Application of assisted reproduction technologies on the indigenous Nguni cows and heifers

By

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DECLARATION

I Ayanda Maqhashu declare that "Application of assisted reproduction technologies on the indigenous Nguni cows and heifers" is my own work, that it has not been submitted before for any degree or examination in any other university, and that all sources I have used or quoted have been indicated and acknowledged by complete references.

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Abstract

The aims of the study were to compare superovulatory (SO) response rate and embryo quality recovered; consequently, correlate sperm motility with fertilization rate on superovulated stud Nguni cows and heifers. Furthermore, compare oestrous synchronization response and pregnancy rate of three breed type cows (Brahman, Bonsmara and Nguni) of different body condition scores following timed artificial insemination in KwaZulu-Natal (KZN) and Limpopo provinces. Nguni stud cows (n= 15) and heifers (n= 10) aged 4-6 and 2-3 years were used as embryo donors. Superovulation of donors involved insertion of a controlled internal drug release device (CIDR) and two injections of FSH daily 12 hours apart for 4 days on a decreasing dosage. Fresh Nguni semen was collected from proven Nguni stud bulls and assessed by computer aided sperm analysis (CASA) before artificial insemination (AI). The doses of AI were prepared and conducted twice, 12 hours apart on synchronized and superovulated Nguni cows or heifers. Embryos were flushed 7 days after AI using a non-surgical technique. Embryos were immediately evaluated under stereo microscope and classified according IETS standard codes (C1, C1- and C2). All transferrable embryos were vitrified. Two pilot study sites were chosen in Eastern Cape – Great kei; (n= 5) and Limpopo - Vuvha; (n=5) provinces for Embryo transfer. Each recipient cow was implanted with one frozen-thawed embryo. For oestrous synchronization, cows (Braman, Bonsmara and Nguni type) were selected in different villages, regardless of parity, age, breed and body weight following pregnancy diagnosis. Cows were grouped according to breed type and body condition scores (BCS) on a scale of 1-5. Group 1 had BCS of $\leq$ 2.5 in KwaZulu-Natal (n=81) and Limpopo n=71), Group 2 had BCS of $\geq$ 3 in KwaZulu-Natal (n=79) and Limpopo (n=100) cows. Cows were synchronized by inserting the controlled intravaginal drug release (CIDR) and removed on Day 8, followed by administration of prostaglandin. The white heat mount detectors (HMD) were placed on the individual cow's tail head as an indicator for

oestrous response if colour changed to red and inseminated twice at 12 hours interval. Pregnancy diagnosis was performed by an ultra sound scanner and rectal palpation 90 days after TAI in embryos and semen recipient cows.

There was no significant difference on the superovulatory response rate between Nguni cows (40%) and heifers (40%). There was a significant difference on the ovary reaction (number of corpus luteum) of cows (11.33±1.41) and heifers (4.00±0.57). There were no significant differences observed on the embryo quality between Nguni cows (2.5±1.00 and 1.25±0.59) and heifers (0.83±0.41 and 1.00±0.36) for excellent (C1) and good (C1-). However, cows had more numbers of unfertilized ova (5.5±1.05 and 1.75±0.47) and degenerate embryos (3.66±1.00 and 1.25±0.39) than heifers. Village cows responded to oestrous synchronization successfully in KZN (100%) and Limpopo (99%) regardless of body conditions and breed type. The lowest pregnancy rate was recorded in Brahman and Bonsmara type cows with BCS of ≤ 2.5 regardless of Province. Interestingly, Nguni type cows with same body condition of ≤ 2.5 had higher average pregnancy rate of 59.5% in Limpopo and 53.5% in KZN. However, cows with BCS of ≥3 had better pregnancy rate regardless of cow breed type, and province. In conclusion, only 40% of both Nguni cows and heifers responded to superovulation. However, Nguni cows had better ovaries reaction compared to heifers. The quality of embryos recovered was similar for both Nguni cows and heifers. Moreover, there was a positive correlation between total sperm motility and fertilization rate bull 1 (93.7%) inseminated the cows (67.5%) and bull 2 (83.5%) inseminated the heifers (53.5%). Higher pregnancy rate (60%) was recorded in Limpopo compared to Eastern Cape (0%). Interestingly, more than 99% of village cows responded to synchronization and inseminated with frozen-thawed semen successfully. Village Nguni type cows were not affected by body condition scoring as they had higher and similar pregnancy rate as those that had body condition of ≥ 3. It is suggested that it is not advisable to breed synchronized Brahman and

Bonsmara type cows with the body condition of ≤ 2.5 except in Nguni cow type as more than 57% average pregnancy rate was achieved.

Dedication

This dissertation is dedicated to my Parents (Mr. Mhlawuli Nelson Maqhashu and Mrs. Kholeka Maqhashu).

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List of abbreviations

AI - Artificial Insemination

ART- Assisted Reproductive Biotechnologies

C (1-) _ Good embryos

C1_ Excellent embryos

C2_ Fair embryos

CIDR - Controlled Internal Drug Releasing Device

CL - Corpus Luteum

E2 -17β - Estradiol-17 β

EC- Eastern Cape Province

eCG - Equine Chorionic Gonadotrophin

FSH - Follicle Stimulating Hormone

FTAI- Fixed time artificial insemination

GnRH - Gonadotrophin Releasing Hormone

hCG - Human Chorionic Gonadotrophin

i.m - Intramuscular

IGF-I - Insulin-like growth factor I

IU - International Units

LH - Luteinizing Hormone

MOET - Multiple Ovulation and Embryo Transfer

pFSH - Porcine Follicle Stimulating Hormone

PGF2α - Prostaglandin F2α

PMSG - Pregnant Mare Serum Gonadotrophin

SO - Superovulation

TM- Total Motility

Chapter 1: General introduction

1.1 Introduction

According to the Food and Agricultural Organization (2012), the world population will increase to nine billion by 2050 and there are also challenges of climate change in the future. Consequently, there will be a need to increase the agricultural food production by 70% in order to feed the growing population (Lutz and Samir, 2010). Food production with sufficient protein in the developing countries would almost double thus imply a significant increase in the production of several key commodities. Production and consumption of meat will also experience an increase. Given the current status of food security and agricultural production in rural households of South Africa, more production of protein source food such as beef will be in demand. Therefore adapted cattle breeds (Nguni) would be the breed of choice for farming as consequences of these challenges in future.

The Nguni cattle breed represent a valuable contribution to the rich biodiversity of the land (Nedambale *et al*., 2008) and possess unique genetic traits such as diseases and tick tolerance, longevity and adaptability in harsh environmental conditions (Ramsay *et al*., 1994). However, Nguni cattle were perceived to be inferior to other cattle breeds mainly due to their smaller body size and varied colour patterns (Ramsay *et al*., 1994). The perceptions are changing as there is an on-going research into the potential of the breed and information on the unique characteristics has added advantages to the breed's adaptability. The use of breeding technologies might assist in conservation and introduction of the good Nguni genetics back to the communities through the use of synchronization, artificial insemination (AI), superovulation (SO) and embryo transfer (ET) technologies.

Superovulation is a term used to describe the drug-induced production of multiple eggs for flushing more embryos to use during assisted reproductive technologies (Gurevich, 2008). The practice involves the administering of external hormones to stimulate production of multiple oocytes that can be ovulated during oestrous (Grimes, 2008). Superovulation in cows and heifers is used to obtain embryos for storage (cryopreservation) or transfer to various cows, especially the ones that face the risk of extinction (Baruselli *et al.,* 2006). Scientists can use superovulation to establish embryo banks for preservation of breeds with good traits such as fertility, adaptation to extreme climatic and nutritional conditions, mothering ability and natural resistance to diseases (Armstrong, 1993; Lopes da Costa *et al.*, 2001).

Assisted reproductive technologies (ART) programmes might help in accelerating reproduction of adaptable cattle and conservation *in situ*. The ART play an important role in genetic improvement and reduction of generation interval (Webb *et al*., 2004). Superovulation of cows using commercially available gonadotrophins has been demonstrated to produce a higher number of oocytes that can be fertilised to produce embryos that can be transplanted and cryopreserved (Kenuya and Greve, 2000). Superovulatory response, embryo quality are reported to be breed specific in cattle and are as a result of endocrine differences at the hypothalamo-pituitary gonadal level (Purohit *et al.*, 2006). Hence the superovulatory protocols give extremely variable responses making it difficult to predict accurately the embryo production from each donor (Silva *et al*., 2009). After recovery, embryos are evaluated for optimal implantation potential and a good quality embryo is the one that has gone through all the development stages without deformities (Van Royen *et al*., 1999). These technologies increase the productivity of both males and females and a large number of offspring are produced from a few genetically superior parents (Lehloenya *et al.,* 2008). To

achieve good results with AI, one needs to be successful in every procedural step from semen collection, dilution, storage and insemination at the optimal time regarding oestrous and ovulation.

In AI practices, an important element is an accurate and precise quality assessment of the motility characteristics of spermatozoa. One possibility of enhancing accuracy and standardization of semen motility assessment is through the use of computer aided sperm analysis (CASA®) system. This provides an opportunity to analyze the fertilizing capacity of bull semen. Evaluation of sperm morphology and progressive motility is the most common method used to assess viability of fresh and frozen-thawed semen before AI. Significant correlations between motility and fertility have been described for cattle (Santos *et al*., 2000). Assessment of *in vivo* fertilizing capacity of semen represents a challenge because it is influenced not only by semen-related factors but also by female fertility such as onset of oestrous and ovulation (Amann and Hammerstedt, 2002).

Oestrous synchronization is a management practice that helps beef producers improves production efficiency and economic returns through fixed time artificial insemination (FTAI). It can help shorten the breeding and calving seasons and help increase calf weaning weights. Its purpose is to control oestrous and ovulation in cycling females, so that breeding can be completed in a short period of time either by AI or natural service. Consequently, using artificial insemination and embryo transfer technologies might bring new genetic materials and changes in the emerging farmer's cattle challenges as the certainty on the quality of the semen and embryo to be transplanted.

1.2 Problem statement

Variability in the superovulatory response and embryo quality of Nguni cows and heifers are still one of the major limiting factors in extensive usage of embryo transfer technology in South Africa. These in turn causes doubts in planning superovulation programmes and hold back the progress in already existing practices because of the expensive chemicals that are used (Nedambale *et al.,* 2008). Information on superovulatory response and embryo quality of Nguni cows has not yet been studied in details. There are many factors that are reported to cause impairments on the embryo quality and the response to superovulation of cattle (Sartori *et al*., 2010), but for Nguni cattle there is no information. A positive correlation between sperm motility characteristics by CASA® and fertility has been reported for most exotic breeds and however there is limited information on the Nguni cattle breed. This poses a challenge in extensive use of good bulls. Similarly good embryo donors are identified by the quality of the corpus luteum (CL) after superovulation (SO) and in terms of Nguni cows and heifers there is no information pertaining to this.

