 21 days. Cows that cycle naturally have 3 chances of ovulation and conceive throughout a 65-day breeding season, but cows who are synchronized and display oestrus within the first few days of the mating season have up to four possibilities.

- Potential benefits for oestrous synchronization

Synchronization of oestrous aids with possible shortened calving intervals, and uniformed calf crop, reduces labour for oestrous detection and improves the use of TIA (Abdelwahid *et al*., 2019), which provides farmers with better herd management and observation of cows/heifers on oestrus. It also aids in the improvement of herd health and management such as consistency in vaccinations, and record keeping.

- Challenges or shortcomings with oestrous synchronization

In general, the major challenges to the success of oestrus synchronization are poor body condition of selected cows/heifers, farmers' understanding of heat detection, silent heat and poor herd management systems (Bilkis *et al*., 2016; Sisay *et al*., 2017; Fekata *et al*., 2020). Moreover, the length of the oestrus cycle may be affected by the cow's age, breed type, body weight, level of nutrition, season of the year, hormonal imbalance, milk production level, and lactation status (Shiferaw *et al*., 2005). According to Xu, (2011), the challenge for oestrus synchronization research has been achieving a high level of synchrony while minimizing the negative impact on conception rate at the synchronized oestrus.

2.7.2 Timed artificial insemination in cattle

The application of AI remains a top and widely available reproductive biotechnology in livestock production (Waberski, 2018), allowing a large number of cows/heifers to be inseminated using semen from a single bull that has been chosen and confirmed to be a market leader for economically important qualities. Meanwhile, the application rate of AI varies from one country to the other. Waberski, (2018) further reported that in countries where controlled breeding programs are practised, AI is applied to more than 80% of dairy cattle, unlike 4% of beef cattle (Vishwanath, 2003; Colazo & Mapletoft, 2014). Kubkomawa, (2018) reported on the control of venereal diseases and

transportation of bulls that are capable of spreading diseases to other cattle herds, AI was originally established to evade such incidences. However, for AI to be effective, the herd man/AI technician needs to assume the role of the herd bull and detect cows/heifers that are ready for insemination (Perry *et al*., 2010). Moreover, a key influence disturbing the chance that a cow/heifer will turn out to be pregnant after AI is the number of sperm dosed for insemination (Flowers, 2002). Likewise, the effective timing of AI helps to attain accepted conception rate in cows/heifers. According to Crites *et al.* (2018), maximizing pregnancy using sexed semen is possible only if there is an interrelation between insemination time and the time of ovulation rather than oestrus appearance.

According to Sales *et al*. (2011), the performance of TAI 60 hours following oestrous synchronization tended to increase P/AI to (50.8%; 99/195) in comparison to 54 hours (42.8%; 83/194) after removal of CIDR, even the semen type used and the insemination timing both have a relation. He further reported that TAI increased P/AI specifically when performed between 0 to 12 hours when sexed sperm was used post-synchronized ovulation. Therefore, AI with the use of sexed semen when performed closer to ovulation and increased P/AI will be attained. The conception rate with sexed semen is influenced positively when AI is performed later (18-24 hours post the onset of observed oestrus) other than insemination 12 hours post oestrus, However, the lifespan functioning of bovine non-sexed semen is longer in comparison with the sexed semen which is shorter (Underwood *et al*., 2010). Protocols used for synchronizing follicle development, CL regression, and ovulation, allowing for TAI, result in higher reproductive success since all cows/heifers are inseminated whether they exhibit oestrus or not (Colazo & Mapletoft, 2014). Additionally, according to Colazo and Mapletoft (2014), TAI protocols have become an important element of reproductive management throughout dairy farms, enabling beef farmers to implement AI in their herds. In Holstein heifers treated to a modified 5-day Co-synch plus PRID regimen, the time of ovulation in relationship to TAI and the type of semen on P/AI were evaluated (Table 2.4).

Table 2.4 Effect of time of ovulation relative to timed-AI and type of semen on P/AI in Holstein heifers subjected to a modified 5-day Co-synch plus PRID protocol.

		P/AI		
Time of ovulation (*h*)	*n* (%)	Non-sexed semen *n*/*n* (%)	Sexed semen *n*/*n* (%)	Overall *n*/*n* (%)
0	20 (17.9)	5/11 (45.4)	4/9 (44.4)	9/20 (45.0)
12	27 (24.1)	6/9 (66.6)	15/18 (83.3)	21/27 (77.7)
24	25 (22.3)	10/13 (76.9)	8/12 (66.6)	18/25 (72.0)
36[a]	38 (33.9)	14/20 (70.0)	9/18 (50.0)	23/38 (60.5)
No ovulation	2 (1.8)	0/2 (0.0)		0/2 (0.0)

[a]P/AI tended to differ (P = 0.1) between sexed and non-sexed semen. Source: (Adopted from: Colazo & Mapletoft, 2017).

- Advantages associated with artificial insemination

The most important reason for AI during the olden days was that it controls the introduction of venereal diseases in cattle, at present; AI has the utmost importance in genetic improvement in cattle herds. The use of sexed semen during AI allows for the advantage of (>90%) results of calves born with the preferred gender either as bulls or heifers (DeJarnette *et al*., 2008), enabling faster progeny testing of bulls to assess genetic diversity. Furthermore, AI helps eliminate bulls that cause injuries to the cows during mating (Sharan, 2015), hence, it also allows the breeding of cattle in different locations, or even after the death of best performing sire. Moreover, AI allows for effective use of oestrous synchronization, as a result, it allows breeding to occur within 7 to 11 days period rather than breeding after 21 days in unsynchronized cows (naturally bred).

- Challenges associated with artificial insemination

Challenges that limit the practice and success of AI are insemination of many cows/heifers on the same day (Abebe & Alemayehu, 2021), timing of insemination (Bilkis *et al*., 2016), site of semen deposition (Gebremedhin, 2008), semen handling practice (Mekonnen *et al*., 2010; Belete *et al*., 2018), poor body condition cows selected for AI program (Fekata *et al*., 2020), and inseminator-related factors (López-Gatius, 2011). According to Morrell *et al*. (2018), good hygiene, method of

thawing, and maintaining temperature between thawing to insemination do contribute to success of AI.

2.7.3 Pregnancy detection in cattle production

2.7.4 Over the past few decades, pregnancy detection has improved, and early pregnancy diagnosis is essential to reduce the amount of time a cow spends not pregnant (Reese *et al*., 2018). For economic reasons, an accurate and convenient pregnancy diagnosis is designed to examine the success of reproduction management in a cattle herd. As a useful tool for fertility management, early detection of cow/heifer pregnancy is necessary. Pregnancy occurs when a sperm and an egg (ovum) unite to produce a zygote in a cows/heifers' fallopian tube (Mphaphathi *et al*., 2021). However, it is possible to identify the embryo when it is located in the uterine horn, which is homolateral to the ovary containing CL in a cow/heifer (Purohit, 2010). Pregnancy detection methods are crucial to management efforts to increase reproductive effectiveness in both dairy and beef production. Early detection of pregnancy helps in recognition of infertility and treatment for reproductive problems. However, various methods for detecting pregnancy in heifers and cows have been improved over time, but there are some advantages and disadvantages to be knowledgeable of, which are divided into visual, clinical, and biochemical testing.

- Visual tests

Non-return to oestrus is a simple and cost-effective method of detecting pregnancy in heifers and cows that have not returned to oestrus after breeding for at least 21 days (Mphaphathi *et al*., 2021). If fusion between a sperm and an ovum does not occur, the non-pregnant heifer or cow will return to oestrus 18 to 24 days after mating or insemination. Non-return to oestrus, on the other hand, necessitates the use of a second method. Allowing for a longer period of observation of additional oestrus can improve the accuracy of using non-return to oestrus.

- Clinical tests

Transrectal hand palpation is the most traditional and economical way to determine pregnancy in cows/heifers (Bond *et al*., 2019). The method aims to accurately identify the location and stage of pregnancy following mating or AI (age of the embryo or foetus). The procedure involves inserting a hand into the rectum and palpating for a foetus in the uterus, feeling the size of both ovaries, one of which has recently ovulated (CL present) and examining for reproductive issues (such as the presence

of ovarian cysts, uterine infections, and uterine adhesions). Palpation of the amniotic vesicle by an experienced technician can diagnose pregnancy as early as 30 to 35 days of pregnancy (Pohler, *et al*., 2020). Transrectal hand palpation and days in pregnancy of each cow can be used as an estimation of the stage of pregnancy (Whittier, 2013), hence, adequate evidence for pregnancy is through palpation of a foetal edge if other uterine findings are normal (Purohit, 2010). Table 2.5 presents the stages of pregnancy and foetal characteristics in cattle.

Table 2.5 Different stages of pregnancy associated with changes in the reproductive tract and foetal size in cattle.

Pregnancy stage	Foetal characteristics	Commonly known adult animals
2 months	Uterine horn is 6,35-8,89 cm in diameter, filled with fluid, and pulled towards pelvic brim into pelvic cavity.	Mouse
3 months	Uterine horns are enflamed (10,16-12,7 cm in diameter), pushed deep into pelvic cavity, and palpation is tricky	Rat
4 months	Uterine horns are 12,7-17,78 cm in diameter. Palpation is simpler and cotyledons are palpable and are 3,81 cm in length.	Small cat
5 months	Enlarged horns (15,24-20,32 cm in diameter) pushed into pelvic cavity and cotyledons are 5,08-6,35 cm in length.	Large cat
6 months	Uterine horns are unreachable. Cotyledons are more expanded. Movement of foetus is induced from sixth month until calving.	Beagle dog

Source (Adopted from: Purohit, 2010).

Transrectal ultrasonography is a type of ultrasound that is considerably easier to learn than rectal palpation and can be used to confirm pregnancy in livestock. An ultrasound scanner can be used as early as 26 days following insemination or mating to assess the anatomy of the uterus and ovaries, as well as embryo viability (Terzano, 2012). The ultrasound scanner offers several benefits over other pregnancy diagnosis methods since it is much simpler to view than it is to feel the reproductive system, although it is expensive. To calculate the estimated age of an embryo, an ultrasound-scanning monitor should capture an image of the embryo, and the size of the embryo must be assessed.

Pregnancy diagnosis with an ultrasound scanner is more accurate in diagnosing the age, sex, and viability of the foetus (at 55-60 days of pregnancy).

- Biochemical testing methods

Biochemical tests require a minimum of 2 mℓ of blood from the heifer or cow's tail coccygeal vein or neck jugular vein to detect pregnancy (Mphaphathi *et al*., 2021). Whenever a heifer or cow has experienced embryonic loss after breeding, the method can produce inaccurate results. Biochemical tests, on the other hand, cannot determine the embryo's age or sex, and pregnancy results are only known after 2 to 4 days due to laboratory testing. Pregnancy-associated glycoproteins (PAG) and P_4 hormones are biochemicals in the blood that are used to determine pregnancy status. The level of P_4 in the heifer's or cow's blood secreted by the CL can also be used to determine pregnancy status 21 to 24 days after AI or mating and analyzed; if the cow/heifer is pregnant, she will have a high P_4 level, because P_4 keeps the pregnancy going until the end. When oestrus occurs, the non-pregnant heifer or cow will have low P_4, as is typical.

The PAG are proteins produced by trophoblast cells in the placenta of a developing embryo and can be used to determine pregnancy status 19 to 21 days after AI or mating (Pohler *et al*., 2020). The trophoblast cells cross the microvillar junction, unite with the uterine epithelium, and transfer placental lactogen and PAG into the uterine stroma, which is absorbed into the maternal bloodstream (Pohler *et al*., 2015). The PAG may help in the processing of growth factors at the placental-uterine interface, play a role in uterine-placental adhesion, or control maternal immune modulation, according to some theories (Wallace *et al*., 2015). However, it has been proven that factors such as parity, postpartum days, oestrus expression, bull, and foetal viability all influence the concentration of circulating PAG (Pohler *et al*., 2016; Franco *et al*., 2018).

The PAG in cow/heifer system, on the other hand, can be detected 60 days after calving. As a result, a postpartum period of 60 to 90 days is required for accurate results in this practice. The PAG has previously been shown to be a good indicator of late embryonic mortality in beef and dairy cattle (Breukelman, *et al*., 2012).

2.7.3.1 Pregnancy loss in cows

In the domestic livestock industry, embryonic and pregnancy loss are still major concerns (Pohler *et al*., 2020). However, production status, genetic composition, environment, and management conditions all influence the timing of embryonic and foetal loss, which further results in managerial and financial challenges for cattle farmers. According to Wiltbank *et al*. (2016) and Reese *et al*. (2020), approximately 70 to 75% of beef cows/heifers and 65 to 70% of dairy cows/heifers inseminated following oestrus expression or bred by TAI on day 7 develop a blastocyst. However, various factors, including genetic fatal mutations, uterine asynchrony, maternal pregnancy recognition, placental inadequacy, and illness, can result in the loss of an embryo and foetus on any day of pregnancy. In addition, Pohler *et al*. (2016) reported that late-embryonic loss could be due to factors including differences in oocyte cytoplasmic maturity at ovulation and the uterine environment or the embryo source (*in vitro* fertilized, cloned by somatic cell nuclear transfer). Furthermore, Pohler *et al*. (2016) reported that embryonic loss might occur early between 28 days of pregnancy or late beyond 28 days of pregnancy. Nevertheless, Producers may be able to reduce expenses related to this cause of reproductive inefficiency by detecting any future pregnancy losses (Ealy & Seekford, 2019). Furthermore, reduced reproductive performance is frequently caused by inadequate oestrous detection and embryonic or fetal losses (Kilany *et al*., 2022). Late embryonic mortality is estimated to affect 5 to 8% of beef cattle pregnancies, while up to 15% of dairy pregnancies may be terminated during this time (Wiltbank *et al*., 2016; Reese *et al*., 2020). However, the causes of late embryonic mortality are less well understood, as most research has focused on the factors that contribute to early embryonic loss (Pohler *et al*., 2020). In Figure 2.6, a depiction of periods of pregnancy development and loss in cattle is presented.

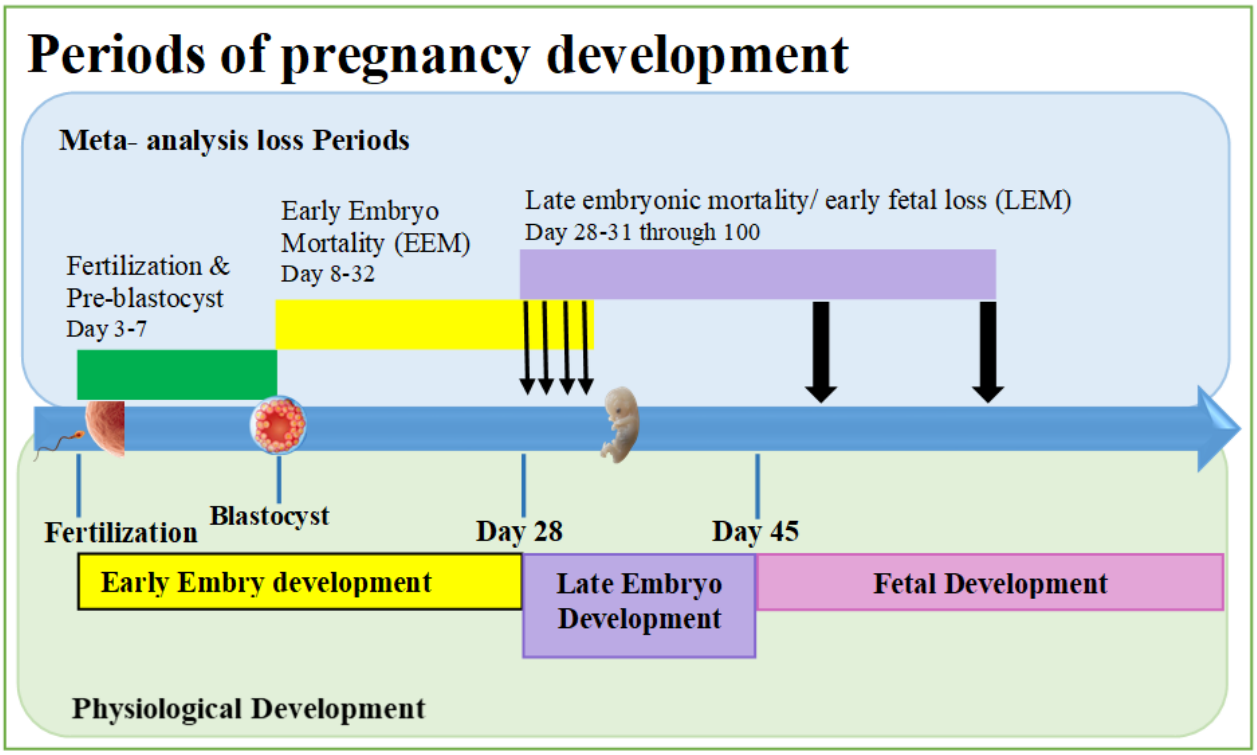

Figure 2.6 The Periods of physiological pregnancy development and loss. Source: (Adapted from: Pohler, 2021).

2.8 Breeding programs in cattle farming

When it comes to the design of cow breeding programs, cattle producers sometimes face an apparent issue. Cattle breeding programs have had some remarkable successes, as seen by the emerging cattle population. However, many breeding programs have already been tried in developing cattle farming sectors, with most of them failing. To further understand why these breeding programs have failed, it is suggested that they be compared to advances that have been implemented by the communal sector (Camara *et al.*, 2019). To enhance the overall reproductive efficiency of a herd a systematic breeding program needs to be implemented. Different farming sectors implement breeding programs based on the type of farming system they practise; breeding methods used in various farming sectors as a percentage are presented in Table 2.6.

Table 2.6 Percentage of breeding methods practised in different farming sectors.

Sector	Uncontrolled %	Controlled %	AI %
Communal	98.1	1.9	0.1
Emerging	63.2	36.8	6.3
Commercial	11.4	88.6	21.9

Note: AI percentage is also included in controlled breeding. Source (Adapted from: Scholtz *et al*., 2008).

2.9.1 Natural breeding program

Herd bulls chosen for commercially important qualities are restricted in the number of cows/heifers they can serve throughout the breeding season when using natural service (Perry *et al*., 2010). The use of bulls for natural mating is a habitually practised breeding method everywhere in the cattle production industry. The program minimises challenges for the need for oestrus detection as the bulls assist with that and is considered a less expensive program, particularly for dairy production. Providing natural breeding oestrus detection is said to be simple since it is the task of the bulls. However, there are variances between bulls. A herd bull's function during breeding season is to recognize heifers/cows in standing oestrus and breed them at the right moment (Perry *et al*., 2010). Half the time synchronization with natural breeding is practised as an alternative to short-term breeding to reduce management demands. Although other farmers utilize AI alongside a bull to try to improve conception and pregnancy rates. Natural breeding raises the greatest risk of venereal disease transmission within a herd and outside. According to Mardones *et al*. (2008), Rae *et al*. (2004) and Rodning *et al*. (2008), the widespread of bovine Tritrichomonas still occurs where natural mating practice is habitual. Therefore, the practice of natural breeding alone has an impact on the intensity of selection and accuracy and genetic variety, as it takes a while to attain genetic progress rate in a herd. Natural mating is thought to have one advantage over AI in that it eliminates the need for oestrus detection (Laven, 2015). Natural service would most likely limit the number of mating per bull to less than 100 per year. Natural breeding is still the most common procedure used in beef cattle operations around the world, although artificial technologies for cattle breeding are rapidly improving and have gradually displaced natural service as the preferred method of breeding in the dairy industries of most developed countries.

2.9.2 Controlled breeding program in cattle

Controlled breeding programs involve the improvement of reproduction performance, which reduces the impact of conception and pregnancy losses. To date, new technology in controlled breeding programs is at the boundary of accelerating the timing and accuracy of genetic decision-making. Hence, techniques such as sexed semen (Chowdhury *et al*., 2019) and sexed embryos (Hirayama *et al*., 2004) have enhanced the interest in manipulation of calf gender to the preferred gender. Furthermore, Holden and Butler, (2018) reported that calves produced from controlled breeding attain an enhanced increased rate of genetic gain, which results in more production and achieving profit. Strategies for a guaranteed controlled breeding program are considered by implementing an effective synchronization protocol that will enable oestrus detection as an important aspect (Quezada-Casasola *et al.*, 2015). Nevertheless, Udin *et al.* (2017) reported that the shortening of postpartum interval, calving season preference, and concentrated and uniform calving could be attained when utilizing an oestrus synchronization program. Therefore, accurate oestrus detection with good observation, TAI and comprehensive record keeping is key to an effective breeding program (Roelofs *et al.*, 2010). Ineffective AI outcomes occur in most cases because of missed oestrus detection. According to Vishwanath, (2003) prevention of venereal diseases, dystocia reduction and more accurate dry-off days are benefits when AI is utilized in controlled breeding, which further allows the use of a single bull consistently throughout the calf crop. It is critical to have good breeding season management to improve the reproductive performance of a breeding herd and its calves' growth, as it has a positive impact on the profit margin of a beef cattle enterprise (Katikati, 2017). Table 2.7 presents summer breeding seasons for different regions of South Africa.

Table 2.7 Time of the year guidelines for a three-month summer breeding season for some regions in South Africa.

Region	Breeding	Calving
Eastern Highveld	November to January	August to October
Western Highveld	December to February	September to November
High rainfall Bushveld	January to March	October to December
Low rainfall Bushveld	February to April	November to January

Source (Adapted from: Bergh, 2004).

