Effects of Ovulation-Inducing Drugs on Pregnancy Rates of Cattle in Rural Areas after Synchronized Oestrus and Artificial Insemination

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DECLARATION

I Luvhengo Dakalo Nethengwe, hereby declare that this dissertation submitted by me in fulfillment of the requirements for the Masters in Rural Development (MRDV) at the Institute for Rural Development, University of Venda has not previously been submitted for a degree at this or any other university, and that all reference material contained therein has been duly acknowledged.

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ABSTRACT

The effect of hormones that play a role during ovulation in the cow, like oxytocin, prostaglandin $F_{2\alpha}$ (dinoprost $PGF_{2\alpha}$) and human chorionic gonadotropin (hCG) were used to increase the ovulation rate, therefore pregnancy rates, when injected intramuscularly at the time of artificial insemination (AI). Frozen-thawed Bonsmara and Nguni semen was used in synchronized cows of different ages, breeds and body condition scores (BCS). The main objective of this study was to evaluate the effect of oxytocin, $PGF_{2\alpha}$ and hCG on the animal's fertility measured as conception rates (CR) and to improve cattle genetics in the rural communities of Vhembe district by the use of AI. A total of 205 beef cattle (142 cows and 63 heifers) were used in this study. Cows and heifers were synchronized in groups by inserting CIDRs in the vagina with 2 mg of oestradiol benzoate (Cidirol) intramuscular injection on day 0 of synchronization. On day 6 in the afternoon, females were injected with 25 mg prostaglandin $F_{2\alpha}$ (dinoprost Lutalyze). On day 7 in the morning the CIDRs were pulled out of the vagina and in the afternoon the females were injected with 2 mg of oestradiol benzoate (Cidirol). During day 8 to 9 signs of possible oestrus were visually observed by cattle owners three times a day for a minimum of 30 minutes a time. From day 9 to 10, AI was performed following the AM/PM rule by the same technician using good quality frozen-thawed semen 12 hours after the beginning of standing oestrus. Cows and heifers were alternately assigned to one of the three ovulation inducing drugs: 300 IU hCG (Chorulon), n = 52; 25 mg $PGF_{2\alpha}$ hormone (dinoprost Lutalyze), n = 49; 50 IU oxytocin hormone (Fentocine), n = 46; or 5 ml sterile isotonic (saline) as a control, n = 58. The pregnancy results between the three ovulation inducing drugs and sterile isotonic saline were statistically analyzed using General Linear Model (GLM) procedures of minitab (minitab 2013) using 2 × 4 factorial in a completely randomized design. The treatment means were compared using Tukey's post hoc test. Significant was set at $p<0.05$. On average the control treatment had the highest pregnancy rate followed by hCG, $PGF_{2\alpha}$ and oxytocin respectively, across all the age groups data, although this was not significantly different ($p>0.05$). Pregnancy rate was lowest in the animals that received oxytocin and was significantly different from the other three treatment ($p<0.05$). Other factors such as semen breed and body condition score did not significantly affected the pregnancy rates ($p>0.05$). Thus, there was no improvement in pregnancy rates among communal beef cattle by administering hCG, oxytocin and $PGF_{2\alpha}$ at the time of artificial insemination in the present study.

Key words: Oestrous synchronization, AI, hCG, $PGF_{2\alpha}$, Oxytocin, BCS and pregnancy rate.

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LIST OF ABBREVIATION

ADH	Anti-diuretic hormone
AI	Artificial insemination
AM	Morning
ARC	Agricultural Research Council
ART	Assisted reproductive technology
AV	Artificial vagina
BCS	Body condition score
BSE	Breeding soundness examination
CBPR	Community-based participatory research
CIDR	Controlled internal drug release
CL	Corpus luteum
CR	Conception rate
DMSO	Dimethyl sulphoxide
FSH	Follicle stimulation hormone
GLM	General linear model
GnRH	Gonadotropin releasing hormone
GSH	Glutathion
hCG	Human chorionic gonadotropin
IU	International unite
MGA	Melengesterol acetate
ml	Millilitre
PG	Prostaglandin
PGF2	Prostaglandin $F_{2\alpha}$
PM	Evening / Night
TAI	Timed artificial insemination
USA	United States of America

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CHAPTER 1

1.1. General Introduction

Rural development is a strategy which enables rural people to gain secure and decent livelihood (Chamber, 1983). The improvement of communal cattle production can be a sustainable way to improve the livelihoods of the rural population in Southern Africa. There is however, little information and research conducted to characterize, understand and develop the communal cattle production systems in Southern Africa (Tavirimirwa *et al.*, 2013). Thus, livestock production in rural development does not only involve improving livestock production for its own sake, but rather enabling rural people to use livestock as a source of survival and well-being, together with all the other activities which make up the livelihood system of the men, women and children who keep these animals (Walters-Bayer & Bayer, 1992).

Communal cattle fulfill multiple role that include milk, manure, (Masikati, 2010; Ndlovu *et al.,* 2004; Khombe, 2002), draught power (Chimonyo *et al.,* 1999), and serve as an indication of one's wealth status (Maburutse *et al.*, 2012), but the largest contribution comes from red meat producing species that include cattle, sheep and goats (Ramsay *et al.,* 2007). Cattle hides are used to make drums, tents and mats, (Musemwa *et al.,* 2007; Mapiye *et al.,* 2006). A survey by Ndebele *et al*. (2007) in Matabeleland revealed that cattle are used as investments and a status symbol. Cattle, thus, generate income among communal households through sales of the animals and their products. Improvement in cattle production and innovative value addition of cattle can create employment for people as individuals are hired to process and sell cattle and their products at various points of the production chain. Cattle play a pivotal role in socio-cultural function such as *lobola* payments and appeasement of ancestors (Maburutse *et al.,* 2012). They are also useful in nutrient recycling in communal rangelands (Tessema *et al.,* 2011). Beef is a high-quality protein that contains all essential amino acids for the human body and also contains additional essential nutrients such as iron, zinc, B vitamins, riboflavin, selenium, choline, and conjugated linoleic acid. Adopting of reproductive technologies at greater rates than currently used is a viable method to dramatically enhance production efficiency of communal beef cattle enterprises (Lamb & DiLorenzo, 2014).

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Animal agriculture is a major factor in household food production and food security in rural areas, and there is vast potential for increased production efficiency and economic activity. This can, however, only be achieved by providing an integrated support infrastructure that includes improved animal husbandry, access to markets, technology transfer and appropriate financial support. It has been estimated that a 50 % improvement in veld and herd management will double the production from the developing and communal areas (Red Meat Status Report, 2005). Agricultural research council, (2014) reported that animal reproductive technology (ART) can have an impact on improving the pregnancy rate of cows in rural areas, since smallholder farmers cannot afford improved genetic bulls for their cows. Livestock farming is a mainstay of South African agriculture, but it is difficult for the emerging black farmers to break in to the market, where a bull costs at least R 5000 .00. Without new genetic material being introduced to the herds of smallholder farmers, the animals can be inbred, compromising their health and ability to reproduce.

Nowers *et al.* (2013) stated that the pregnancy rate with natural breeding in the rural areas is around 30 %. This was also observed by ARC, (2014) that the calving percentage for beef cattle is 35 % in the communal sector and 62 % in the commercial sector. More emphasis should therefore be put on increasing fertility. Nedambale, (2014) reported that this is why the ARC, which is funded by the South African National Department of Science and Technology and the National Department of Agriculture, Forestry and Fisheries, developed the mobile laboratory for artificial insemination and embryo transfer to improve animal fertility in rural areas for the smallholder farmers.

Ramsay *et al.* (2007) reported that matching animals to both environment and the production system is a key factor in sustainable animal agriculture and by integrating information on the environment with information on species and breeds. Thus, stock owners will be able to make more informed choices on the breeds and combinations best suited to individual production inputs (Red Meat Status Report, 2005). Artificial insemination is the most important single technique devised for the genetic improvement of animals in communal farming system. This is possible because a few highly selected males produce spermatozoa to inseminate thousands of females per year, whereas only relatively few progeny per selected female can be produced per year (Hafez, 1993).

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Artificial insemination, the act of mechanically and unnatural depositing of semen into the female reproductive tract with the goal of achieving conception, was truly the first ART to be practiced. As the most frequently used ART procedure today, AI has had and continues to have a tremendous impact on the various animal breeding industries (Althouse, 2007). In production animals, AI is a way to increase reproductive efficiency and production. Artificial insemination has proven to be a very effective reproductive technology that selectively increases genetic gain through increased selection pressure on males. In Holstein cattle, for example, AI supported selection for the milk production trait and within 40 years milk production has nearly doubled (Heise, 2011). Farm animals, male as well as females, are usually chosen for breeding programs based on breeding soundness examinations (BSEs). These BSEs determine suitability and likelihood of females or males to participate successfully in breeding programs. Animals that do not fulfill certain criteria are identified. These "problem" animals are excluded from insemination programs (Heise, 2011).

The oestrous cycle of females can be manipulated to establish efficient insemination programs. With the use of oestrous synchronization programs, large groups of females can be inseminated at the same time. This does not only have the advantage of concentrating work on specific days during breeding, but will ultimately also simplify the herd management before and after the offspring are born nearly all at the same time. Group feeding of pregnant animals, parturition observation, vaccination programs for calves and tail docking of lambs are just a few examples of improved herd management practices through the group effect achieved through oestrous synchronization of female animals (Heise, 2011). Another reason for AI is to ensure the effectiveness of the use of semen. An increased number of offspring from a superior sire can be produced when AI is employed. For example, a bull's ejaculate can be sufficient to inseminate 200 to 250 cows when split into doses instead of impregnating one cow. Ram ejaculates can also be split into up to 15 or more fresh AI doses. Overuse of males is prevented and commercial distribution of superior bull semen is facilitated (Heise, 2011).

Other important aspects are the prevention of venereal disease transmission that plays a major role in the economic system of offspring production, and increased safety for valuable breeding animals as mating related injuries are avoided. Venereal diseases that play a major economic role in cattle production are for example Trichomonosis and Campylobacteriosis, both of which decrease reproductive efficiency through decreased pregnancy rates, high return rates to oestrus and increased pregnancy losses, therefore remain a hindrance to increased production

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efficiency of cattle production. In general, shipping fresh and frozen semen nationally and internationally involves fewer health risks and welfare implications than transporting live animals (Foote, 2002).

Import and export of frozen semen is a huge economic market. Semen of a specific bloodline, a specific individual male or breed can be imported. This is especially important in countries where breeds were introduced and are somewhat isolated with a small genetic pool. To eventually prevent breeding setbacks due to inbreeding and to expand the genetic pool, imported semen is used. Furthermore, semen can be used from males that have died or are not physically available for mating due to distance or physical inability. A great advantage of frozen semen in general is that it can be stored indefinitely and has the potential to outlive the male donor animal by years (Heise, 2011).

The advantage of AI in the rural areas is that there is no need of maintenance of a breeding bull for a herd; hence the cost of maintenance of breeding bull is avoided. Artificial insemination enables the widespread use of outstanding sires and dissemination of valuable genetic material even to small farms in the rural areas. Artificial insemination facilitates progeny testing under a range of environmental and managerial conditions, thereby further improving the rate and efficiency of genetic selection. Artificial insemination leads to improved performance and potential of the national herd and permits coordination of a breeding policy on a national basis, and it also permits crossbreeding to change the production emphasis, such as switching from milk to beef. Artificial insemination can accelerate the introduction of new genetic material through the import of semen. Artificial insemination reduces international transport cost and permits the use of semen from incapacitated or oligospermic males. Artificial insemination also reduces the risk of spreading sexually transmitted diseases such as Trichomonosis and Campylobacteriosis in the rural areas, since all the equipment used are sterile (Hafez, 1993).

Althouse (2007) reported that some of these advantages can be considered disadvantages, given that this technology can help spread genetic defects and it requires an increased level of training and intensive breeding management to successfully integrate the technique into a system. The majority of breeding in modern dairy cattle, poultry and swine production system is performed by AI. Artificial insemination was the first assisted reproductive techniques applied to control and improve reproduction as well as genetics. Foote (2002) stated that the first

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successful insemination was performed by the Italian physiologist and priest Abbe Lazzaro Spallanzani 1784 in a dog which whelped three pups 62 days later.

The establishment of AI as a practical procedure was initiated in Russia in 1899 by Ivanov who studied AI in domestic farm animals, dogs, foxes, rabbits and poultry. He also developed semen extenders. Milanov, another Russian scientist and successor of Ivanov, started large scale breeding programs for cattle and sheep, and designed and produced artificial vaginas (AV). Horse breeding programs and research was initiated at the same time in Japan even though translation of the original research only became available to the western world after 1958. Some AI work, especially in horses and cattle, had been done in Denmark in the early 1900s. It was Danish veterinarians who established the method of rectovaginal fixation of the cervix for insemination in cattle which enabled semen deposition deep into the cervix or into the uterine body (Foote, 2002). This technique is still used today. Another Danish invention was the straw for packaging semen. These straws have been further developed and modified by the French and are now used worldwide for processing and storage of frozen semen (Foote, 2002).