1.3 Justification

Nilchuen *et al*. (2011) reported that different breeds of cattle respond differently to superovulation. The Nguni cattle is a type of breed that is well adapted to extreme climatic conditions, highly fertile, disease resistant, no calving difficulties, higher proportion of total weight and survives well in low quality forage (Scholtz, 2010). Interestingly, all of the factors that impair embryo quality seem not to be a great challenge to the Nguni cattle since it is known of being disease resistant and has a good reproduction performance. It could be potentially profitable to apply more reproductive technologies to improve the breed numbers (Kafi and McGowan, 1997; Bester *et al*., 2003; Musemwa *et al*., 2008; Nedambale *et al*., 2011).

The dose for successfully superovulating the *Bos indicus* is reported to be lower than that of *Bos taurus* (Barati *et al.,* 2006). The smaller doses of superstimultory treatments for *Bos indicus* cattle are reported to be as a result of small ovarian structures such as the follicles and *corpus luteum* (Silva *et al.,* 2009). Since the chemicals used for superovulation are low it can be economical to practise more superovulation on the Nguni. Proper studying of the response to superovulation and factors that influence the quality of the embryos produced can contribute to better planning of embryo transfer programmes with more attention given to the most relevant factors.

1.4 Objectives

1.4.1 Major objective

1.4.1.1. The major objective of the study was to compare superovulatory response rate between Nguni cows and heifers; consequently, correlate sperm motility with fertilization rate.

1.4.2 Specific objectives

The specific objectives of the study were:

1.4.2.1 To compare number of *corpus luteum* following ovulation between Nguni cows and heifers.

1.4.2.2 To correlate the sperm motility evaluated by Sperm Class Analyser technology with fertilisation rate; and,

1.4.2.3 To compare recovered quality of embryos and pregnancy rate following embryo transfer.

1.4.2.4 To compare oestrous response and pregnancy rate following timed artificial insemination on village cows with different body conditions in KZN and Limpopo

1.5 Hypothesis

1.5.1 Nguni cows will respond to superovulation treatments better than heifers.

1.5.2 Nguni cows will yield similar amount of embryos as the heifers.

1.5.3 High sperm motility rate will positively correlate with fertilization rate following artificial insemination.

1.5.4 Cows will respond to oestrous synchronisaation and give better results on pregnancy after timed artificial insemination.

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Chapter 2: Literature review

2.1 Introduction

Superovulation is a term used to describe the drug-induced production of multiple eggs for use during animal improvement through assisted reproductive technologies (Gurevich, 2008). The practice involves the administering of external hormones to stimulate production of multiple oocytes that can be ovulated during oestrous (Grimes, 2008). Superovulation is used to obtain embryos for storage (cryopreservation) or transfer of various female species, especially the ones that face the risk of extinction (Baruselli *et al.,* 2006). Scientists can use superovulation to establish embryo banks for preservation of breeds with good traits such as fertility, adaptation to extreme climatic and nutritional conditions, mothering ability and natural resistance to diseases (Lopes da Costa *et al.*, 2001).

The objective of superovulation is to encourage a high number of ovulations and expected fertilisation rate without disturbing the normal physiological functions in the reproductive tract for ova and embryo development (Kafi and McGowan, 1997). The response to superovulation is measured according to the number of ova flushed out of the animal, and the quality of embryos extracted. The variation in the response to superovulation indicates that 15-20% of all superovulated female animals do not produce transferable embryos; consequently, this was reported to be as a result of gonadotrophin treatment (Greve *et al*., 1995).

Embryo quality refers to the viability and morphological attributes of an embryo (Greve *et al.,* 1995). At collection embryos are evaluated under a microscope to determine their stage of development and quality (Grimes, 2008). Quality of embryos is critical for transfer and for the evaluation of the treatment that was used for stimulation of the animal. Embryo quality is

affected by a number of factors such as higher superovulatory response, gamete quality, *corpus luteum* quality and progesterone concentration (Nilchuen *et al.,* 2011).

2.2 Superovulatory response of cows and heifers

Superovulatory response refers to the reaction of animals to hormonal stimulation. Economically it is the measure of live offsprings born per donor. Variations in superovulatory response represent a major limitation to the routine production of embryos from cattle (Sartori *et al*., 2010). However no significant difference have been reported between cows and heifers (Baruselli *et al.,* 2006). Superovulatory response of indigenous cows is said to be unpredictable because of inefficient conventional superovulation protocols (Lopes da Costa *et al.,* 2001). Superovulation treatments can induce pre-ovulatory endocrine disturbances, follicle morphology, function deviation, decrease sperm transport, cause low fertility and oocyte maturation abnormalities (Lopes da Costa *et al.,* 2001).

The response of animals to superovulation can diverge with the amount of the hormonal treatment administered. In a study that was conducted by Yaakub *et al.* (1998) animals were treated with 800 UI, 600 UI and 400 UI pFSH and it was discovered that the ones treated with 800 IU pFSH had greater numbers of corpora lutea, ova and embryos recovered. This contradicts a study that was done on Holstein cows that were given different dosages of superovulatory treatments and showed no difference in ovarian response (Kanuya *et al.,* 1996). The higher doses of hormones such as the follicle stimulating hormone and gonadotrophin were reported to put animals at risk of low fertilisation rates (Sartori *et al*., 2010).

Superovulated animals had lower fertilisation rates when compared with single-ovulated animals (Sartori *et al*., 2010). The lower rates of fertilisation are associated with disturbances in the transport of spermatozoa and or ova and poor quality of oocytes. Return to natural cycling after superovulation has been reported to be disturbed greatly in many animals. According to Kanuya *et al*. (1996), 72% of the animals superovulated returned to their normal cycle but after insemination only 3% conceived and the ones that never returned to oestrous developed a disease called cystic ovarian disease.

2.3 Factors affecting superovulatory response

2.3.1 Effect of the dominant follicle

Cattle are known to be monovulatory animals (Webb *et al*., 2004). The process of egg development in cow ovaries is termed the oestrous cycle and it is typically 20 to 21 days in length (Whittier, 1993). Superovulatory response has been studied in cattle and there are many variations associated with the stage of follicular growth at the time of treatment administration (Yaakub *et al*., 1998). In cows, the growth of follicles is a well organised process that changes with time. It begins with the development of a group of follicles. This is a species dependant process; in cows only one follicle is selected for further development and this occurs in two or three phases during oestrous cycle. A good superovulatory response results in quite a high number of follicles developing and not undergoing atresia. According to Lucy (2007), the rate of ovulation is closely related to capacity of the dam to sustain gestation and adequately support her young. Two important structures are present within the ovaries namely the follicle and CL. Each of these structures undergo development and regression phase during the egg development cycle (Noseir, 2003). Follicles start their

development in the ovaries and they grow as a group of many follicles that consist of germ cell surrounded by a layer of flattened cells.

A (species) specific number of follicles is selected from each group of follicles and continues differentiation to a size that is suitable for ovulation. The mechanism that controls the initiation of groups and the number of elemental follicles that start to grow is still not known. Stock *et al*. (1995) also indicated that there are no details on the mechanism controlling the selection of follicles that are to continue developing in a follicular wave. The germ cell is the one that has the potential to mature into an egg (Whittier, 1993). Webb *et al*. (2004) reported that follicular growth in cattle occurs in waves. The development of follicles is primarily controlled by gonadotrophins and growth factors. Follicle development is influenced mostly by nutrition and can have a great influence on the development of oocytes quality and hence fertility.

The one follicle that is selected and recruited to grow and develop is termed the dominant follicle (DF) (Lucy *et al.,* 1992). Dominant follicle is dominant because it suppresses the growth of other recessive follicles and Lucy *et al*. (1999) also reported that the DF is dominant because it survives and joins the pool of antral follicles compared to recessive counterparts. The dominant follicle plays an important role in that it nurtures and releases the oocyte, furthermore it produces hormones that control reproduction (Lucy, 2007). The dominant follicle of the first follicular wave remains active until the middle of oestrous cycle and poses a challenge for superovulation treatments because research findings from an ultrasonography indicate that the growth of other follicles is inhibited once there is a dominant follicle.

In the follicular development each wave of follicles has emergence selection phase and dominance phase and during the dominance phase recessive follicle growth is suppressed, this suppression is as a result of increase in follicle stimulating hormone (FSH) level. It has been discovered that the presence of a dominant follicle during superovulation reduces the superovulatory response and embryo yield (Staigmiller *et al.*, 1994).

The methods of monitoring the development of a dominant follicle for example transrectal ultrasonography have provided some information on how the stages of the oestrous cycle at the time of gonadotrophin treatment initiation affect superovulatory response (Kafi and McGowan, 1997). Kafi and McGowan (1997), also discovered that the presence of a dominant follicle has some deleterious effects on the time of commencement of superovulatory treatment. Superovulatory response is affected by ovary characteristics at the beginning of treatment and this has led to researchers concluding that the presence of a growing dominant follicle has a depressive effect on treatment response because greater number of small follicles results to favourable treatment response (Callejas *et al*., 2007).

2.3.2 Effect of age of an animal

Studies to evaluate the effect of age in cattle have been conducted and debatable results have been reported (Mapletoft and Hastler, 2005). Kafi and McGowan (1997) reported that age did not have effect on the total number of embryos recovered, in a study conducted by Greve

(2000) on cows older than nine years the percentage of transferable embryos was reported to be low. Additionally, a study that was conducted on goats revealed that older animals responded poorly to superovulation in comparison with younger animals (1-2 years) (Lehloenya and Greyling, 2010; Sartori *et al.*, 2010). Although these young animals have a good response to superovulation their ovaries show low fertilisation rates as well as embryo recovery and survival rates (Lehloenya and Greyling, 2010). The decline in superovulatory response of older cows is associated with the decline in the number of follicles that are capable of responding to gonadotrophin treatment. Lehloenya and Greyling (2010) reported that in cattle the effect of age on superovulatory response is not always considered as it always shows small or no difference on the number of transferable embryos recovered.

2.3.3 Effect of breed on superovulatory response

The breed of an animal has not been found to be an important factor affecting superovulatory response. However different responses to gonadotrophin treatments among various breeds were reported to vary even within the same breed (Sartori *et al*., 2010). Animals constitute an important factor of variation and is mostly prolific in breeds such as dairy breeds show a greater response to multiple ovulation, yielding more transferable embryos and offspring than unimproved beef breeds (Kafi and MacGowan, 1997).