2.9 Spermatogenesis in bulls

The primary functions of the testes are to produce endocrine and exocrine factors. The endocrine factors include the generation of testosterone into circulation by the Leydig cells, which are located near the seminiferous tubules. Thereafter, testosterone will begin the spermatogenesis process, which is similar to oogenesis in cows. The exocrine factors include the generation of sperm cells in the seminiferous tubules and migration to the epididymis. Spermatogenesis is a lengthy and logical process that lasts for 61 days. It is divided into spermatocytogenesis (mitosis) 21 days, meiosis 23 days, and spermiogenesis 17 days (Staub & Johnson, 2018). After a succession of mitotic cell divisions, Type A1 spermatogonia eventually transform into Type B spermatogonia (Figure 2.7). Thereafter, Type B spermatogonia go through a last round of mitosis to generate primary spermatocytes (only 2 cells are visible), which then go through meiosis (Rahman & Pang, 2020). According to Wang *et al*. (2021), several events of meiosis have been studied in spermatocytes, whereby primary spermatocyte yields 2 secondary spermatocytes and cellular division of primary spermatocytes into secondary spermatocytes divides into four round spermatids that contain either the X or Y-chromosomes. Furthermore, meiotic recombination is required for the crossover between the X and Y-chromosomes to occur in the pseudoautosomal region (Ma *et al*., 2022). During this entire process, the sperm cells migrate from the basement membrane toward the lumen of seminiferous tubules of the testis in a process called spermiation (Staub & Johnson, 2018). Following spermiation, sperm move to the epididymis, where they grow physically and morphologically, allowing the sperm to reach an egg and develop into an embryo (Oliva & Castillo, 2011). The process is dynamic due to sperm DNA replication occurrence. Givens, (2018) reported that semen contamination could occur resulting from infections related to any of the bull's reproductive tract features (testicle, epididymis, vas deferens, seminal vesicle, prostate gland, urethra, and penis) and/or blood cells infected travelling into the bull's reproductive tract. Singh *et al*. (2015) reported that from the 50% X-chromosome and 50% Y-chromosome carried during spermatogenesis, the same sperm cells can go through a scientific logic of sperm separation (sexed semen) and be able to produce an embryo through AI or IVF. Except for their DNA content, most recent studies have found no significant differences between the X and Y sperm types (You *et al*., 2017).

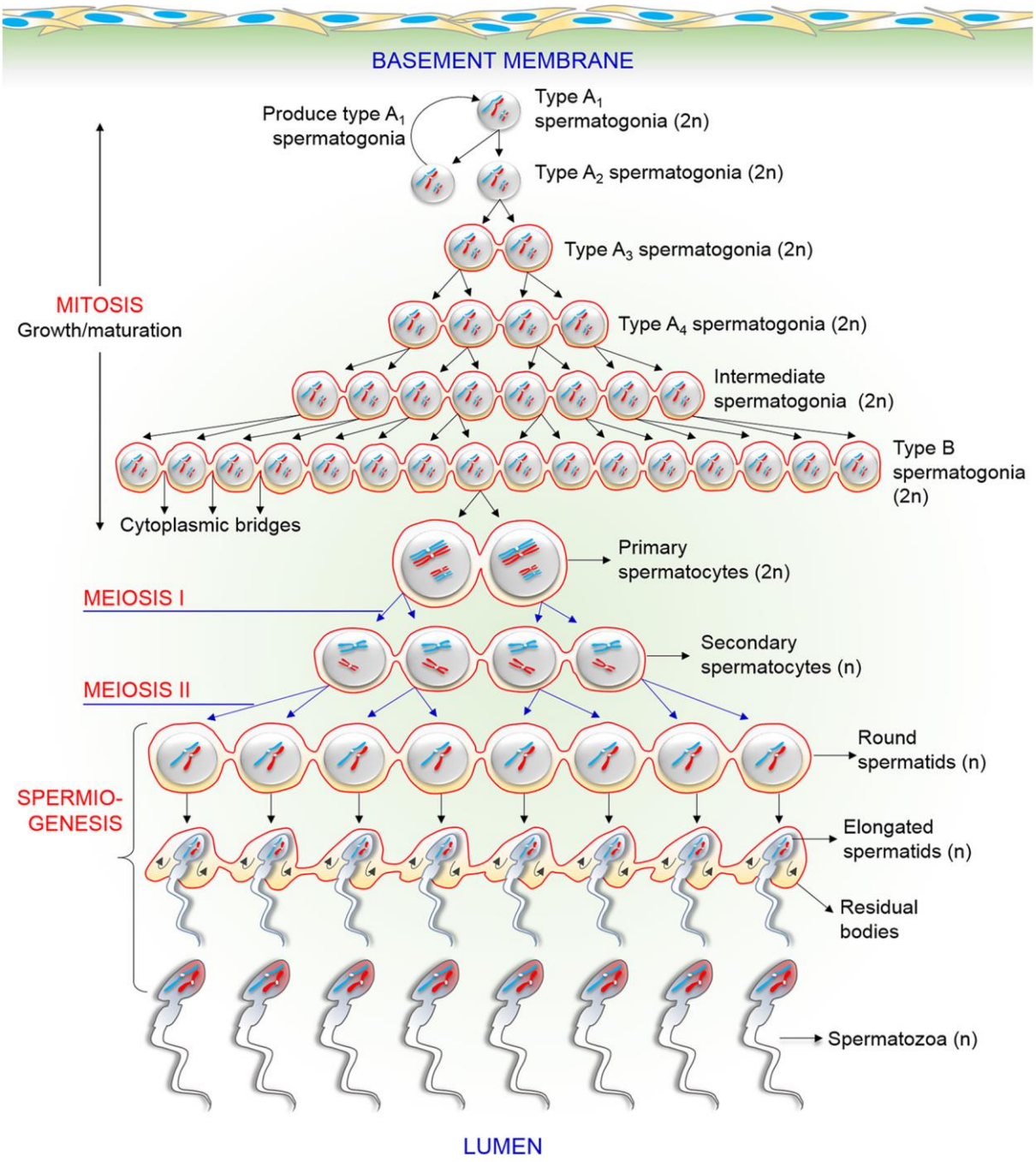

Figure 2.7 Schematic diagram of various processes in male germ cells during spermatogenesis and spermiogenesis. Source: (Adapted from: Rahman & Pang, 2020).

Chapter 3

Materials and methods

3.1 Equipment and hormones

Equipment and hormones for oestrous synchronization and AI were purchased from ANB Vet, Randburg and Embryo Plus®, Brits, Republic of South Africa.

3.2 Animal ethics

Ethical approval for all experimental procedures in this study was obtained from University of South Africa (UNISA), College of Agriculture and Environmental Sciences animal research ethics committee approval number 2019/CAES_ARC/153.

3.3 Study sites

The study was conducted in emerging dairy and beef cattle farms from four district municipalities of Gauteng province in South Africa between January 2021 to June 2022. The province is the smallest (18 178 Km^2) of all nine provinces in South Africa, which makes it a landlocked province without foreign borders and four provinces border Gauteng, the Free State on the southern border, North West on the western border, Limpopo on the north border and Mpumalanga on the east border (General Overview of Gauteng, 2007). Four districts were identified (City of Tshwane, City of Johannesburg, Sedibeng and West rand) since most livestock agricultural activities are established there (Figure. 3.1) and are classified as important agricultural sites in the province (Nesamvuni *et al.*, 2016).

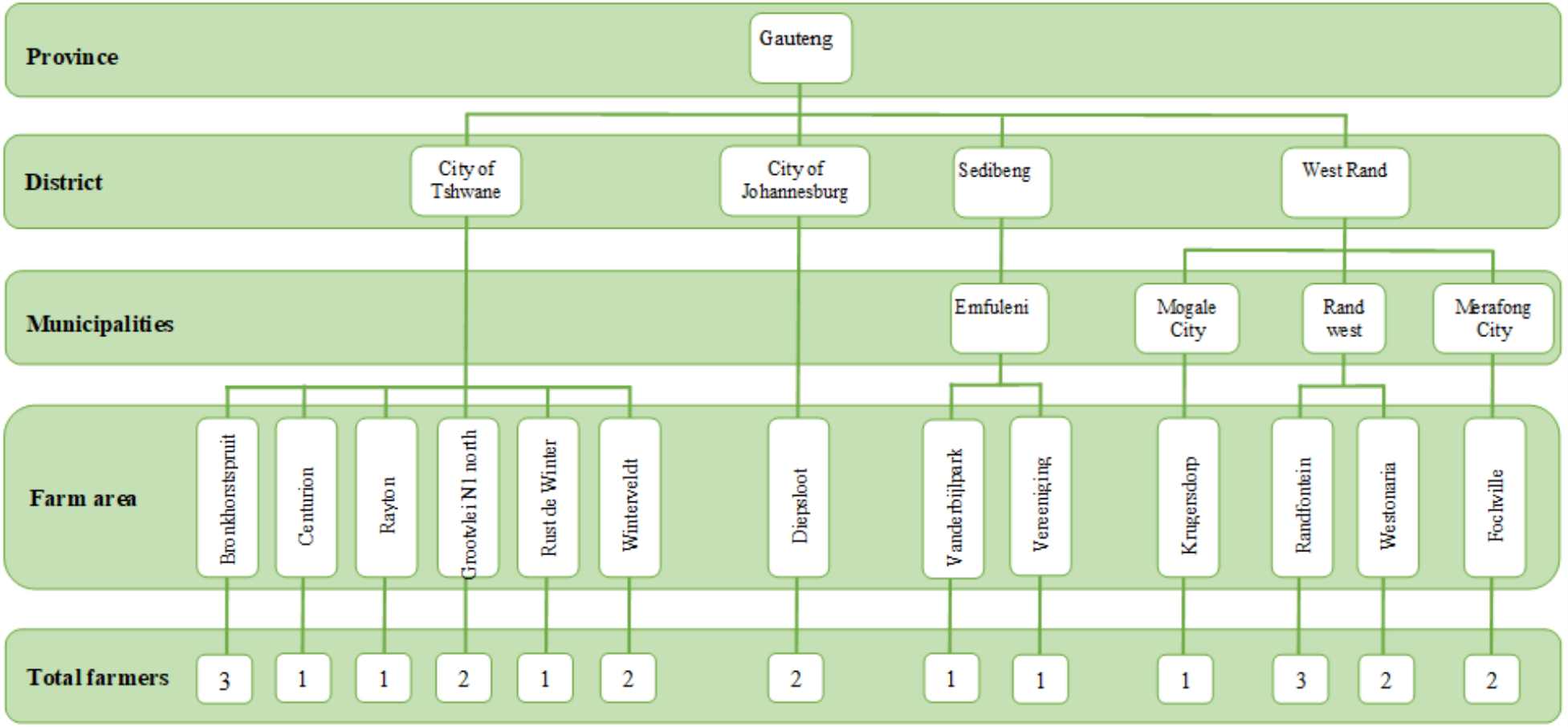

Figure 3.1 Study site districts, municipalities and farm areas in Gauteng province.

3.3.1 GameteTek Cryo-Mobile laboratory Truck

The GameteTek Cryo-Mobile laboratory was deployed to the site of emerging cattle farmers and it carried the liquid nitrogen (LN_2) tanks with frozen semen straws, CASA-SCA® system and thawing unit. The laboratory activities (frozen semen thawing and semen evaluation) were performed inside the mobile laboratory (Figure 3.2). The GameteTek Cryo-Mobile laboratory was established to render livestock reproduction services to livestock farmers at their farm's place (Nedambale, 2014).

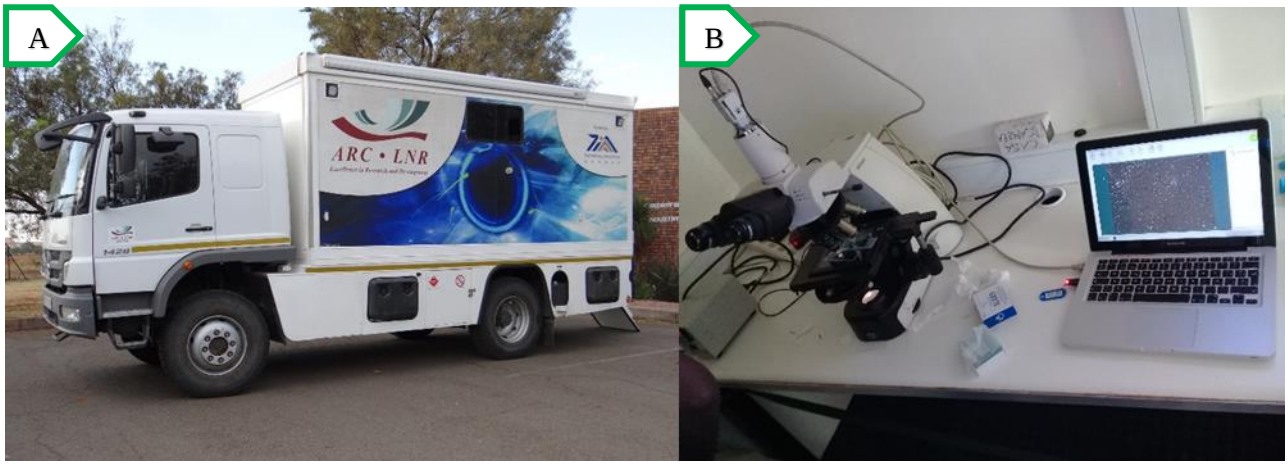

Figure 3.2 The exterior of the GameteTek Cryo-Mobile laboratory truck **(A)** and the interior exhibiting the CASA-SCA® **(B)**. Source: (Personal collection: TL Magopa).

3.4 Screening and selection of dairy and beef cows for oestrous synchronization

The dairy (*n* = 149) and beef (*n* = 137) cows were screened purposefully as presented by the farmers and based on accessibility of farm handling facilities where advanced reproductive biotechnologies such as AI, oestrous synchronization and pregnancy diagnosis were achievable. The cattle types were determined based on their phenotypic traits of resemblance to dairy and beef cattle types.

3.4.1 Selection of dairy cows

A total of *n* = 136 dairy cows (lactating or dry) were selected with the requirement that they were aged between 3 to 8 years, BCS of ≤ 2.5 and above (1-5 scale), non-pregnant cows, at least 90 days postpartum, with parity 1 to ≥ 5, and negative to contagious abortion. Throughout the study, all selected dairy cows were ear-tagged for easy identification and kept in their natural habitats (the owner's farms) where they were maintained on semi-extensive pasture with supplementary feeding.

3.4.2 Selection of beef cows

A total of *n* = 97 beef cows (lactating or dry) were selected with the requirement that they were aged between 3 to 8 years, BCS of ≤ 2.5 and above (1-5 scale), non-pregnant cows, at least 90 days postpartum, with parity 1 to ≥ 5, good mothering ability and negative to contagious abortion. Throughout the study, all selected beef cows were kept in their natural habitats (the owner's farms) and maintained on an extensive production with natural pasture and were ear tagged for easy identification. Supplementary feeding was not given to any of the cattle.

3.4.3 Body condition scoring dairy and beef cows

All dairy cows were assigned a BCS according to a condition scoring chart developed by Edmonson *et al*. (1989) (Figure 3.3) and the BCS for all beef cows was assigned using the Queensland government chart (Figure 3.4) from 1 (emaciated) to 5 (extremely fat) using 0.5 increments and cows were classified as score ≤ 2.5, 3 or ≥ 3.5. For each cow, 5 skeletal checkpoints were observed for fat reserves by visual and palpation methods, which include backbone, short ribs, hook bones, tail head and pin bones. The same experienced person recorded the condition scores before and after oestrous synchronization.

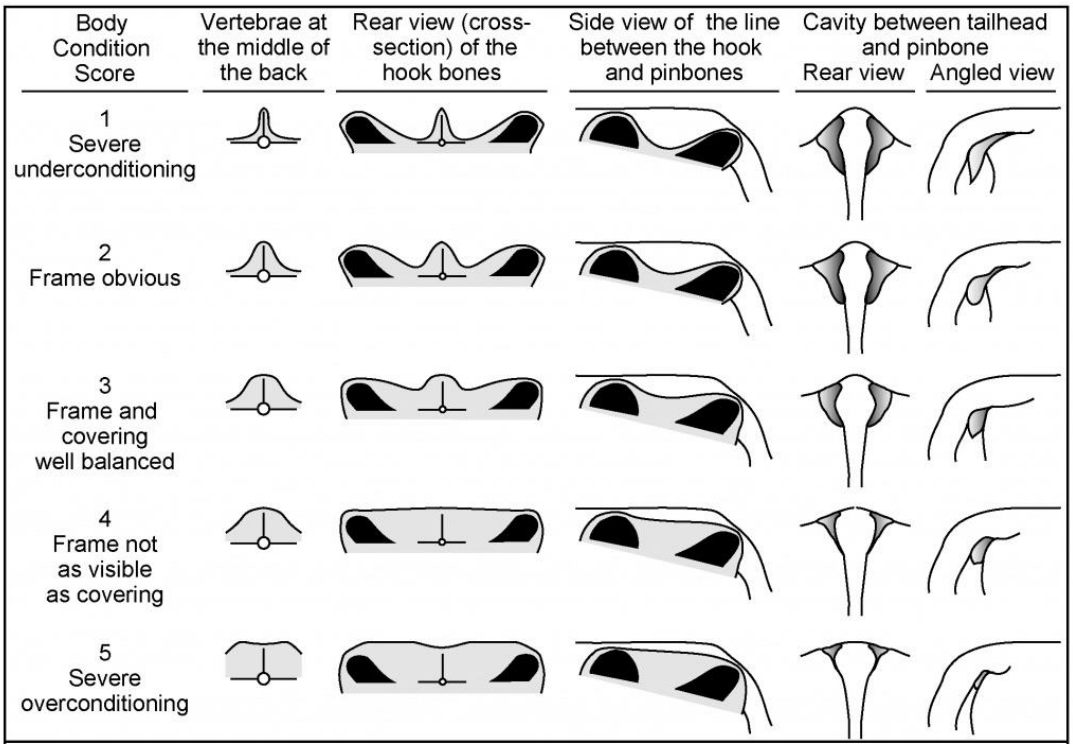

Figure 3.3 Body condition scoring chart for dairy cows. Source (Adopted from: Edmonson *et al*., 1989).

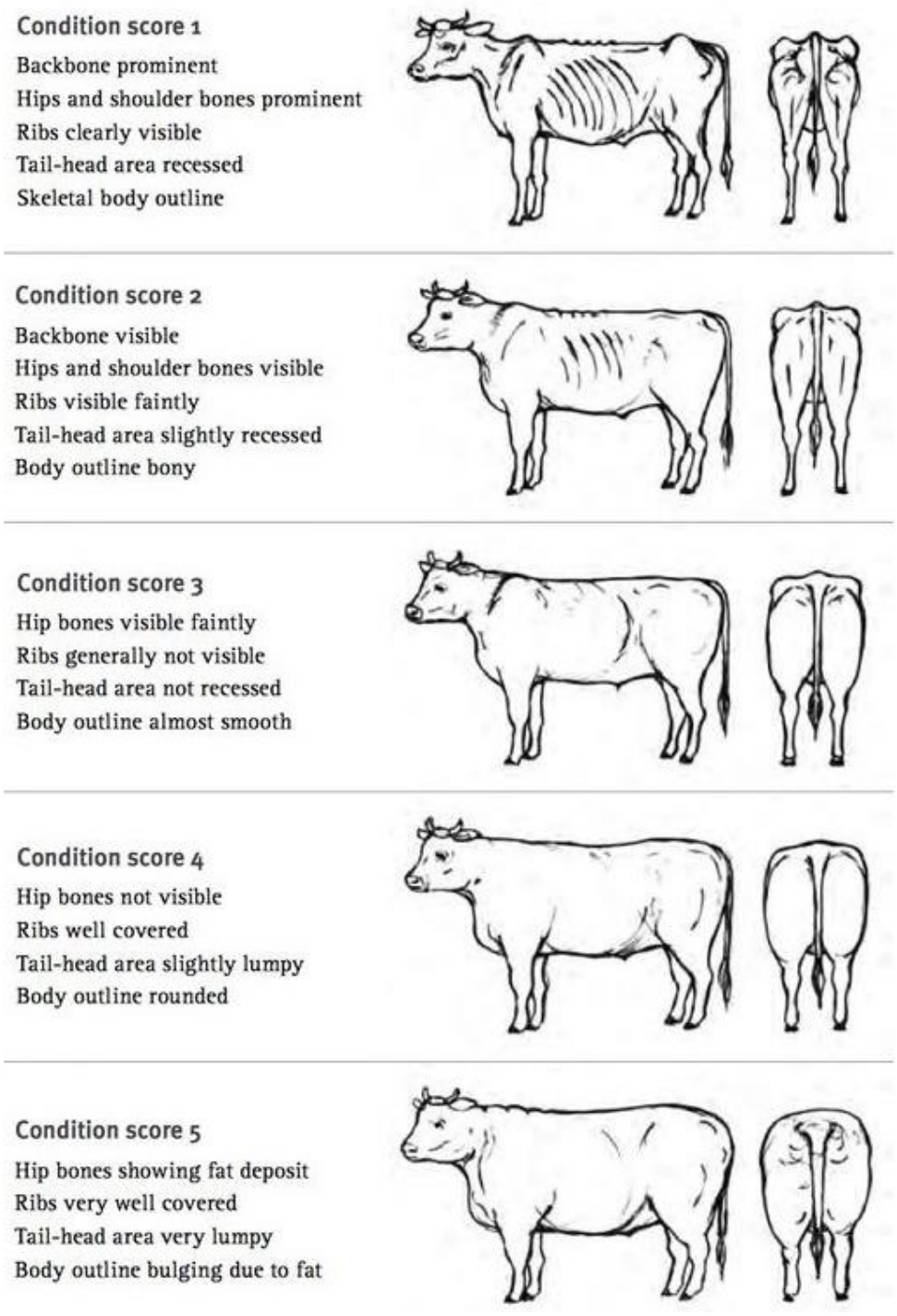

Figure 3.4 Body condition scoring chart for beef cows. Source (Adopted from: Queensland Government, online).