Research on artificial insemination in Italy led to the development of an artificial vagina for dogs in 1914 and to the establishment of the "International congress on AI and Animal Reproduction" in 1948. This congress is held every four years since 1948 (Foote, 2002). Rapid development of AI in dairy cattle occurred in the USA in the 1940s. One of the important milestones was the establishment of the "Dairy breeding research center" on the campus of the Pennsylvania State University in 1949 to assist in the development of artificial insemination in dairy cattle. Interest in and development of frozen semen started with successful cryopreservation of gametes from a variety of animal species after discovery of the protective action of glycerol by scientists in Cambridge, England in 1949 (Amann & Pickett, 1987).

The role of AI in rural areas is to combine adaptability, hardiness, disease resistance and heat tolerance of local beef breeds with the high quality genetic bull semen with faster growth rate, higher milk production potential and feed conversion efficiency as well as short inter-calving period of 365 days between the first and second calf (Kedede, 1992). AI can enable rural stockowner to improve their livestock production, management skills and their living standard and therefore generate income on a more regular basis (Red Meat Status Report, 2005).

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1.2. Problem Statement

Livestock in Vhembe district, namely cattle, goats and sheep, shows clear sign of inbreeding (mating of related animals) over decades of farming practices. The indications include petit body size, slow average daily weight gain, decreased fertility, and loss of natural resistance against disease and parasites as well as extended calving intervals. Lack of controlled breeding in communal areas has caused inbreeding, which results in poor growth rate in cattle (Webb & Mamabolo, 2004).

There are no structured breeding systems and appropriate infrastructure such as paddocks in the communal land and, therefore, cows and bulls of unknown genetic merit and bloodlines run together all year round. Traits that used to measure reproductive performance of cattle such as calving rate, calving interval and age at first calving under communal management are extended due to inbreeding and calf mortality is very high (Tavirimirwa, *et al,* 2013).

An extremely uncharacteristically high mortality among calves and slow growth among those that survive are the major constraints to cattle production in communal areas. Low pregnancy rate is one of the major causes of economic loss to cattle farmers (Mashoka *et al.*, 2007), and is mainly due to lack of good genetic bulls as most farmers cannot afford to buy highly genetic improved bulls to accelerate animal's fertility in Vhembe district.

1.3. Justification

A well-planned artificial insemination program that introduces new genetic materials in the Vhembe district can address this shortcoming in animal production, but the limitation of land and separation of bulls from cows during oestrous synchronization due to a lack of farming infrastructure, will hamper such a scheme. The utilization of frozen spermatozoa from improved genetic bulls for AI could be a valuable tool to combat the presently prevailing inbreeding and increase the genetics and cost value of offspring in the cattle production of the target communities in the Vhembe district. This will alleviate and help to eliminate venereal diseases among the cattle.

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1.4. Main objectives

- To improve the genetic value of livestock by the use of AI with highly improved genetics bulls semen after oestrous synchronization in Vhembe district. This will increase milk and beef production which will improve the livelihoods system of smallholder farmers in the rural communities.
- To provide technical skills to rural livestock breeders with proper animal breeding principles, production management and reproduction.

1.4.1. Specific objective

- To evaluate the effect of three ovulation induction drugs (oxytocin, dinoprost prostaglandin $F_{2\alpha}$ and human chorionic gonadotropin (hCG)) on the animal's fertility measured as pregnancy rates.

1.4.2. Hypothesis

- The pregnancy rate obtained with frozen thawed semen purchased from a reputed AI company can be higher than normal breeding practices, when synchronized cows of different age, condition scores and breeds are inseminated in the Vhembe district of the Limpopo province.
- Drugs that play a role during ovulation in the cows, like oxytocin, dinoprost prostaglandin $F_{2\alpha}$ and human chorionic gonadotropin (hCG) will increase the ovulation rate, and therefore pregnancy rate, when injected intramuscularly at the time of insemination.

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CHAPTER 2: LITERATURE REVIEW

2.1. Pregnancy rates in smallholder cattle in the communal farming system

The livestock industries in developing countries are highly dualistic with commercial, emerging and communal sectors (subsistence or small-scale farmers) all co-existing. While the off-take (slaughter rate) from the commercial sector is at an acceptable level, the off-take from the other sectors is still low in certain countries as a result of low fertility and high mortality; which are mainly the result of diseases and parasites, lack of feed resources and poor rangeland management (Chimonyo *et al.,* 2000; Montshwe, 2006; Musemwa *et al.,* 2007). The major discrepancies in production and throughput between the various sectors are linked to aspects such as pre-weaning mortality, herd composition and calving percentage (Scholtz & Bester, 2010). Mokantls, (2004) revealed that the fertility of beef cattle in communal farming system is said to be low, taking calving percentage as a measure of production. Studies involving structured interview techniques estimated the calving percentage of beef cattle in communal farming systems in South Africa at 14.9 % and 41 % (Bembridge & Tapson, 1993; Nthakheni, 1993). Studies done in communal grazing areas of Zambia recorded calving percentages of 44 %, 88 % and 27.9% (Perry *et al.*, 1984). In Botswana a survey that combined the structured interview technique with rectal pregnancy diagnosis and monthly recording of calving estimated the calving percentage of cows on communal farms at 36 % and 50% (Reed *et al.*, 1974).

The optimal level of performance in the commercial sector should yield a calving percentage of 95 % to 99 % and the target should be 98 % (Mokantla *et al.*, 2004). In South Africa, the Brahman Cattle Breeder's Society has set a target calving percentage of 70 % for stud herds (Editorial, 1994). Calving percentage is the number of calves born per number of female cattle exposed to a bull expressed as a percentage (Chenoweth, 1994; Mossman, 1984; Youngquist, 1997). This is also called effective calving percentage. Calving percentage does not relate to the dates of birth or when calves were born during the calving season. All full-term calves are included in the number of calves born, even if they are dead on arrival. Calving percentage is a good indicator of breeding performance and herd fertility (Collett, 1998; Mossman, 1984; Youngquist, 1997). Calving percentage is influenced by pregnancy rate and pregnancy loss percentage. A low calving percentage indicates that a problem exists in a herd, but does not

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indicate the cause of the problem and where it occurs. A low calving percentage may indicate that bull fertility is inadequate, the nutritional programme is inadequate, that there is disease causing pregnancy loss, or that there is a mismatch between herd genetics and the environment (Mickelson, 1990; Vanroose *at al.*, 2000). Only when these management factors are addressed, it will be the formulation of breeding objectives or strategies that will have an impact in the emerging and communal sectors (Scholtz *et al.,* 2013). Furthermore, subsistence farmers in Africa keep livestock for multiple purposes and rural households depend on livestock for milk, meat, hides, horns, fertilizer and income (Chimonyo *et al.,* 1999; Dovie *et al.,* 2006). Livestock is therefore central to the livelihoods and wellbeing of rural communities. This makes performance recording difficult since the breeding objectives may include many traits, some of which cannot be easily measured or quantified, as they are related to social or cultural practices (Scholtz *et al.,* 2011). The formulation of breeding objectives and strategies in the local African breeds requires an infusion of good improved bulls genetic to increase their reproduction potential. This has been successfully achieved through artificial insemination in many countries, and recently AI was combined with synchronization of oestrous (sexual receptivity) in Ethiopia (Alastairs, 2013).

The Animal reproductive technology with the greatest potential to enhance rate of genetic improvement is AI in the communal beef cattle. The other reproductive technologies, with the exception of embryo transfer, only have a minor impact on genetic improvement in the rural communities because of technical limitations such as which occurs with decreased fertility and offspring viability with use of these technologies (Basrur & king, 2005). Genetic variables such as genetic gain per unit of time, progeny superiority as a results of the increased selection differential, decreased generation intervals and extent of inbreeding resulting from the use of AI. These genetic variables are indicators of the impact that the use of AI can have on improving genetics in communal beef cattle (Evans, 1991). This technology has, therefore, been used in the beef industry in a manner that has not increased inbreeding to the extent that detrimental impacts such as decreased reproduction have occurred (Bradley, 1998). Artificial insemination was the first assisted reproductive technique applied to control and improves reproduction as well as genetics (Foote, 2002). Artificial insemination has been and still is the most used reproductive technique in animals (Heise, 2011).

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2.2. Semen Handling in the Liquid Nitrogen Tank (LN_2)

Success of AI is dependent on the quality of fresh semen and its capacity for dilution and storage with minimum loss of fertilizing ability. The best preservation technique to date of post-thaw survival is restricted to about 50% of the sperm population (Watson, 1995). The final cryopreservation goal of semen is not only to maintain the initial motility, but also to maintain the necessary metabolism to produce energy and plasma proteins to survive in the female reproductive tract during fertilization. In addition, the acrosomal enzymes for the penetration of the ovum, capacity of progressive movement and to prevent any damage which reduces life span of spermatozoa and its fertilizability (Palacio-Ango, 1994). The major factor affecting the results of insemination with frozen-thawed semen is the addition of cryoprotectants (Aboagla & Trade, 2004), extension media, extension rate and spermatozoa. The damage to the spermatozoa is due to the formation of internal ice crystals. And then, to an increase in solute concentration in the extension media or interaction of both physical factors (Leboeuf *et al.*, 2000).

Webb, (2008) report that bull semen could be successfully frozen and stored for an indefinite period has revolutionized AI in cattle. In 1949, British scientists discovered that addition of glycerol to the semen extender improved resistance of sperm to freezing. Glycerol acts to remove water from the sperm cell prior to freezing and prevents the formation of intra-cellular ice crystals which would damage the sperm (Sudeep & Kanimozhi, 2009). The cryoprotectants are added to extenders to protect the sperm from damage during the freezing process (Singh *et al.*, 1995).

The level and type of cryoprotectants in semen diluents influence these events and their effects on the sperm cells during freezing. Most semen preservation protocols still favor glycerol as the cryoprotective medium (Abdel-Khalek *et al.*, 2008). In certain instances, other cryoprotectants are possibly better, for example, dimethyl sulphoxide (DMSO) was preferred for bovine and buffalo bulls (Gabr, 2009). Also, ethylene glycol (Rodrigues *et al.*, 2004), acetamide and lactamide (Nagase *et al.*, 1972) provided good protection to bull spermatozoa during freezing. However, little information is available on the combinations of extenders for bull semen. On the other hand, mammalian sperm cells are susceptible to lipid per-oxidation by O_2 and H_2O_2 due to their high content of polyunsaturated fatty acids in their membranes and the lack of significant cytoplasmic components containing antioxidants which result in a decreased sperm motility and

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viability (Statish & Das, 2005). One of enzymes that are distributed in sperm cells is reduced glutathion (GSH) where one of its functions is to protect cell against the destructive effect of reactive oxygen species (Meister, 1983; Lewis *et al.*, 1997). Bernstein and Petropavlovsky were the first to describe the use of a 9.2 % glycerol solution for cryopreservation of mammalian spermatozoa including bull, boar, guinea, pig, rabbit, ram, stallion as well as avian, fowl and duck spermatozoa (Pesch *et al.*, 2007).

There are two methods of freezing and storing semen namely dry ice-alcohol (-73 ºC) and liquid nitrogen (-196 ºC). Liquid nitrogen is preferred because there is no evidence of fertility deterioration with age. Fertility gradually declines in semen stored in dry ice-alcohol (Foote, 1974). Frozen semen can be stored indefinitely if a proper temperature is maintained. A previous article reported of a calf born from frozen semen stored for 16 years. Fresh, liquid semen can be successfully stored for 1 to 4 days at 4 ºC (Grove & North, 1965). Semen is usually stored in French straws and several AI organizations have gone to this method exclusively. Artificial colouring is frequently added to semen extenders in order to distinguish one breed from another. Complete identification of the bull is required on each individual semen canister as well as the LN_2 tank container (Foote, 1974).

When extended semen cools during the freezing process, micro-environments are created within the semen package. Each chemical component of extended semen freezes or solidifies at a different temperature. Water freezes as temperatures drop below 0 ºC forming ice crystals which remain somewhat unstable at temperatures above -44 ºC (O'Connor, 2013). This instability may be due to recrystallization of the ice. Also, as water is converted to ice, the sperm are exposed to the remaining concentrated solution of salts and other components of extender which freeze at temperatures considerably below the freezing point of water (O'Connor, 2013). Instability of ice and concentrated solutions are harmful to sperm. Fortunately, incorporating glycerol as a cryoprotective agent and improving freezing rates have minimized sperm damage, however, semen must be kept at temperatures well below critical temperatures where the recrystallization of ice begins to occur (Michael, 2012).