2.3.4 Effect of nutritional stress on superovulatory response

In ruminants there is a relationship between nutrition and reproduction, this relationship is reported to be inconsistent and complex (O'Callaghan *et al.,* 2000). Kafi and McGowan (1997) discovered that underfeeding in beef cows resulted in a decreased serum concentration of oestradiol and delayed follicular growth. This is reported to result from hormonal disturbances at the level of ovary, anterior pituitary gland and hypothalamus (Kafi and McGowan, 1997). Diet alters endocrine signalling pathways, resulting in oocyte abnormalities in superovulated cattle. *Ad-libitum* feedingbefore mating in superovulated animals reduced the number of quality embryos recovered while in non-stimulated animals embryos produced were of high quality (O'Callaghan *et al.,* 2000). Siddique *et al.* (2002) reported that stress from nutritional deficiency negatively influenced the follicular growth, insulin like growth factor, concentration in the follicular fluid, luteinizing hormone and pulse frequency in cows that are on a multiple ovulation programme. However oocyte development rate and embryo quality are negatively affected by a high protein diet (Yaakub *et al*., 1998). Diets that limit body condition score to 2.5-3 in beef cows before superovulation treatments resulted in quality embryos with no cysts growing on the ovaries (Siddiqui *et al.,* 2002). Animals on a high nutrition diet appear to be at risk of recruiting fewer follicles during exogenous gonadotrophin treatment (Barati *et al.,* 1998).

2. 3.5 Effect of season on superovulation

Weather patterns of the same season differ from year to year and from region to region (Kafi and McGowan, 1997). Research findings by (Kafi and McGowan, 1997; Nowshari and Ali,

2005) reported that season has no effect on the superovulatory response of non-seasonal breeders such as camels and cattle. Research findings from Barati *et al*. (2006) also indicated that season did not affect superovulatory response in cows that were given different doses of superovulation treatment. In smallstock the observations on the ovarian response to superovulation, *corpus luteum* formation and number and quality of embryos showed to be higher in the breeding season than the non-breeding season that season hence they are called seasonal breeders (Lehloenya and Greyling *et al.,* 2008; Mitchelle *et al.,* 2002). Seasonal effect is associated with the breed and/or location of the animal. A study on Portuguese Saloia breed of cattle showed no seasonal effect on the donor superovulatory response or fertility (Bettencourt *et al*., 2007).

2.3.6 Effect of thermal stress

Heat stress is reported to reduce the reproductive performance of beef and dairy cattle (Boyles, 2013). Higher temperatures are reported to have a negative impact on follicular growth, *corpus luteum* function, expression of oestrous behaviour, superovulatory response and embryo quality (Barati *et al*., 2006). Thermal stress in superovulated heifers after insemination resulted in the recovery of a higher number of abnormal and retarded embryos. In heat stressed animals the size of first ovulatory size follicle is reduced and *corpus luteum* from these follicles produced small amounts of progesterone and also affected oocyte maturation process (Nowshari and Ali, 2005). In cattle exposed to high humidity and environmental temperature it was discovered that low quality embryos were recovered from superovulated cows. Heat stress can act in more than one way to reduce fertility in cows. Heat Stress can reduce dry matter intake to indirectly inhibit GnRH and LH secretion from

the hypothalamo-pituitary system (dashed lines). However, it is not clear if heat stress can also directly influence the hypothalamo-pituitary system (thin solid line) to reduce GnRH and LH secretion. Heat stress can directly compromise the uterine environment (solid lines) to cause embryo loss and infertility. The effect of heat stress is diagrammatically represented in **Figure 2.1**.

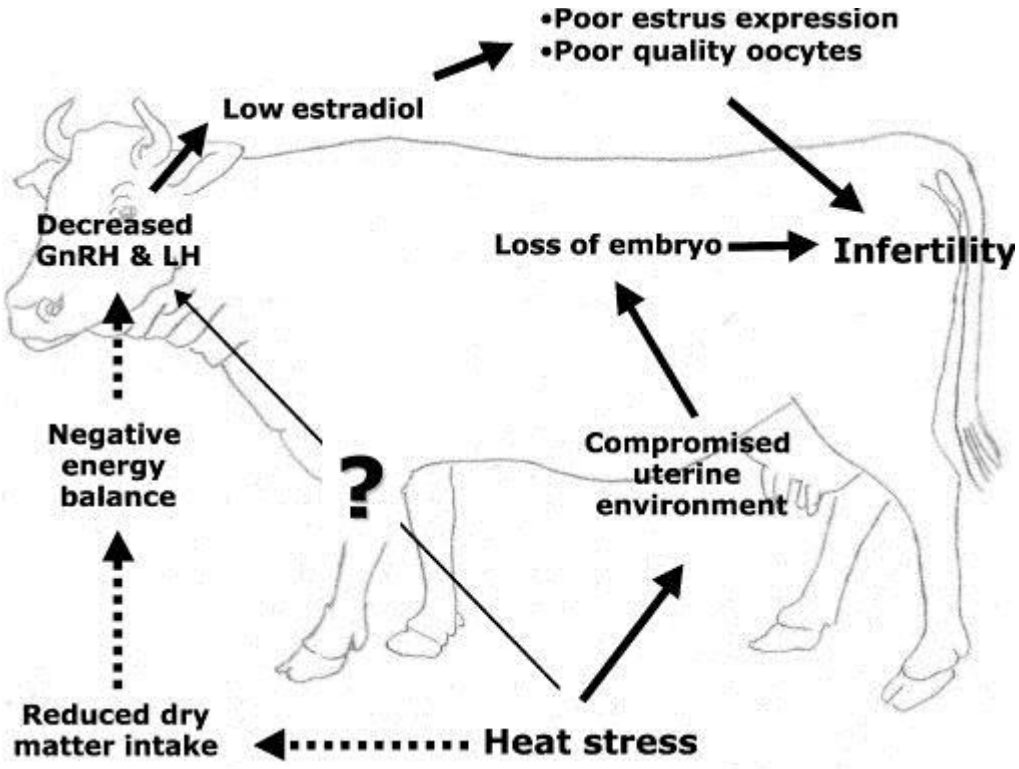

FIGURE 2.1. A schematic description of the possible mechanisms for the effect of heat stress on reproduction of cows (Boyles, 2013).

2.3.7. Transportation stress

After superovulatory treatment transportation of animals reduces the response to superstimulation. In a study where beef heifers were subjected to transportation every 12 h during the 4 d of treatment with FSH, plasma levels of cortisol were increased and ovulation

rate reduced compared to non-transported heifers (Nawshari and Ali, 2005). Kafi and McGowan (1993) reviewed the effects of stress on the reproductive performance of farm animals and concluded that exposure to stressors in the environmental, management and animal derived during the early postpartum period or immediately before the luteinizing hormone (LH) surge resulted in maximum suppression of gonadotrophin secretion.

2.3.8 Effect of cow parity and lactation status

Owing to the limited number of desirable breeding animals available and the inherently low rate of reproduction in cattle, attempts have been made to superovulate both heifers and cows using commercially available gonado-trophic preparations (Kanuya and Greve, 2000). There is very little information on the effects of parity on superovulation. During lactation energy is required for maintenance and for milk production and this results in negative energy balance. Negative energy balance is said to be the same as under nutrition and causes pulsatile secretion of luteinising hormone and during the early postpartum period increases ovarian inactivity in cows and reduced the size of follicles. The reduction in follicle size is as a result of increase in LH pulse which is necessary for growth of ovarian follicles. This indicates that cow parity does not affect superovulation until an animal is exposed to secondary factors such as nutrition and hormonal disturbances.

2.3.9 Effect of superovulation on the endocrine system

Domesticated animals are superovulated with a lot of commercial products (exogenic hormones). Most commonly used products are equine chorionic gonadotrophin (eCG) and follicle stimulating hormone (FSH) (Driancourt and Fry, 1992).These exogenic hormones are partly purified from mare's serum and porcine pituitary glands. Their major function is to bind to and activate FSH receptors on granulose cells of follicles, and to stimulate the continued growth of these follicles by inhibiting apoptosis (Armstrong, 1993).

The commercial products tend to have side effects; the eCG has an inherent LH bioactivity and enhances deposition of lean tissue. Equine chorionic gonadotrophin has been reported to enhance cell growth in bovine species. Effect of eCG on bovine in vivo-cultured granulosa cells have been studied and the results have been variable. The immunization against growth hormone-releasing factor decreases serum eCG and insulin-like growth factor, and delays puberty in beef heifers (Driancourt *et al*., 1992). Faults have been found with the use of FSH, it has been discovered to be contaminated with luteinizing hormone and causes pre ovation and reduces multiple ovulations. Gonadotrophins increase the Insulin like growth factors.

The progesterone profiles of a gonadotrophin treated animal tend to be low. Premature decline in circulating progesterone following the initial gonadotrophin administration is a condition that is unsuited with a normal preovulatory LH release. It is most often associated with impaired oocyte maturation and ultimately reduced embryo quality (Price *et al.*, 1999).

2.3.9.1 Effect of superovulation on steroid secretion

Equine chorionic gonadotrophin is known to increase the plasma progesterone and estrodiol concentration (Alcivak *et al.*, 1992). During the luteal phase of the cycle progesterone concentration increases, and with eCG superovulated animals it remains higher even after prostaglandin-induced luteolysis. With FSH that is containing LH contamination, progesterone concentrations are not significantly affected. There is no adequate information on the effect of superovulation on plasma testosterone and androstenedione concentration. These bulk of steroids are not secreted directly into the blood but into the granulose cells for conversion into estradiol (Driancourt *et al.*, 1994).

2.3.9.2 Effect of superovuation on other hormones

Two follicular hormones have been measured after superovulation in cattle, the first being inhibin, which is a protein that selectively inhibits FSH secretion. There are very few reports of inhibin secretion in ruminants (Price *et al.*, 1999). Inhibin concentration is however increased by the superstimulation protocols. The second follicular hormone is gonadotrophin surge attenuating factor which is also a protein that attenuates magnitude of the preovulatory LH flow. In cattle FSH induced superovulation increases gonadotrophin surge attenuating factor (Donaldson, 2003). The increase in the number of follicles increases these hormones.

Luteinising hormone has been reported to be completely inhibited during the preovulatory process, the reason for this is related to the observed low percentage of LH receptors and

increased gonadotrophin surge attenuating factor. The period between LH surge and prostaglandin-induced luteolysis is shorter in superovulated animals (Soumano and Price 1997). Luteinising hormone pulse is significantly reduced by superovulatory protocols. During superstimilation in cattle, plasma progesterone, estradiol, and inhibin concentration are increased and the secretion of LH is reduced greatly but the cause of this reduction is unknown (Driancourt *et al*., 1994). There is still a need to study these endocrine variations on the reproductive system and the superovulatory protocols.

2.3.10 Superovulatory protocols

The efficiency of superovulation of animals is mostly dependant on the protocol that was used. Superovulation is the basis of embryo transfer which is the biotechnology to increase the progeny per genetically superior donor cow (Bo *et al*., 2010). Superovulatory protocols have improved greatly since the early days of embryo transfer. Traditionally the superovulatory protocols consisted of a single administration of the hormones that were not purified. During this era gonadotrophin treatments were initiated during the mid-luteal phase 9-11 days after oestrous around the time of emergence of the second follicular wave (Mapletoft *et al*., 2002). Detection of oestrous was required and the treatments required donors to be in oestrous at the same time in order to commence treatment at the same time (Bo *et al*., 2010).