3.5 Oestrous synchronization and oestrus observation in dairy and beef cows

Synchronization of oestrous was carried out with the aid of CIDR (Pfizer Laboratories, South Africa) in selected dairy (*n* = 136) and beef (*n* = 97) cows, containing 1.9 g of P_4 hormone, subjected to a 9-day Ovsynch + CIDR protocol (Figure 3.5). The protocol included the insertion of CIDR device into the reproductive tract (vagina) of each cow on any random day of oestrous cycle (Day 0) with 2 mℓ intramuscular (i.m.) administration of Estradiol benzoate (EB, VTech, South Africa). Removal of CIDR device, 2 mℓ i.m. administration of $PGF_2\alpha$ (Estrumate®, Intervet (Pty) Ltd, South Africa) and placement of adhesive tail-head HMD patch (Kamar®, Steamboat Springs, USA) was done on Day 8. 1 mℓ i.m. administration of EB was done on Day 9. Oestrus synchronization expression for all cows was detected and recorded at the time of AI based on the HMD patch colour either as are red (oestrus/activated patch) or white (no oestrus/ not activated patch). Activities for oestrous synchronization in dairy and beef cows are depicted in Figures 3.6 and 3.7.

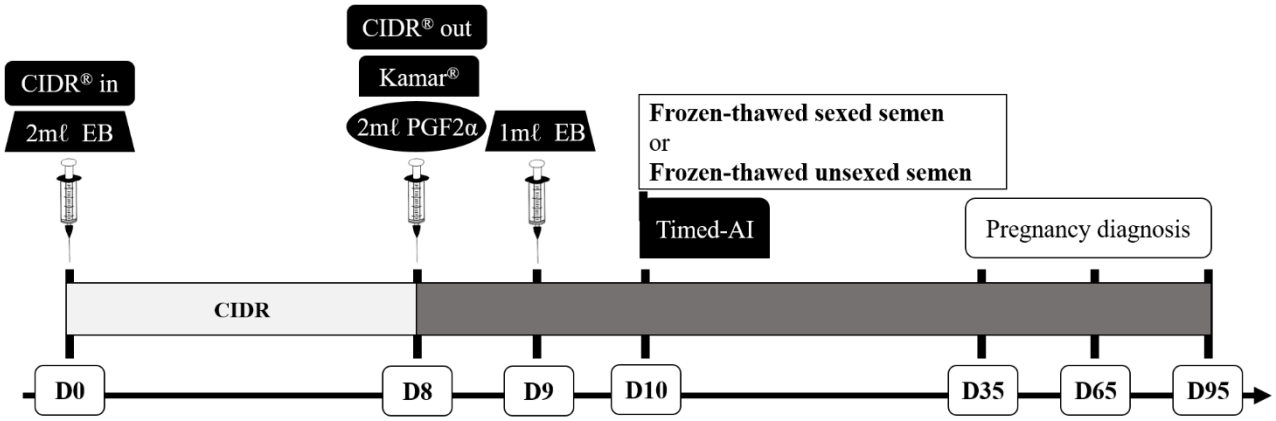

Figure 3.5 Depiction of a 9-day Ovsynch + CIDR protocol and TAI 55 hours following CIDR removal, used to induce oestrous cycle in dairy and beef cows. Source: (Personal collection: TL Magopa).

Figure 3.6 Depiction of oestrus synchronization in cows: **(A)** CIDR insertion, and **(B)** placement of adhesive HMD patches. Source: (Personal collection: TL Magopa).

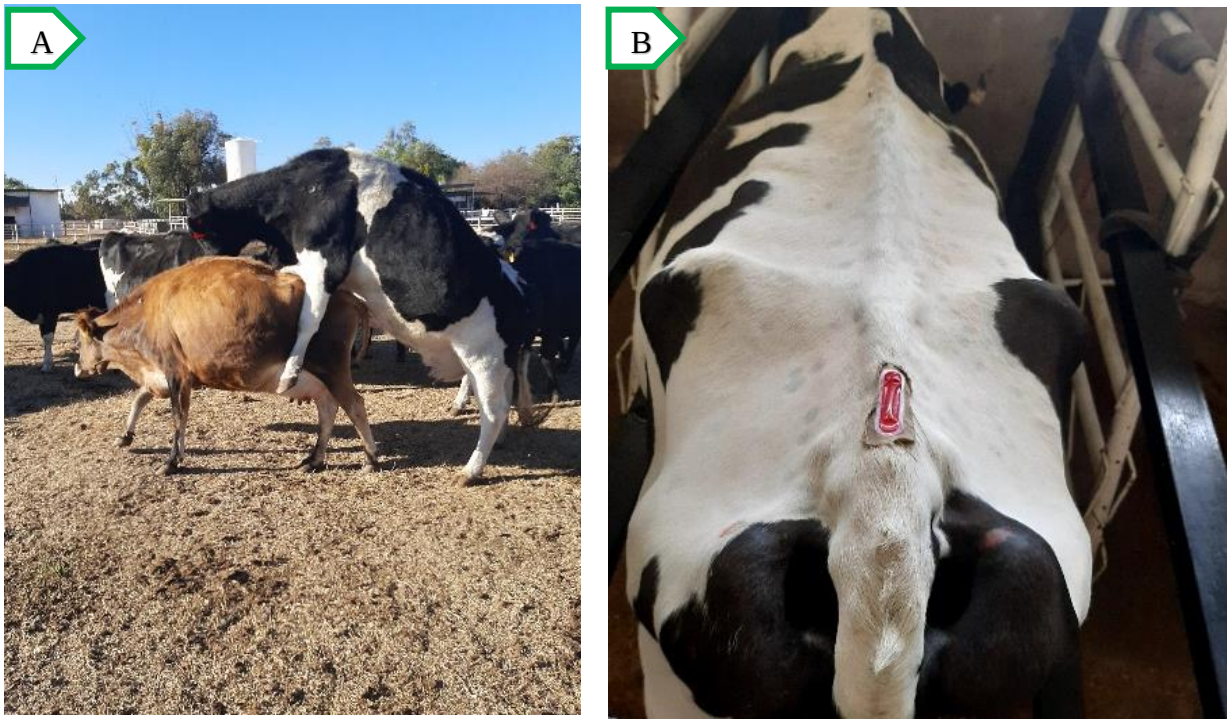

Figure 3.7 Depiction of oestrus synchronization expression: **(A)** cows mounting each other (oestrus sign) and **(B)** cow with an activated HMD patch. Source: (Personal collection: TL Magopa).

3.6 Evaluation of frozen-thawed bull semen

Frozen sexed and non-sexed semen straws for dairy (Holstein Friesian) and beef (Angus) bulls were purchased from ABS Global South Africa (Pty) Ltd, Western Cape, South Africa. The semen straws were stored in separate LN_2 semen tanks (-196 ºC) until use. Semen thawing and evaluation were carried out in the GameTek Cryo-mobile truck laboratory. Frozen semen straws were removed from LN_2 tank and air dried for 10 seconds then plunged in a thawing flask (CITO thawer 12/220V) containing warm water (37 ºC) for 1 minute. Thereafter, semen straws were dried with a paper towel to dry off the water. A pair of scissors was used to cut the end tip of the straw (powdered side) and evaluated for post-thaw sperm motility, morphology and membrane integrity. The semen straws comprising 40% and above sperm motility were used during AI (Joint, 2005).

3.6.1 Sperm motility and velocity evaluations in dairy and beef bull's semen

Sperm motility and velocity evaluations for frozen-thawed sexed and non-sexed semen of dairy and beef bulls were assessed with the aid of the CASA- SCA® system. Total sperm motility analyses were divided into non-progressive motility, and/or progressive motility and rapid, medium, or slow, whereas sperm velocity analyses were curvilinear, straight-line, average path, linearity, straightness, wobble, amplitude of lateral head displacement, and beat cross frequency (Ariagno *et al*., 2017). Depiction of bull sperm progression categories by CASA system are presented in Figure 3.8. A drop of 5 µℓ frozen-thawed semen was placed on a warmed microscope glass slide (~76 × 26 × 1mm-7101, Globalroll, China), covered (22 × 22 mm- 7101 microscope slide cover glass, China) (Tuncer *et al*., 2010). The individual sperm motility and velocity were observed and recorded at 10 × magnification objective lens (Nikon®, Japan) connected with an Apple MacBook Pro (A1278, California) containing SCA® software. The CASA-SCA® system settings for evaluation of bull sperm motility and velocity are listed in Table 3.1.

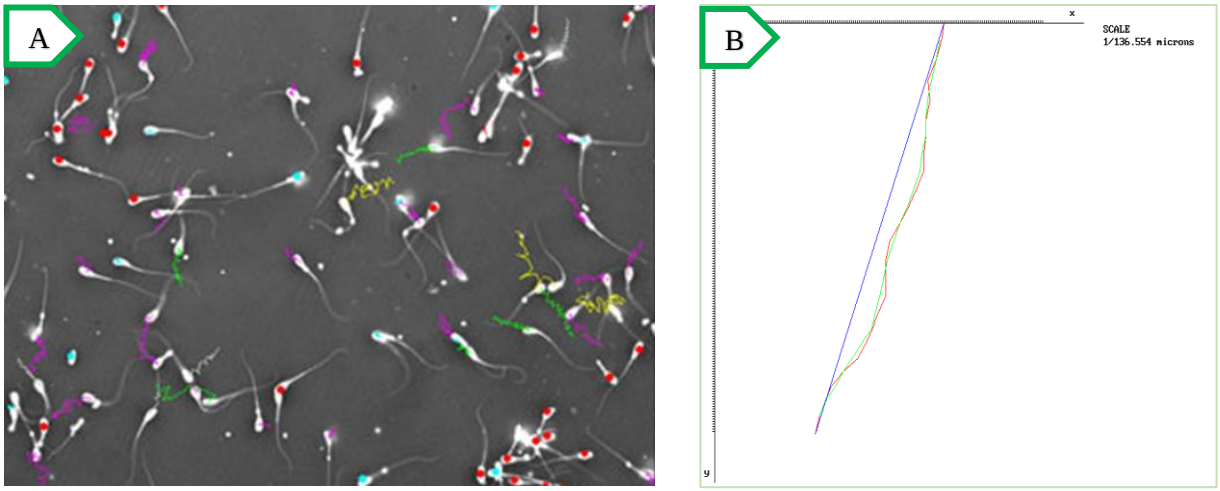

Figure 3.8 Depiction of **(A)** different progression categories for bull sperm and **(B)** individual sperm linearity percentage by CASA system. Source: (Personal collection: TL Magopa).

Table 3.1 The settings for CASA-SCA® (V.5.2.0.1) used in this study to analyse dairy and beef bull sperm motility and velocity parameters.

Parameters	**Settings**
Brightness	300
Image per second	50
Optics	Ph-
Chamber	Cover slide
Frame rate (Hz)	60
Scale	10 ×
Particle area (μm^2)	5 < 70
Slow (μm per second)	< 10
Medium (μm per second)	< 25
Rapid (μm per second)	< 100
Progressivity %	> 70 of straightness
Circular %	< 50 of linearity
Connectivity	12
Number of images	50

Source: (Personal collection: TL Magopa).

3.6.2 Sperm morphology evaluation in dairy and beef bulls' semen

Sperm morphology for frozen-thawed sexed and non-sexed semen of dairy and beef bulls was evaluated with the aid of a microscope (Olympus ® Corporation BX51FT, Tokyo, Japan) at 100 × magnification after staining. At least 2 slides per thawed semen straw were prepared. A 0.6 mℓ Eppendorf micro-centrifuge tube (Simport, Canada) was used to add and mix 7.0 µℓ of semen with 20 µℓ of eosin-nigrosin staining (University of Pretoria, Faculty of Veterinary Sciences Pharmacy, Onderstepoort, South Africa). Depiction of bull sperm morphology and viability in frozen-thawed semen is presented in Figure 3.9. A drop of 5 µℓ from the mixture (semen and eosin-nigrosin staining) was placed and smeared across the clear end of a microscope slide and allowed to air dry at room temperature, before evaluation (Mphaphathi, 2017). A total of n = 200 sperm were counted per replicate (n = 6), recorded and categorized as live normal sperm (sperm with a clear colour since they abstained the stain), dead normal sperm (sperm with a darker colour since they permeated the stain) and live sperm with abnormalities (Oliveira *et al*., 2012).

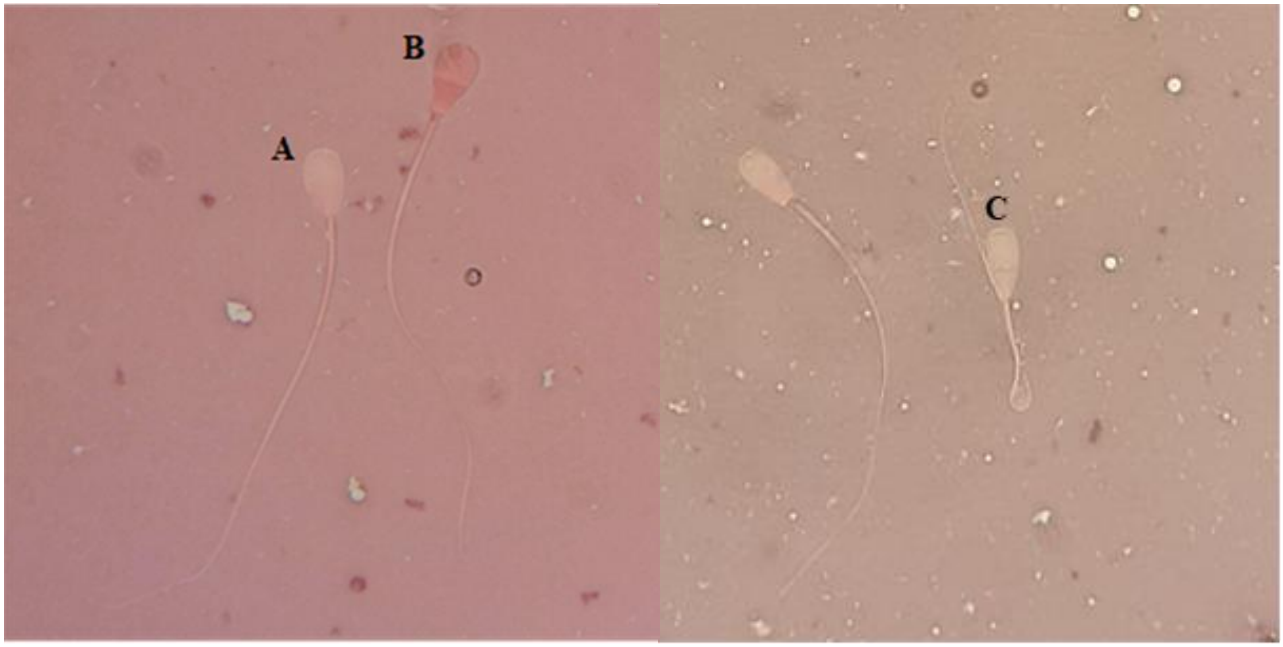

Figure 3.9 Depiction of bull sperm morphology and viability in frozen-thawed semen; **(A)** live normal sperm, **(B)** dead normal sperm and **(C)** live sperm with tail abnormality. Source: (Personal collection: TL Magopa).

3.6.3 Sperm plasma membrane integrity evaluation in dairy and beef bulls' semen

Sperm membrane integrity for frozen-thawed sexed and non-sexed semen of dairy and beef bulls was evaluated with the aid of a microscope (Nikon®, Japan). A total of 2 slides per thawed semen straw were prepared. Depiction of hypo-osmotic swelling test for bull sperm membrane integrity in frozen-thawed semen is presented in Figure 3.10. A drop of 10 µℓ frozen-thawed semen was added and mixed with 100 µℓ of Hypo-osmotic swelling test solution (HOST) (0.735 g of Sodium citrate and 1.351 g of Fructose, into 100 mℓ volume of sterile water [SABAX, Adcock Ingram, Midrand, South Africa]) in a 0.6 mℓ Eppendorf micro-centrifuge tube (Simport, Canada) (Palomar Rios *et al*., 2018). Thereafter, the mixture was incubated (Forma Scientific, 3158 Water Jacketed CO_2 Incubator, USA) at 37 ºC for 30 minutes. Immediately after the incubation, a drop of 5 µℓ from the mixture was placed in the middle of the microscope glass slide, covered, and evaluated. A total of *n* = 200 sperm were counted per slide (*n* = 6), recorded and categorized as membrane intact (swollen tail sperm/live) and membrane damaged (unswollen tail sperm/dead) (Ugur *et al*., 2019).

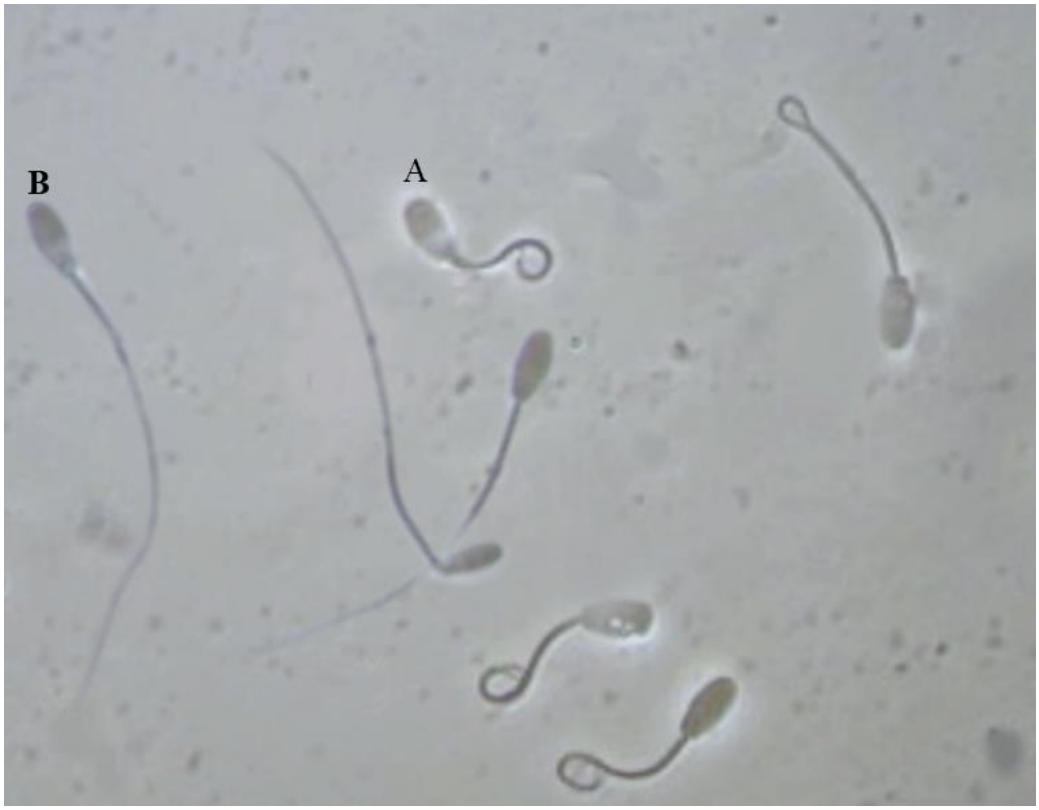

Figure 3.10 Depiction of hypo-osmotic swelling test for bull sperm membrane integrity in frozen-thawed semen; **(A)** sperm with intact membrane, and **(B)** sperm with damaged membrane. Source: (Personal collection: TL Magopa).

3.6.4 Pedigree for dairy (Holstein Frisian) and beef (Angus) bulls used for artificial insemination

Table 3.2 represents the pedigree information for Holstein Frisian bulls with sexed or non-sexed semen used for artificial insemination.

Table 3.2 Dairy sexed and non-sexed bull's pedigree and traits information.