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2.3. Semen Tank Management

The semen storage tank is a large vacuum-sealed metal bottle with an extremely efficient insulation system. Because of the vacuum bottle construction, the temperature can remain at -196 °C (liquid nitrogen temperature) as long as at least 4.8 cm of liquid nitrogen is present. Technical advances in design and construction have produced storage tanks with a liquid nitrogen holding time of 6 to 9 months (Pesch *et al.*, 2007). Although semen storage tanks are well constructed, they are still susceptible to damage from mishandling. Semen tanks should be kept in clean, dry and well ventilated areas. Avoid excessive movement of the tank. The inner chamber, which contains liquid nitrogen, is suspended from the outer shell by the neck tube. Any abnormal stress on the neck tube caused by sudden jarring or an excessive swinging motion and can crack the tube. This results in vacuum loss from the outer chamber and rapid evaporation (Michael, 2012).

To increase holding time, keep the tank in a cool location away from direct sunlight. Avoiding drafts from furnaces and outside air also helps prevent excessive liquid nitrogen evaporation (Lucero, 2015). However, make sure there is sufficient ventilation in the room to prevent possible suffocation which can be caused by excessive nitrogen gas in the air you breathe. Protect the tank from corrosion by keeping it elevated above concrete or wet floors. Use boards or pallets and pick a location that is safe from children and vandals, but do not hide the tank. It must be placed where it can be seen daily and where it can be monitored routinely for nitrogen level (Selk, 2012). Finally, always be watchful for a lid that is left off and for frost or sweat on the tank. Give particular attention to the neck and vacuum fitting (O'Connor, 2013). Frost indicates that the vacuum insulation has been lost and liquid nitrogen has been or is evaporating rapidly (Lucero, 2015). If you suspect this has happened, use a wooden yardstick to measure the amount of liquid in the tank. If the tank is losing liquid nitrogen, the semen must be transferred to a good tank immediately. Should the tank be empty of liquid nitrogen it is doubtful that the semen is viable and it should be evaluated before it is used (Selk, 2012).

2.4. Thawing Semen

A recommendation of thawing semen varies among AI organizations, each of which has a specific method for diluting, cooling, packaging, and freezing semen in straws. The total

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processing system determines the optimal rate of thaw. As a result of considerable research, it is generally concluded that warm water thaw at 35 ºC results in improved sperm cell recovery compared with other methods of thawing (Taminaude Agricultural University, 2008). Success of warm water thaw is due to the fact that sperm are exposed to critically dangerous temperatures between -44 ºC and 0 ºC for only a brief amount of time (Mottershead, 2000). The rise in temperature is rapid enough to minimize sperm damage. A major criticism and concern for the warm water thaw is the danger of cold shock caused by mishandling the straw following thawing (Michael, 2012). Cold shock is the permanent injury to sperm caused by a sudden decrease in semen temperature after thawing. It can occur during preparation of the inseminating device or transport to the cow. If precautions are taken to prevent cold shock, the advantage of warm water thaw will be realized (Michael, 2012). It is important that the temperature of the thaw water be checked immediately before removing the straw from the tank. Use an accurate, easy-to-read thermometer. The length of thaw should be at least 40 seconds. Some organizations recommend the pocket thaw for straws. This method is successful for semen processed and packaged by their system. However, the pocket thaw should not be used for semen packaged in straws from other organizations (Selk, 2012).

2.5. Body condition scores (BCS)

Body condition scoring is a "hands-on" method of determining the amount of fat an animal is carrying. BCS is a management tool that can be used to predict herd fertility and to determine the effectiveness of feeding programs throughout the year (Ziegler *et al.,* 2013). Research and field experiments have shown that body condition influences productivity, reproduction, health and longevity. Thinness or fatness can be a clue to underlying nutritional deficiencies, health problems and/or improper herd management. If done on a regular basis, body condition scoring can be used to troubleshoot problems, improve the health and productivity of the animals herd (Heinrichs & Isler, 2015).

Body condition is determined by determining fat cover over four major locations on the animal's body: back bone (spine or topline), short ribs, and hip bones (hooks and pins) and around the tail head (Oberem *et al.*, 2009; Hunt, 2013; Heinrichs & Isler, 2015). In South Africa the body condition scoring is based on a five point scale: 1 represent an extremely thin animal and a score of 5 a grossly fat animal. A cow with a score of 3 is considered to be in a trim condition. In the UK and USA, a 9 point scale is used (ARC, 2013).

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BCS 1: The animal is very emaciated, starving and weak. However, the entire body is extremely thin, and all skeletal structures are prominently visible. No muscle tissue is evident and no external fat is present. All the skeletal structures are visible and very sharp to the touch. The hair coat appears to be very dull and survival during stress is a doubt. BCS 2: The animal is thin. The vertebrae along the topline are prominent. Muscle tissue is evident, but not abundant. Individual vertebrae can be felt, but are not as sharp. The short ribs can be identified individually when touched, but they feel sharp rather than very sharp. Individual ribs can be identified visually and there is some tissues covers around the hook and tail head (Ziegler, 2013; Heinrichs & Isler, 2015).

BCS 3: an animal increased fat cover over ribs and ribcage is only slightly visible. There are obvious fat deposits behind the front shoulder. Areas on each side of the tail head are fairly well filled but not round. BCS 4: the animal is moderately fat and the bone structure is no longer noticeable. The skeletal structure is difficult to identify. Individual short ribs cannot be felt even with firm pressure. Folds of fat are beginning to develop over the ribs and thurl area of the animal. Fat cover around the tail head is evident on both sides as slight "rounds" that are soft to the touch (Heinrichs & Isletr, 2015).

BCS 5: Very fat or obese. The animal has a "blocky" appearance. The bone structure is not noticeable. The back bone has a flat appearance and cannot be felt even with pressure. Folds of fat are apparent over the ribs, thurl and thighs. The hip bones and tail head to pin area on both sides are completely buried in fat. The animal's mobility is impaired by the large amounts of fat (Ziegler, 2013).

2.6. Oestrous Synchronization

Synchronization of oestrus means manipulating the oestrous cycle of beef females in a herd to allow breeding to occur at an approximately the same time. Synchronization protocols use products that mimic hormones naturally produced to alter the timing of oestrus by affecting structures on the ovaries (follicles and corpus luteum [CL]). Oestrous synchronization can be useful in both AI and natural service programs. By synchronizing oestrous, a producer can schedule labour for a concentrated time during the breeding and calving season (Heise, 2011).

For oestrous synchronization protocols to be successful, females must be healthy, on an adequate plane of nutrition, and, for some protocols, exhibit oestrous cycles before the protocol

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begins. This means that oestrus and ovulation are occurring during a normal 21 day (19 day for heifers) oestrous cycle. Some oestrous synchronization protocols can induce an oestrous cycle in anestrous (non-cycling) females that are close to initiating an oestrous cycle. However, regardless of the oestrous synchronization program, the protocols are not a substitute for a good nutrition and herd health program (Rasby & Funston, 2010). Cows need to be at a body condition score (BCS) 3 or better (out of 5) during the breeding season and be at least 50 days post calving to help increase the success of the synchronization protocol. If there are young, thin and or late calving cows in the herd, it is likely they are not cycling. The addition of a progestin or progesterone such as a CIDR® in the protocol will assist in jump starting some of these non-cycling cows. However, caution needs to be taken, as the CIDR® or other progestin are not the "cure all" for thin, young and late calving cows and one should look at whether it is cost effective to synchronize these cows (Salverson, 2012). In the herd non-pregnant cows or replacement heifers (12 to 16 month of age), will be at various stages of their 21 day oestrous cycle or will be in anestrus. Under normal conditions, about 5 percent of the cycling females will be in oestrus on any given day. Cyclic females may be grouped into one of the three categories based on structures present on their ovaries (Rasby & Funston, 2010). The first group is females with an active corpus luteum present. It includes females that are somewhere between day 6 and day 17 of the oestrous cycle. About 60 percent of cycling females will have an active CL on their ovary. The remainder of the cyclic females will either be developing a new CL (day 1 to 5) or regressing a CL (day 18 to 21). There will also be a group of females that are in anestrus.

During the oestrous cycle, two to four waves of follicles will grow and regress on the ovary until one emerges as the dominant follicle, from which the ova will be ovulated. This will take place when the progesterone secreted by the CL is regressing due to the death of the CL. Two hormones are produced in the ovulation process: estrogen and progesterone. The primary hormone produced by the follicle is estrogen. Progesterone is produced by the CL on the ovary (Johnson *et al,* 2010). To combat the problem of oestrus detection, and to minimize the labour involvement in AI in cattle, drugs have been developed to synchronize oestrous cycles in females. In this manner, several females can be given the proper treatment to cause ovulation to occur simultaneously (Cole & Garrett, 1980).

The majority of oestrous synchronization programs use one or a combination of the two basic methods that work with the physiology of the cow's normal oestrous cycle: prostaglandin

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($PGF_{2\alpha}$) injections that cause CL regression and oestrus in 1 to 5 days after the injection, unless the cow or heifer is in the first 4 to 5 days of her oestrous cycle when her CL is not responsive to $PGF_{2\alpha}$ (Rodning *et al.*, 2012). The administration of PG alone is commonly utilized to synchronize an ovulatory oestrus in oestrous cycling cows. However, this method is ineffective in anestrous females and variation among animals in the stage of the follicular wave at the time of PG injection directly contributes to the variation in onset of oestrus during the synchronized period (Johnson, *et al.*, 2010).

The progesterone or progestins, released from controlled internal drug release inserts (EAZIBREED CIDR®) or ingested in feed by feeding melengesterol acetate (MGA®), mimic the effects of the cow's natural progesterone by preventing oestrus from occurring as long as they are present in the body. Once removed, the cow or heifer typically comes into oestrus in 1 to 3 days. However, a heifer is sub-fertile during the first oestrus following MGA treatment due to ovulation of an older oocyte, so the heifer should be bred on a subsequent, synchronized oestrus. Gonadotropin-releasing hormone (GnRH) injections promote and synchronize follicle growth and induce ovulation. A GnRH injection administered approximately 48 hours after a prostaglandin injection provides a more concise synchrony of ovulation (Rodning *et al.*, 2012).

2.7. Ovulation Inducing Drugs

The Prostaglandins (PG_S) was first isolated from accessory male sex gland fluids, were termed prostaglandins because of their association with the prostate gland. They are secreted by almost all body tissues. The prostaglandins, derived from arachidonic acid, are short-acting. Some forms never appear in the blood, whereas others are degraded after they circulate throughout the liver and lungs. $PGF_{2\alpha}$ is the natural luteolytic agent that terminates the luteal phase (corpus luteum) of the oestrous cycle and allows for the initiation of a new oestrous cycle in the absence of fertilization. $PGF_{2\alpha}$ is particularly potent in terminating early pregnancy (Reeves, 1987). PGs are found in the form of at least 6 parent compounds that induce various pharmacologic responses (Hafez, 1993).

All PGs are 20-carbon unsaturated hydroxyl fatty acids with a clopentane ring (cyclopentano perhydro phenanthrene ring structure). They are divided into PGE, PGF, PGA and PGB groups. Arachidonic acid, an essential fatty acid, is the precursor for the PG most closely associated with reproduction, mainly prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$) and prostaglandin E_2 (PGE_2). PG's are

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involved in control of blood pressure, lipolysis, and gastric secretion, blood clotting, processes including renal and respiratory function. Blood level of PG's are generally low, but are elevated in certain conditions such as parturition (DeSilva & Reeves, 1985; Murdock & Dunn, 1983). PG's are involved in ovulation in the ewes and cows when ovulation is blocked by the administration of indometacin, an inhibitor of prostaglandins synthesis. LH release is not affected, so the action and synthesis of PG is at level of ovarian follicle involving $PGF_{2\alpha}$ and/ or $PGE_{2\alpha}$.

$PGE_{2\alpha}$ stimulate contraction of the uterus, dilates blood vessels but has no luteolytic action. $PGF_{2\alpha}$ stimulates contraction of uterus, aid in sperm transport in the male and female, cause's constriction of blood vessel, and has luteolytic properties. Venoconstrictive effects of $PGF_{2\alpha}$ induce hypoxia, which in turn leads to luteolysis. If the uterus is removed, the CL will not regress for at least the length of the respective gestation. The mechanism by which $PGF_{2\alpha}$ transport from uterus to the ovary is that $PGE_{2\alpha}$ passes directly through the walls of utero-ovarian vein into the ovarian artery and directly to the CL (McCracken, 1984). An increase in estrogen, which increases myometrium growth in the uterus, stimulates $PGF_{2\alpha}$ synthesis and release. If the female animal becomes pregnant, some signal (Btp1) is sent from embryo to uterus preventing $PGF_{2\alpha}$ release, thus allowing CL of cycle to become CL of pregnancy. $PGF_{2\alpha}$ will not cause regression of CL during its 5 days of life, because the CL does contain $PGF_{2\alpha}$ receptors on the luteal cells. In the sow, it will not cause regression until day 12 of cycle. Various treatments with PG are used to synchronize oestrus for AI (McCracken, 1984). Cows are treated twice at 10-days intervals, after which they will all theoretically exhibit oestrus 3 days after second injection. PG used in timed breeding in mares and abortion in cattle. Trade names of these compounds are Lutalyse and Estrumate for the cows and mares. $PGF_{2\alpha}$ used in treating infected uterus of dairy cows. Mechanism of action is twofold: (a) Regression of CL, if present; induction of follicle growth and estrogen production and (b) contraction of uterus, under the action of estrogen (McCracken, 1984).