The improvement in the protocols now ensures that the emergence of the follicular wave and timing of ovulation occur at the same time in the donors and a fixed artificial insemination is

guaranteed regardless of the phase of oestrous (Baruselli *et al*., 2006; Bo *et al*., 2010). The uniformity that the protocol improvement has brought is reported to have positive effects on commercial embryo transfer and breeding programmes. The complexity that is sometimes found on the superovulation protocols brings about variability in the superovulatory response (Mapletoft and Bo, 2012).

Most commonly used protocols in cattle have not eliminated the variability in the superovulatory response but they have a positive impact on application by permitting the initiation of treatments at a self-appointed time and being now easier to implement on farm. For superovulation to be optimal synchronisation of follicular wave emergence has to be done in cattle progesterone (P4)-releasing devise and estradiol are used (Lane *et al*., 2008). The most commonly used protocols are follicle stimulating hormone (FSH), gonadotrophin releasing hormone (GnRH) and equine chorionic gonadotrophin (eCG). All these are members of the family of glycoproteins and are the hormones to superovulate the donor cows.

2.3.11 Gonadotrophin releasing hormone GnRH

Gonadotrophin releasing hormone has been reported to synchronise the follicular wave emergence and stimulates the ovulation of the dominant follicle so that after a day or two new follicular wave emerges (Mapletoft and Bo, 2012). Ovulation rates after GnRH had been administered were shown to be 44.3% to 85% at different stages of the oestrous cycle. The interval between GnRH treatment and follicle emergence is not constant (Pursely *et al.*,

1995). Furthermore, some negative responses after superovulating cows using this method were reported. In beef heifers the response to superovulation was 56% and 62.4% in another study that was done in dairy cows (Colazo *et al*., 2009; Bo *et al*., 2010 Mapletoft and Bo, 2012).

2.3.10.2 Follicle stimulating Hormone

Superovulation using FSH requires a lot of attention when handling the animals because two injections are given to the donors in one day this may results in undue stress to the animals (Bo *et al.*, 2010). Mishandling and treatment errors may lead to failures. Stressors and errors are likely to cause a decrease in the response to superovulation. Single or two intramuscular injections of FSH were used to try and avoid stressing the animal, there was no difference in superstimulation when single and twice injections were administered to the animals (Colazo *et al*., 2009). Barati *et al*. (2006) and Nilchuen *et al.* (2011) reported this hormone as better than the rest as it gives a higher number of embryos.

2.3.10.3 Equine chorionic gonadotrophin

Equine chorionic gonadotrophin is a complex glycoprotein that is used as a traditional way of superovulating the donor cow. The use of eCG has been reported to be very quick and effective (Bo *et al*., 2010). A single administration of eCG was reported to induce superovulation. However neutralising of this treatment with antibodies is necessary at the

time of insemination to avoid continuing ovarian stimulation, this is because eCG has a long half-life. Use of this treatment may result in large unovulated follicles at the time of embryo collection and decreased embryo collection efficiency and quality (Colazo *et al.*, 2009).

The conveniences of superovulatory protocols are that they control the follicular wave emergence, they are practical and easy to perform and they do not require accurate oestrous detection. The use of all the treatments is profitable but GnRH when used with presynchronization with progesterone releasing devise it undoubtingly improves the response to superovulation during the first follicular wave.

2.4 Oestrous synchronization and Artificial insemination

Oestrous synchronization is manipulation of females to be on heat at the same time, it improves AI submission rates, decrease intervals between AI, reduce days open, and improve overall reproductive performance. Oestrous synchronization programs have an advantage of allowing cows to be bred after completing the fixed time artificial insemination (FTAI) protocol, even though signs of oestrous are not observed. However, some disadvantages include increased cost for treatments. Oestrous cycle is 21 days long, the herd bull can only expect to catch about 1/3 of the cycling animals in heat during the first week of the breeding period if oestrous synchronization is not used. Regardless of whether the animals are inseminated naturally or artificially, only 65 to 70% of the cows are expected to conceive to a given insemination (DeJarnette *et al.,* 2011).

Most of the oestrous synchronization protocols can induce 75 to 90% of the cycling animals to display oestrous within 5 days period. Additionally, many protocols can induce a fertile heat in as much as 50% of the anoestrous cows (DeJarnette *et al.,* 2011). The benefits of these FTAI protocols can result in 40 to 50% of the cows pregnant following one single day of breeding with zero hours spent for heat detection.

2.5 Embryo quality

Multiple ovulation programmes are aimed at maximizing of the production of transferable embryos (Siddiqui *et al.*, (2002). Animals are superovulated for the purpose of recovering many embryos from a superior donor female. After recovery embryos are tested for optimal implantation potential and a good quality embryo is the one that has gone through all the development stages without deformities (Van Royen *et al*., 1999). They have an effect on final oocyte maturation, follicular steriodogenesis, sperm transport, fertilisation and development of embryos in the oviduct and uterus until they are recovered (Greve *et al.*, 1995). The changes that these gonadotrophins have are mostly observed in the plasma concentration of progesterone and Luteinizing hormone. Most deleterious effects of gonadotrophins are mostly found on oocyte, early embryo and their respective microenvironments (Alcivar, 1992). Embryos are produced *in vitro* and *in vivo*, there are some differences between these two productions and the quality of embryos varies. Differences between these embryos have been observed and the causes for these differences are unknown (Lehloenya and Greyling, 2010).

According to Maddox-Hyttel *et al*. (2002) one useful measure of quality of the embryo is the ability of the embryo to survive. In an *in vivo* fertilisation, the oviduct is the site of

fertilisation and early embryo development. The oviductal environment can support embryonic growth up to the blastocyst stage across a wide range of species before transfer (Ferguson *et al.,* 1990). Fertilized ova are scored as excellent, good, fair, poor or degenerate and are also called grades of embryo. Unfertilized ova, and poor and degenerate embryos, are considered to be non-transferable, and excellent, good and fair embryos are considered to be transferable (Maddox-Hyttel *et al*., 2002).

2.6 Conclusion

The use of reproductive technologies is to try and increase production, reproductive efficiency and rates of genetic improvement. Superovulation of animals forms the basis of embryo transfer to increase the embryos of an animal enough for flushing. The response of animals to superovulatory treatments has a lot of variation and a lot of studies have not clearly brought out the cause for this. A compromise in animal welfare causes an imbalance in the endocrine system and exposure to stressors were discovered to be the major causes in variation on embryo quality and superstimulation response.

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Chapter 3.

Superovulation response and embryos quality recovered following flushing Nguni heifers and cows

Abstract

The aims of the study were to compare superovulatory (SO) response rate and embryo quality recovered and consequently, correlate sperm motility with fertilization rate on superovulated stud Nguni cows and heifers. Nguni stud cows (n= 15) and heifers (n= 10) aged 4-6 and 2-3 years were used as embryo donors. Superovulation of donors involved insertion of a controlled internal drug release device (CIDR) and two injections of FSH daily 12 hours apart for 4 days on a decreasing dosage. Fresh Nguni semen was collected from two proven Nguni stud bulls and assessed by computer aided sperm analysis (CASA) before artificial insemination (AI). The doses of AI were prepared and conducted twice, 12 hours apart on synchronized and superovulated Nguni cows or heifers. Embryos were flushed 7 days after AI using a non-surgical technique. Embryos were immediately evaluated under stereo microscope and classified according to IETS standard codes (C1, $C1^{-}$ and C2) and all transfarable embryos were vitrified for storage. Data was analysed using one way ANOVA (SAS, 2003). There was no significant difference on the superovulatory response rate between Nguni cows 40% and heifers 40%. There was a significant difference on the ovary reaction (number of *corpus luteum*) of cows (11.33±1.41) and heifers (4.00±0.57). There were no significant differences observed on the embryo quality recovered between Nguni cows (2.5±1.00 and 1.25±0.59) and heifers (0.83±0.41 and 1.00±0.36) for excellent (C1) and good ($C1^{-}$). However cows had more numbers of unfertilized ova (5.5±1.05 and 1.75±0.47) and degenerate embryos (3.66±1.00 and 1.25±0.39) than heifers. There was a positive correlation between total sperm motility and fertilization rate bull 1 (93.7%) inseminated the

cows (67.5%) and bull 2 (83.5%) inseminated the heifers (53.5%). In conclusion, only 40% of both Nguni cows and heifers responded to superovulation. However, Nguni cows had better ovaries reaction compared to heifers. The quality of embryos recovered was similar for both Nguni cows and heifers. The total sperm motility was observed to be positively correlated to fertilisation rate and cows had higher fertilization rate than heifers because of the bull used which had higher total motility.

Key words: *Corpus luteum*, embryo quality, sperm motility.

3.1 Introduction

Superovulation (SO) of cattle is aimed on obtaining maximum number of fertilized and transferable embryos with a high probability of producing pregnancies (Mapletoft *et al*., 2002). The SO protocol uses exogenous doses of hormones during the mid-luteal phase to overcome the natural mechanism that would normally allow one follicle to be dominant and ovulate at the end of the cycle (Bertens, 2013). Thus multiple productions of offspring from a single genetically superior dam at once rather than having one calf in a year are achieved (Hasler, 2010; Marquezini *et al.,* 2013). Superovulation has a potential of helping in rapid genetic improvement and conservation of breeds with reduced biodiversity such as Nguni cattle breed. Information on the superovulatory response of Nguni cattle is crucial in programs of *in situ* and *ex situ* conservation of the Nguni cattle breed. The response to superovulation is measured by the numbers of CL found on the ovaries and ova/embryos that are recovered after embryo flushing (Barati *et al*., 2006; Silver *et al*., 2006).

Unpredictability of superovulatory response after gonadotropin treatments and fertilization continues to be a challenge for the embryo transfer industry (Baruselli *et al.,* 2006). Poor response to oestrous synchronization and superovulation are reported to be as results of unique reproductive physiology and stress from handling in the females (Lopez da Costa *et al*., 2001). Furthermore, Otava (2010) reported that reduction in the conception rate is not caused only by female factors but also reduced fertilizing capacity of the spermatozoon due to preservation procedure or poor quality bulls. The spermatozoon can be analyzed for motility and viability using CASA® system before AI. Sperm motility has been reported to be a good predictor of conception rates (Otava, 2010; Hilary and Smith, 2000).

In countries such as South Africa and other tropical countries, there has been an increasing demand to multiply the genetics of valuable *bos indicus* breeds that are adapted to harsh environments. Due to global warming and climate change that have been reported to affect growth rate, milk production, reproduction, health and well-being of livestock (Sirohi and Michaelowa, 2007). This might exacerbate the shortage of food in future. With all the factors reducing livestock production it is thus essential to improve production of hardy animals such as Nguni cattle breed. The Nguni cattle breed is an indigenous breed of Southern Africa. In a process of conserving this breed, embryo biotechnology are adopted as one of the possible ways of enhancing reproduction and genetic improvement for the existing population. Superovulation is a rarely used but vital method for speeding genetic improvements in animals.