Traits	Sexed				Non-sexed			
Bull	Blake: 94HO17739 		Samaritan: 29HO18691 		Kipling: 29HO18548 		Aventador: 29HO17612 	
Sire	DE-SU 521 BOOKEM-ET		BACON-HILL PETY MODESTY-ET		S-S-1 MONTROSS JETT-ET		LADY-MANOR MAN-O-SHAN-ET	
Dam	VAL-BISSON SHOTTLE IMELDA-ET		MORNINGVIEW SUPER MEGAN-ET		DE-SU FLAME 3903-ET		WALKUP SHAMROCK AURORA-ET	
DOB	2011-08-06		2012-10-19		2016-07-24		2013-07-24	
Production								
Milk	-228.2 Kg	99% Rel	+374.2 Kg	99% Rel	+195.5 Kg	91% Rel	-299.4 Kg	91% Rel
Pro	+3.6 Kg	+0.09%	+10.4 Kg	-0.01%	+11.3 Kg	+0.04%	+1.8 Kg	+0.09%
Fat	+6.4 Kg	+0.12%	+10.4 Kg	-0.03%	+9.5 Kg	+0.02%	+8.2 Kg	+0.16%
Health & Fertility								
PL	+0.2	99% Rel	-0.8	99% Rel	+0.5	83%	+1.5	88%
DPR	-2.2	99% Rel	+0.8	99% Rel	+0.6	82%	+1.3	86%
SCS	2.67	99% Rel	2.81	99% Rel	2.89	90%	2.77	90%
CCR	-2.5	99% Rel	+0.8	99% Rel	+0.9	82%	0.0	82%
Calving traits								
SCE	2.7%	99% Rel	2.6%	99% Rel	1.8%	89%	1.6%	79%
DCE	2.6%	99% Rel	2.1%	99% Rel	2.4%	72%	1.5%	77%
SSB	7.1%	99% Rel	6.6%	99% Rel	6.9%	76%	6.3%	73%
DSB	6.2%	99% Rel	4.0%	99% Rel	6.2%	65% Rel	5.6%	73%
Conformation								
BD	2.39	Deep	1.76	Deep	0.38	Deep	0.23	Deep
FLS	1.15	High	0.65	High	-0.24	Low	-0.08	Low
UH	2.12	High	2.64	High	1.73	High	0.98	High
UC	0.94	Strong	1.27	Strong	-0.24	Weak	1.24	Strong
UD	1.84	Shallow	1.07	Shallow	1.47	Shallow	1.82	Shallow
TL	0.67	Long	0.59	Long	-1.58	Short	-0.66	Short

DOB = date of birth; Pro = protein; PL = productive life; DCR = daughter pregnancy rate; SCS = somatic cell score; CCR = cow conception rate; SCE = sire calving ease; DCE = daughter calving ease; SSB = sire stillbirth; DSB = daughter stillbirth; BD = body depth; FLS = feet & legs score; UH = udder height; UC = udder cleft, UD = udder depth; TL = teat length.
Source: (Adapted from: ABS South Africa website).

Table 3.3 represents the pedigree information for Angus bulls with sexed or non-sexed semen used for artificial insemination.

Table 3.3 Beef sexed and non-sexed bull's pedigree and traits information.

Traits	Sexed						Non-sexed					
Bull	No Worries: 629AR0262 			Reserve: 629AN1852 			No Worries: 29AR0262 			Americano: 29AN1889 		
Breed type	Red Angus			Black Angus			Red Angus			Black Angus		
Sire	BROWN JYJ REDEMPTION Y1334			NETHERTON MR READER J527 (ET)			BROWN JYJ REDEMPTION Y1334			B/R NEW DAY 454		
Dam	LSF CRYSTAL R5154 X0105			NETHERTON ANNIE H474 (ET)			LSF CRYSTAL R5154 X0105			SANDPOINT BLACKBIRD 8809		
DOB	1/31/2014			9/21/2012			1/31/2014			1/31/2011		
	EPD	ACC	RANK	EPD	ACC	RANK	EPD	ACC	RANK	EPD	ACC	RANK
CED	+15	.69	23%	+10	.88	25%	+15	.69	23%	-	-	-
BW	-4.9	.83	8%	+0.6	.96	35%	-4.9	.83	8%	-	-	-
WW	+60	.78	51%	+49	.95	70%	+60	.78	51%	-	-	-
YW	+106	.74	31%	+91	.93	70%	+106	.74	31%	-	-	-
ADG	+.29	.74	16%	.00			+.29	.74	16%	-	-	-
DMI	+2.15	.40	95%	+.80	.69	50%	+2.15	.40	95%	-	-	-
MILK	+29	.28	19%	+21	.90	75%	+29	.28	19%	-	-	-
ME	+1	.67	52%	-	-	-	+1	.67	52%	-	-	-
HPG	+12	.39	36%	+11.1	.66	45%	+12	.39	36%	-	-	-
CEM	+6	.40	59%	+6	.87	75%	+6	.40	59%	-	-	-
MW				+43	.79	65%				-	-	-
STAY	+16	.33	46	-	-	-	+16	.33	46	-	-	-
MARB	+.72	.51	7%	+.76	.71	25%	+.72	.51	7%	-	-	-
YG	+0.05	.46	43%	-	-	-	+0.05	.46	43%	-	-	-
CW	+19	.61	51%	+25	.74	85%	+19	.61	51%	-	-	-
REA	+.06	.59	54%	+.81	.67	15%	+.06	.59	54%	-	-	-
FAT	+.01	.46	35%	-.034	.71	10%	+.01	.46	35%	-	-	-
GM	+59		17%	+10	.88	25%	+59		17%	-	-	-
SC	36.5 cm			39.6 cm			36.5 cm			-		

DOB = date of birth, CED = calving ease direct, BW = birth weight; WW = weaning weight; YW = yearling weight; ADG = average daily gain; DMI = dry matter intake; ME = maintenance energy; HPG = heifer pregnancy; CEM = calving ease maternal; MW= mature weight; STAY = stability; MARB = marbling; YG = yield grade; CW = carcass weight; REA = rib-eye area; GM = grid master index; SC = scrotum circumference. Source (Adapted from ABS South Africa website).

3.7 Timed artificial insemination in dairy and beef cows following oestrous synchronization

The AI was performed 55 hours following the removal of CIDR by an experienced inseminator from ARC-AP in synchronized dairy and beef cows. In brief, a lubricant (Kyron Laboratories (Pty) Ltd, South Africa) was applied to a hand protected by a long shoulder-length glove (OB, International Veterinary Supplies) and inserted with gentle pressure within the rectum of a cow to remove excess dung, and then the cow vulva was wiped with a paper towel. A frozen-thawed female-sexed (X-sexed) or non-sexed semen straw was analysed and placed inside the AI rod (Brass AI Pistolette, Shivam Pharma, India) that was cleaned with a paper towel. An AI sheath (IMV Technologies, France) was placed over the rod to lock the straw inside the rod, and a sanitary sleeve (SBS Cryo. Tec, Argentina) was also used after placing the sheath to cover the rod and the sheath to optimize hygiene. The rod was inserted gently inside the reproductive tract (vaginal) of the recipient cow and went past the cervix, depositing 0.25 mℓ of semen into the uterine body (intrauterine insemination). After removal of the rod, the cow's vulva was massaged. In Figure 3.11, an AI rod and AI in a cow are depicted.

Figure 3.11 Depiction of **(A)** an artificial insemination rod and **(B)** artificial insemination in a cow. Source: (Personal collection: TL Magopa).

3.8 Pregnancy diagnosis in dairy and beef cows

Pregnancy diagnosis was performed on Days 35, 65 and 95 following TAI with the aid of a transrectal ultrasound scanner (Ibex pro™, E.I. Medical Imaging, USA) and transrectal hand palpation. During transrectal ultrasound scanner procedure, a disinfected probe (transducer - L7HDi - Linear transducer/probe [5.0 MHz], 12 cm depth) connected to the Ibex pro™ monitor and applied with an ultrasound gel (Clinica®, South Africa) was gently inserted inside the rectum on individual cows and positioned above the uterine body (Mphaphathi, 2017). Pregnancy was determined by looking at the embryonic fluid, presence of the embryo, or the embryo's heartbeat.

During transrectal hand palpation procedure, a hand covered with a long shoulder-length glove (OB, International Veterinary Supplies) was applied with a lubricate (Kyron Laboratories (Pty) Ltd, South Africa) and introduced with gentle pressure inside the rectum. Pregnancy was determined by palpating the reproductive tract located on the pelvic floor to assess the presence of the corpus luteum (CL) in the ovaries or the embryo in the uterus. The pregnant and non-pregnant cows were recorded. Depiction of how both methods were done is presented in Figure 3.12.

Figure 3.12 Depiction of **(A)** a portable Ibex pro™ ultrasound scanner, **(B)** transrectal ultrasound scanner pregnancy diagnosis on a cow, and **(C)** transrectal hand palpation pregnancy diagnosis on a cow. Source: (Personal collection: TL Magopa).

3.9 Data analysis

All data were analyzed by general linear model (GLM) procedure of Statistical Analysis System (SAS, 9.3). Analysis of variance (ANOVA) was tested to compare treatment means for semen quality (sexed vs non-sexed semen), bull (n = 8) and treatment × bull as a fixed effect and presented as mean ± standard deviation (SD) and percentage. A set of variables were evaluated in the statistical model to determine their effect on oestrous synchronization expression (cattle type), conception rate and pregnancy loss (cattle type and semen type). Chi-square test was used to determine significant differences for equal proportions. A binary logistic regression model was constructed with oestrous synchronization expression, conception rate and pregnancy loss as dependent variables. It was observed that breed of cow and herd were found non-significant, hence removed from the analysis. For all analyses, differences between the variables were considered to be statistically significant at $p < 0.05$ and as a tendency at $p < 0.10$.

Chapter 4

Results

4.1 Oestrus expression in dairy and beef cows following oestrous synchronization

The proportion of oestrus expression by BCS and lactation status in dairy and beef cows are presented in Table 4.1. Oestrus rates by BCS of ≤ 2.5 (79.0%), 3 (89.0%) and ≥ 3.5 (92.6%) were higher in dairy cows compared with ≤ 2.5 (68.4%), 3 (61.1%) and ≥ 3.5 (70.8%) in beef cows ($P < 0.05$). Lactating (86.2%) and dry (81.5%) dairy cows had higher oestrus rates, as compared with beef lactating (67.7%) and dry (59.4%) cows ($P < 0.05$).

Table 4.1 Effect of body condition score and lactation status on oestrus expression in dairy and beef cows.

Variables	Cattle type					
	Dairy (*n* = 136)			Beef (*n* = 97)		
	Oestrus expression[a]		Total (*n*)	Oestrus expression[a]		Total (*n*)
	Oestrus *n* (%)	No oestrus *n* (%)		Oestrus *n* (%)	No oestrus *n* (%)	
BCS[b]						
≤ 2.5	49 (79.0)	13 (21.0)	62	13 (68.4)	6 (31.6)	19
3	40 (89.0)	5 (10.6)	47	33 (61.1)	21 (38.9)	54
≥ 3.5	25 (92.6)	2 (7.4)	27	17 (70.8)	7 (29.2)	24
Total (*n*)	116	20		63	34	
Lactation status						
Lactating/suckled	94 (86.2)	15 (13.8)	109	44 (67.7)	21 (32.3)	65
Dry	22 (81.5)	5 (18.5)	27	19 (59.4)	13 (40.6)	32
Total (*n*)	116	20		63	34	

[a] Oestrus expression = Number of cows detected in oestrus/ no oestrus ÷ number of cows assigned to oestrous synchronization × 100.

[b] BCS = Body condition score.

The proportion of oestrus expression by age and parity in dairy and beef cows is presented in Table 4.2. Dairy cows had the highest oestrus rate by age compared with beef cows in age groups of 4 (100% vs 57.1%), 5 (87.2% vs 70.6%), 6 (87.2% vs 61.8%), ≥ 7 (85.2% vs 73.7%) and 3 years (64.7% vs 61.5%; $P < 0.05$). Oestrus rates by parity were higher in dairy cows compared with beef cows in parity of ≥ 5th (92.9% vs 66.7%), 2nd (87.2% vs 64.7%), 3rd (86.4% vs 66.7%), 4th (83.3% vs 75.0%) and 1st (76.2% vs 58.3%; $P < 0.05$).

Table 4.2 Effect of age and parity number on oestrus expression in dairy and beef cows.

Variables	Cattle type					
	Dairy (*n* = 136)			Beef (*n* = 97)		
	Oestrus expression[a]		Total (*n*)	Oestrus expression[a]		Total (*n*)
	Oestrus *n* (%)	No oestrus *n* (%)		Oestrus *n* (%)	No oestrus *n* (%)	
Age (years)						
3	11 (64.7)	6 (35.3)	17	8 (61.5)	5 (38.5)	13
4	14 (100.0)	0 (0.0)	14	8 (57.1)	6 (42.9)	14
5	34 (87.2)	5 (12.8)	39	12 (70.6)	5 (29.4)	17
6	34 (87.2)	5 (12.8)	39	21 (61.8)	13 (38.2)	34
≥ 7	23 (85.2)	4 (14.8)	27	14 (73.7)	5 (26.3)	19
Total (*n*)	116	20		63	34	
Parity						
1st	16 (76.2)	5 (23.8)	21	14 (58.3)	10 (41.7)	24
2nd	34 (87.2)	5 (12.8)	39	22 (64.7)	12 (35.3)	34
3rd	38 (86.4)	6 (13.6)	44	14 (66.7)	7 (33.3)	21
4th	15 (83.3)	3 (16.7)	18	9 (75.0)	3 (25.0)	12
≥ 5th	1 (92.9)	1 (7.1)	14	4 (66.7)	2 (33.3)	6
Total (*n*)	116	20		63	34	

[a]Oestrus expression = Number of cows detected in oestrus/ no oestrus ÷ number of cows assigned to oestrous synchronization × 100.

The proportions of oestrus synchronization expression in dairy and beef cows are presented in Figure 4.1. The proportion of oestrus expression was higher in dairy (85.3%) cows than in beef (65.0%) cows ($P < 0.05$). The overall non-oestrus expression was 23.2% for both dairy and beef cows.

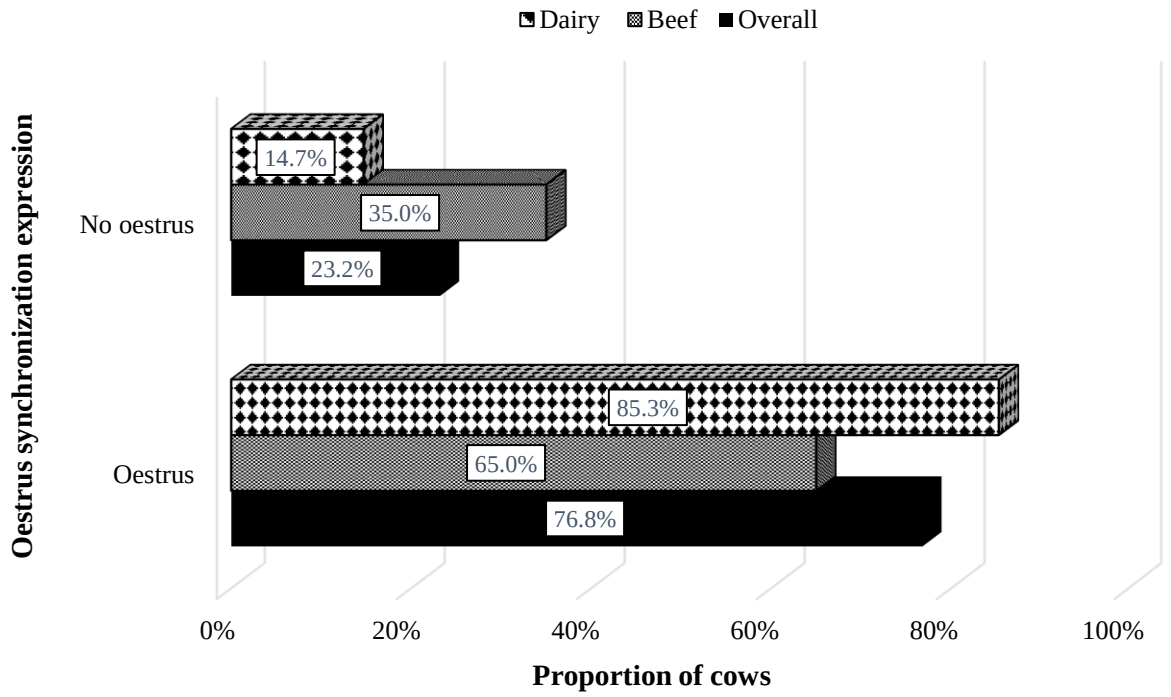

Figure 4.1 The proportion of oestrus synchronization expression (oestrus and no oestrus) in dairy and beef cows subjected to 9-day Ovsynch + controlled intravaginal drug release (CIDR) protocol.

4.2 Sperm quality in frozen-thawed X-sexed and non-sexed semen from dairy and beef bulls

The sperm motility parameters evaluated in frozen-thawed sexed and non-sexed semen from dairy and beef bulls are presented in Table 4.3. The average sperm TM in dairy (X-sexed; 66.85% vs non-sexed; 70.7%) and beef (X-sexed; 58.8% vs non-sexed; 83.9%) bulls were recorded ($P < 0.05$). The percentage of average sperm PM was high for beef non-sexed (55.2%) semen when compared with X-sexed (24.8%) semen ($P < 0.05$). However, for dairy, X-sexed (42.8%) semen recorded the highest average sperm PM when compared with non-sexed (38.6%) semen ($P < 0.05$). Consequently, lowest average sperm NPM was recorded in dairy X-sexed (24.0%) semen when compared with non-sexed (32.0%) semen ($P < 0.05$). However, in beef, non-sexed (28.8%) semen recorded the lowest average sperm NPM in comparison with X-sexed (34.0%) semen ($P < 0.05$).

Table 4.3 Sperm motility parameters (mean ± SD) evaluated in frozen-thawed sexed and non-sexed semen from dairy and beef bulls.

Bull type	Semen type	Bulls (n = 8)	Bull name	TM (%)	PM (%)	NPM (%)	IM (%)	RAP (%)	MED (%)	SLW (%)
Dairy	X-sexed	1	Blake	67.0±8.3^{b}	33.1±11.3^{b}	33.9±12.6^{a}	33.0±8.4^{b}	17.7±11.5^{bc}	31.8±10.5^{bcd}	11.5±7.5^{bc}
		2	Samaritan	66.7±4.1^{b}	52.6±3.5^{bc}	14.1±8.5^{b}	33.3±4.1^{b}	21.7±14.2^{ab}	31.3±13.7^{bcd}	7.6±6.7^{c}
		Average	Average	66.8±6.2	42.8±11.2	24.0±10.5	33.1±6.2	19.7±12.8	31.5±12.1	9.5±7.1
	Non-sexed	3	Kipling	60.4±3.8^{bc}	52.6±11.1^{a}	33.6±4.2^{a}	36.6±4.8^{ab}	17.2±3.6^{bc}	23.2±4.3^{d}	20.0±3.6^{a}
		4	Aventador	81.0±4.8^{a}	50.5±1.5^{a}	30.4±5.4^{a}	19.0±4.8^{c}	29.1±5.2^{a}	35.3±5.3^{bc}	16.5±3.7^{ab}
		Average	Average	70.7±4.3	38.6±2.5	32.0±4.8	27.8±4.8	23.1±4.4	29.2±4.8	13.9±5.4
Beef	X-sexed	5	No worries	58.2±8.8^{c}	21.3±3.1^{c}	36.9±10.0^{a}	41.8±8.8^{a}	8.9±4.5^{cd}	30.9±6.8^{bcd}	18.4±3.5^{a}
		6	Reserve	59.5±8.5^{bc}	28.4±10.2^{bc}	31.1±10.6^{a}	40.5±8.5^{ab}	6.5±4.1^{d}	28.9±7.2^{cd}	18.6±8.7^{a}
		Average	Average	58.8±8.6	24.8±6.6	34.0±10.3	41.1±8.6	7.7±4.3	29.9±7.0	18.5±6.1
	Non-sexed	7	No worries	81.4±5.8^{a}	53.6±11.2^{a}	27.8±7.3^{a}	18.6±5.8^{c}	29.9±6.2^{a}	40.2±7.7^{b}	11.3±3.2^{bc}
		8	Americano	86.5±9.9^{a}	56.8±10.1^{a}	29.8±11.1^{a}	13.4±9.9^{c}	15.1±6.8^{bcd}	60.2±11.9^{a}	11.2±3.8^{bc}
		Average	Average	83.9±8.2	55.2±10.6	28.8±9.2	16.0±7.8	22.5±6.5	50.2±9.8	14.9±4.8

$^{a-d}$ Values in the same column with the same superscripts are not significantly different (P > 0.05).

TM = total motility; PM = progressive motility; NPM = non-progressive motility; IM = immotile; RAP = rapid; MED = medium; SLW = slow.

Frozen-thawed X-sexed and non-sexed sperm velocity parameters from dairy and beef bulls are presented in Table 4.4. The averages in sperm VCL (μm/s) for dairy (X-sexed; 75.3 μm/s vs non-sexed; 82.2 μm/s) bulls and beef (X-sexed; 63.3 μm/s vs non-sexed; 85.6 μm/s) bulls were recorded ($P < 0.05$). X-sexed semen in beef bulls had the lowest velocity parameters, including average sperm VSL (21.9 μm/sec), VAP (34.2 μm/sec) and STR (57.0 %) compared with others ($P < 0.05$). No significant difference in sperm velocity parameters (VCL, VSL, VAP, LIN, STR, WOB, ALH, BCF, and HPA) was found across bull type, semen type, and among bulls.

Table 4.4 Sperm velocity parameters (mean ± SD) evaluated in frozen-thawed X-sexed and non-sexed semen from dairy and beef bulls.