Oxytocin and vasopressin are synthesized in the hypothalamus and stored in the neurohypophysis (posterior pituitary). Arginine vasopressin, also called ADH. Lysine vasopressin is secreted in domestic pigs (Hafez, 1993). Oxytocin and ADH are synthesized in the supraoptic and paraventricular nuclei of the hypothalamus and are only stored and released from the neurohypophysis. These hypothalamic hormones (neurohypophyseal hormones) are synthesized together with the carrier proteins called neurophysins. As with other

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neurosecretions, oxytocin and vasopressin are transported in small vesicle enclosed by a membrane down the axon of the neuron. These secretory vesicles flow down in to the hypothalamic-hypophyseal nerve axons by axoplasmic streaming. They are stored at the nerve endings next to the capillary beds in the neurohypophysis until their release into the circulation (Hafez, 1993).

Oxytocin is also produced in the corpus luteum. Thus oxytocin has two sites of origin, the ovary and the hypothalamus. Oxytocin has several functions: contraction of uterine muscle, increased contraction frequency in the oviduct. Oxytocin transport both female and male gametes in the oviduct, it induces female to let-down milk after parturition and it aids delivery in young animals when the period of labour is extended (Hafez, 1993). Estrogen enhances responsiveness of smooth muscle to oxytocin. The lactating female becomes conditioned to visual and tactile stimuli associated with suckling or milking. This conditioning induces the release of oxytocin into the circulation, to act on the myoepithelial cells (smooth cells) that surround the alveoli in the mammary gland, resulting in milk let-down. Hafez, (1993) report that ovarian oxytocin is involved in luteal function by acting on the endometrium to induce prostaglandin $F_{2\alpha}$ release, which has a luteolytic action (regression of the corpus luteum).

The glycoprotein human chorionic gonadotropin (hCG), consists of α-and β-subunits with a molecular weight of 40.000 daltons. The α-subunit has 92 amino acids and 2 carbohydrate chains. The α-subunit of hCG is similar to the α-subunits of human, porcine, ovine, and bovine LH. The β-subunit has 145 amino acids and 5 carbohydrate chains (Hafez, 1993). Human chronic gonadotropin, synthesized by the syncytiotrophoblastic cells of the placenta of primates, is found in both the blood and urine. It is detected in the urine 8 days after conception by sensitive radio immunoassays (Hafez, 1993). Human chorionic gonadotropin appears early in human pregnancy, detection of hCG in the urine is the basis of immunologic human pregnancy tests. In addition, the LH-like action of hCG has made it the hormone for treatment of cystic ovaries in cattle. The hCG treatment of a cow with cystic ovaries usually requires 5,000 to 10,000 IU of hCG, after which the cystic follicle will either ovulate and forms CL or more often, luteinizes. In either case, the luteal structure produces progesterone and CL is functional for 20 days and regresses normally, allowing the cows to cycle 21 days after treatment. At that time she is expected to breed almost as successfully as a non-cystic cow. Certain malignancies, such as chorioepithelioma and hydatiform moles in women are associated with high level of urinary gonadotropins (Hafez, 1993).

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2.8. Artificial Insemination Techniques

The technique of inseminating a cow is a skill requiring adequate knowledge, experience and patience. Improper AI techniques can negate all other efforts to obtain conception. Semen must be deposited within the tract of the cow at the best location and at the best time to obtain acceptable conception rates. Early methods of AI with frozen-thawed semen involved deposition of the semen in the vagina, as would occur in natural mating. Those methods are not satisfactory. Fertility is low and greater numbers of sperm are required. Another method which gained popularity was the "speculum" method. This method is easily learned, but proper cleaning and sterilizing of the equipment between successive female animals is necessary, making it more impractical to inseminate than with the rectovaginal technique which is the most widely used AI method today (Dejarnette & Nebel, 2010).

In the recto-vaginal technique a sterile, disposable catheter containing the thawed semen is inserted into the vagina and then guided into the cervix by means of a gloved hand in the rectum. The inseminating catheter is passed through the spiral folds of the cow's cervix aided by hand in the rectum into the uterus. Part of the semen is deposited just inside the uterus and the remainder in the cervix as the catheter is withdrawn, or all of the semen is deposited in the uterine body. Expulsion of the semen should be accomplished slowly and deliberately to avoid excessive sperm losses in the catheter (Taminaude Agricultural University, 2008).

The body of the uterus is short; therefore, care should be taken not to penetrate too deeply which might cause physical injury. In animals previously inseminated, the catheter should not be forced through the cervix since pregnancy is a possibility. Since research data show little variation in conception rates when semen is placed in the cervix, uterine body or uterine horns, some people recommend incomplete penetration of the cervical canal and deposition of semen in the cervix (Dejarnette & Nebel, 2010). The recto-vaginal technique is more difficult to learn and practice is essential for acceptable proficiency but the advantages make this method of insemination more desirable than other known methods. With practice, the skillful technician soon learns to thread the cervix over the catheter with ease. If disposable catheters are used and proper sanitation measures are followed, there is little chance of infection being carried from one cow to another (Taminaude Agricultural University, 2008).

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2.9. Timing of Artificial Insemination for Maximum Conception

Timing of Artificial insemination (TAI) based on observed standing oestrus, and the actual times will vary depending on the length of standing oestrus, but the goal is to inseminate near the end of the standing oestrus (Kasimanickam, 2006). Cows ovulate approximately 4 to 16 hours after the end of standing oestrus. Inseminating near the end of oestrus provides time for the sperm to undergo capacitation, which gives sperm the ability to fertilize before they encounter the egg (Taminaude Agricultural University, 2008). In general, it is better to have the sperm waiting for the ovum, rather than the ovum waiting for the sperm, because the ovum has a shorter lifespan (Rodning *et al.,* 2012).

Some things to consider when timing AI include the following: Good oestrus detection is critical for successful AI. Observe for standing oestrus at least 30 minutes twice a day. Early morning, late afternoon, and evening are the best times for oestrus detection (Keown & Kononoff, 2007). Maximum conception rates for AI occur when animals are bred near the end of standing oestrus. Traditional AI has therefore followed the AM/PM rule. An animal first observed in oestrus in the AM should be inseminated that PM. An animal first observed in oestrus in the PM should be inseminated the next AM (Rodning *et al.,* 2012).

Some producers may consider using timed AI, in which insemination occurs at a predetermined time following an appropriate synchronization program. Timed AI allows for a more regimented schedule (Bridges *et al.*, 2008). Recent improvements in timed AI and oestrous synchronization protocols have increased pregnancy rates while allowing for easier scheduling of labour resources and less cattle handling (Perry *et al.,* 2002). If a timed AI program is used with oestrous synchronization, the need for oestrus detection can be eliminated (Bridges *et al.*, 2008). Another method is to combine AI and natural service with the use of cleanup bulls to boost overall pregnancy rates and reduce the amount of oestrus detection and drug expense. Commonly, oestrous synchronization and AI are used for one AI service, and then cleanup bulls are turned out for the remainder of the breeding season (Rodning *et al.,* 2012).

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CHAPTER 3: RESEARCH METHODOLOGY

3.1. Design of the Study

The study was conducted in the Vhembe district, 180 kilometers north east of Polokwane. The climate is tropical, the temperature is generally between 16 °C to 40 °C and it is usually moderately cold in winter and hot in summer. Winter is from May to July; it is a dry season, usually cold but rarely reaches the freezing point. Summer is from November to February; it is usually hot, summer rainfall occurs within these months but decrease drastically in January. Humidity is 60%. Agricultural research council, (2013) reported that the average rainfall is 530 mm per annum and ranges from 200 mm to 2 500 mm per annum. The climate conditions are suitable for livestock and crop farming. A total of 205 beef breed animals of different breeds, age and body condition scores in the rural communities of Vhembe district municipality, Limpopo province of South Africa, were used in this study. Cluster sampling procedures were used to select villages for the study and wards were randomly selected. Reflection and implication of social preparation for experimental artificial insemination of cattle was discussed in Chapter 7 in the appendix section. Ethical clearance was obtained from the University of Venda Ethics Committee with a project registration number (SARDF/13/ANS/03/1809) for permission to do this study.

3.2. Pregnancy Diagnosis

Screening of cows and heifers was done by pregnancy diagnosis (rectal palpation) twice in the space of six weeks. A left arm-length gloved arm was lubricated with cooking oil and then inserted through the anus into the rectum. The faeces in the rectum were removed with a left gloved arm. However, it was seldom necessary to remove all the faeces from the bowel. Instead, the left gloved arm kept flat and opens against the floor of the rectum, allowing the faeces to pass over the top of a left gloved hand. While the left gloved arm was handling the cervix, the rectal contraction rings attempted to force the left gloved arm from rectum. The left gloved hand was then immediately placed through the center of the rings and massaged back and forth with two fingers of the left gloved hand. The contraction rings eventually relaxed and then pass over the left gloved arm and the technician continue with the palpation.

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The reproductive tract was palpated through the rectal wall to detect the size of the uterine horns, if there were any discrepancy in the diameter of the uterine horns and the presence of fluid and membranes or cotyledons. Therefore, the membrane slip and palpation of the amniotic vesicle were positive signs of pregnancy. The corpus luteum, although present, was not conclusively the sign of pregnancy, more especially in the early stages of pregnancy. In cows that were in a later stage of the pregnancy, the cervix was located cranial of the pelvic rim and the uterus could not be retracted. Initial diagnosis was done to isolate non-pregnant cows and heifers from the bulls and pregnant females, so that the cattle owners can free the bulls and pregnant females, while feeding non-pregnant cows and heifers intensively in a secluded area. A second diagnosis after six weeks was done for the confirmation of the positive signs of pregnancy from cows that could not be diagnosed pregnant in the initial diagnosis.

In the meantime empty cows and heifers were rated for body condition score using a scale of 1-5 (Oberem *et al.*, 2009). Body condition was determined by assessing the subcutaneous fat reserves over the loins and around the pin-bone of the animals. Score 1: Individual short ribs have a thin covering of flesh while the bones of the chine, loin, and rump regions are prominent. Hook and pin bones protrude sharply, with a very thin covering of flesh and deep depressions between bones. The animal was showing severe depression below the tail head and between pin bones with bony structure protrudes sharply, and ligaments and vulva are prominent. Score 2: Individual short ribs can be felt but are not prominent. The ends of ribs are sharp to touch but have a thicker covering of flesh. Score 3: Short ribs can be felt by applying slight pressure. The backbone appears as a rounded ridge and firm pressure is necessary to feel individual bones. Hook and pin bones are rounded and smooth. The areas between pin bone and around tail head appears smooth, without signs of fat deposit. Score 4: Individual short ribs are distinguishable only by firm palpation and short ribs appear flat or rounded, with no overhanging shelf effect. The ridge formed by backbone in chine region is rounded and smooth while the loin and rump regions appear flat. Hooks are rounded and the span between them is flat. The area of tail head and pin bones is rounded, with evidence of fat deposit. Score 5: Bony structures of backbone, short ribs, and hook and pin bones are not apparent. Subcutaneous fat deposit is evident and the tail head appears to be buried in fatty tissue. The age of all the cows and heifers that were inseminated was done by counting the number of adult teeth. Heifers that were having 1 or 2 pairs of adult permanent incisors and cows that were 3 to 10 years older, with all four pairs of permanent incisors, were identified, grouped separately and ear-tagged before they were synchronized and artificially inseminated.

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3.3. Oestrous Synchronization Procedures

A total of 205 female animals were synchronized with 2 mg of Oestradiol benzoate (Cidirol) intramuscular injection and a controlled internal drug release vaginal insert (CIDR®, Pfizer Animal Health, New York) on day 0 of oestrous synchronization. The white plastic insert device with two legs in the front and a blue nylon string at the back is impregnated with progesterone and designed to continuously release progesterone once inserted intra-vaginally. The CIDR was inserted intra-vaginally with a CIDR applicator (Figure 3.1). Veterinary disinfectant (F10®SC) was used as an antiseptic solution for dipping the CIDR applicator before re-using it. Cooking oil was also used to lubricate the applicator before insertion in the vagina. One CIDR was placed into the applicator so that the short legs folded and only the tips of the legs were protruded from the front of the applicator. The tail of the animals was lifted and the vulva lips were cleaned and wiped with disposable paper towel.