In superovulated females the ovulated oocytes are fertilized to become embryos. These embryos are then recovered 7 days after AI and are observed for optimal implantation potential using their morphological characteristics. A good quality embryo is the one that has gone through all the development stages without deformities (Van Royen *et al*., 1999). Scientists use SO to establish embryo banks for preservation of breeds with good traits such as fertility, adaptation to harsh climatic and nutritional conditions, mothering ability and natural resistance to diseases and also propagation of superior genes to areas of degraded genes through ET (Lopes da Costa *et al*., 2001; Mpletoft *et al*., 2002).

3.2 Materials and Methods

3.2.1 Experimental site

The study was conducted at the Agricultural Research Council's (ARC) Animal Production Institute (API), Irene, Republic of South Africa. The location of this campus is 25° 53' 58'' South; 28° 11' 52'' East and is in the Highveld region of South Africa at an altitude of 1524 m above sea level. The climatic conditions in this environment range from hot days to cool nights in summer to a moderate temperature on winter days with cool nights. The average temperatures at Irene range from 4.6°C to 28.0°C. The region is coldest during July when the temperature drops to an average temperature of 1.7°C.

3.2.2 Embryo donor cows and heifers

Experimental animals were cared for according to the guidelines of the ARC- API ethics committee (APIEC13/007). Nguni stud cows (n = 15) and heifers (n= 10) aged 4-6 and 2-2.5 years respectively were selected following pregnancy diagnosis and examination of the reproductive tract for abnormalities and diseases. The cows and heifers had an average body weight of 350 kg and 210 kg, respectively.

3.2.3 Oestrous synchronization and superovulation (SO) of Nguni cows and heifers

Nguni donor cows and heifers followed the same oestrous synchronization and superovulation protocol whereby a controlled internal drug releases (CIDR®) (1.9g, Pfizer

(Pty) ltd, Sandton, Republic of South Africa (RSA) was placed into the vagina of each cow and heifer on Day 0. An intramuscular injection (i.m) of Cloprostenol Sodium (263µg, Estrumate®, Isando, RSA) was administered (i.m) to the cows and heifers after CIDR® removal on Day 8 of oestrous synchronization followed by (i.m) injection of estradiol benzoate (1g Pfizer (Pty) ltd, Republic of South Africa) on Day 9. Heat was observed with the aid of heat mount detectors (Kamar®, RSA) which were glued on the tail head of each donor cow and heifer. Day 0 was repeated and a new CIDR was inserted three days after heat observation. On Day 4, two injections of FSH, Folltropin-V® (20mg, Armidale, Australia) administered at 12 h intervals initiated every day for 4 days on a decreasing dosage, plus two injections of prostaglandin 12 h apart on the last two days of Folltropin®. Oestrous detection was performed every 8 h for 3 days starting 24 h after the second prostaglandin injection. Oestrous detection was done with the aid heat mount detector after superovulation. Cows and heifers were artificially inseminated three times (at 12, 24 and 36 hours) after detection of standing oestrous with diluted raw semen from stud Nguni bull of proven fertility. The sperm motility rate (non-progressive, progressive, slow, medium, and rapid) and the velocity on the curve line were evaluated and recorded using a Sperm Class Analyzer® (SCA-Microptic®, Spain) system before AI.

3.2.4 Embryo flushing, grading and vitrification

Rectal palpation was done on both ovaries on day 7 after AI to determine the number of *corpus luteum* (CL) present. Embryo recovery was performed 7 days after AI, with a standard non-surgical technique to flush the uterine horns. An epidural anaesthesia (Lidocaine®,Beyer (Pty) Ltd, Isando, RSA) was injected in the intervertebral space between the vertebrae of the

tail-head region. The vulva and rectum were thoroughly cleaned before flushing. Uterine flushing was conducted with a Complete Flush medium, a silicone two-way Foley catheter and a long flushing tube. Each horn was repeatedly flushed with the Complete Flush medium for retrieval of the embryos. Retrieved embryos were transferred into an embryo filter containing holding medium and evaluated using a stereo-microscope (Olympus SZ40, Olympus, Japan). Embryos were evaluated for the viability and graded excellent, good, fair and degenerate C1, C1-, C2 and C3) according to the International Embryo Transfer Society standards codes their stage of development (i.e., ova, 2–16 cells, early morula, compacted morula, early blastocyst, blastocyst and expanded blastocyst) and quality (i.e., excellent, good, fair, poor and degenerated). Excellent and good quality embryos were considered as transferable and others were classified as non-transferable embryos. All transferrable embryos were washed in trypsin, washed again in M199 + 20 % FBS, rinsed and afterwards put in base solution for 3 minutes. Embryos were equilibrated in 17.5% ethylene glycol for 3 minutes at room temperature and were transferred into drops of vitrification solution. Drops of 1-2 µl containing embryo were carried into a pre cooled metal box covered with aluminium foil. 1.5ml cryovial tubes were used to put embryos after freezing and were stored into liquid nitrogen tank (-196°C) until use.

3.2.5 Embryo recipients

Two study sites were chosen (Eastern Cape – Great Kei; n = 5 and Limpopo Province - Vuvha; n = 5 cows) in South Africa. Embryo recipients were synchronized for oestrous to the same stage as the embryo donors. Rectal palpation was done on both ovaries on Day 7 of standing heat to determine the development of CL on the recipient's cows. Embryo transfer was then performed by placing the warmed embryo on the horn that had developed the CL.

3.2.6 Data analysis

The data was analyzed using the Proc (GLM) implemented in the SAS software of V9.3 of 2010. The means were compared using one way analysis of variance (ANOVA). The difference between the two treatments was evaluated using the student t- test. The total ova/embryos, transferable embryos and stages of embryo development were tested by Chi-square test for the significant difference between the treatments ($P<0.05$). Results are expressed as means, standard errors of the means and percentages. Pearson correlations were used for sperm motility and fertilization rate.

3.3 Results

The motility characteristics of the spermatozoa from each bull were analysed for progression and velocity as shown in Table 3.1. Total sperm motility of 93.7, 83.5, and 78.9% was observed for bull 1, 2 and 3 respectively. Bull 1 had the highest number of rapidly moving sperm (47.9%) than bull 2 (30%) and bull 3 (45%); however, progression was higher in bull 2 (67%) than bull 1 (62.8%) and bull 3 (59.6%). Bull 3 had highest number of dead sperm (21.1%) than bull 1 (6.3%) and bull 2 (16.6%). Only bull 1 and 2 were used for AI bull 1 inseminated cows and bull 2 heifers. Average Fertilization rates of 67.6% and 53.5% were observed following AI with bull 1 and 2 semen respectively. The Pearson correlation revealed a positive correlation between total motility (TM) and progressive motility (0.378), a negative correlation was observed between TM and non-progressive motility (-3.19), a negative correlation was again observed between TM and static sperm (-0.996), A positive correlation was observed on the rapid sperm and TM; however, it was negatively correlated with medium, slow and static sperm. There was a positive correlation between TM and fertilization rate for cows and heifers combined as shown in Table 3.2.

Superovulation response of 40% (heifers 4/10 and cows 6/15) was obtained from both cows and heifers and embryo quality characteristics were observed in the Nguni cows and heifers following flushing. Embryos were classed according to their quality as shown in Table 3.3.There was a difference on the ovary reaction between cows (11.33±1.41) and heifers (4.00±0.57) ($P<0.05$). There were higher numbers of recovered unfertilized ova in cows (5.50±1.05 compared to heifers (1.75±0.47). The cows had more number of degenerated embryos (3.66±1.00) compared to heifers (1.25±0.39). The mean number of all the embryos classed as C1, C1- and C2 in both cows and heifers were similar as indicated on the table 3.1.

Table 3.1: Sperm motility characteristics with fertilization rate on superovulated Nguni cows and heifers (%).

Bulls	Total Motility	Progressive	Non-progressive	Rapid	Medium	Slow	Static	Fertilization rate	
Bull 1	93.7	62.8	30.9	47.9	36.8	9.0	6.3	67.6	Cows
Bull 2	83.5	67.6	15.9	30.0	46.2	11.0	16.6	53.5	Heifers
Bull 3	78.9	59.6	19.3	45.0	24.6	8.8	21.1	-	

Table 3.2: Correlation of sperm motility characteristic with fertilization rate in Nguni cows and heifers.

Parameters	TM	P	NP	RAPID	MD	STATIC	Fertilization Rate
TM		0.378^{ns}	0.751^{*}	0.460^{ns}	-0.556^{ns}	-0.990^{***}	0.874^{***}
P			-0.319^{*}	0.973^{***}	-0.953^{**}	-0.283^{*}	0.763^{**}
NP				-0.235^{ns}	0.120^{ns}	-0.806^{*}	0.340^{ns}
RAPID					-0.960^{**}	-0.367^{ns}	0.830^{*}
MID							-0.873^{*}
						0.488^{*}	
STATIC							-0.823^{*}
Fertilization Rate							

***correlation is significant at 0.001, **correlation is significant at 0.01, *correlation is sig at 0.05, ^{ns} correlations are not significant.TM= total motility, P= progressive, NP= Non-progressive motility, MED= medium and FR= Fertilization Rate.

Table 3.3: Number of superovulation response and embryo quality between Nguni cows and heifers (Means±SE).

Treatments	Animals (n)	SO response (%)	Ovary reaction (#CL)	#Ova	#Deg embryos	Blastocyst (n)		
						#C1	#C2	#C1-
Cows	15	(6/15) 40	11.33±1.41[a]	5.50±1.05[a]	3.66±1.00[a]	2.5±1.00[a]	0.83±0.41[a]	0.16±0.04[a]
Heifers	10	(4/10) 40	4.00±0.57[b]	1.75±0.47[b]	1.25±0.39[b]	1.25±0.59[a]	1.00±0.36[a]	00±00[a]

[a,b]Means within the same column with different superscript (a and b) are statistically different at ($P<0.05$). CL= *Corpus luteum*, Deg= Degenerating embryos, C1= Excellent embryos, C2= Good embryos, C1-= Fair embryos.