Bull type	Semen type	Bulls (*n* = 8)	VCL (µm/s)	VSL (µm/s)	VAP (µm/s)	LIN (%)	STR (%)	WOB (%)	ALH (µm)	BCF (Hz)	HPA (%)
Dairy	X-sexed	1	74.0±26.6^{bcd}	26.2±8.5^{bc}	38.9±12.1^{c}	32.7±8.6^{bc}	62.7±7.2^{ab}	53.3±9.2^{b}	2.6±0.7^{bc}	14.3±3.3^{cd}	1.5±3.7^{c}
		2	76.6±15.6^{bcd}	34.6±20.6^{bc}	52.4±7.9^{a}	31.2±11.7^{bc}	63.7±12.4^{ab}	48.9±10.0^{b}	2.0±0.3^{a}	18.6±2.0^{ab}	5.3±5.9^{ab}
		Average	75.3±21.1	30.4±14.5	45.6±10.0	31.9±10.1	63.2±9.8	51.1±9.6	2.3±0.5	16.4±2.6	3.4±4.8
	Non-sexed	3	75.7±4.7^{bcd}	29.0±3.0^{abc}	39.6±2.2^{bc}	32.4±6.6^{bc}	60.0±6.4^{bc}	49.8±5.8^{b}	2.6±0.1^{cd}	13.8±0.8^{cd}	6.2±2.1^{a}
		4	88.8±5.8^{ab}	38.2±1.1^{a}	49.6±1.8^{a}	39.8±3.4^{ab}	68.5±3.9^{ab}	55.7±2.7^{ab}	2.8±0.2^{bc}	18.9±1.5^{ab}	4.5±3.5^{abc}
		Average	82.2±5.2	33.6±2.0	44.6±2.0	36.1±5.0	64.2±5.1	52.7±4.2	2.7±0.1	16.3±1.1	5..3±2.8
Beef	X-sexed	5	61.1±8.2d^{d}	18.2±2.9^{c}	32.1±3.2^{c}	28.4±2.9^{c}	52.6±4.3^{c}	52.2±2.7^{b}	2.3±0.2^{cd}	15.7±1.2^{e}	1.0±1.4^{c}
		6	65.6±23.4^{d}	25.6±9.5^{bc}	36.3±10.4^{c}	29.3±11.5^{bc}	61.5±9.5^{abc}	52.6±9.5^{b}	2.0±0.8^{d}	19.7±2.9^{d}	3.0±2.4^{abc}
		Average	63.3±15.8	21.9±6.2	34.2±6.8	31.8±7.2	57.0±6.9	52.4±6.1	2.1±0.5	17.2±2.0	2.0±1.9
	Non-sexed	7	92.3±5.6^{a}	36.1±6.8^{ab}	50.1±5.9^{a}	38.0±6.0^{ab}	66.1±6.6^{ab}	55.3±3.6^{ab}	3.0±0.1^{b}	16.6±2.3^{bc}	1.8±1.2^{bc}
		8	78.9±7.0^{bcd}	38.1±12.0^{a}	47.3±3.2^{ab}	45.7±9.1^{a}	69.2±7.4^{a}	63.1±6.4^{a}	2.8±0.2^{cd}	20.1±3.1^{a}	1.2±1.8^{c}
		Average	85.6±6.3	37.1±9.4	48.7±4.5	41.8±7.5	67.6±7.0	59.2±5.0	2.9±0.1	18.3±2.7	1.5±1.5

$^{a-d}$ Values in the same column with the same superscripts are not significantly different ($P > 0.05$).

VCL = curvilinear velocity; VSL = straight-line velocity; VAP = average path velocity; LIN = linearity; STR = straightness; WOB = wobble; ALH = amplitude of lateral head displacement; BCF = beat cross frequency; HPA = Hyperactive.

Morphological parameters of frozen-thawed X-sexed and non-sexed sperm from dairy and beef bulls are presented in Table 4.5. There was no significant difference in live sperm percentages between X-sexed dairy (57.2±2.5) and beef (58.2±2.8) semen. Similarly, dairy (69.2±2.8) and beef (71.1±4.9) non-sexed semen indicated no significant difference in live sperm proportion ($P < 0.05$). Furthermore, no significant difference was observed in the average proportion of tail defects between X-sexed (dairy; 2.9±1.4 and beef; 1.7±1.1) and non-sexed semen (dairy; 1.7±1.7 and beef; 2.3±1.1; $P < 0.05$).

Table 4.5 Morphological parameters of dairy and beef bull's frozen-thawed X-sexed and non-sexed sperm (mean ± SD).

			Viability		Live sperm with abnormalities
Bull type	Semen type	Bull (n = 8)	Live normal sperm (%)	Dead normal sperm (%)	Tail defects (%)
Dairy	X-sexed	1	57.6±2.0^{c}	38.7±1.8^{a}	3.7±1.7^{a}
		2	56.8±3.0^{c}	41.0±2.4^{a}	2.2±1.2^{abc}
		Average	57.2±2.5	39.8±2.1	2.9±1.4
	Non-sexed	3	71.9±3.2^{a}	26.6±2.4^{c}	1.5±1.8^{bc}
		4	66.6±2.4^{b}	31.4±2.7^{b}	2.0±1.6^{bc}
		Average	69.2±2.8	29.0±2.5	1.7±1.7
Beef	X-sexed	5	58.2±3.0^{c}	39.5±3.0^{a}	2.2±1.1^{abc}
		6	58.2±2.6^{c}	40.4±2.6^{a}	1.3±1.1^{bc}
		Average	58.2±2.8	39.9±2.8	1.7±1.1
	Non-sexed	7	72.7±4.5^{a}	26.7±4.5^{c}	0.6±0.8^{c}
		8	69.6±5.4^{ab}	27.6±4.0^{c}	4.1±1.5^{ab}
		Average	71.1±4.9	27.1±4.2	2.3±1.1

$^{a\text{-}c}$ Values in the same column with the same superscripts are not significantly different ($P > 0.05$).

The percentage of sperm plasma membrane integrity in frozen-thawed X-sexed and non-sexed semen from dairy and beef bulls are presented in Table 4.6. The average percentage of membrane intact sperm in X-sexed dairy (51.0±3.0) and beef (52.3±2.1) semen were comparable ($P < 0.05$). Both non-sexed dairy (64.2±3.3) and beef (65.2±2.4) semen had comparable average percentages of intact sperm membrane ($P < 0.05$). However, overall X-sexed semen had the highest average percentage of membrane damaged sperm compared to non-sexed semen for both dairy and beef bulls ($P > 0.05$).

Table 4.6 Membrane integrity in frozen-thawed X-sexed and non-sexed sperm dairy and beef bulls (Mean ± SD).

Bull type	Semen type	Bull ($n = 8$)	Hypo-osmotic reaction	
			Membrane intact (swollen tail) (%)	Membrane damaged (unswollen tail) (%)
Dairy	X-sexed	1	51.3±3.1[d]	48.7±3.1[a]
		2	50.7±2.9[d]	49.3 ± 2.9[a]
		Average	51.0±3.0	49.0±3.0
	Non-sexed	3	67.5±3.2[a]	32.5±3.2[d]
		4	60.8±3.5[c]	39.2±3.5[b]
		Average	64.2±3.3	35.8±3.3
Beef	X-sexed	5	52.7±1.7[d]	47.3±1.7[a]
		6	52.0±2.6[d]	48.0±2.6[a]
		Average	52.3±2.1	47.7±2.1
	Non-sexed	7	66.7±2.5[ab]	33.3±2.5[cd]
		8	63.7±2.4[bc]	36.3±1.8[bc]
		Average	65.2±2.4	34.8±2.1

[a-d] Values in the same column with the same superscripts are not significantly different ($P > 0.05$).

4.3 Conception rates in dairy and beef cows artificially inseminated with sexed and non-sexed semen

Conception rates and pregnancy losses by cattle type during the first (between Days 35 and 65) and second (between Days 66 and 95) periods of pregnancy diagnosis are presented in Table 4.7. Conception rates on Day 35 were high in dairy (X-sexed; 61.9% and non-sexed; 62.0%) cows when compared with beef (X-sexed; 56.0% and non-sexed; 52.2%) cows ($P < 0.05$). Similarly, on Day 95, the dairy (sexed; 41.4% and non-sexed; 48.5%) cows had higher conception rates than beef (sexed; 38.0% and non-sexed; 37.0%) cows ($P < 0.05$). Pregnancy/embryo losses between Days 35 and 65 in dairy (X-sexed; 33.3% and non-sexed; 18.2%) cows and beef (X-sexed; 28.6% and non-sexed; 29.2%) cows were recorded ($P < 0.05$). Pregnancy losses were high in dairy (X-sexed; 7.7% and non-sexed; 8.3%) cows between Days 66 and 95 when compared with beef (X-sexed; 5.0% and non-sexed; 0.0%) cows ($P < 0.05$).

Table 4.7 Conception rates and losses in dairy and beef cows following timed artificial insemination with frozen-thawed X-sexed and non-sexed semen during the first (between Days 35 and 65) and second (between Days 66 and 95) periods of pregnancy (Proportion/percentage).

Cattle type	Semen type	Cows inseminated (*n*)	Conception rate[a]			Pregnancy loss[b]	
			Day 35 *n*/*n* (%)	Day 65 *n*/*n* (%)	Day 95 *n*/*n* (%)	Day 35-65 *n*/*n* (%)	Day 66-95 *n*/*n* (%)
Dairy	X-sexed	64	39/63 (61.9)	26/60 (43.3)	24/58 (41.4)	13/39 (33.3)	2/26 (7.7)
	Non-sexed	72	44/71 (62.0)	36/70 (51.4)	33/68 (48.5)	8/44 (18.2)	3/36 (8.3)
Beef	X-sexed	51	28/50 (56.0)	20/50 (40.0)	19/50 (38.0)	8/28 (28.6)	1/20 (5.0)
	Non-sexed	46	24/46 (52.2)	17/46 (37.0)	17/46 (37.0)	7/24 (29.2)	0/17 (0.0)

[a] Conception rate = Number of pregnant cows ÷ number of cows assigned to TAI × 100.

[b] Pregnancy loss = Number of embryo/foetal mortalities in a given period ÷ initial number of pregnant cows × 100.

Conception rates and losses by semen type in the first (between Days 35 and 65) and second (between Days 65 and 95) periods of pregnancy diagnosis are presented in Table 4.8. Overall conception rates on Day 35 in X-sexed semen was 59.3% compared with 58.1% in non-sexed ($P < 0.05$). The overall conception rate on Day 65 in X-sexed semen was 41.8% compared with 45.7% in non-sexed semen ($P < 0.05$). On Day 95, overall conception rates in X-sexed semen was 38.0% compared with 43.9% in non-sexed semen ($P < 0.05$). Pregnancy/embryo loss between Days 35 and 65 in X-sexed semen was 31.3% when compared with 22.1% in non-sexed semen ($P < 0.05$). However, the overall pregnancy/embryo losses between Days 66 and 95 in X-sexed and non-sexed semen was 6.5% and 5.7% respectively ($P > 0.05$).

Table 4.8 Conception rates and losses according to semen type (X-sexed and non-sexed) of dairy and beef cows during the first (between Days 35 and 65) and second (between Days 66 and 95) periods of pregnancy diagnosis following timed artificial insemination (Proportion/percentage).

Semen type	Cattle type	Cows inseminated (*n*)	Conception rate[a]			Pregnancy loss[b]	
			Day 35 *n*/*n* (%)	Day 65 *n*/*n* (%)	Day 95 *n*/*n* (%)	Days 35-65 *n*/*n* (%)	Days 66-95 *n*/*n* (%)
X-sexed	Dairy	64	39/63 (61.9)	26/60 (43.3)	24/58 (41.4)	13/39 (33.3)	2/26 (7.7)
	Beef	51	28/50 (56.0)	20/50 (40.0)	19/50 (38.0)	8/28 (28.6)	1/20 (5.0)
	Overall	115	67/113 (59.3)	46/110 (41.8)	43/108 (39.8)	21/67 (31.3)	3/46 (6.5)
Non-sexed	Dairy	72	44/71 (62.0)	36/70 (51.4)	33/68 (48.5)	8/44 (18.2)	3/36 (8.3)
	Beef	46	24/46 (52.2)	17/46 (37.0)	17/46 (37.0)	7/24 (29.2)	0/17 (0.0)
	Overall	118	68/117 (58.1)	53/116 (45.7)	50/114 (43.9)	15/68 (22.1)	3/53 (5.7)

[a] Conception rate = Number of pregnant cows ÷ number of cows assigned to TAI × 100.

[b] Pregnancy loss = Number of embryo/foetal mortalities in a given period ÷ initial number of pregnant cows × 100.

The proportions of pregnancy following TAI on Days 35, 65 and 95 in dairy and beef cows are presented in Figure 4.2. The overall proportion of pregnancy following TAI in dairy and beef cows was high on Day 35 (58.7%) compared with Days 65 (43.8%) and 95 (41.9%) of pregnancy ($P < 0.05$). The proportions of pregnancy in dairy (Day 35; 61.9%, Day 65; 47.7%, and Day 95; 45.2%) cows were high compared with beef (Day 35; 54.2%, Day 65; 38.5%, and Day 95; 37.7%) cows in all days of pregnancy ($P < 0.05$).

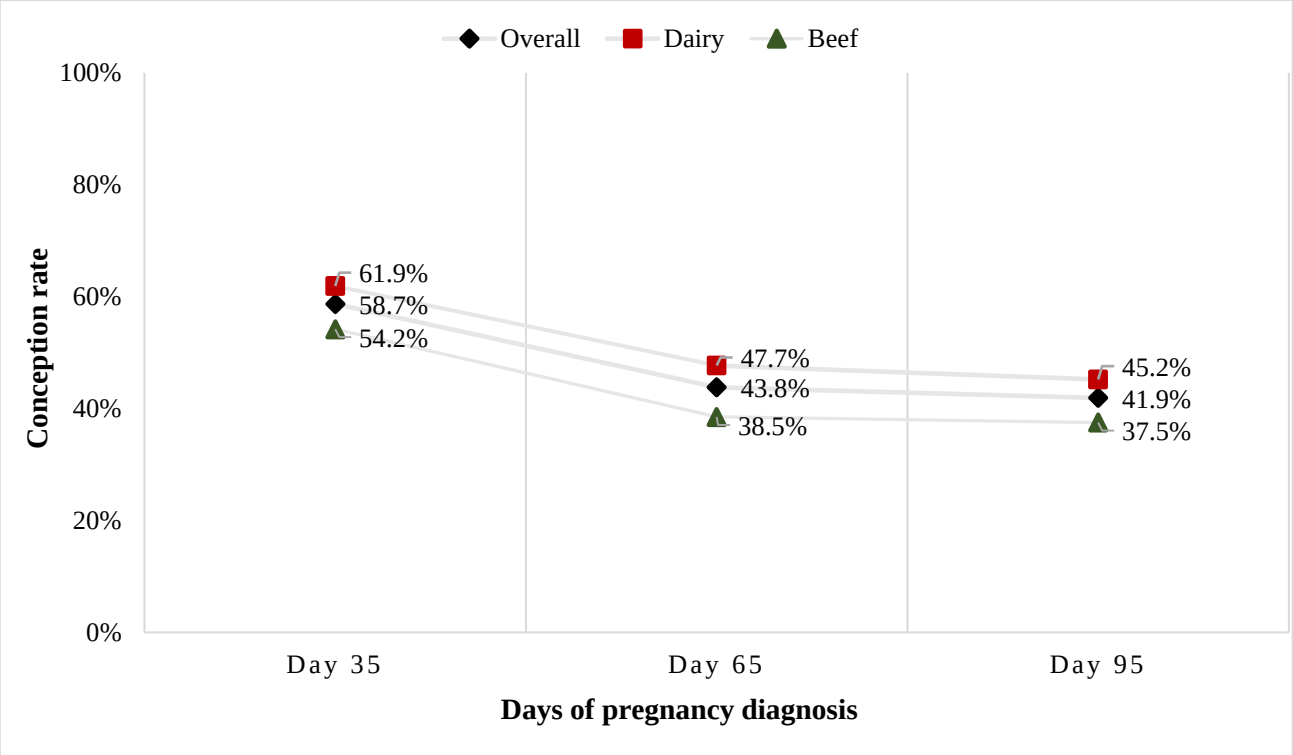

Figure 4.2 The proportion of pregnancy in dairy and beef cows on different days (35, 65 and 95) of pregnancy diagnosis following timed artificial insemination.

The proportions of pregnancy losses in different periods (first and second) of pregnancy in dairy and beef cows are presented in Figure 4.3. The overall pregnancy/embryo loss was high between Days 35 and 65 (26.7%) period compared with Days 66 and 95 (6.1%) period in dairy and beef cows. However, between Days 66 and 95 dairy cows (8.1%) had a higher proportion of pregnancy loss compared with beef (2.7%) cows ($P > 0.05$).

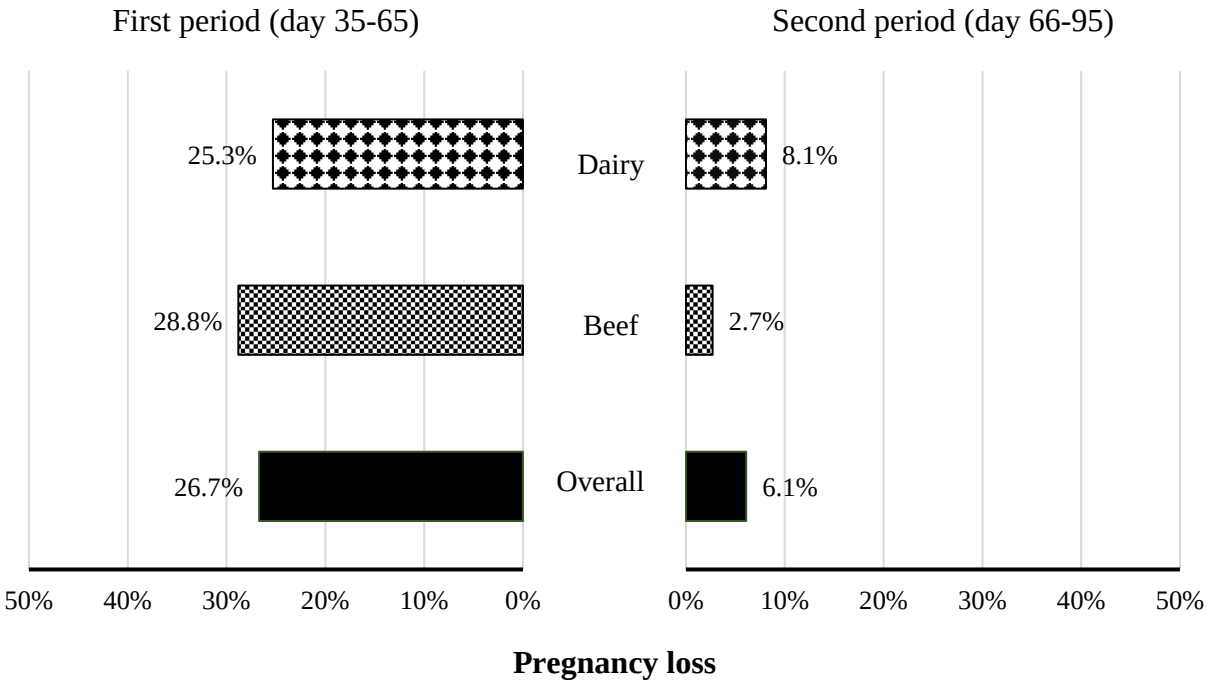

Figure 4.3 The proportion of first-period pregnancy losses between Days 35 and 65 and second-period pregnancy losses between Days 66 and 95 of pregnancy in dairy and beef cows.

The conception rates in dairy and beef cows by lactation status when sexed or non-sexed semen was used are presented in Figure 4.4. Lactating dairy cows inseminated with X-sexed (42.5%) or non-sexed (50.0%) semen, had higher conception rate compared with beef (X-sexed; 31.2% and non-sexed; 34.4%) ($P < 0.05$). However, conception rate in dry cows was higher in beef (X-sexed; 47.4% and non-sexed 46.1%) cows compared with dairy (X-sexed; 36.4% and non-sexed; 36.4%) ($P < 0.05$).

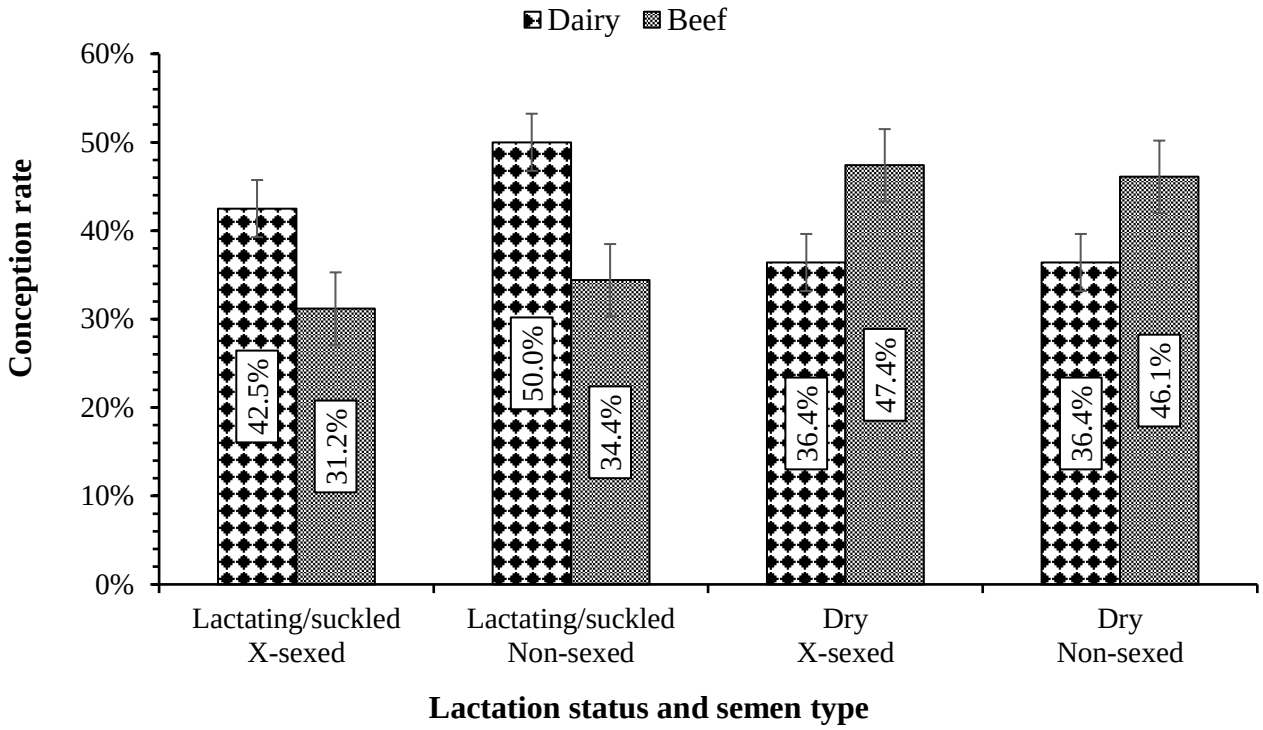

Figure 4.4 Conception rates by lactation status (lactating or dry) in dairy and beef cows on Day 95 following timed artificial insemination with X-sexed or non-sexed semen.