With the tail lifted, the loaded applicator was inserted sloping slightly upwards through the vulva and then forward without forcing it into the anterior vagina. Thereafter, the removal string was released while pressing the handle of the plunger and easily pulling the barrel of the applicator backwards. If the CIDR was correctly placed with the front legs open in the anterior portion of the vagina, only the blue removal string was hanging from the vagina. The blue removal string was trimmed by a scissor to about the level of the vulva lips to prevent inadvertent removal during grooming practices by the animal (Figure 3.2). On day 6 in the afternoon the heifers and cows were injected with 25 mg dinoprost prostaglandin $F_{2\alpha}$ (Lutayze). On day 7 in the morning the CIDRs were pulled out of the vagina and in the afternoon the cows were injected with 2 mg of Oestradiol benzoate (Cidirol). During day 8 to 9 visual detection of oestrus was done by cattle owners three times a day for a minimum of 30 minutes a time.

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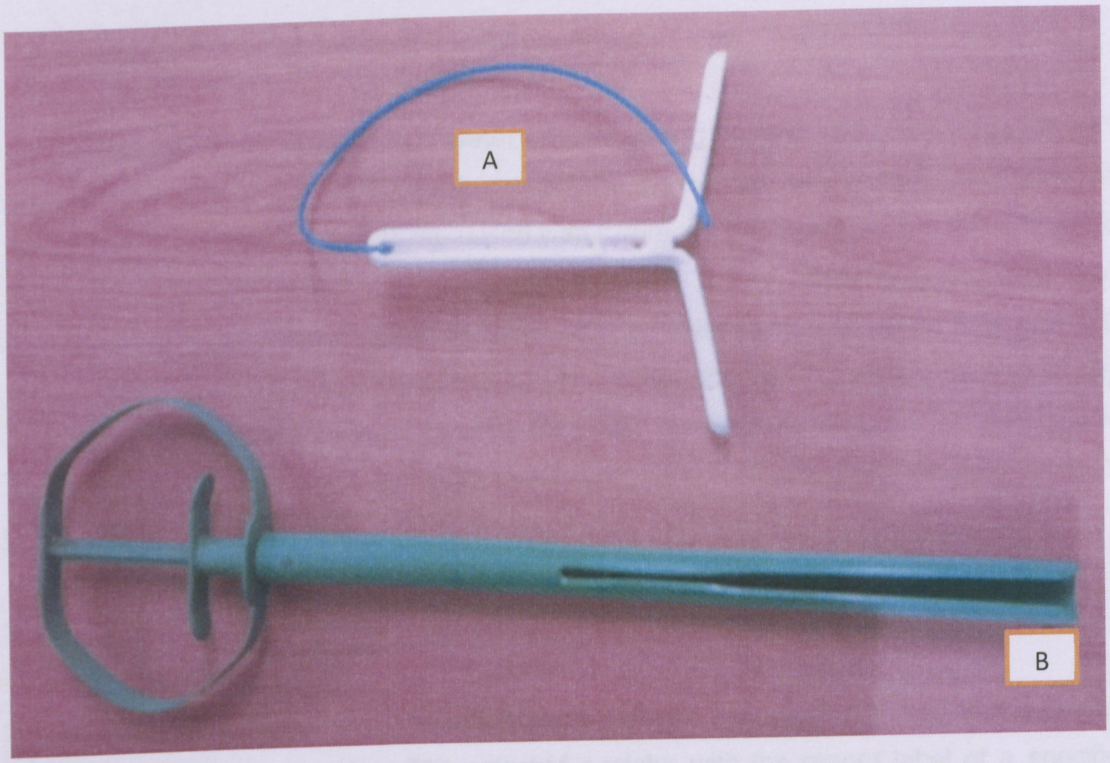


Figure 3.1: CIDR (A) and (B) CIDR applicator.

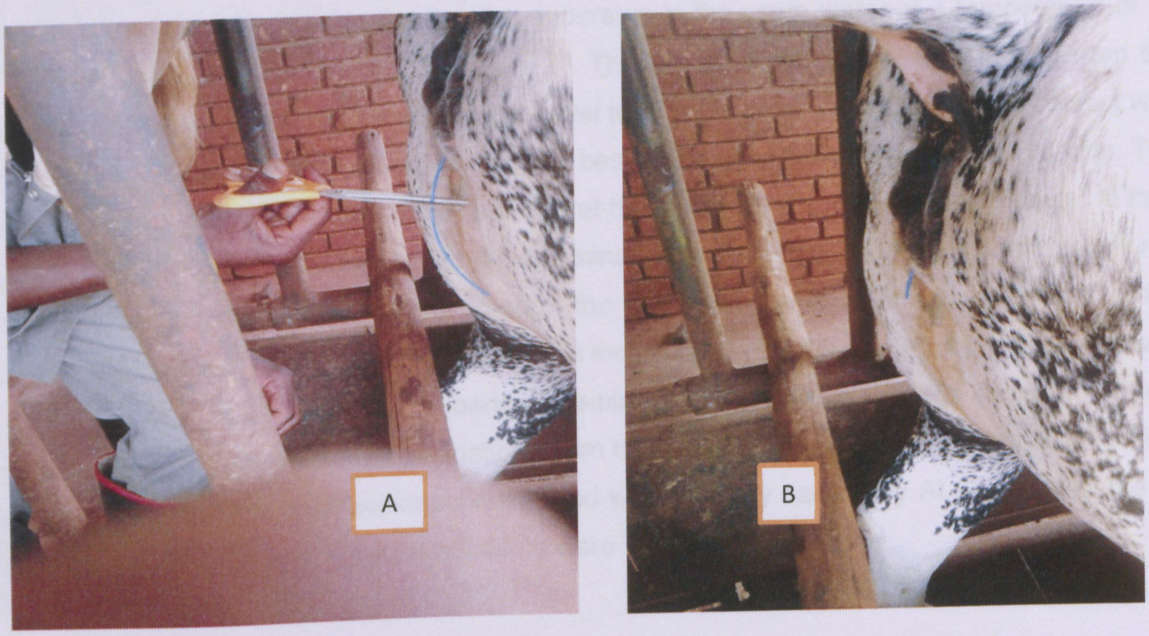


Figure 3.2: Trim the blue removal string at the level of the vulva lips (A) before trimming the blue removal string and (B) after trimming the blue removal string.

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3.4. Preparation of Automatic Semen Apparatus

An electric kettle was used to heat water into the boiling point of 100 °C at University of Venda experimental farm and then the water poured into the thermo vacuum flask, and taken along to the AI site. Thereafter, at the AI site boiled water from the thermos flask poured into the thawing apparatus and then mixed with cold water from the 2 liter sterile bottle to drop the water temperature from boiling point into warm water. The water temperature was measured at 35 °C by a thermometer in the thawing apparatus. Thereafter, the thawing apparatus was plugged into a cigarette lighter hole of a car to keep the water temperature constant at 35 °C. The water temperature was monitored every 15 minutes or before thawing another straw from the LN_2 tank.

3.4.1. Thawing of Semen

The LN_2 full of canisters that contained semen straws was placed closed to the car with the plugged-in thawing apparatus. The selected canister with the record label of a specific bull's semen was lifted to the neck of LN_2 tank and a single semen straw was removed within 5 seconds and then placed into the thawing apparatus in the warm water at a temperature of 35 °C for the period of 30 seconds (Figure 3.3). The semen straw was then removed from the thawing apparatus and dried off with paper towel to prevent water from coming into contact with the semen. The AI rod was inside the car just beside the thawing apparatus and LN_2 tank. The AI rod plunger was pulled back to allow space at the front end of the rod for the straw to fit into. A single semen straw was placed into the insemination rod with the plug end inside of the AI rod and then cut off by a clean scissor through the middle of the end sticking out. A protective plastic sheath was pulled over the rod and then locked over with the ring. A chemise plastic was used as a condom by covering the loaded insemination rod together with the sheath to protect the tip of the AI rod against contaminations from the vulva and vagina until the rod entered the cervix. Thereafter, the artificial insemination rod was carefully carried by AI assistant to the AI technician at the site where the cows/heifers were inseminated.

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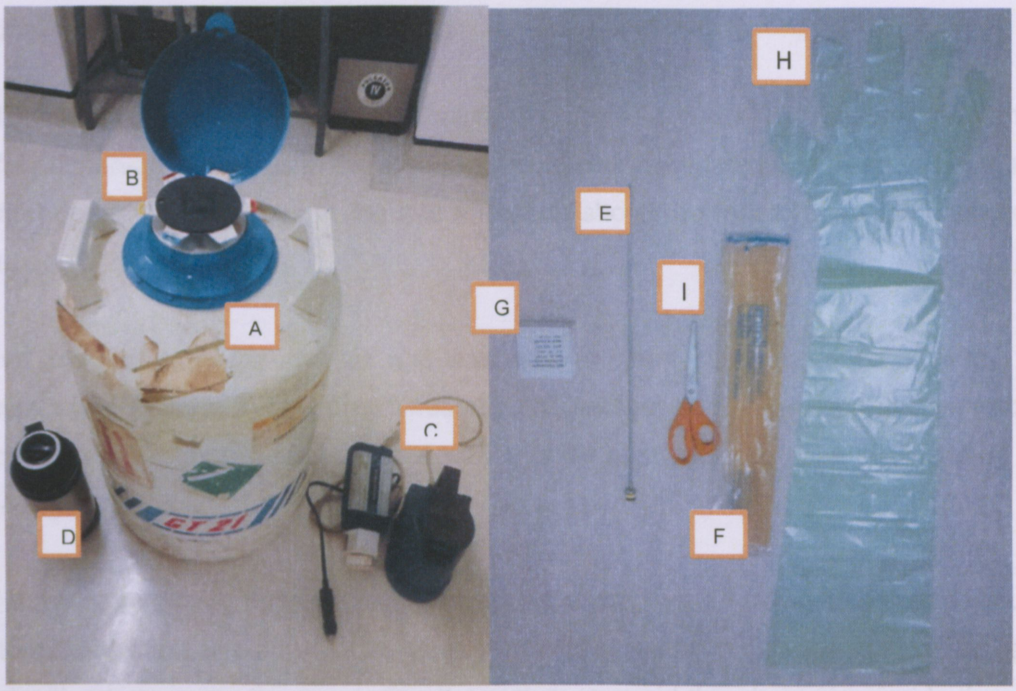


Figure 3.3: AI equipment: (A) nitrogen tank, (B) canister, (C) thawing apparatus, (D) thermo vacuum flask, (E) insemination rod, (F) protective sheath, (G) chemise plastic, (H) arm-length glove and (I) scissor.

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3.5. Artificial Insemination

Artificial insemination was performed following the AM/PM rule. The rule specifies that an animal first observed in oestrus in the morning should be inseminated that late afternoon and an animal first observed in oestrus in the late afternoon should be inseminated the next morning (Rodning *et al.*, 2012).

The vulva and rectum of the animals was washed with clean water and dried off with a clean disposable paper towel to avoid contamination of the anterior part of the reproductive tract. The outermost part of the anus was lubricated with cooking oil in order to ease the insertion of the left arm-length gloved arm.

The left arm-length gloved hand forming a cone shape was twisted slowly to enter the anus into the rectum. Once the left arm-length gloved arm was inside, the faeces in the bowel was removed without removing the arm inside the rectum to avoid the air coming inside the rectum so as to avoid the wall of the rectum from becoming hard and unworkable. The gloved hand in the rectum was kept open and flat against the floor of the rectum, allowing faeces to pass over the top of the gloved arm. The cervix was then palpated through the rectal wall and located. Thereafter, the loaded insemination rod with its chemise sheath was inserted through the opening of the vulva lips and passed to the external end of the cervix by manipulating the cervix forward to straighten the vaginal folds. Using the same hand inside the rectum, the cervix was held tightly and in good position as the tip of the AI rod was carefully guided into the cervix by twisting and bending the cervix until the rod passes the third ring of the cervix and the left gloved hand could feel the AI rod into the uterine body. The plunger was pushed slowly to drop the semen directly into the uterine body. The cervix and clitoris of the animal was massaged immediately after semen deposition, to accelerate semen transport and to ensure that semen reaches both uterine horns through the contraction of the oviduct and uterine muscles.

3.6. Ovulation Inducing Drugs

Cows and heifers were alternately assigned to one of the three ovulation inducing drugs and a control immediately after insemination: (1) 300 IU hCG (Chorulon) injection under the tail into the vein, n = 52; (2) 25 mg dinoprost $PGF_{2\alpha}$ hormone (Lutalyze) intramuscular injection, n = 49;

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(3) 50 IU oxytocin hormone (Fentocin) intramuscular injection, n = 46 and (4) 5 ml sterile isotonic saline intramuscular injection as a control, n = 58.

3.7. Pregnancy Diagnosis

Two months after insemination, cows and heifers were palpated for early pregnancy diagnosis. A left arm-length gloved arm was lubricated with cooking oil and then inserted into the rectum. Faeces in the bowel were removed from the rectum, without removing the arm from the rectum to avoid the air entering the bowel and cause the rectal wall to be hard and difficult to locate the cervix. The reproductive tract was palpated through the rectal wall to detect the asymmetry of the uterine horns, a lower tone of the pregnant horn and fluctuation of the pregnant horn (later both horns). The membrane slip and presence of the amniotic vesicle were positive signs of pregnancy and the results were recorded.