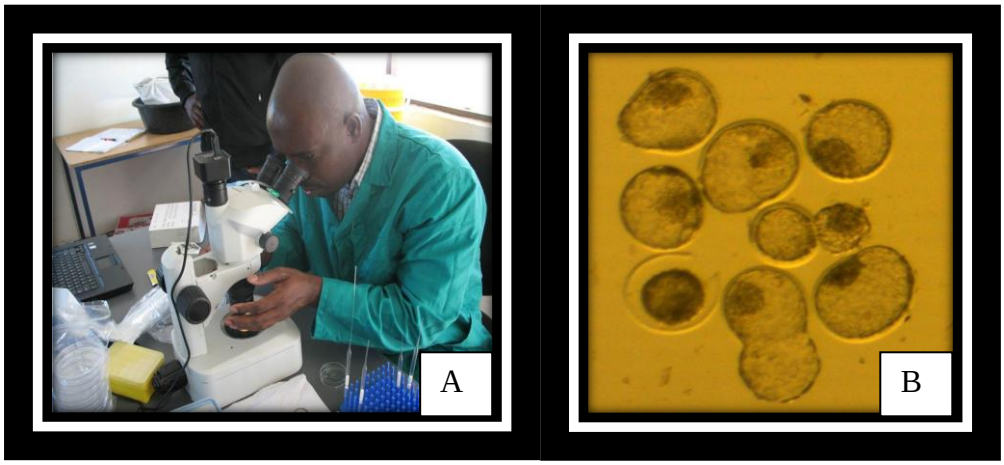

Figure 3.1: (A) Stereo microscope and (B) C1 embryos at blastocyst stage.

All cows, in Limpopo (n = 5) and Eastern Cape (n = 5) provinces, were transferred a viable embryo. Pregnancy diagnosis revealed 60% and 0% of cows conceived in Limpopo and Eastern Cape provinces, as shown in Table 3.4. All conceived cows gave birth to healthy calves.

Table 3.4: Pregnancy rates following embryo transfer in Nguni cows (%)

Province	N	Pregnancy rate %	Calving rate
Eastern Cape	5	0	0
Limpopo	5	60	60

3.4 Discussion

This study demonstrated that there was a positive correlation of total sperm motility, rapid, progressive and non-progressive with fertilization rate. It was not surprising to observe a negative correlation of non-progressive (static) and medium (MED) movement sperm with fertilization rate. Furthermore Nguni cows and heifers can be superovulated with a recovery rate of reasonable number of transferable embryos. However, response rate was not different between cows and heifers although it is still low. Dalton *et al*. (2000) reported higher fertilization rate when donors were inseminated 24 hours after the onset of oestrous compared to inseminations at 0 or 12 h, in this study cows and heifers received three doses of semen at 0, 12 and 24h. It was speculated that these differences might have occurred due to decreasing fertilization potential of sperm before ovulation occurred. Furthermore, Dalton *et al*. (2000) and Otava (2010) reported that it is still unclear at which time AI, with conventional thawed semen, yields optimal embryo quality.

The present study used diluted raw semen from two bulls after analysis of sperm motility by CASA®. The results are comparable to the results of the sperm motility that was observed from these bulls using CASA® as shown in Table 3.1. Similar results were obtained by Otava (2010) where sexed and conventional sperm were analysed by CASA® before AI and lesser total motility of sexed sperm were reflected by lower fertilization rate which was lower (31.81%) while 75% conception rate was recorded on the conventional sperm that had higher total motility.

The results for fresh semen are usually higher depending on the fertility of the bulls used. Poor motility is reported as a good predictor of failure in fertilization (Vasan, 2011). The higher percentage of fertilization rate in the rapid movement of sperm is similar to the results by Bo *et al.* (2008) where females were inseminated with rapidly moving sperm and within 5 minutes after insemination, sperm were present within the Fallopian tubes, and the number of sperm found there was proportional to the number inseminated. Similarly Fotrow *et al.* (2007) got a conception rate of 55-60% when using conventional semen of high total motility than of 35-40% when using the sexed semen.

The results on the response of Nguni cows and heifers to superovulation showed no significant difference as it was (40%) on both. Similar results were reported on the dairy cows and heifers where there was no difference on the SO response (Silva *et al*., 2002). No significant differences were observed amongst the Nguni cows and heifers when the numbers of transferable embryos were compared. Similarly, Nilchuen *et al*. (2011) and Silva *et al.* (2002) reported that there were no significant differences in the quality of embryos flushed from Kamphaeng Sean cows and heifers (15.50±3.4; 13.50±3.40), (14.25±3.61; 11.75±3.61) (9.75±3.01; 7.75±3.01), after receiving FSH treatment. The large difference on the number of embryos flushed could be due to breed differences. The ovary reaction (number of CL), total number of unfertilized ova and degenerate embryos was greater on the Nguni cows than heifers even though overall SO response was similar among these two groups. The greater number of CL was reported to be mostly in heifers in dairy cattle since their progesterone levels are higher than lactating cows that

are distress from negative energy balance. Cows used in this study were not lactating and in an acceptable body condition (3-4) for breeding on a scale of 1-5. In the present study cows and heifers received similar doses of Follotrophin-V®. Individual animal variations and the unnatural process of superovulation might have an effect on the different SO response because under normal breeding programs the physiology of a cow allows one egg to be released per cycle (Stroud and Hastler, 2006). In superovulation females are provided with FSH in order to recruit high numbers of follicles that will be selected at ovulation, this happens when FSH is bound on the receptors in the ovary. According to Nilchuen *et al.* (2011), when the dosage is higher it results in less ovary reaction since there are fewer receptors to withstand the reaction. The heifers used in the present study weighed less than the cows and yet received similar amount of FSH, chances of the dose being unsupported by the ovary were high. Overstimulation has been reported to result in anaovulatory follicles and hence reduced ovary reaction and fertilization rate since the *bos indicus* breeds require less dosages of superovulation treatments than *bos taurus* (Satori *et al*., 2009; Gonzalez *et al*., 1997). Furthermore, a lesser dose 200 and 240 mg were used for heifers and cows respectively in a study on Brahman cattle and 200 mg was reported to be the appropriate dose as better results were obtained (Barati *et al*., 2006). According to Barati *et al*. (2006), a greater number of CL was expressed by the heifers that received 200mg of Follotropin. However, this is a lesser dose than the recommended 400mg for *bos taurus* breeds. Heifers require less dosage of FSH than cows because of the body weight and size. The Sanga breeds are reported to have delayed puberty than the exotic breeds and Sanga heifers are not mated until three to four years and heifers used in this study were 2, 5 years and this might have affected the reduced ovary reaction (Schoeman, 1989). According to Scholtz (1985), Nguni heifers that were mated at 15 months obtained 37% pregnancy rate under natural mating. The *Bos indicus* breeds

are generally small framed with also very small body organs with heifers the ovaries are very small even after superovulation the chances of miscounting the CL during rectal palpation were maximized, in Nelore cows and heifers the CL was reported to be small (Figueiredo *et al*., 1997). The most commonly used protocols are FSH, gonadotrophin releasing hormone (GnRH) and equine chorionic gonadotrophin (eCG) and human chorionic gonadotrophin (hCG). Ovulation rates after GnRH had been administered were shown to be 44.3% to 85% at different stages of the oestrous cycle (Pursely *et al.,* 1995; Colazo *et al*., 2009; Mapletoft *et al*., 2002 and Bo *et al*., 2010; Bo, 2012). Superstimulation using FSH requires a lot of attention because two injections are given to the donors in one day this may results in undue stress to the cows and stress might result in lower responses in donors that are not used to being handled daily such as Nguni cattle breed (Bo *et al*., 2010).

The eCG is a complex glycoprotein that is used as a traditional way of superovulating the donor cow. The use of eCG has been reported to be very quick and effective (Bo *et al*., 2010). A single administration of eCG was reported to induce ovarian superovulation and thus minimize stress. In the present study, FSH protocol was used because of improvement in the protocol that ensures that the emergence of the follicular wave and timing of ovulation occur at the same time in the donors and a fixed AI is guaranteed (Bo *et al*., 2010; Baruselli *et al*., 2006). The uniformity that the protocol improvement has brought has been reported to have positive effects on commercial embryo transfer and breeding programmes (Lane *et al*., 2008). The complexity that is sometimes found on the superovulation protocols brings about variability in the superovulatory response (Mapletoft and Bo, 2012).

In Eastern Cape Province, all recipient cows did not conceive. This might be contributed by drought that occurred during the trial. The lower results might be as a result of poor nutrition that

reduced the cows' body condition scores to 2 on a scale of 1-5 and management and thus compromised conception. The poor quality of the CL at the time of embryo transfer, as determined by palpation is reported to affect pregnancy (Hasler, 2013). Lower progesterone levels on the recipient cows on the time of ET results in lower pregnancy rates. The quality of embryos is reported to be the major factor reducing pregnancies however variables from females can also be observed (Hastler, 2013). Krininger *et al*. (2003) reported that conception rates of more desirable quality embryos (excellent or good) are greater than embryos qualified as fair or poor quality; therefore, conception rates would increase if a greater percentage of grade 1 (excellent) embryos are harvested and transferred after collection. The high pregnancy percentage Limpopo might have been as a results of good management and recipient fertility.

3.5 Conclusion

There was a positive correlation between total sperm motility and fertilization rate. Furthermore Nguni cows and heifers can be superovulated with a recovery rate of reasonable number of transferable embryos; however, response rate was not different between cows and heifers although it is still low. Ovary reaction was higher on the cows; however it did not make difference in the number of embryos recovered as most were unfertilized and degenerate from the cows. Moreover the conception rate in synchronised, superovulated cows and heifers can be improved by use of fresh-thawed semen from bulls with higher sperm motility rate analysed by computer aided sperm analysis before AI.

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Chapter 4

Comparison of oestrous synchronization response and pregnancy rate on village cows following timed artificial insemination in KwaZulu-Natal and Limpopo provinces

Abstract

Economic loss from reproductive inefficiency is related to failure of detection and unexpressed oestrous and low conception rates in cattle. The objectives of the current study were to evaluate the oestrous response and pregnancy rate following timed artificial insemination (TAI) with frozen-thawed semen in cows. The study was carried out in cows at different villages of KwaZulu-Natal (KZN; n=160) and Limpopo Province (L; n=171). Cows were selected randomly as presented by the farmers, regardless of parity, age, breed and body weight following pregnancy diagnosis. The cows were grouped according to breed type and body condition scores (BCS) on a scale of 1-5. Group 1 had BCS of ≤ 2.5 in KZN (n=81) & L (n=71) and Group 2 had BCS of ≥ 3 in KZN (n=79) & L (n=100) cows. All the cows followed the same oestrous synchronization protocol. The cows were inserted controlled intravaginal drug release (CIDR) and removed on Day 8, followed by administration of prostaglandin. The heat mount detectors (HMD) were placed on the individual cow's tail head as an indicator for oestrous response. The HMD were originally white when placed but turned red when the cows were mounted. Cows were then inseminated twice at 12 hours interval. Pregnancy diagnosis was performed by an ultra sound scanner and rectal palpation 90 days after TAI. Data was analyzed by SPSS version 20. Oestrous responses were 100% in KZN and 99% in LP. The lowest pregnancy rate was recorded in Brahman and Bonsmara type cows with BCS of ≤ 2.5 regardless of Province. Interestingly, Nguni type cows with same body condition of ≤ 2.5 had higher average pregnancy rate of 59.5% in Limpopo and 53.5% in KZN. However, cows with BCS of ≥3 had better pregnancy rate

regardless of cow breed type, and province. In conclusion village cows can be synchronized successfully and inseminated with frozen- thawed semen; however the pregnancy rates are still low in cows with lower body condition. Village Nguni type cow was not affected by body condition scoring as they had higher and similar preganancy rate as those that had body condition of ≥ 3.