The proportions of pregnancy in dairy and beef cows by BCS, when X-sexed or non-sexed semen was used are presented in Figure 4.5. The proportion of pregnancy was high for dairy (X-sexed; 50.0% and non-sexed; 41.4%) cows with BCS ≤ 2.5 compared with beef (X-sexed; 27.3% and non-sexed; 25.0%) cows ($P < 0.05$). For X-sexed semen, there was a high proportion of pregnancy in beef (41.9%) cows with BCS 3 when compared with dairy (31.6%) cows, however, for non-sexed semen, dairy (45.8%) cows with the same BCS had a higher proportion of pregnancy when compared with beef (40.9%) cows ($P < 0.05$). There were high proportions of pregnancy in dairy (X-sexed; 36.4% vs non-sexed; 64.3%) cows with BCS ≥ 3.5 when compared with beef (X-sexed; 33.3% vs non-sexed; 40.0%) cows ($P < 0.05$).

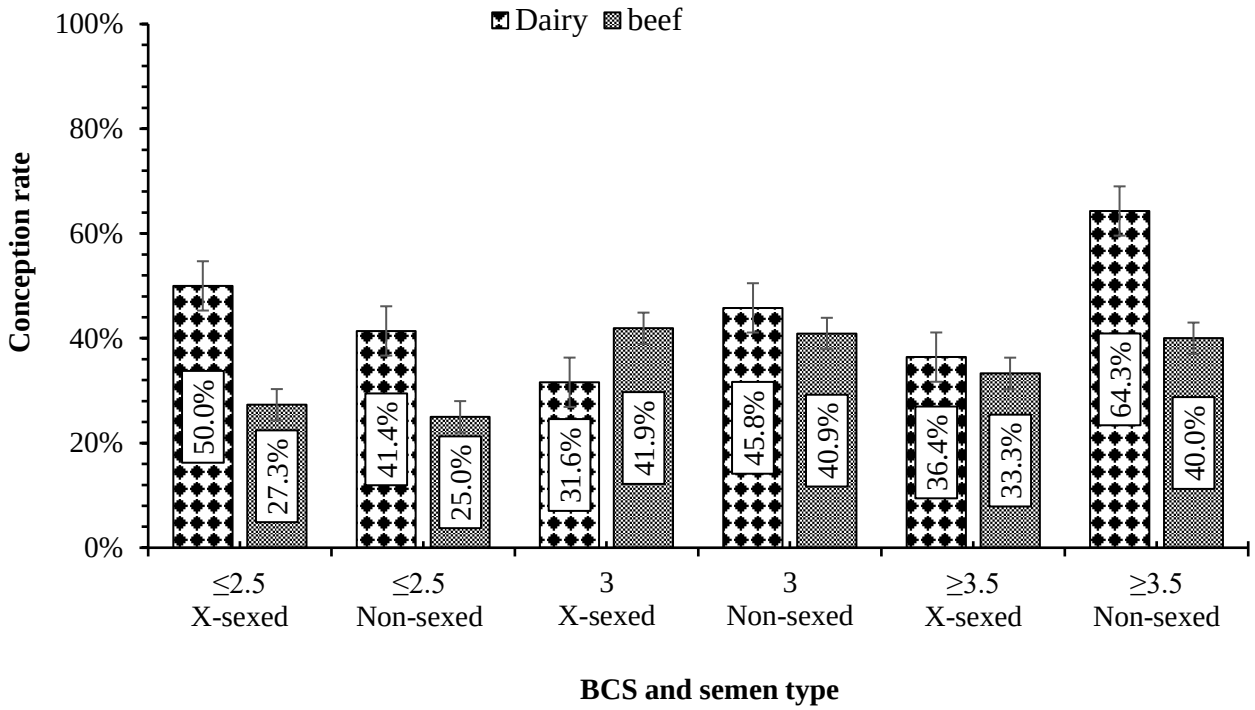

Figure 4.5 The proportions of pregnancy by body condition score (≤ 2.5, 3 or ≥ 3.5) on Day 95 following timed artificial insemination with X-sexed or non-sexed semen in dairy and beef cows.

The proportions of pregnancy in dairy and beef cows by age with X-sexed or non-sexed semen following TAI are presented in Figure 4.6. In age group of 3 years, X-sexed semen in dairy (66.7%) cows had higher proportion of pregnancy when compared with beef (33.3%) cows. However, for non-sexed semen in the same age group beef (57.1%) cows had higher proportion of pregnancy when compared with dairy (40.0%) cows ($P < 0.05$). There were high proportions of pregnancy in age group of 4 years for dairy (X-sexed; 50.0% vs non-sexed; 44.4%) cows when compared with beef (X-sexed; 42.9% vs non-sexed; 33.3%) cows ($P < 0.05$). There were high proportions of pregnancy in age group of 5 years dairy (X-sexed; 42.1% vs non-sexed; 57.9%) cows when compared with beef (X-sexed; 36.4% vs non-sexed; 16.7%) cows ($P < 0.05$). Dairy (X-sexed; 45.0% vs non-sexed; 55.5%) cows aged 6 years had higher proportions of pregnancy when compared with beef (X-sexed; 42.9% vs non-sexed; 30.0%) cows ($P < 0.05$). However, the proportions of pregnancy in age group of ≥ 7 years were high in beef (X-sexed; 30.8% vs non-sexed; 66.7%) cows when compared with dairy (X-sexed; 11.1% vs non-sexed; 31.5%) cows ($P < 0.05$).

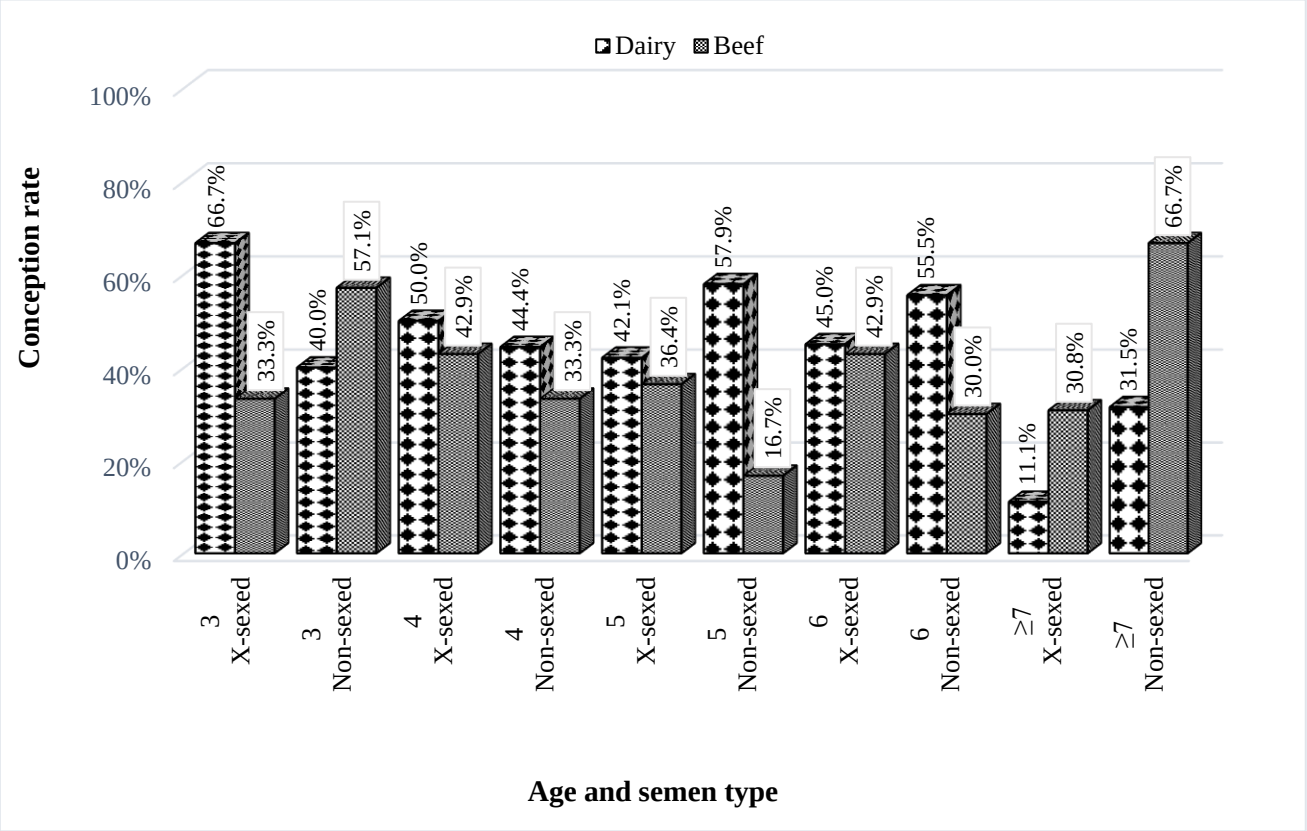

Figure 4.6 Conception rates on Day 95 by age (3 to ≥ 7 years) in dairy and beef cows artificially inseminated with X-sexed or non-sexed semen.

Conception rates by parity in dairy and beef cows inseminated with sexed or non-sexed sperm are presented in Figure 4.7. For 1st parity, dairy (X-sexed; 40.0% vs non-sexed; 50.0%) cows had higher proportions of pregnancy when compared with beef (X-sexed; 30.8% vs non-sexed 14.3%) cows ($P < 0.05$). There were high proportions of pregnancy in 2nd parity for dairy (X-sexed; 47.6% vs non-sexed; 50.0%) cows when compared with beef (X-sexed; 43.7% vs non-sexed; 33.3%) cows ($P < 0.05$). There were high proportions of pregnancy in 3rd party for dairy (X-sexed; 47.6% and non-sexed; 57.1%) cows when compared with beef (X-sexed; 45.4% vs non-sexed; 40.0%) cows ($P < 0.05$). High proportions of pregnancy were observed in 4th parity for dairy (X-sexed; 33.3% and non-sexed; 50.0%) cows when compared with beef (X-sexed; 14.3% vs non-sexed; 40.0%) cows ($P < 0.05$). However, there were higher proportions of pregnancy for ≥ 5th parity in beef (X-sexed; 66.7% vs non-sexed; 50.0%) cows when compared with dairy (X-sexed; 0.0% vs non-sexed; 12.5%) cows ($P < 0.05$).

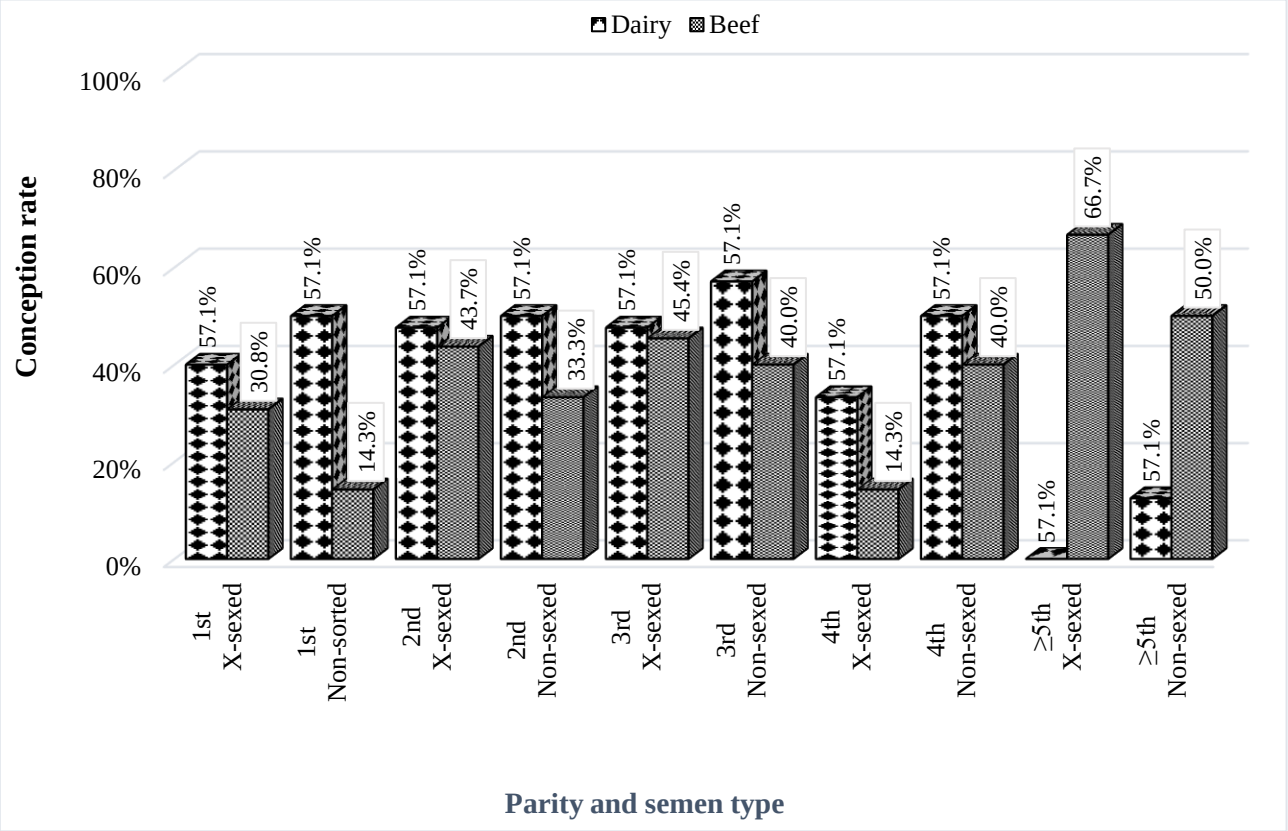

Figure 4.7 The proportions of pregnancy by parity (1st to ≥ 5th) on Day 95 in dairy and beef cows inseminated with X-sexed or non-sexed semen.

The proportions of pregnancy in dairy and beef cows by oestrus expression are presented in Figure 4.8. The overall proportion of pregnancy was higher in cows that expressed oestrus (42.3%) than in cows that did not express oestrus (39.2%; $P < 0.05$). There was a high proportion of pregnancy in dairy (46.3%) cows that expressed oestrus when compared with beef (35.5%) cows ($P < 0.05$). However, due to occurrence of sub-oestrus, beef (41.2%) cows that did not express oestrus had a higher proportion of pregnancy than dairy (35.3%) cows ($P < 0.05$).

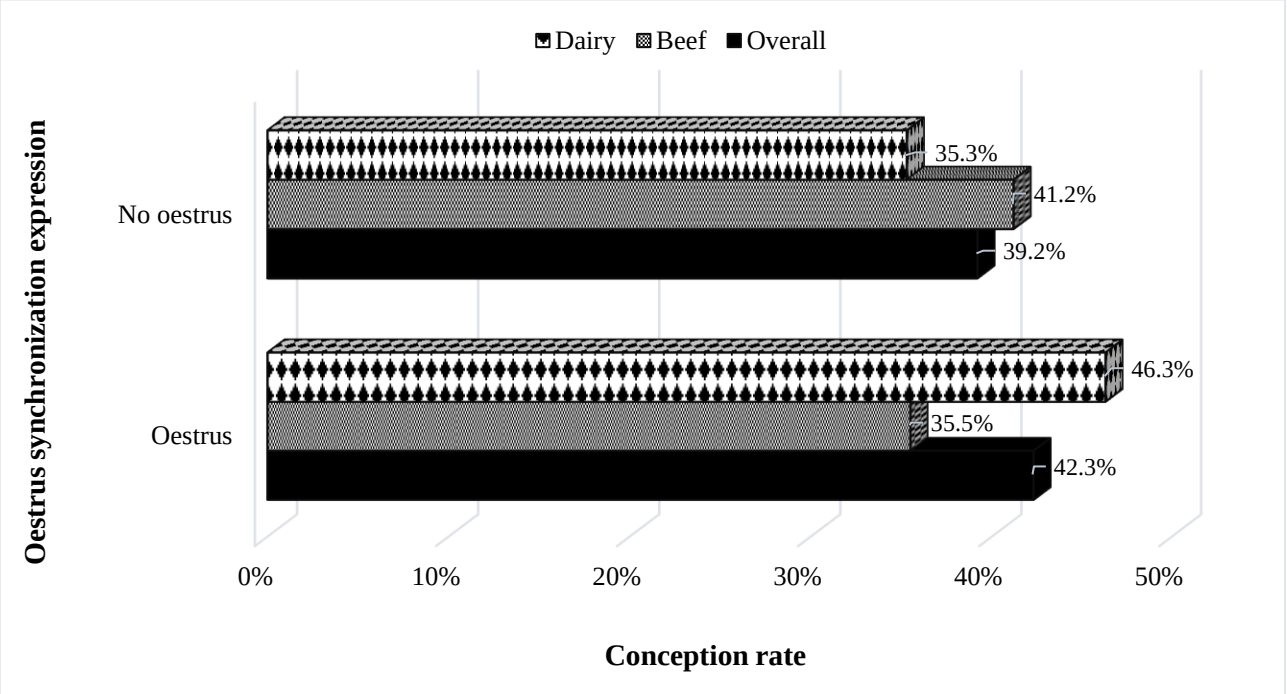

Figure 4.8 Conception rates by oestrus expression (oestrus or no oestrus) in dairy and beef cows on Day 95 following timed artificial insemination.

In Figure 4.10, the proportion of pregnancy by oestrus expression and semen type in dairy and beef are presented. There was a high conception rate in dairy cows that expressed oestrus (oestrus/activated patch) and inseminated with non-sexed (50.9%) semen compared with X-sexed (41.2%) semen ($P < 0.05$). However, there were comparable conception rates in beef cows inseminated with X-sexed (34.3%) or non-sexed (37.0%) semen ($P > 0.05$).

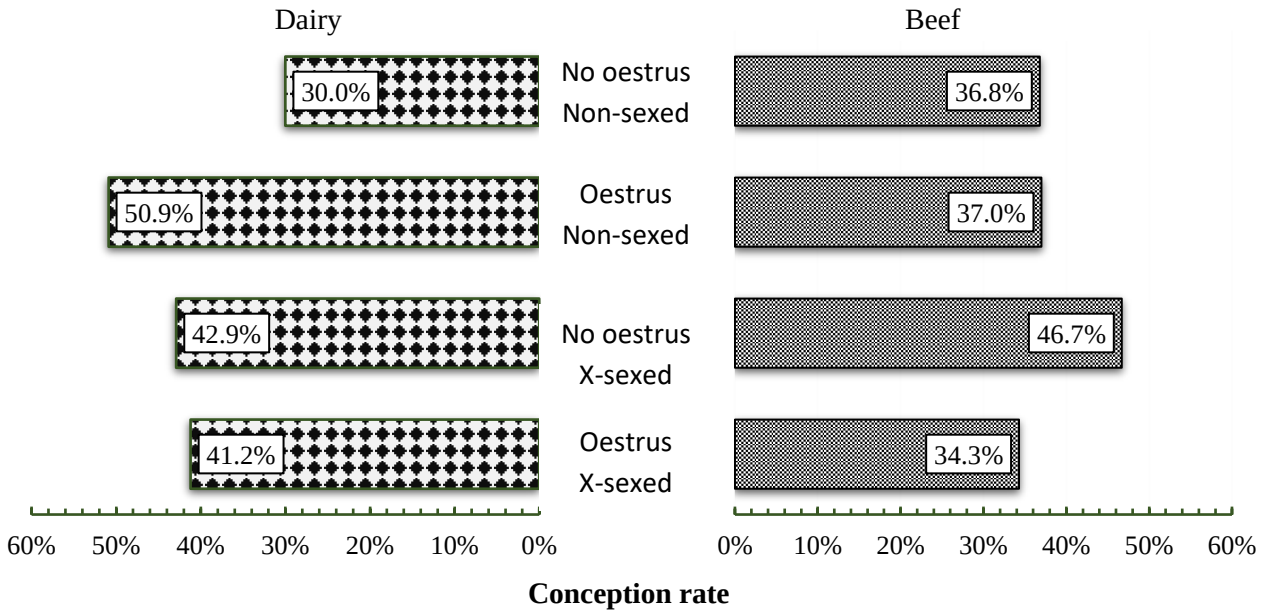

Figure 4.9 The interaction of oestrus expression (oestrus and no oestrus) and semen type (X-sexed or non-sexed) on Day 95 of pregnancy in dairy and beef cows following timed artificial insemination.