3.8. Data Analysis

The pregnancy results between the three ovulation inducing drugs and sterile isotonic saline as a control with the use of frozen-thawed semen were statistically analyzed using General Linear Model (GLM) procedures of minitab (minitab 2013) using 2×4 factorial in a completely randomized design. The treatment means were compared using Tukey's post hoc test. Chi-square analysis using the PROC FREQ procedure was used to compare the pregnancy rate among the treatment groups and pregnancy rate between semen breeds, Significant was set at $P<0.05$. The following model was used:

$Y_{ij} = \mu + S_i + D_j + \varepsilon_{ijk}$

Y_{ij} = data obtained from the K^{th} cows on the j^{th} ovulation inducing drugs and inseminated with the i^{th} semen.

μ = overall mean

S_i = effect of the i^{th} semen (a. Bonsmara semen and b. Nguni semen)

D_j = effect of the j^{th} ovulation inducing drugs (a. human chronic gonadotropin (hCG), b. prostaglandin $F_{2\alpha}$ and c. oxytocin).

ε_{ijk} = error

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CHAPTER 4: RESULTS

4.1. Pregnancy Status

The outcome of the distribution of the samples assigned to the four treatments after artificial insemination with Bonsmara and Nguni semen is summarized in Table 4.1.2. The pregnant status of 205 female animals inseminated was observed and describes as the pregnancy rate. A total of 205 female animals of which 142 were cows and 63 were heifers were used in this study Table 4.1.1.

4.2. Pregnancy Rate of Cows and Heifers after AI with Bonsmara and Nguni semen treated with four different Treatments.

On overall the control treatment had the highest pregnancy rate followed by hCG, $PGF_{2\alpha}$ and oxytocin respectively, across all the age groups data, although this was not significantly different ($p>0.05$) between sterile isotonic saline as a control and hCG and $PGF_{2\alpha}$ treatment groups. Pregnancy rate was lowest in the animals that received oxytocin and was significantly different from the other three treatment ($p<0.05$). Pregnancy rate was highest in cows that were inseminated with Nguni semen and treated with the saline injection as a control, but it was not significantly different from the animals inseminated with Bonsmara semen and treated with $PGF_{2\alpha}$ and hCG groups ($p>0.05$). The cows that were inseminated with Nguni semen and injected with $PGF_{2\alpha}$ recorded the lowest rate of pregnancy which was, however, not significantly different from the cows inseminated with Nguni semen and treated with hCG and oxytocin treatment ($p>0.05$), but significantly different to the control group which was inseminated with Nguni semen ($p<0.05$). The pregnancy rate in heifers was highest in the animals inseminated with Bonsmara semen with hCG injection and heifers inseminated with Nguni semen and treated with $PGF_{2\alpha}$ injection, followed by heifers inseminated with Bonsmara semen with $PGF_{2\alpha}$ treatment and heifers inseminated with Nguni semen and treated with oxytocin treatment respectively. There was no significant different ($p>0.05$) between heifers inseminated with Bonsmara semen with hCG, $PGF_{2\alpha}$ treatment and heifers inseminated with Nguni semen with $PGF_{2\alpha}$ treatment, although the Bonsmara semen and hCG group in heifers had a significant different ($p<0.05$) when compared to a control group (Table 4.1.2.). However, the control group

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inseminated with Nguni semen in heifers had a significant different to a group of heifers treated with oxytocin treatment ($p<0.05$). Thus, the $PGF_{2\alpha}$ in heifers with both Nguni and Bonsmara semen positively influenced the pregnancy rate ($p<0.05$). Heifers on the oxytocin treatment had the lowest pregnancy rate compared to the other treatments ($p<0.05$).

Table 4.1.1 Number of entries based on age and treatment

Age		Treatment groups			
Female's category	Total	hCG	$PGF_{2\alpha}$	Oxytocin	Control
Overall	205	52	49	46	58
Cow	142	37	37	30	38
Heifer	63	15	12	16	20

Table 4.1.2.The effect of bull semen and ovulation inducing drugs on pregnancy rates

Age and bull semen		Treatment Groups							
		hCG		$PGF_{2\alpha}$		Oxytocin		Control	
Female category	Semen Breed	n	%	n	%	n	%	n	%
Cows	Bonsmara Semen	26	73.1^{a}	26	76.9^{a}	19	52.6^{b}	25	72.0^{a}
	Nguni semen	11	45.5^{b}	11	36.4^{b}	11	45.5^{b}	13	84.6^{a}
Heifers	Bonsmara Semen	8	87.5^{a}	7	71.4^{a}	8	37.5^{b}	11	45.5^{b}
	Nguni Semen	7	57.1^{b}	5	80^{a}	8	25^{c}	9	66.7^{b}
Overall		52	67.3^{a}	49	67.3^{a}	46	43.5^{b}	58	69.0^{a}

$^{a.b.c}$ means within the row with different superscripts differed significantly ($p<0.05$), n= number of entries, hCG= human chorionic gonadotropin, $PGF_{2\alpha}$= prostaglandin $F_{2\alpha}$, % = percentages of pregnancy rate on each treatment groups within the row.

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4.4. The effect of Age and Body Condition Score on Pregnancy Rate in Cows and Heifers

On overall the BCS 5 had the highest pregnancy rate followed by BCS 4 and BCS 3 respectively among all the age groups, although this was not significant ($p>0.05$). Body condition score 2 showed the lowest pregnancy rate and this was significantly different from the other three higher body condition scores ($p<0.05$). There were not enough cows in the BCS 1 to calculate any differences. Pregnancy rate was numerically highest in cows that were in BCS 5, BCS 4 and BCS 3 compared to the BCS 2 and it significantly decreased ($p<0.05$) the pregnancy rate in cows (Table 4.1.3). In addition, the pregnancy rate in heifers was highest in BCS 3 and BCS 4 followed by BCS 5. However, BCS 2 had a lower pregnancy rate and this was statistical different ($p<0.05$) when compared to the heifers in BCS 3 and BCS 4. Good genetic Bonsmara and Nguni semen were both used to inseminate cows and heifers of different breeds, age and body condition score. Cows and heifers were artificially inseminated according to the choice and preference of semen breeds by the cattle owners and good pregnancy rate were achieved, which leads to more calves born from good superior bulls (Figure 4.1).

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The effect of age and body condition scores (BCS) on pregnancy rate in cows and heifers is shown in Table 4.1.3.

Table 4.1.3. The effect of age and body condition score on pregnancy rate.

Age	Body condition score (BCS)					
Female's category	BCS 1	BCS 2	BCS 3	BSC 4	BCS 5	Significant level
Cows	0.0 (0/1)^{c}	8.3 (1/12)^{b}	68.0 (34/50)^{a}	69.0 (40/58)^{a}	81.0 (17/21)^{a}	0.000
Heifers	-	40.0 (6/15)^{b}	66.7 (18/27)^{a}	63.6 (7/11)^{a}	50.0 (5/10)^{ab}	0.362
Overall	0.0 (0/1)^{c}	25.9 (7/27)^{b}	67.5 (52/77)^{a}	68.1 (47/69)^{a}	71.0 (22/31)^{a}	0.001

$^{a.b.c}$ means within the row with different superscripts differed significantly ($p<0.05$), ^{ab} means within the row with different superscripts not differed significantly ($p>0.05$), BCS= body condition score.

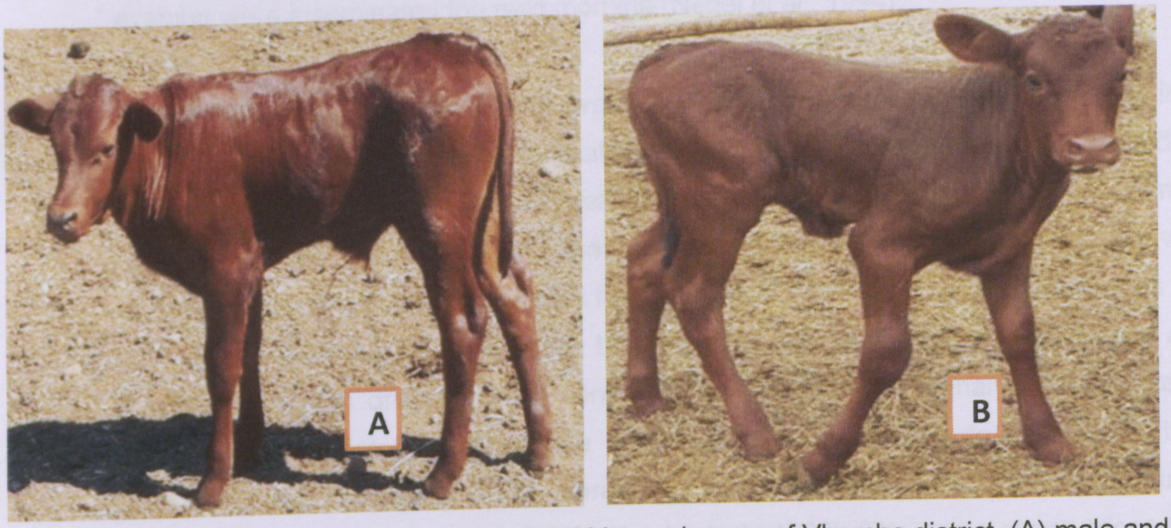


Figure 4.1: Two Bonsmara calves born after AI in rural areas of Vhembe district, (A) male and (B) female.

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CHAPTER 5: DISCUSSION

Detecting oestrus in heifers and cows followed by AI 12 hours later can improve conception rate over timed AI (Rood *et al.,* 2011). Although conception rates may be reduced in cows inseminated either soon after the onset of oestrus (less than 4 hours) or more than 14 –16 hours after the onset of oestrus, quite acceptable conception rates can be achieved in herds practicing once-daily insemination. Cows should be inseminated at the next opportunity after standing oestrus is detected (Morton, 2014). In this study the pregnancy rate of 62% was achieved in cows and heifers that were inseminated 12 hours after detecting the first sign of oestrus in the group of cows/heifers synchronized, following the AM/PM rule. The rule specifies that where cows and heifers were first detected in oestrus in the morning they were inseminated that late afternoon or evening, while cows first detected in oestrus in the late afternoon or evening were inseminated the next morning (Nebel *et al.,* 1994).

One objective of the present study was to improve the genetic value of livestock in the rural communities of Vhembe district by evaluating the effect of three ovulation induction drugs hCG, $PGF_{2\alpha}$ and oxytocin on the animal's fertility measured as conception rates. Two semen breeds (Bonsmara and Nguni) alongside the aforementioned ovulation induction drugs with sterile isotonic saline as a control were used for the artificial insemination in the two groups (cows and heifers) of female cattle. On average the results of the present study showed that among the ovulation induction drugs and the control group, the highest pregnancy rates were achieved from the sterile isotonic saline as a control. This could be due to changes in the uterine contractility and possibly to the acceleration of sperm transport in the reproductive tract of cattle (Sayre *et al*., 1997; King *et al.,* 2004; Hawk *et al.,* 1983). The same observations have been made by Gumen *et al.* (2011) who concluded that the administration of hCG, $PGF_{2\alpha}$ and oxytocin at the time of AI does not increase pregnancy rates in lactating dairy cows. Moreover, oxytocin treatment at the time of AI decreases pregnancy rates in lactating dairy cows showing spontaneous oestrus and therefor it is not recommended for these animals. The results of the present study are in agreement with Chenault, (1990) who found that the use of hCG at the time of AI does not affect conception rate in beef cattle. On the other hand, Bonsmara semen and hCG treatment group at the time of AI affected the pregnancy rate in cattle. These findings are similar to the observation of (Jaswal & Singh, 2013) who concluded that hCG at the time of AI increases conception rate in dairy cows. Although, unequal numbers of semen straws were used for the artificial insemination in the present study and this was due to the cattle owners'

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choice of semen breed for the artificial insemination. Human choronic gonadotropin is a gonadotropin with luteinizing hormone (LH) activities. Human choronic gonadotropin has a LH action in cows and can be used to stimulate the developing follicle towards maturation and to induce ovulation and to bring about luteinization of the granulosa cells, to maintain the functional life of the corpus luteum and to increase progesterone secretion from luteinized cells. Human choronic gonadotropin also augments the action of follicle stimulation hormone (FSH) on ovarian growth (Broers, 1995). In this study, in overall sterile isotonic saline treatment as a control had the highest pregnancy rates followed by hCG treatment, although this was not significantly different ($p>0.05$).