Keywords: synchronization, village cows, body condition, pregnancy diagnosis

4.1 Introduction

Cattle from villages are an important but an unproductive asset for South Africa as they comprise 42% of the national herd and contribute only 5% to South Africa's gross domestic product from beef industry. There are about 13 million of cattle in South Africa with over 5.5 million located in the villages and are comprised of different breed types (Nedambale, 2012). Furthermore the village cattle genetic information is unknown. Therefore, the need for livestock improvement has become more urgent in recent years with the increasing focus of the government and industries on food security (Hanotte *et al.,* 2002; Tada *et al.,* 2013). Increase in the beef industry and the rest of its value chain relies in the interventions that target this unproductive herd (village cattle). So achieving improvement, profitability and long-term viability of the emerging beef sector requires accessibility of the sector to all existing knowledge and technologies for better reproductive performance.

Good management practices and assisted reproductive technologies (ART) such as oestrous synchronization, artificial insemination and superovulation are one of the rarely used but vital practices for productive and profitable cattle farming (Wildeus, 2000). Oestrous synchronization assists in the accomplishment of faster livestock improvement programmes such as fixed time artificial insemination (FTAI) and superovulation of cows, thus minimizes costs, time and labor required for oestrous detection in cows and buying superior dams and sires. In this way, the transfer of genes by AI into populations of decreased biodiversity is made possible, uniform and less costly (Lopez da Costa *et al.,* 2001; Webb *et al.,* 2004). However, these ARTs are limited by a lot of intrinsic (individual animal variation, lactation status and health status) and extrinsic

(nutrition and weather condition and management practices) factors. Body condition score (BCS) of an animal may be used as a management tool for assessing the physiological states of cattle (Ciptadi *et al.,* 2012). The BCS is the most practical method of evaluating the energy reserves by the proportion of body fat all over the body. It is a management tool that can be used in estimating returns from livestock farming. It is reported to have a strong relationship with a cow's calving interval and the numbers of days open (Berry *et al.,* 2003; Suzuki *et al*., 2006 and Ciptadi *et al.,* 2012).

Village farmers rear cattle that are crosses between *Bos indicus* and *Bos taurus* that display a difference behaviorally and likely to lose the body condition unpleasantly in dry season (Ciptadi *et al.,* 2012). Improvement of reproductive performance of cows in the emerging sector has the potential to increase herd size, replacement and off take rates from smallholder farms. The indigenous knowledge and socio-economic situations alone might lead to inbreeding and unplanned genotypes the farmer has neither experience nor resources to manage (FAO, 2013).

The Nguni cattle breed represents a valuable contribution to the livelihoods of resource limited farmers of Southern Africa. It possesses exceptional genetic traits such as diseases and tick tolerance, longevity, adaptability in harsh environmental conditions and requires no supplements in dry seasons. Indigenous cattle breeds are currently subjected to fast degradation and dilution because of unplanned breeding, crossbreeding and introduction of exotic germplasm (Scholtz, 2005). Long postpartum oestrous period, low fertility, high number of services per conception, low birth weight and low average daily gain and lower weaning weight is a very common problem with small holder reared cows. Progesterone or a progestin analogue is generally used to

synchronize oestrous in cows during the breeding and non-breeding seasons. Therefore, the objectives of the present study were to evaluate the oestrous response and pregnancy rate following FTAI with frozen-thawed semen on different breeds of cattle found in emerging farmers of KwaZulu Natal and Limpopo Province.

4.2 Materials and Methods

4.2.1 Location and experimental cows

The study was carried out on small holder village cattle farmers of KZN (Nogejane, Besters, Mphuzanyni, Manzabilayo, Driefontein, Fitty Park, Machibini and Mthandi) (n=160) and Limpopo (Vhalinavho, Vuvha, Muyexe, Ga-Nkidikitlana, Dikgokgopeng, Taulomme, Bilangfontein, Ha- Masekona, Legrwareng and Prospect) (LP n=171). Cows were selected randomly, with the criteria of being non-pregnant and have a normal reproduction cycle, given birth before, regardless of parity, milk yield, age, breed and body weight. Selected cows were synchronized and fixed time artificial insemination (FTAI) during the December - March breeding season. The cows were grouped according to breed type and BCS into two groups on a scale of (1-5) where 1 is severely emaciated and 5 too fat. The types of breeds were identified at the time of oestrous synchronization and BCS were recorded before and after synchronization. The breeds were identified by their phenotypic traits of resemblance of Nguni type (phenotypically resembled Nguni cattle breed), Brahman type (phenotypically resembled Brahman breed) and Bonsmara (phenotypically resembled Bonsmara cattle breed).

4.2.2 Oestrous synchronization

Experimental cows in all the groups were synchronized using an ovisynch protocol that utilizes a Gonadotropin releasing hormone (GnRH) and prostaglandin (PGF2α). A controlled internal drug release (CIDR®) device containing 1.9g progesterone was inserted in the vagina of each cow on Day0 with an intramuscular injection (i.m) of estradiol benzoate (EB). On Day5, cows were

injected with a GnRH i.m to assist in the follicular growth. The CIDR was removed on the 8th day as per protocol and a PGF2α (Estrumate®, Schering-Plough Animal Health, USA) injection was administered i.m. Another i.m injection of EB that is half of the one administered at Day0 was given to the cows on Day9. Heat detector device that are white in color were mounted with glue on the tail head of each cow. These heat detector devices change color to red when the cow was mounted (indicating that the cows responded to the synchronization protocol).

4.2.3 Artificial insemination

The fixed time artificial insemination (FTAI) of the cows was performed 12 hours after the estradiol benzoate injection at the time of standing heat. Frozen thawed semen of registered Nguni bulls of superior fertility were used. Cows were inseminated twice at 12 hours interval.The sperm motility rate (non-progressive, progressive, slow, medium, and rapid) and the velocity on the curve line were evaluated and recorded using a Sperm Class Analyzer® (SCA-Microptic®, Spain) system before freezing and FTAI. Bulls with sperm motility results of ≥75% were used for AI.

4.2.4 Pregnancy diagnosis

Pregnancy status was diagnosed after 90 days following FTAI by transrectal ultrasonography of the reproductive tract and rectal palpation. Observations of embryonic fluid, appearance of the embryo or embryonic heartbeat were used as determinants of pregnancy. Pregnancy per AI was

calculated per province after FTAI. The side of *corpus luteum* (CL) formation was palpated in all pregnant cows.

Figure 4.1 Pregnancy diagnoses with an ultrasound scanner.

4.2.5 Data analysis

Frequencies of pregnant and non-pregnant cows in each breed type and BCS were analyzed using SAS version 9.3 2010 .

4.3 Results

In KZN Province, there was a 100% response of oestrous synchronization in all the breeds types (Nguni, Brahman and Bonsmara type cows) while Limpopo Province there was 99% response as shown in **Table 4.1**. The pregnancy rate was lowest on the cows with BCS ≤ 2.5 among the breed types; Brahman (25%), Bonsmara (29%) and an exception was observed with the Nguni type cows as (57%) pregnancy was observed in Limpopo. Cows with BCS of ≥3 had better pregnancy rate of 60, 61and 62% in Limpopo. Similar results were observed in KZN, lower pregnancy rates were on the cows with BCS≤2.5 (36, 35 and 45 Brahman, Bonsmara and Nguni breed types), but better in cows with BCS≥3 in 45, 52 and 60% in Brahman, Bonsmara and Nguni breed types.

Table 4.1. The pregnancy percentage amongst different breeds type after oestrous synchronization and artificial insemination by frozen-thawed semen in Limpopo and KwaZulu-Natal provinces.

Provinces	Breed types	Body conditions score	Cows (n)	Synchronization response (%)	Pregnancy rate (%)
Limpopo	Brahman	≤2.5	24	96	25
	Brahman	≥3	15	100	60
	Bonsmara	≤2.5	28	100	29
	Bonsmara	≥3	21	100	61
	Nguni	≤2.5	19	100	57
	Nguni	≥3	65	100	62
KwaZulu Natal	Brahman	≤2.5	30	100	36
	Brahman	≥3	20	100	45
	Bonsmara	≤2.5	31	100	35
	Bonsmara	≥3	19	100	52
	Nguni	≤2.5	20	100	45
	Nguni	≥3	50	100	60

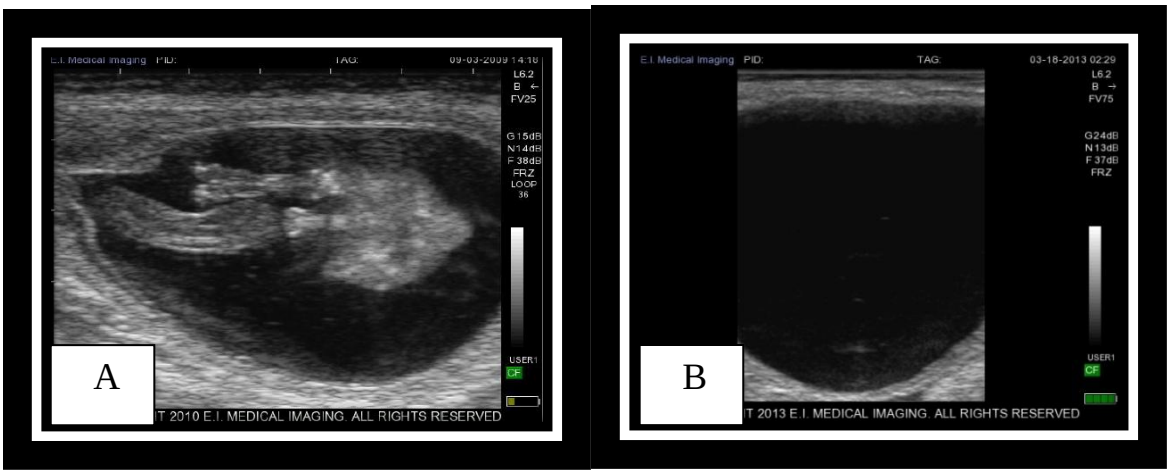

Figure 4.2: (A) Pregnant cow (B) Non pregnant cow as observed on the scanner.