The overall contribution of dairy (X-sexed = Bull 1 and 2; non-sexed = Bull 3 and 4) and beef (X-sexed; Bull 5 and 6, non-sexed; Bull 7 and 8) bulls used for artificial insemination to conception rates and pregnancy losses for the first (between Days 35 and 65) and second (between days 66 and 95) periods of pregnancy diagnosis are presented in Table 4.9. On Day 35, Bull 1 (78.4%) accounted for a high conception rate per bull when compared with all other bulls. However, on the same day/period Bull 2 (38.5%) accounted for the lowest conception rate per bull ($P < 0.05$). There was no difference in conception rate per bull for Bull 3 (62.9%), Bull 4 (61.1%), Bull 5 (64.0%), and Bull 7 (62.5%) on Day 35 ($P > 0.05$) of pregnancy. Concurrently, Bull 1 (55.9%) accounted for a high conception rate per bull on Day 95, with Bull 2 (20.0%) accounting for lowest conception rate per bull ($P < 0.05$). However, there was no difference in conception rate per bull on Day 95 for Bull 3 (48.5%), Bull 4 (48.6%), Bull 5 (36.0%), Bull 6 (40.0%), and Bull 7 (45.8%; $P > 0.05$). A higher incidence of pregnancy loss per bull was recorded between Days 35 and 65 of pregnancy in Bull 2 (50%). There was no difference in the incidence of pregnancy loss per bull between Days 35 and 65 for Bull 1 (27.6%), Bull 3 (22.7%), Bull 5 (37.5%), Bull 7 (26.7%), and Bull 8 (33.3%; $P > 0.05$). Three bulls (Bull 1; 9.5%, Bull 4; 10.5% and Bull 5; 10.0%) had a higher incidence of pregnancy loss per bull between Days 35 and 65 ($P > 0.05$). However, there was no incidence of pregnancy loss per bull between Days 66 and 95 on the four bulls (Bulls 2, 6, 7 and 8).

Table 4.9 Classification of dairy and beef bulls used for artificial insemination based on contribution to conception rates and pregnancy losses for the first (between Days 35 and 65) and second (between Days 66 and 95) periods of pregnancy following timed artificial insemination (Proportion/percentage).

Cattle type	Semen type	Bulls (*n* = 8)	Conception rate[a]			Pregnancy loss[b]	
			Day 35 *n*/*n* (%)	Day 65 *n*/*n* (%)	Day 95 *n*/*n* (%)	Day 35-65 *n*/*n* (%)	Day 66-95 *n*/*n* (%)
Dairy	X-sexed	1	29/37 (78.4)	21/35 (60.0)	19/34 (55.9)	8/29 (27.6)	2/21 (9.5)
		2	10/26 (38.5)	5/25 (20.0)	5/24 (20.0)	5/10 (50.0)	0/11 (0.0)
	Non-sexed	3	22/35 (62.9)	17/35 (48.6)	16/33 (48.5)	5/22 (22.7)	1/17 (5.9)
		4	22/36 (61.1)	19/35 (54.3)	17/35 (48.6)	3/22 (13.6)	2/19 (10.5)
Beef	X-sexed	5	16/25 (64.0)	10/25 (40.0)	9/25 (36.0)	6/16 (37.5)	1/10 (10.0)
		6	12/25 (48.0)	10/25 (40.0)	10/25 (40.0)	2/12 (16.7)	0/10 (0.0)
	Non-sexed	7	15/24 (62.5)	11/24 (48.5)	11/24 (45.8)	4/15 (26.7)	0/11 (0.0)
		8	9/22 (40.9)	6/22 (27.3)	6/22 (27.3)	3/9 (33.3)	0/6 (0.0)

[a] Conception rate = Number of pregnant cows ÷ number of cows assigned to TAI × 100.

[b] Pregnancy loss = Number of embryo/foetal mortalities in a given period ÷ initial number of pregnant cows × 100.

Conception rates per bull on Day 35 according to cow oestrus expression at the time of artificial insemination with semen from dairy (sexed = Bull 1 and 2; non-sexed = Bull 3 and 4) and beef (sexed; Bull 5 and 6, non-sexed; Bull 7 and 8) bulls are presented in Figure 4.10. There was no difference in conception rates per bull when cows expressed oestrus for Bull 1 (89.7%), Bull 2 (90.0%), Bull 3 (86.4%), Bull 4 (90.9%) and Bull 5 (81.3%; $P > 0.05$). However, there was a tendency of Bull 8 (oestrus; 44.4% vs no oestrus; 55.6%) to account for a high conception rate per bull when cows did not express oestrus.

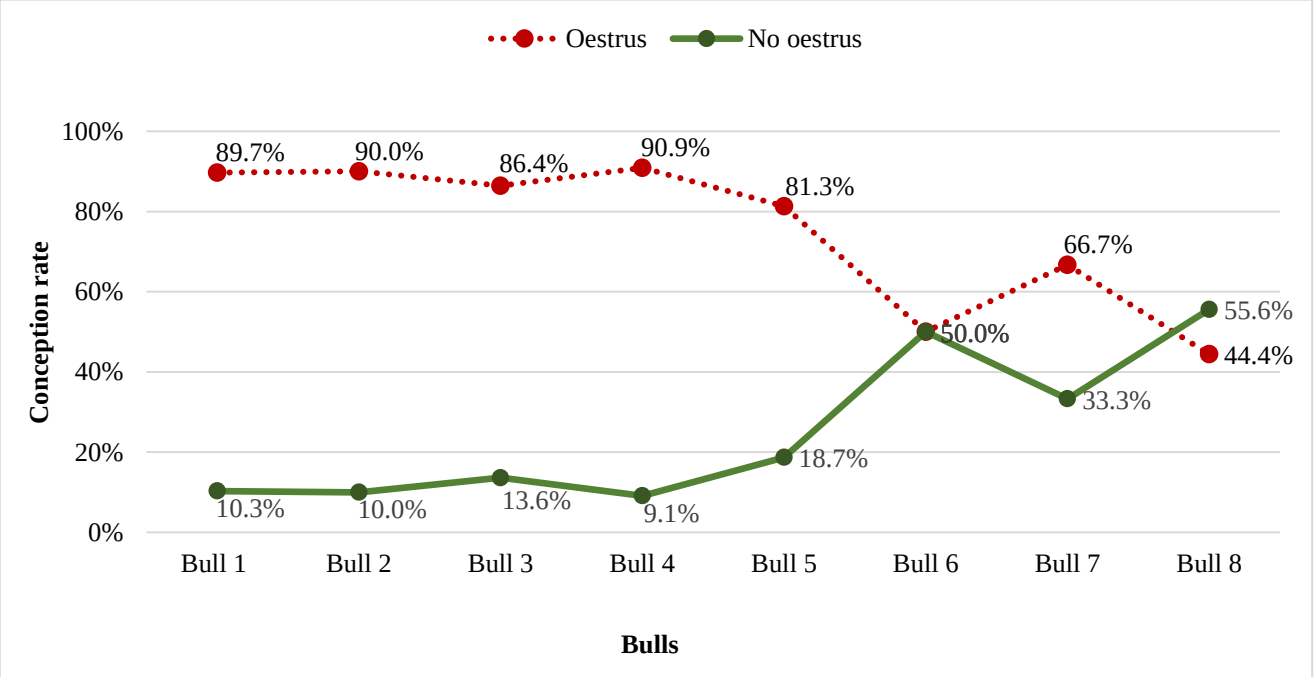

Figure 4.10 Conception rates per bull on Day 35 based on oestrus expression (oestrus or no oestrus) of cows at the time of artificial insemination.

Pregnancy loss per bull between Day 35 and 65 according to cow oestrus expression at the time of artificial insemination with semen from dairy (sexed = Bull 1 and 2; non-sexed = Bull 3 and 4) and beef (sexed; Bull 5 and 6, non-sexed; Bull 7 and 8) bulls are presented in Figure 4.11. The incidence of pregnancy loss per bull (Bull 1; 100%, Bull 2; 80.0%, Bull 3; 80% and Bull 5; 83.3%) did not differ when cows expressed oestrus ($P > 0.05$). There was no difference in the incidence of pregnancy loss per bull for Bull 6 (oestrus; 50.0% vs no oestrus; 50.0%) and Bull 7 (oestrus; 50.0% vs no oestrus; 50.0%) when cows expressed oestrus compared with no oestrus expression ($P > 0.05$). However, there was a tendency for Bull 4 (100%) to have a high incidence of pregnancy loss per bull when cows did not express oestrus.

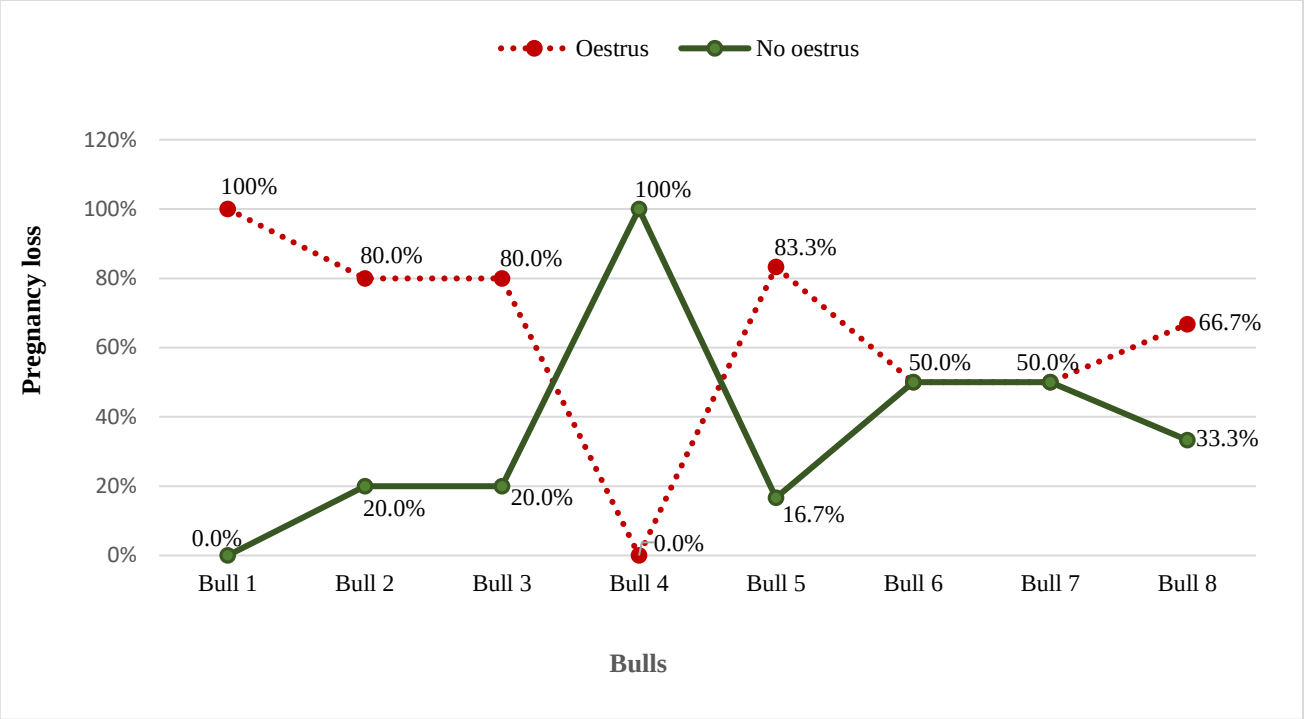

Figure 4.11 Pregnancy loss per bull between Days 35 and 65 based on oestrus expression (oestrus or no oestrus) of cows at the time of artificial insemination.

In Figure 4.12, empty cow uterine body and embryo/foetal development at different stages of pregnancy are presented. An empty cow uterine body was detected in cows that were found not pregnant on all days of pregnancy diagnosis (Figure 4.12A). On Day 35 of pregnancy diagnosis, an embryo was detected in the uterine body cow (Figure 4.12B). A foetus was detected on Day 65 with a change in shape and size (Figure 4.12C). A longer uterine body and larger foetus with heartbeats were detected on Day 95 (Figure 4.12D).

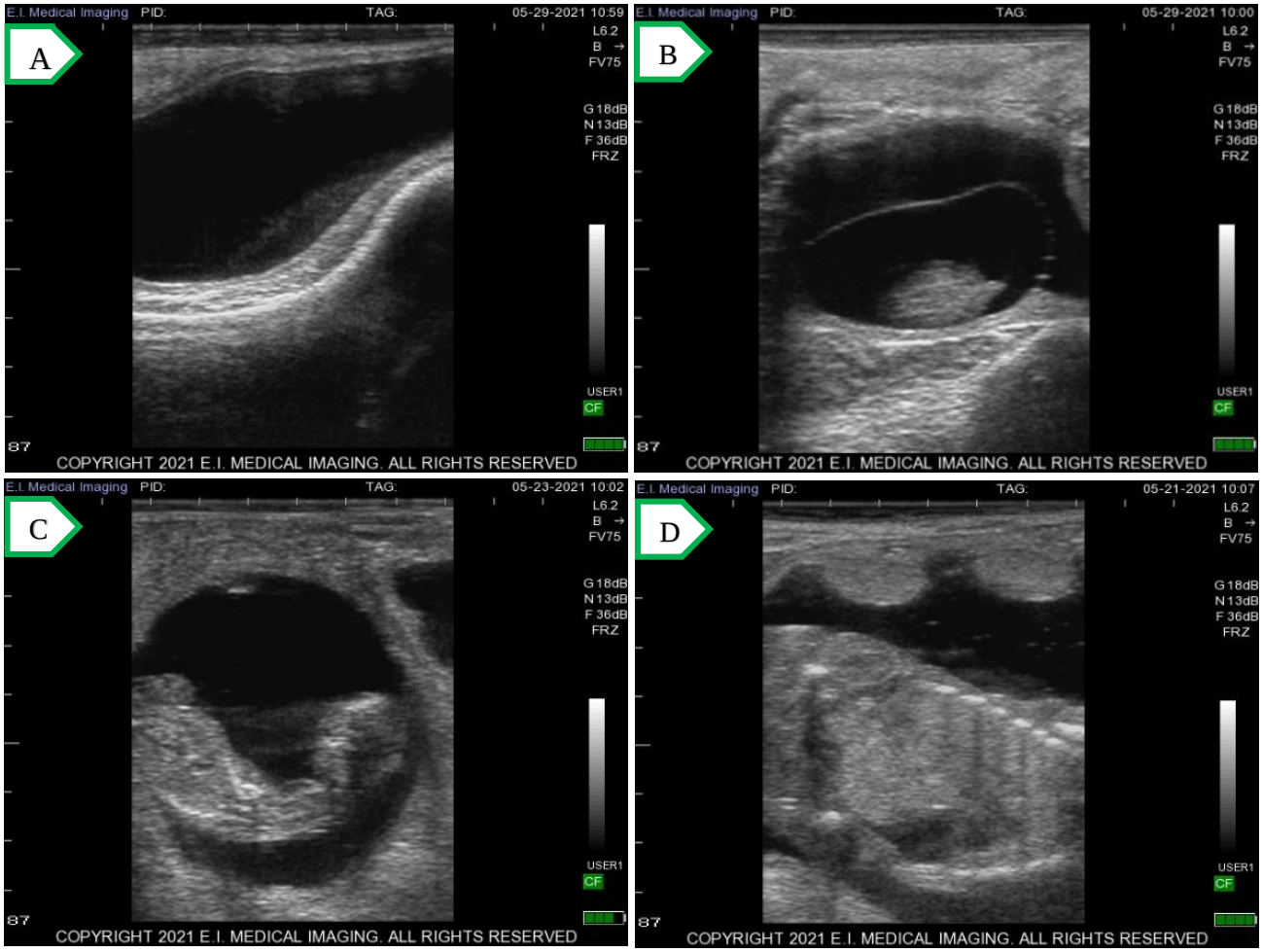

Figure 4.12 Ultrasound depiction of a cow uterine body **(A)** Day 0 cow with empty uterine body/non-pregnant cow, **(B)** Day 35 cow pregnancy, **(C)** Day 65 cow pregnancy, **(D)** Day 95 cow pregnancy. Source: (Personal collection: TL Magopa).

Chapter 5

Discussions

5.1 Oestrus expression in dairy and beef cows following oestrous synchronization

In the present study, overall oestrus expression of 85.3% in dairy cows and 65.0% in beef cows were recorded. This finding was in agreement with previous studies where 80% oestrus expression was recorded in dairy cows by Karki *et al*. (2018), 83.3% in dairy cows by Hirole *et al*. (2018), and 75.0% in beef cows by Raphalalani *et al*. (2020) when Ovsynch protocol was used. According to Bisinotto *et al*. (2015) and Nowicki *et al*. (2017), this protocol assumes that the first GnRH administration induces the ovarian follicle to ovulate, which leads to development of the CL. The efficacy of the first GnRH administration in inducing ovulation differs from 66 to 85% (Perry *et al*., 2005) and depends on the follicle maturation phase (Bello *et al*., 2006) at the time of administration. One of the effects of supplementing P_4 with TAI is enhanced oestrous synchronization (Lima *et al*., 2009; Chebel *et al*., 2010). e Silva *et al*. (2021) reported that supplementation of P_4 concentration with CIDR insertion results in increased ovulation since it reduced the frequency of LH pulses and promoted healthy development, and prevented premature oocyte maturation. As a result, P_4 inhibits LH secretion, resulting in decreased follicle growth, E_2 production, and ovulation rates (Dadarwal *et al*., 2013). However, in contrast, a study by Bisinotto *et al*. (2015) observed that supplementation of P_4 through CIDR insertion (87.1%) on the same day, as the first GnRH for 7 days had no influence on oestrous cycle synchronization in dairy cows compared with control cows without CIDR insertion (87.2%). According to Nowicki *et al*. (2017), administration of $PGF_2\alpha$ into the Ovsynch protocol stimulates luteolysis while still allowing the dominant follicle of the next wave to continue to develop. Furthermore, Nowicki *et al*. (2017) explained that the second GnRH administration on Day 9 of the protocol predicts when the follicle will ovulate, preparing the heifers/cows for insemination 16 to 24 hours later.

According to Darbaz *et* al. (2021), Ovsynch protocol has been used to synchronize ovulation in TAI of cows during the first service. Studies performed by Stevenson *et al*. (2008), Wiltbank and Pursley, (2014) reported that Ovsynch protocol had poorer reproductive results in heifers. However, heifers in Ovsynch group had 35.1% conception rates, whereas in the $PGF_2\alpha$ group it was 74.4%. The rates in cows, on the other hand, were nearly comparable, with 37.8% in the Ovsynch group and 38.9% in the control group. According to Nowicki *et al*. (2017), this was highlighted by the fact that heifers had lower P_4 levels in their blood on the day after $PGF_2\alpha$ administration than cows since there was a

lack of ovulation in heifers following administration of the first GnRH. In addition, Souza *et al*. (2008) and Stevenson *et al*. (2008) suggested that this shows that in heifers, another strategy, such as double Ovsynch, might improve reproductive success.

In this study, oestrus expression was measured using tail-head pressure sensor HMD, which needs a cow to remain standing and mounted by another cow to change the colour. Based on oestrus expression of dairy and beef cows in the present study, the results were influenced by BCS (Madureira *et al*., 2015), lactation status (Cedeño *et al*., 2021), age (Orihuela, 2000), parity (Stevenson *et al*., 2008). It was observed in the present study that dairy and beef cows with BCS of ≥ 3.5 (92.6% vs 70.8%) revealed greater oestrus expression followed by BCS of 3 (89.0%) in dairy cows and BCS of ≤ 2.5 (68.4%) in beef cows. The least oestrus expression in dairy cows was observed in BCS of ≤ 2.5 (79.0%) whereas in beef cows were observed in BCS of 3 (61.1%). Nishimura *et al*. (2018) reported that cows with a lower BCS revealed smaller follicles and lower ovulation rates. Contrastingly, some studies found no differences in oestrus expression of beef cows with different BCS (3.0, 3.5, and 4.0; Meneghetti *et al*., 2009; Centurion-Castro *et al*., 2013). In addition, the return to oestrous cycle in cows during the postpartum phase is largely influenced by their BCS. The BCS is a subjective visual and tactile measurement of body condition that is used to track the nutritional and health status of high-producing cows throughout their productive cycle (Berry *et al*., 2008). It has been associated with reproductive function both phenotypically (Buckley *et al*., 2003) and genetically (Berry *et al*., 2003), indicating that nutrition condition has an impact on reproductive efficiency.