Earlier studies have been cited in several publications (Chenault, 1990; Lewis *et al.*, 1990; Mee *et al.*, 1990; Swanson & Young, 1990). After concluding that the mechanism of LH-like action of hCG for improving pregnancy in dairy cattle remains unknown, Stevenson *et al.*, (1984) suggested that hCG administration during oestrus may affect the time of ovulation, fertilization rates, corpus luteum development, progesterone secretion, and (or) embryonic survival. As asynchrony of LH release and oestrus may cause delayed ovulation in sub fertile cows (Maurer & Echternkamp, 1982), then exogenous hCG administered at the onset of oestrus might hasten ovulation and increase fertilization. Tanabe *et al.* (1949) reported there was no evidence that ovulation was delayed in untreated sub fertile cows or that hCG treatment at the time of insemination affected ovulation time in sub fertile cows. Thus, there was no evidence that the hCG treatment improved the synchronization of ovulation. Variability in pregnancy rate among the different studies might be associated with the potency of LH-like action of hCG on gonadotropin release or the timing of hCG (Mee *et al.,* 1990) and AI relative to the onset of oestrus (Stevenson *et al.*, 1984; Mee *et al.*, 1990). The hypothesis of the current study was not fully supported by the findings. Therefore, on overall the results of this study represent that the administration of hCG at the time of AI did not affect the pregnancy rates.

The results of this study indicated that there was a reasonably higher pregnancy rates achieved in heifers inseminated with Nguni and Bonsmara semen and treated with $PGF_{2\alpha}$ treatment. This results is in disagreement with the results obtained by Lopez-Gatius *et al.* (2004) showed that $PGF_{2\alpha}$ administered at the time of fixed timed insemination had no effect on pregnancy rate in cows with acceptable reproductive performance. The results of the present study indicated that $PGF_{2\alpha}$ had the significant effect on the pregnancy rate in cows and heifers. This findings were not similar to those reported in lactating dairy cows in which the administration of $PGF_{2\alpha}$ at the

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time of AI following spontaneous oestrus did not have any beneficial effect (Gumen *et al.,* 2011) and this is in agreement with our overall finding, that there was no improvement in pregnancy rates among heifers and cows by administering an injection of $PGF_{2\alpha}$ at the time of artificial insemination in the present study.

$PGF_{2\alpha}$ is used in cows and heifers virtually without side-effects. Provided that a corpus luteum of more than five days is present in the ovary, $PGF_{2\alpha}$ can readily causes luteal regression which is followed by follicle growth, oestrus and ovulation (Broers, 1995). The administration of $PGF_{2\alpha}$ at the time of AI stimulates contraction of the uterus and aids in sperm transport in both male and female. Thereafter, causes contraction of the blood vessels and has luteolytic properties in domestic animals (Hafez, 1993). $PGF_{2\alpha}$ and its analogues are widely used as a luteolytic effect on corpora lutea. Its uterotonic effect is additionally useful for pathological conditions of the uterus (Broers, 1995).

Oxytocin causes the release of pituitary gonadotropin in both sexes of many species, especially the cows (Donaldson *et al.,* 1965). Oxytocin is used to increase pregnancy rate by improving the sperm transport in the female reproductive tract of several species, and to aid in ovulation (Sayre & Lewis, 1997; Yildiz, 2005). Oxytocin causes the release of LH from the pituitary which causes ovulation in the cows. These results also suggest that LH is the luteotropic hormone in the cows and is necessary for normal corpus luteum development and function, but at least two period of luteotropic stimulation are necessary for function. One of these is at ovulation and the other after the fourth day of the cycle (Lasley, 1968). However, in the present study, the administration of oxytocin at the time of AI did not improve the pregnancy rate in cows and heifers. Clitoral massage which probably releases oxytocin following artificial insemination increased pregnancy in beef cows (Cooper *et al.*, 1985). The administration of oxytocin following AI also increased conception rate in lactating dairy cows (Yildiz, 2005), but in this study and other study it had hardly any effect on pregnancy in cows and heifers (Hays *et al.,* 1958). Yildiz, (2005) indicated that the pregnancy rate increased in lactating dairy cows after oxytocin administration just before AI. This could be due to changes in uterine contractility and possibly to the acceleration of sperm transport in the reproductive tract of ewes (Sayre & Lewis, 1997; King *et al.,* 2004; Hawk *et al.,* 1983). Oxytocin possibly exerted its influence by stimulating prostaglandin production (Kittok & Britt, 1977; McCracken *et al.,* 1980).

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In this study Bonsmara and Nguni semen were used for artificial insemination in different agro-ecological zones of Vhembe district. On overall the semen from the two breeds did not have an effect on the pregnancy rates in both cows and heifers after the treatment. However, Bonsmara semen with hCG treatment in heifers showed higher pregnancy rate than the Nguni semen and oxytocin treatment in the present study ($p<0.05$). Livestock owners in Vhembe district of Limpopo province preferred Bonsmara and Nguni breeds (Table 4.1.2), because they are indigenous cattle breeds and they can able to utilise low quality feeds. They are more heat resistant and can walk for longer distances in search for water and feed during the dry period of the year in Vhembe district, when compared to the imported breeds.

An investigation by Ndebele *et al.* (2007) revealed that the Nguni breed is indigenous to Southern Africa and is known for its high fertility, short calving intervals and long reproductive lifespan. It is tolerant to tropical diseases as well as internal and external parasites. Nguni breed is also highly adaptable to poor quality grazing and conditions of excessive heat and humidity. This adaptability provides the Nguni with the unique potential to produce high quality meat and hides under ecologically controlled free ranging conditions without the use of chemicals.

Bonsmara SA (2012) reported that Bonsmara strictly selected for economic traits such as fertility, milk production, growth and adaptability are still practiced even today. This has contributed to the reality that Bonsmara proudly succeeded in becoming the strongest and most professionally administered beef breed in South Africa. Bonsmara, the most prominent of beef breeds in South Africa, currently have more than 120 000 registered Bonsmara cattle. Opperman, (1962) reported that the Afrikaner breed outnumbers all other purebred beef and dairy cattle and forms the basis of crossbreeding in the ranching areas of South Africa. It contributed to the development of two new breeds namely the Bonsmara and Tauricus. Almost 70% of all beef cattle slaughtered in South Africa are either pure bred or carry Afrikaner blood (Opperman, 1962).

The results of this study demonstrate that the highest pregnancy rate was in cows that were in body condition 5 and heifers that were in BCS 3 at the time of artificial insemination, but it was not significantly different ($p>0.05$) with heifers that was in BCS 4 and cows that was in BCS 4, respectively. However, substantial differences were recorded between cows that were in body condition score 2 and 5. In addition there was no significant different ($p>0.05$) in pregnancy rate in cows that were in BCS 1 and BCS 2. The ARC (2013) reported that in South Africa the

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condition scoring is based on a five point scale: 1 represent an extremely thin animal and a score of 5 a grossly fat animal. A cow with a score of 3 is considered to be in a trim condition. In the UK and the USA, a 9 point scale is used. The results of the present study are consistent with common practices in dairy herds where the optimum for calving is 3.0 to 3.5 if the 5-point scale is used; lower calving BCS is associated with lower production and reproduction, whereas calving BCS of more than 3.5 is associated with a reduction in early lactation feed intake and milk production and an increased risk of metabolic disorders (Roche, 2011).

Steenkamp *et al.* (1975) compared the pregnancy rates of cows of similar weight that differed in condition score and found that condition at mating was more important than weight. This agrees with the findings of Van Niekerk (1982), who observed a calving rate of 78 % for cows in optimum condition compared with just 8 % for animals in the poorest condition and similar to our findings (Table 4.1.2). The reproductive performance of beef cattle under communal farming conditions is low, taking calving percentage as a measure of production (Bembridge, 1987; Bembridge & Tapson, 1993; Nthakheni, 1993; Mokantla *et al.,* 2004; Scholtz & Bester, 2010). Nowers *et al.* (2013) reported that the average calving percentage of 35.7% in the Communal farming and was similar to the findings of Clark *et al.* (2006) who reported calving percentages in the communal sector in the region of 40 %, but lower than the pregnancy rate of this study (62 %) for inseminated communal cows and heifers and are also in agreement with the calving percentages of between 43 % and 64 % reported by Madzivhandila *et al.* (2007).

The feed costs of maintaining the animals in this better condition are more than covered by increased reproductive performance. Thus animals should be fed well to promote good reproductive performance. Kahan *et al.* (1998) reported that the better performance with regard to the reproductive and productive efficiency of the heifers and cows included age at first service and calving, parturition to service, calving interval, gestation length, daily and total milk yield, and age and body weight of cows that influence the onset of oestrus and the subsequent fertility after calving. It is more efficient to feed animals to maintain good body condition than to allow them to lose weight in the hope that it can be regained before the mating season. Wright (1985) estimated that the loss of one unit in condition score would represent 3200 MJ of metabolisable energy, while restoring the animal's condition score would require about 6500 MJ of dietary metabolisable energy. This agrees with Van Niekerk (1982) who concluded that the feed cost of maintaining a cow at a condition score of 3.0 was half that required to raise a cow's condition from 1.5 to 3.0. The benefits of feeding animals well in terms of better reproductive performance

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are often easily appreciated by peasant farmers. However, smallholders usually have only small supplies of supplementary feed and will need advice through awareness programs and extension services, which animals to feed, how much to feed and when to feed them.

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CHAPTER 6: CONCLUSION AND RECOMMENDATIONS

6.1. Conclusions

The present study showed that drugs that play a role during ovulation in cows and heifers like oxytocin, prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$) and human chorionic gonadotropin (hCG), did not increase the pregnancy rate when injected 12 hours after the onset of standing oestrus and at the time of AI in cows and heifers of different age, condition score and breeds. It, however, increased the pregnancy rate of cows and heifers compared to natural breeding success in the rural areas. AI will quickly increase the offspring genetics and livelihood of cattle farmers within the rural areas of Vhembe district municipality of Limpopo province of South Africa. The hypothesis of the present study was not fully supported by the findings. Livestock owners in the rural area of Vhembe district encountered serious problem with separation of cows from bulls for synchronization programs.

These people are farming in the communal land, where they cannot control and isolate bulls from cows due to lack of farming infrastructure like a fence to separate their grazing land into different camps. Cattle handling facilities in the Vhembe district were old and were not in the adequate condition to administer injection or insert and remove the CIDR during synchronization program. Most of all, the synchronization programs require cattle to be handled often multiple times to administer injections and/or to insert or remove vaginal devices. Having adequate, low stress working facilities ensures that the recommended protocol is followed and cattle respond better to the synchronization protocol.

In conclusion, technical skills were provided to rural livestock breeders such as pregnancy test by rectal palpation, detection of standing oestrus and the optimum time for mating. Good genetic Bonsmara and Nguni bull semen were used to inseminate cows and heifers and good pregnancy rate were achieved on the first insemination, which will lead to more calves born from good superior genetic bulls. This will enable livestock production to contribute more to the cash economy in terms of sales for slaughter to the market in the agro-ecological zones of Vhembe district of Limpopo province.

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6.2. Recommendations

Artificial insemination could be the foremost valuable tool to consolidate and maximize utilization of rural recipient cows that could be selected and inseminated with superior bull semen if managed at a satisfactory standard. Based on the current study it is suggested that the National Government or the Provincial Department of Agriculture should assist the communal and the emerging sector or cattle owners with a cattle compound in which cows can be separated from the bulls during period of synchronization of oestrus. Cattle owners in the rural areas can construct their own cattle compound with a basic livestock working facilities, only if they unite and become one community. Separation of cows from the bulls and limited land is the most challenging factor faced in this study including farmers' commitment and assurance. AI is a very specialized assisted reproductive technology that requires special commitment throughout the program. AI has been and still is the most used reproductive technique in animals. A lot of research needs to be done and constantly livestock management on the reproduction and production rates of beef cattle in the communal areas of Vhembe district can improve the livelihood of rural people.