4.4 Discussion

It was demonstrated that village cows regardless of breed type, body condition and province can be successfully synchronized and artificially inseminated with frozen-thawed sperm and carry pregnancy to calving. Heat mount detectors were helpful to obverse which cows came to heat. However, pregnancy and calving rate was low in cows with body condition score of ≤2.5. Heat detection is reported to be a tough and a labor intensive task that most breeders miss and end up not inseminating cows in time (Bruno *et al.*, 2013). For an economic beef production high pregnancy rates are vital. Infertility in cattle is mostly associated with female failing to show signs of heat and releasing an egg after every 21 days (oestrous cycle). Bruno *et al*. (2013) reported that with good oestrous detection method it is almost impossible to miss cows that responded to the synchronization protocols. Results from a similar study where two oestrous synchronization treatments were used oestrous was detected in 88% of cows from G1 where P4 was not supplemented and 95% from G2 P4 supplemented. Pregnancy rate results showed 75% of cows from G1 and 85% from G2, this indicates that a good oestrous synchronization program can result to higher response and resulting pregnancy rates in well managed cows (Santos *et al*., 2000). The results on the current study are similar to the observations found in the Madura beef cows where the response was high (75%) for oestrous synchronization but overall conception was lower (66.6%) due to most cows that had low BCS (Ciptadi *et al*., 2012). Yamada *et al*. (2003) reported that nutritional status of cow peripartum after oestrous synchronization influenced conception rates and postpartum ovarian cycles. Cows with BCS ≥4 and ≤2.5 are reported to have a delayed onset of oestrous (Captadi *et al.*, 2012). Cows with BCS of 2.5 or less have displayed conception rates vividly lower with the exception of Nguni type in both

Provinces and in all the three breeds. The Nguni cows had higher pregnancy rates than Bonsmara and Brahman, the majority of the Bonsmara and Brahman cattle had very low BCS in these villages and also their conception rates were reduced (Nqeno *et al.,* 2009). Reproduction characteristics are affected by loss of condition in cows before calving the best performances were recorded in females with medium BCS 2.75 to 3.50 (Mouffok *et al.,* 2011).

Similar results were reported by Captadi *et al*. (2012) where BCS was highly related to reproductive performance: thinner cows showed lower reproductive performance, whereas higher BCS had positive associations with days to first oestrous, interval to first service and conception rate at first service. The results of the current study for lower pregnancy rates might be due to diseases and nutrition but the communal pasture setting provides a unique environment for the study of disease and herd problems (Stewart *et al.,* 1998). It represents a combination of cows from a variety of management systems (vaccinated and non-vaccinated), brought together as a group for the breeding and grazing season. It also poses a challenge when it comes to controlling infectious disease, because of the intermingling of animals from different herds (Stewart, 1998). Hence it makes it difficult to identify whether cows get adequate feed or not. In addition to low nutritional status and BCS, there was no strict separation of calves from their mothers due to lack of camps and limited grazing lands. The farmers allowed the calf to suckle the mother until it was naturally weaned. In dry season, this practice is harder for the cows as they do not get enough nutrient supply for milk synthesis. As a consequence, nutrients reserve in the body is mobilized to compensate nutrient required for milk production, this results in cows losing their body condition during lactation. The higher pregnancy was observed in Nguni type cows in the villages of both Provinces Nguni cattle breed has been reported to be resistant to

infection such as *trypanosomiasis*, *opthalmia*, heart water and other tick-borne diseases and tick infestations (Muchenje *et al*., 2008b; Marufu *et al.,* 2010). The breed show exceptionally good fertility under harsh conditions, with excellent reproductive performance (Nqeno *et al*., 2009). Nguni cattle are less susceptible to dystocia, this being ascribed to their small uterus and low birth weight (Rani *et al*., 2011). Ngunis are excellent foragers and will graze and browse on steep slopes and in thick bush. They fatten well on natural grazing (Muchenje *et al.,* 2008a). They have long productive lives; cows calve regularly and produce 10 or more calves. The experiment was only limited to cows from parity 1-6. Similar results were observed on Madura cowswhere the highest AI success was achieved in cows with BCS 3.5 (75.0%). Cows on acceptable BCS 3-4 that do not conceive after oestrous synchronization and AI are problem cows its either they are sick, late calvers and infertile. Cows that calve early will have more days postpartum before the beginning of the next breeding season. Thus, compared to late calving cows, early calving cows will have resumed normal oestrous cycles and fertility by the beginning of the next breeding season (DeJarnette, 2004). Increased fertility for cows artificially inseminated upon oestrous detection has been reported (Jordan *et al*., 2002; Kasimanickam *et al*., 2005; Bruno *et al*., 2013) and is most likely due to the cows completing the FTAI protocol. Most of the cows that did not conceive are cows that were not identified in oestrous because they experienced some issue such as false oestrous, negative energy balance, uterine or metabolic diseases, asynchrony to the reproductive program. However, even though cows were detected on oestrous pregnancy rate were highly affected by the body condition of cows. As reported by Wardynski (2013) cows that do not maintain adequate body condition going into the calving and breeding seasons, will usually have reduced conception rates and consequently, higher culling rates. However, lower BSC is generally related to the lower progesterone levels in the blood and over conditioned cows

have been reported to have a higher risk of dystocia and metabolic disorder (Schroeder and Staufenbiel, 2006).

4.5 Conclusion

The village cows can be synchronized successfully for oestrous regardless of the breed, BCS and the geographical area. Pregnancy rate was lower in breed types that had BSC less than 2.5 on a scale of 1-5. Frozen- thawed semen can be used for FTAI if the BCS is improved. The results that were observed in the study show that FTAI has a potential of increasing productivity in the small holder cattle herds and improve the genetic material if only the BCS can be improved by reducing the number of older animals, vaccinating the whole herd, culling infertile animals and reducing the stocking rate in accordance to the carrying capacity of the veld so that the BCS of cows improves for the best reproductive performance.

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Chapter 5

5.1 General Discussion

In Chapter 3 Nguni stud cows (n= 15) and heifers (n= 10) aged 4-6 and 2-3 years were used as embryo donors, it was observed that the superovulation and fertilization rate were not different between Nguni cows and heifers. Lower response of Superovulation (SO) might be due to intra group and individual animal variations. The present study used diluted raw semen from two bulls after motility analysis with CASA® system. High Fertilization rate was obtained from the bull that had higher percentage of total sperm motility (93.7 %) which was used to inseminate the cows followed by bull 2 (83.5%) which was used to inseminate heifers. However the bull that had sperm with lesser number of progression and velocity was not used for AI. The results of fertilization rate were comparable to the results of the sperm motility obtained after analysis with CASA®, higher total motility bull semen resulted in higher fertilization rates and the correlation results indicated a strong correlation between total sperm motility and fertilization. Better results were obtained by Otava 2010 when using conventional over sex sorted semen were analysed by CASA® before AI and lesser total motility and viability of sexed sperm were reflected on the conception rate which was lower (31.81%) while 75% conception rate was recorded on the conventional sperm.

In the current study, Nguni cows showed better ovary reaction measured by number of CL present compared to heifers. Cows had a higher number of unfertilized ova and degenerate embryos, even though overall SO response was similar among these two groups. The results of the present study were in contrasts with findings from the dairy breed where the heifers had a greater number of CL than cows. This is due to individual animal variations, negative energy

balance on the dairy cows imposed by lactation. Usually low response means less ovulations and low number of *corpus luteum* (CL) in the Nguni cows some had a higher number of *corpus luteum* but no ovulation. In the ewes it was reported that the response to ovarian stimulation is affected by the breed being used, nutrition and season of the year (Bettencourt *et al.,* 2008). Failure of cows to conceive has been reported to be as closely associated with the Graafian follicle development and maturation, oestrous onset, successful coitus, ovulation, fertilization, implantation, and the development and delivery of the foetus and its membranes. Anything interfering with these routines, such as diseases, poor nutrition, inadequate herd management, hereditary and congenital factors, hormonal disturbances or environmental changes, makes the animal infertile (FAO, 2013).

In Chapter 4 it was observed that neither body condition nor breed affected the oestrous synchronization response in both provinces KZN (100%) and Limpopo (98%). Bruno *et al*., (2013) reported that with good oestrous detection method it is almost impossible to miss cows that responded to the synchronization protocols. Even though cows responded very well on oestrous synchronization conception was lower.The results on the current study are similar to the observations found in the Madura beef cows where the response was high (75%) for oestrous synchronization but overall conception was (66.6%) due to most cows of low BCS (Captadi *et al*., 2012). Yamada *et al*., (2003) reported that nutritional status of cow peripartum after oestrous synchronization influenced conception rates and postpartum ovarian cycles. Cows with BCS >4 and < 2.5 are reported to have a delayed onset of oestrous and its longer (Captadi *et al.*, 2012). Cows should be routinely assigned a BCS so that becoming too fat or thin is avoided as it results in health problems and reduced conception. Cows with BCS ≤2.5 or less have displayed conception rates vividly lower in both Provinces and in all the three breeds. The Nguni cows had

higher pregnancy rates than Bonsmara and Brahman type. The majority of the Bonsmara and Brahman type cows had very low BCS in these villages and hence their conception rates. Reproduction characteristics are affected by loss of condition in cows before calving the best performances were recorded in females with medium BCS 2.75 to 3.50 (Mouffok *et al.*, 2011).

5.2 Conclusion

Nguni cows responded to superovulation and had better ovaries reaction compared to heifers although response rate was lower for both cows and heifers. The quality of embryos recovered was similar for both Nguni cows and heifers. Moreover, there was a positive correlation between total sperm motility and fertilization rate bull 1 (93.7%) inseminated the cows (67.5%) and bull 2 (83.5%) inseminated the heifers (53.5%). Pregnancy diagnosis of 60% and 0% were recorded in Limpopo (LP) and Eastern Cape EC. Interestingly, more than 99% of village cows responded to synchronization and inseminated with frozen- thawed semen successfully. Village Nguni type cow was not affected by body condition scoring as they had higher and similar pregnancy rate as those that had body condition of ≥ 3. It is suggested that it is not advisable to breed synchronized Brahman and Bonsmara type cows with the body condition of ≤ 2.5 except in Nguni cow type as 51% average pregnancy might still be achieved. The village cows can be synchronized successfully for oestrous regardless of the breed, BCS and the geographical area.

5.3 Recommendation

It is therefore recommended to further investigate the response to superovulation variation and factors that influence the quality of the Nguni embryos. It is recommended that more research should be done to investigate the precise dose suitable for Nguni cows and heifers. It is suggested that Nguni heifers be excluded from superovulation and flushing due to their small cervix that is difficult to pass during AI and flushing of the uterine horns. Furthermore, embryo flushed should be transferred to a large number of recipients and the conditions should be as homogeneous as possible.

The results that were observed in the study show that FTAI has a potential of increasing productivity in the small holder cattle herds and improve the genetic material if only the BCS can be improved by reducing the number of older animals, vaccinating the whole herd, culling infertile animals and reducing the stocking rate in accordance to the carrying capacity of the veld so that the BCS of cows improves for the best reproductive performance. It is also recommended that selection of cows for ART be selected before the breeding season so that superior cows can be used to maximize the pregnancy rate.

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