The present results obtained revealed higher oestrus expression in lactating dairy (86.2%) cows compared to dry (81.5%) cows, similarly lactating/suckled beef (67.7%) cows revealed higher oestrus expression when compared with dry (59.4%) cows. In contrast to the present results, previous studies by e Silva *et al*. (2021) in beef revealed greater oestrus expression in dry cows than in lactating cows. However, Endo *et al*. (2012) reported similarities in oestrus rate in lactating and dry dairy cows. Moreover, in the present study, it was observed that lactating dairy cows had higher oestrus expression than lactating beef cows, similarly dry dairy cows revealed higher oestrus expression than dry beef cows. According to Short *et al*. (2021), the rate of LH pulses in postpartum cows is significantly inhibited by a suckling calf. An influence of age and parity on oestrus synchronization expression was observed, dairy cows aged 4 years and cows in ≥ 5 parity revealed higher oestrus expression, whereas in beef cows higher oestrus expression was revealed in cows aged ≥ 7 years and cow in 4th parity. These results are in accordance with those previously reported by Raphalalani *et al*. (2020) in beef cows aged ≥ 8 years and parity ≥ 4. Contrary to the current results, Belay *et al*. (2016) reported a higher oestrus expression in dairy cows aged 7 years. According to Aziz and Abdel-Wahab,

(2017); Salar and Bastan, (2018) the expression of primiparous and multiparous cows to oestrous synchronization protocols differs. Furthermore, respond better to synchronization procedures (Roche *et al*., 2009). In addition, the prolonged return to oestrus in cows following postpartum may explain differences in oestrus expression by parity (Opsomer *et al*., 2002).

5.2 Sperm quality in frozen-thawed sexed and non-sexed semen from dairy and beef bulls

In this study, following thawing of sexed semen, an average sperm TM of 66.8% and 58.8% for dairy and beef bulls were recorded. In this case, the differences might have been the effect of breed and cattle type. Spilman (2019) recorded a post-thaw sperm TM of 64.0 % in dairy bulls, which was proportionally lower than the current findings. The post-thaw average sperm TM of 62.8% and 77.3% for X-sexed and non-sexed semen were recorded. These differences might indicate that sperm sexing process results in a lower proportion of sperm motility in the population before cryopreservation, or least number of sperm that become progressively motile after cryopreservation as a response to processes that occur during sperm sexing.

In the present study, frozen-thawed sexed and non-sexed semen from dairy and beef bulls were quantified to compare sperm parameters that could be markers for conception rate. Interestingly, some dissimilarities in sperm quality between bull type and semen type were observed. Semen from AI bulls passed normal commercial semen testing and fulfilled all the study's minimum requirements. One possible disadvantage of sexed semen is the increase in cell debris deposited in every insemination dosage as a result of the sexing process (Perry *et al*., 2020). The key quality for evaluating a specific semen sample is sperm motility rate (Dorado *et al*., 2011). According to Al-Badry (2012), thawing straws at 37 °C for 30 seconds increased sperm motility in Friesian bulls by 65.2%. The value of CASA system is that constant and reliable sperm motility and velocity data is presented, whereas subjective evaluation results in discrete speculation of sperm motility percentages with increments of 5-10% (Broekhuijse *et al*., 2011).

During the sexing process, the stain, laser, or electric charges and physical forces applied to sperm droplets may have influenced sperm motility parameters (Carvalho *et al*., 2010). In addition, Kurykin *et al*. (2016) found dissimilarities in sperm TM for frozen-thawed sexed and non-sexed semen. In comparison to our results, DeJarnette *et al*. (2011) also reported that non-sexed semen had a relatively higher overall sperm TM after thawing compared with sexed semen. According to Morrell *et al*. (2018), there is a numeric difference in sperm TM between beef and dairy bulls, with beef bulls having higher sperm TM. Comparable results were obtained in the study whereby sperm TM of

83.9% and 70.7% for non-sexed semen of beef and dairy bulls, respectively. Evaluation of frozen-thawed semen in bulls has historically relied on post-thaw sperm motility (Mathur *et al*., 2015). According to Steele *et al*. (2020) not only does sperm sexing decrease sperm motility and velocity, but it also compromises future embryonic development.

Hoflack *et al.* (2007) found that Holstein bulls had considerably higher sperm TM than a particular Belgian Blue, beef bulls. This was not in agreement with the current study, which found beef bulls to have high sperm TM. Besides reduced motility, in the present study sexed semen in beef bulls showed decreased VAP. According to Steele *et al*. (2020), lower sperm VAP (μm/s) indicates that their *in vivo* progress through the oviduct was slower. The CASA sperm HAP (%) in the current study was higher in dairy bulls' sperm although they showed lower LIN (%) than beef bulls' sperm. In contrast, beef bull sperm showed a higher BCF (Hz) and lower ALH (μm) than dairy bull sperm. However, sexed sperm also had a lower ALH (μm), indicating that they move straighter and slower than non-sexed sperm.

Results from the present study indicated that sperm membrane damage was high in sexed semen in contrast with non-sexed semen, which had higher proportions of sperm membrane intact. The high proportion of membrane damage in sexed sperm resulted from the sperm sexing process. Spilman (2019) reported an average viable normal sperm of 67.6% and 66.3% for non-sexed and sexed semen in post-thaw semen, which were proportionally comparable to our findings. The integrity of sperm plasma membrane is critical in assessing the possible viability of bull sperm samples (Puglisi *et al*., 2010; Oliveira *et al*., 2014).

There were differences observed in individual bulls' sperm TM (58.2 to 86.5%). These differences were attributes of semen type (sexed vs non-sexed) and bull type (dairy vs beef). In the current study, it was also observed that sperm sexing had little impact on sperm motility and velocity parameters of other bulls. In principle, the quality of frozen-thawed sperm in bulls differs significantly across bulls of the same breed, between breeds, and between cattle types. Because of this inconsistency, determining bull fertility *in vivo* and *in vitro* is difficult (Maicas *et al*., 2019). Furthermore, Utt (2016) and Harstine *et al*. (2018) reported inconsistency in sperm quality between different ejaculates from the same bull and between straws within the same ejaculate. The fact that inseminations are applied with separate ejaculates in most existing research on sexed semen is a clear problem. Borchersen and Peacock, (2009) observed that whereas sperm sexing has reduced the fertility of some bulls, it has had little effect on the fertility of other bulls.

5.3 Conception rate in dairy and beef cows artificially inseminated with sexed and non-sexed semen

The current study was conducted to compare conception rates of dairy and beef cows inseminated with sexed or non-sexed semen after TAI. In this study, overall conception rates with sexed semen were 59.3% on Day 35, 41.8% on Day 65 and 39.8% on Day 95 after TAI, whereas conception rates with non-sexed semen were 58.1%, 45.7% and 43.9%, on Day 35, 65 and 96, respectively. Corroborating previous studies that reported low conception rates with sexed semen when compared with non-sexed semen in dairy or beef cows (Sá Filho *et al*., 2012; Karakaya *et al*., 2014). In this study, conception rates with sexed semen in dairy cows were 61.9% on Day 35, 43.3% on Day 65 and 41.4% on Day 95, concurrently, conception rates with non-sexed semen in dairy cows were 62.0%, 51.4% and 48.5%. These results are in accordance with previous studies in dairy cows by Sá Filho *et al*. (2012) who reported low conception rate with sexed semen when compared with non-sexed semen. Conception rates with sexed semen in beef cows were 56.0% on Day 35, 40.0% on Day 65 and 38.0% on Day 95, while conception rates with non-sexed semen were 52.2%, 37.0% and 37.0%. These results were in agreement with previous studies in beef cows done by Sá Filho *et al*. (2012) who reported conception rates of 45.9% (sexed) and 54.7% (non-sexed) and Sales *et al*. (2011) who reported conception rates of 40.9% (sexed) and 55.3% (non-sexed). In this study, conception rates were higher than that reported in previous TAI studies by Bodmer *et al*. (2005) in dairy cows (sexed; 27.6% and non-sexed; 28.1%), Andersson *et al*. (2006) in dairy cows (sexed; 21%), Karakaya *et al*. (2014) in dairy cows (sexed; 25.7% and non-sexed; 39.0%),

Previous studies also observed low conception rates with sexed semen when compared with non-sexed semen in heifers. In dairy heifers, Bodmer *et al*. (2005) reported conception rate of 33.3% (sexed) and 59.3% (non-sexed), Seidel Jr and Schenk (2008) reported 42.1% (sexed) and 62.0% (non-sexed) conception rate. Whereas in beef heifers, Deutscher *et al*. (2002) reported conception rate of 54.0% (sexed) and 67.0% (non-sexed) and Thomas *et al*. (2017) reported 51.7% (sexed) and 60.0% (non-sexed) conception rate. Previous studies also observed low conception rates with sexed semen when compared with non-sexed semen in other breeds. Sales *et al*. (2011) reported conception rates of 31.4% (sexed) and 51.4% (non-sexed) with Jersey bull semen. de Oliveira Marques *et al*. (2018) reported conception rate of 42.8% (sexed) and 52.0% (non-sexed) with Nelore bull semen.

Results of the present study revealed that sexed semen in dairy cows with BCS of ≤ 2.5 and 3 higher conception rate, whereas beef cows with BCS of 3 were inseminated with sexed semen and had a higher conception rate. Sales *et al*. (2011) reported lower conception rates in beef cows (Bos indicus)

that were treated with TAI with BCS of < 2.75 in comparison to those with BCS of > 2.75. However, studies that focused on dairy cattle have revealed that changes in postpartum metabolism pose a larger threat to the health and fertility of cows with high BCSs (Wathes *et al*., 2007a; 2007b). There are contradictions in the effects of BCS on oestrus expression and conception rate reported. However, Donovan *et al*. (2003), observed no relationship between BCS and pregnancy at first insemination. Furthermore, it was indicated that when bred in summer (Donovan *et al*., 2003) and winter (Searle, 2019), pelvic size, and skeletal size, had a greater correlation to conception rate.

From this study, it was revealed that conception rate was also influenced by lactation status of the cows. Lactating dairy cows recorded higher pregnancies than dry cows inseminated with either sexed (42.5% vs 36.4%) semen or non-sexed (50.0% vs 36.45) semen. Conception rate of dry beef cows was higher than in lactating cows inseminated with sexed (47.4% vs 31.2%) semen or non-sexed (46.1% vs 34.4) semen. According to Stevenson *et al*. (2008), lactating dairy cows treated with Ovsynch + CIDR insertion maintained a greater concentration of P_4 before AI and had an improved conception rate. This study's findings on low fertility in dry cows are associated with insufficient CL and reduced P_4 concentrations, which are responsible for pregnancy establishment and maintenance. Endo *et al*. (2012) reported higher P_4 concentrations in lactating cows when compared with dry cows. In addition, changes in metabolism caused by lactation enhance P_4 metabolic rate in the liver (Sangsritavong *et al*. 2002; Wiltbank *et al*., 2006). However, in the current investigation, it is possible that lactating cows had greater P_4 concentrations in the CL. Moreover, Maicas *et al*. (2019) also reported low conception rates in lactating cows inseminated with fresh sexed (1×10^6; 37.6% or 2×10^6; 38.9%) semen than in non-sexed (48.0%) semen. In other research, conception rates of 23.8% (Bodmer *et al*., 2005), 21% (Andersson *et al*., 2006), and 30% (DeJarnette *et al*., 2008) were reported in lactating dairy cows inseminated with sexed semen at TAI, which was lower than those reported in the present study (42.5%).

Interestingly, this reproductive behaviour deviates from the tendencies that are reported in previous studies on dairy and beef cattle. Sexed semen in dairy cows aged 3 and 4 years had higher conception rates, whereas sexed semen in beef cows had higher conception rates in age groups 4 and 6 years compared to other age groups. Non-sexed semen conception rates were high in dairy cows aged 5 and 6 years, but beef cows aged 3 and 7 years showed high conception rates. de Moraes *et al*., (2019) reported that age negatively influences pregnancy in cows, further suggesting that a cow's fertility is greatest between the ages of 4 and 9 and reduces after that. Contrary to our results, conception rates were compared by semen type and cattle type in each age group.

In the present study, low conception rate associated with parity for sexed semen in dairy cows was greater for 4th and ≥ 5th parities than for 1st, 2nd and 3rd parities, whereas sexed semen in beef cows, revealed low conception rate in 1st and 4th parities than 2nd, 3rd and ≥ 5th parities. For non-sexed semen in dairy cows, low conception rate was observed in 4th and ≥ 5th parities than 1st, 2nd and 3rd parities, whereas non-sexed semen low conception rate in beef cows was greater for 1st than 2nd, 3rd, 4th and ≥ 5th. These results are inconsistent with previous observations by Schenk *et al*. (2009) in dairy heifers and cows, who reported that conception rate decreases as parity increases for sexed semen. In addition, previous studies revealed that heifers had a higher conception rate in comparison to lactating cows when sexed semen was used (DeJarnette *et al*., 2010; Norman *et al*., 2010). However, results from the present study revealed that both sexed and non-sexed semen conception rates in dairy and beef both decreased and increased as parity increased.

The present study revealed that dairy cows that expressed oestrus had higher conception rate than cows that did not express oestrus. However, beef cows that did not express oestrus recorded higher conception rates than cows that expressed oestrus. Considering the results of this study, it is uncertain why beef cows with no oestrus expression revealed higher conception rate than cows that expressed oestrus. This may indicate that cows with higher conception rates that did not express oestrus synchronization experienced sub-oestrus (silent heat). According to Nowicki *et al*. (2017), sub-oestrus is an increasing problem on many cattle farms. In addition, Kumar *et al*. (2014) reported that sub-oestrus in most cases is caused by insufficient E_2 release by mature follicles or a high E_2 level to develop oestrus signs in the central nervous system of a cow/heifer. However, this issue affects 10 to 40% of dairy farms (Zduńczyk *et al*., 2005).

In this study, it was observed that when HMD patches were not activated (no oestrus) conception rates ranged from 35.3 to 41.2%, whereas when HMD patches were activated (oestrus), conception rates ranged from 35.5 to 46.3%. It was anticipated that sexed semen in cows that did not express oestrus would result in lower pregnancy. This is consistent with a current meta-analysis of various studies, cows that expressed oestrus before fixed-TAI had 26% higher conception rates than those that did not express oestrus (Richardson *et al*., 2016). In contrast to the present results, Perry *et al*. (2020) observed lower conception rates with sexed semen among beef cows and heifers that did not express oestrus. The E_2 directly controls the biological clock in the uterus (Nakamura *et al*., 2006) as well as oviductal glycoproteins produced in the bloodstream (Buhi, 2002). In this regard, it has been shown that sperm movement to the place of fertilization (fallopian tube) is enhanced when cows are in oestrus or exposed to E_2.

From this study, it was revealed that conception rate among individual bulls was different within sexed and non-sexed semen in both dairy and beef cows. In contrast to the current results, DeJarnette *et al*. (2010) reported that in sexed and non-sexed semen of both cows and heifers, the range of conception rates across individual bulls was comparable. Contrarily, DeJarnette *et al*. (2009); Frijters *et al*. (2009) reported highest conception rates across individual bulls with sexed semen than for non-sexed semen.

Individual bulls resulted in dissimilar conception rates at different stages of pregnancy (Days 35, 65 and 95), highlighting the need to diagnose pregnancy at different stages of pregnancy when categorising bull fertility. In contrast, previous reports for sexed semen of European breed bulls; resulted in comparable conception rates (Seidel Jr. & Schenk, 2008; Schenk *et al*., 2009). Differences among bulls' contribution to pregnancy have been well reported in several studies for both sexed (Bodmer *et al*., 2005) and non-sexed (Donovan *et al*., 2003; Franco *et al*., 2018) semen. It has been reported that reduced number of sperm (2.1 vs 20 × 10^6 sperm) per insemination in sexed semen influences lower fertility in sexed semen (Cerchiaro *et al*. 2007; Frijters *et al*., 2009). Further causes for the lower fertility in sexed semen include sperm injury during the sexing process (Seidel Jr & Schenk, 2008) and possibly reduced viability of sexed sperm in the cow reproductive tract (Schenk *et al*., 2009). El-Zarkouny *et al*. (2004), Wiltbank and Pursley, (2014) also reported that conception rates might range from 35.0 to 60.0% with the same protocol.

A recent meta-analysis found that between days 16 and 32, beef cows lost 15% of their pregnancy (Reese *et al*., 2020). Consequently, the present study was unable to record the early phase of pregnancy loss (days 16 to 24), which is comparable with our results for first period (days 35 to 65). The specific processes that cause pregnancy loss at this period are uncertain, however, they might be associated with embryo development or a lack of supplementary embryonic membrane production since it occurs around the time of embryo attachment and placentation beginning (Pohler *et al*., 2016). However, in the current study, it was observed that pregnancy loss was more in dairy cows than in beef cows; moreover, sexed semen had more pregnancy loss than non-sexed semen.

Another issue that needs consideration is pregnancy loss. In the current study, there was an observation of difference in the proportions of pregnancy loss across cows inseminated with different bulls. Cows inseminated with sexed semen bulls had accounted for higher rates of pregnancy loss between day 35 and 65 following TAI, which is contrary to studies from Tubman *et al*. (2004), Borchersen and Peacock, (2009) that there are no variations between sexed and non-sexed semen on pregnancy loss. According to De Rensis and Scaramuzzi, (2003) pregnancy loss was found as a result

of heat stress in cattle between days 35 and 41 following insemination. In dairy cattle, a 1°C increase in rectal temperature was associated with a reduction in conception rate from 61 to 45% (Carwell, 2010). Karakaya *et al*. (2014) reported comparable results to the present study, showing that sexed semen in dairy cows increased pregnancy loss more than in non-sexed semen.

Only a few research have looked at the impact of bull on embryonic mortality (López-Gatius *et al*., 2002; Starbuck *et al*., 2004), with the majority of research concentrating on maternal and environmental elements that influence pregnancy success after fertilization (Bilodeau-Goeseels *et al*., 2003; Hansen *et al*., 2004). In this study, a large difference was observed in pregnancy loss percentage across individual bulls in both embryonic/foetal development periods. It is unclear if oocyte, sperm, or both contribute to causing an effect on embryo development. There is limited information on influence of different breeds/cattle types on pregnancy establishment and maintenance after day 35 since most studies focused on the prenatal embryogenesis of *in vitro* produced embryos. In this study, comparison of conception rate and pregnancy loss using TAI with sexed and non-sexed semen of dairy and beef bulls was observed. In a study by DeJarnette *et al*. (2008) effect of same bull sperm dose was evaluated in heifers whereby a dose of 5×10^6 resulted in a 13.1% greater conception rate compared with doses of 2.1×10^6 with one bull, however, it was observed that semen dose had no influence on conception rate across bulls.

With the observed difference in conception rate on day 35 following TAI, there was also a large difference in pregnancy loss between bulls that greatly affected conception rate (20.0 to 55.9%) on day 95 in both dairy and beef cows inseminated with sexed and non-sexed semen. In other research, bulls were found to be positively associated with pregnancy with both types of semen (Healy *et al*., 2013). A similar observation was reported by Franco *et al*. (2018) who revealed variation in pregnancy loss per bull ranging from 3.9 to 7.2% in beef cows. In corroborating, López-Gatius *et al*. (2022) reported that pregnancy loss (between Days 38 and 90 of pregnancy) ranged from 3.2 to 17.6% among AI bulls in dairy cows. To our knowledge, this was the first study in South Africa to compare conception rates on different days (35, 65 and 95) of pregnancy for dairy and beef cows subjected to TAI (Ovsynch) protocol with sexed or non-sexed semen.

Chapter 6

Conclusion and recommendations

6.1 Conclusion

In conclusion, sexed semen can be efficiently utilized in TAI procedures in cows irrespective of cattle type. The acceptable oestrus synchronization expression and conception rates of dairy and beef cows were achieved. The results revealed that dairy and beef cows have different oestrus synchronization expression and conception rates, although they were both synchronized using the same program. The 9-day Ovsynch + CIDR protocol and TAI used was an effective synchronization protocol with sexed semen. Semen parameters assessed indicated that both bull type (beef vs dairy) and semen type (sexed vs non-sexed) affected sperm quality. The non-sexed frozen-thawed semen tends to have higher conception rates than X-sexed semen between dairy and beef cows following AI. Thus, the results of this study have contributed to a better understanding of the distinctions between sperm cells of sexed and non-sexed semen.

6.2 Recommendations

It is recommended that sexed semen can be successfully utilized through advanced reproductive biotechnologies in an organized emerging cattle farming system. Further research is warranted to assess the ideal timing for insemination in relation to ovulation when using sexed semen in dairy and beef cows, as well as to determine any differences in bull fertility that may exist following the sexing process.

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Appendix 1: Study ethical clearance from University of South Africa

UNISA university of south africa

UNISA-CAES ANIMAL RESEARCH ETHICS COMMITTEE

Date: 15/03/2022

NHREC Registration # : N/A
REC Reference # : 2019/CAES_AREC/153
Name : Mr TL Magopa
Student #: 63088622

Dear Mr Magopa

Decision: Ethics Approval Renewal after Second Review from 11/03/2022 to 28/02/2023

Researcher(s): Mr TL Magopa
luthermagopa@gmail.com

Supervisor (s): Mr ML Mphaphathi
masindim@arc.agric.za; 012-672-9337

Ms T Mulaudzi
mulaut@unisa.ac.za; 011-670-9588

Working title of research:

Application of artificial insemination in synchronized dairy and beef cows using sex-sorted semen in Gauteng Province

Qualification: MSc Agriculture

Thank you for the submission of your progress report to the UNISA-CAES Animal Research Ethics Committee for the above mentioned research. Ethics approval is confirmed for the three-year period, **subject to submission of yearly progress reports**. **Failure to submit the progress report will lead to withdrawal of the ethics clearance until the report has been submitted.**

Due date for progress report: 28 February 2023

*The **high risk application** was originally **reviewed** by the UNISA-CAES Animal Research Ethics Committee on 24 January 2020 in compliance with the Unisa Policy on Research Ethics and the Standard Operating Procedure on Research Ethics Risk Assessment.*

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