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CHAPTER 7: Appendix

REFLECTION AND IMPLICATION OF SOCIAL PREPARATION FOR EXPERIMENTAL ARTIFICIAL INSEMINATION OF CATTLE IN RURAL COMMUNITIES OF VHEMBE DISTRICT

7.1. Background

Community-based participatory research (CBPR) is a collaborative approach to research that equitably involves all partners in the research process and recognizes the unique strengths that each brings. CBPR begins with a research topic of importance to the community with the aim of combining knowledge and action for social change to improve community (Minkler & Wallerstein, 2003). Community-based breeding system is a breeding program that involves local communities and institutions in the design implementation and ownership of breeding strategies. Its main objective is to improve the productivity of local breeds and thereby improve the income of rural farmers by ensuring access to improved animals that respond to improved feeding and management (Negussie, 2011). Developing and implementing a community-based breeding program involves a series of interconnected activities and includes a description of the production system, definition of breeding goals, evaluating market access and policies, development and implementation of a locally adapted breeding strategy (Frans *et al.,* 2010). Participatory rural appraisal is often described as a tool kit of methods that include semi-structured interviews, scoring, ranking, and visual tools like participatory maps and timelines (Chambers, 2007). The emphasis is on using flexible approaches that allow the participants to express themselves in their own knowledge system and provide direction to the interview process. This is very different from the structured interview process used in other epidemiological methods, where questions are formulated from the interviewer's frame of reference (Mariner *et al.,* 2003). Community or village-based breeding programs are intended to overcome the problems related to genotype-environment interaction, to avoid the genetic lag between the nucleus and the village populations, and are also appropriate for in situ conservation of indigenous animal genetic resources. Village-based breeding programs also help to bridge the gap between the skills of the breeders and the farmers (Negussie, 2011). Currently village or community-based breeding programs have gotten wide popularity and they are being implemented in a number of developing countries in Asia and Africa mainly for the genetic improvement and conservation of small ruminants (Negussie, 2011). Community-based

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and participatory surveillance methods do not replace conventional surveillance and analytical capacities. They extend the capabilities of the system with additional information and enhance the ownership of data collection activities by communities (Mariner *et al.,* 2003). Artificial insemination (AI), embryos transfer (ET) and semen sexing are some examples of reproductive biotechnologies. AI is the process of collecting sperm cells from a male animal and manually depositing them into the reproductive tract of a female. AI is the first reproductive technique that had a major impact on animal breeding schemes worldwide. In combination with pedigree registration and milk recording, AI offers the opportunity to obtain accurate estimates of breeding values of young bulls and results in a genetic progress that is much higher than natural mating. This is due to the high selection intensity and accuracy arising from AI since only the top bulls are selected for use in producing numerous offspring in many herds (Van Arendonk, 2011). Although there is now a considerable body of published research on indigenous types of ruminants and non-ruminants livestock in tropical areas of Africa, much of the work published has the disadvantage of having been carried out under controlled conditions at research stations. Consequently, results are often not applicable to communal production systems in rural areas (Webb & Mamabolo, 2004). The purpose of this chapter was to describe how community-based participatory research was applied to the cattle genetics improvement through the use of artificial insemination in the rural areas of Vhembe district.

7.2. Ethical clearance

In conducting the research I initially acquired ethical clearance from the University research board, which was a permitting document that attached my study to the University in general towards the community and the stakeholders. Before outreach the communities as a livestock researcher I were familiar with ethical precepts and policies. Animals were protected from research designs that involve pain, illness, isolation, mutilation and/or premature death. Animal based research address an important question relevant to the University's objectives in advancing knowledge, education, science and human and animal welfare through research, be based on plausible hypothesis and had a reasonable prospect of yielding good results. Therefore, using animals for experimentation, pregnant diagnosis and teaching was my responsibility that the animals that were used were affording the highest level of welfare and protection from the abuse and violation.

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7.3. Awareness creation and securing for the research

Getting permission to conduct the study took different tolls since there were quite a number of parties to be consulted prior to my community outreach as a researcher. Initially I consulted the Vhembe District Department of Agriculture, chiefly because the agricultural activities within the district are registered under their sector. It was from this department that I managed to get the rightful channels to the legible cattle farmers in each community through their traditional leaders. Through their traditional leaders I managed to get direct access to the cattle farmers in their communities after various cattle insemination awareness events, which we presented right from the Limpopo Department of Agricultural to individual farmers I worked with throughout the research, although it was not without its own weaknesses.

7.4. Building relationships and securing commitment of farmers to participate

Although it was difficult initially to gather the farmers for a single large formal meeting in each community, I managed to run awareness meetings on an individual basis which each one of the participating community members created a platform to fully understand and participate in the research which in turn secured commitment to all the processes throughout regardless of the fears and doubts in the members. Regardless of the fact that it was time consuming and difficult to call upon the famers for a single gathering the individual meetings helped largely in creating a concrete feedback mechanisms because we from then onwards communicated directly telephonically, and farm visits which was the greatest contribution in monitoring and evaluating the cattle we used for the research.

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Table 7.1.1: Sequence of events

Date	Milestone		Significance of the Milestone
	Activities	Results	
October 2012	Meet Vhembe district municipality's Local Economic Development (LED).	Advised to meet the District Manager for the Limpopo Department of Agriculture in Vhembe District. Further advised to meet the head of department (HOD) of the Limpopo Department of Agriculture (LAD)	This was necessitated by the need to obtain strategic institutional permission and support. The meetings revealed that there was another organization also carrying out almost similar work on artificial insemination and embryo transfer in the Vhembe District.
November 2012	Gave Presentation on livestock improvement through the use of artificial insemination to the Head of Department Limpopo Department of Agriculture by my supervisor Prof Barry, Head of Biotechnology Laboratory of Center of Excellent in Animal Assisted Reproduction (CEAAR),	Got go ahead after 2 months, as the Head of the Department of Limpopo Department of Agriculture first had a meeting with the organization that was doing the similar research project with us.	This was necessary to obtain go ahead and support from the head office of Limpopo Department of Agriculture.

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February 2013	Meet District Limpopo Department of Agriculture	The District Department of Agriculture organized a meeting with National Emergent Red-Meat Producer Organization (NERPO).	This was important to outline the objectives of the research and revitalize the memorandum of understanding between the university of Venda and Limpopo District Department of Agriculture.
March 2013	Meet Vhembe NERPO chair person. Invited to the community meeting by National Emergent Red-Meat Producer Organization.	Members of the National Emergent Red-Meat Producer Organization were interested on the research and actively participated on the meeting by suggesting some ideas on how the research can uplift rural cattle owner.	This was important to inform the National Emergent Red-Meat Producer Organization about the research and building relationship with farmer's organization.
March 2013	Meet head men, extension officers and cattle owners. Artificial insemination awareness and secure farmers commitment.	Farmers asked many questions about the research in order to understand exactly when, how and what is the benefit of the community on artificial insemination and what will happen after the insemination if the maybe some complications in their cows.	This was important to get data base of farmers who were interested in the research. Further get permission by head man and go ahead by farmers. Farmers registered their names and contact details to ease communication between farmers and researcher.
April 2013	Calling farmers on the register and for farm visit in order to Identifying animals, farming	Many cattle handling facilities were not in good working condition were one may insert vaginal insert and	This was necessitated by the need to know the type of breed that a farmer was farming with and the choice of the

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	area and cattle handling facilities within the farm and/or community. Further checking on the cattle handling facilities if there were in the good working condition.	administered synchronization injection. However, some of the farmers did their are best to fix the cattle handling facilities before the synchronization and insemination time.	type of the bull semen (Bonsmara & Nguni) a farmer will prefer.
July 2013	Farm visit and Pregnancy diagnosis by rectal palpation and separation of cows & heifers from the bulls	Many cows did not diagnose for pregnant status due to poor and old public cattle handling facilities. But for the farmers who were having their own cattle handling facilities, cattle were diagnosed.	This was mostly important to identifying pregnant and non-pregnant cows. Non pregnant cows were separated from the bulls for the period of 6 weeks before they were synchronized and artificially inseminated.
July 2013	Farm visit and synchronization of cows and heifers	Cows and heifer were synchronized were there was cattle handling facilities.	This was done to manipulate the oestrous cycle of the cows in order to bring them in oestrus at the sometime.
August 3013 to June 2014	Farm visit and artificial insemination	A total of 205 female animals were inseminated.	This was important for the deposition of semen into the uterus of the cows with the aim of achieving conception and thereafter pregnancy.
November 2013 to September 2014	Farm visit and pregnant diagnosis.	Data was collected 2 months after insemination from all cows and heifers that were inseminated.	This was important to detect which cows were pregnant or not pregnant by rectal palpation 2 months after

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				insemination and data was collected and recorded.

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Case study 7.1.2

Dear sir/madam

RE: REPORT ON THE UNIVERSITY OF VENDA [UP] CATTLE IMPROVEMENT PROJECT

On December 2013 I was phoned by one researcher from the University of Venda, who told me that he was from the University of Venda and he asked if we were farming with Bonsmara cattle and then I confirmed to him that we farmed with Bonsmara cattle. Then, the researcher said he was running a Univen project (UP) that aims to improve cattle genetic in Vhembe district and we could benefit from it and I provisionally agreed that it could be done. Thereafter I inform our family members about the UP that is improvement cattle genetics through artificial insemination with the use of good quality Bonsmara and Nguni bull's semen. On the 18th of December 2013, we had an appointment, and he came and gave us information about separation of cows from the bulls after pregnant diagnosis by rectal palpation. Further explained that the semen breed he will use to inseminate our cows is our own choice, and we recommended that we will use the Bonsmara semen of two different bulls due to fear of further inbreeding and the researcher agreed. The screening of pregnancy status, the researcher merely used his hand covered by plastic into the vagina of the female to identify whether it was pregnant or not. How precise this could be possible to inform him weather the females were pregnant or not. How precise this could be done and detect that the females were pregnant or not, while we could not determine. About 33 females were targeted to be used by UP and all the females were ear tagged. All females were tagged with UP tag numbers and those numbers were suggested by the researcher. Then, the Y-shaped plastic was inserted into the vagina of the 33 females and an injection was also administered in order to abort the unborn calf, in case it was pregnant, a few days after the above procedure was done we saw blood coming out through the vagina from the cattle that have been prepared for artificial insemination. On the day he should have arrived with the semen to inseminate he did not come. We even sent a driver to go to University to fetch him but he could not come. We now discussed and found that the UP was a disaster. Then he came back to us with excuses that "he had an accident" and repeated the same procedure to let them be on heat again and he never came back again. When I phoned him that he should come he said in Tshivenda, *"ndi khou ya u thogomela kholomo dza khotsi a nga"* and that was all about the UP project. We, as family and farmers engaging upon cattle farming as an economic activity have now come to the conclusion that the UP has been a waste of time and other economic resources, abusive to animals and dangerous to their health and very costly. What we are now engaging upon is

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an attempt to control the damage caused by this project.

Kind Regards,

Yours faithfully

Cattle owner

7.2.3. Case study

A letter of complaint sent by one of the cattle farmers in Vhembe District against the research to the University due to a misunderstanding between the researcher and the farmer on the expectations and results of research. The farmer wanted the processes to be done at his call as a way of reaping quick results, with less consideration of the consequences and without any understanding of animal assisted preproduction. And I did not insert my left arm through the vagina, but through rectum and palpate the reproductive tract through the rectal wall of the cow. The farmer in this case had too much expectation that the experiment could sow, hence I was forced to terminate my project with his cattle which led to perhaps writing such a complaining letter to the University, because upon scientific tests and facts the cattle did not bleed (abort). His letter was written based on assumptions since I injected the preparatory drugs to the cattle and I did not answer to his inseminating calls, because he did not want to comply with the scientific timing expected, because he had calculated on the results more than the process. Such cases brought about skepticism among some of the famers, because the farmer in concern could not be honest in his explanations to the other farmers, at the same time wanted to manipulate the University in a form of compensation although his claims were void. One of the challenges was that farmers with an example of the above did not trust student researcher abilities and understanding of the field of study, for instance the use of pregnant diagnosis by rectal palpation. It shows that the community expects scientific machineries to give accurate results from researchers, which is not always the case. Although the experience was more negative it also brought about awareness that I later used in the next encounters with the farmers who agreed to participate in the project.

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7.5. Experimentation with artificial insemination

I managed to artificial inseminate 205 female animals of which 142 were cows and 63 were heifers. In this study, by using community-based participatory research Artificial insemination achieved 63 % pregnancy rate in the rural communities of Vhembe district with the use of good genetic quality semen of Bonsmara and Nguni bulls. These female animals were synchronized and artificially inseminated from individual farmers within the rural areas of Vhembe district. Cattle farmers made this research work possible by fully participating through creating good opportunities to separate bulls from cows by feeding and keeping non pregnant cows and heifers in the safe intensive place. Animal feeds were formulated in the university of Venda and sold at an affordable price to the farmers could not afford to purchase commercials feeds from a reputed feed company. More calves were born from good superior genetic bulls in the rural communities. Our design was to inseminate 800 cows within Vhembe district communities. But separation of cows from the bulls and limited land was the most challenging factor faced in this study including farmer's commitment and assurance. AI is a very specialized assisted reproductive technology that requires special commitment throughout the program. This could be possible if there were a cattle compound in which cows and heifers could be separated from the bulls during the period of synchronization of oestrus. My animal science curriculum from undergraduate played a crucial role chiefly because it is from there where I leant how, when and why to examine animals without machinery (rectal palpation) and the principles of animal physiology.

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7.6. Conclusion

The community based participatory research approach, above all the methods made the research much easier than expected chiefly because the cattle farmers also played a crucial role in the process of oestrus detection which requires human dedication and thus the success or failure of AI depends on how well this task performed. Community involvement to cattle genetic improvement by the use of artificial insemination in the rural areas of Vhembe district was possible due to fully participation by the cattle owners. Which at the end of the day, pregnant rate of 62 % results were reaped although it is not without its own weaknesses. Regardless of the challenges that I encountered as a researcher, the research process related me as an individual to the community of Vhembe district such that there were lessens leant from both parties but most of it all the farmers are now aware of the artificial insemination process, animal management and reproduction which they can use in future for their livestock production in their herd. On the other hand, I as a researcher I am now aware of the ills and abilities of artificial insemination in the resource-poor rural areas. Therefore a great relationship between the University and the community in concern has been cultivated through the community-based participatory research (CBPR).

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