USE OF UNIQUE COLLECTION DEVICE IMPROVES CONCEPTION RATES OF BOVINE AND EQUINE

by

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ABSTRACT

While the practice of artificial insemination may date back eight centuries, there is still a need for improved techniques for semen handling. Previous research from this laboratory using a canine or equine model, demonstrated that semen collected in a modified collection device, the Device for Improved Semen Collection (DISC), remained fertile for longer periods as compared to samples collected using standard techniques. The object of the present study was to perform controlled breeding trials involving cattle and horses comparing semen collected in the DISC to a traditional control (TC). All sires were collected in both the DISC and the TC. Following collection, all semen samples were processed using standard techniques designed to produce breeding doses consistent with industry standards. Cells were then held a minimum of 24 hrs prior to breeding. In two separate trials, cattle were synchronized with a standard 2-shot prostaglandin protocol. Horses were bred using cells that had been held for periods of 24, 48 or 72hrs post extension. Data collected from the present study supports earlier work, demonstrating extended motility (and in theory fertility) from semen collected in the DISC. Pregnancy data from all three fertility trials demonstrate higher conception rates in animals bred with sperm collected in the DISC unit. Further, to date, no birth defects have been recorded. These data indicate that the DISC to be a superior system for semen collection, resulting in higher conception rates without increased risk of birth defects.

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CHAPTER I

INTRODUCTION

There were approximately 14,100,000 head of cows and calves in the State of Texas, and 97,102,000 head in the United States in 2006 (Texas Cattle Feeders Association, 2006). However, data suggest less than 5% of US producers were reported to be utilizing artificial insemination (AI) in their management practices (Perry, 2002). Typically producers want to be profitable. Pounds of calf weaned and overall increases in animal production in general can be measured as increased profitability. In theory, by utilizing reproductive technologies, such as AI, beef producers are able to monitor the cows more closely, identify problem cows sooner, can condense their calving season to a shorter period, preferably sixty days, and have a more uniform calf crop. Uniformity is becoming a major marketing tool for cow/calf producers. Cattle that look alike and are approximately the same age and weight will be more marketable than those that do not. Unfortunately, the high cost of feed has made an already difficult business increasingly more difficult. Recently, the Chicago Mercantile Exchange had August 2008 live cattle at $98.00/cwt and the Chicago Board of Trade had corn priced at $5.68/bu in September 2008. Such high production costs coupled with low profitability; result in breakevens almost impossible for a routine cattle operation. Therefore, the ability to produce superior cattle coupled with an aggressive marketing plan will allow producers to withstand volatile markets. The uses of advanced reproductive technologies such as AI have made improvements in beef cattle reproduction leading to superior animals. Not only does AI improve reproductive management situations, but it also assists managers in making marketing decisions before the breeding season instead of at sale time.

Currently there are many biotechnologies available for the reproduction of cattle; however, the most widely used remains AI. Artificial insemination has been a reproductive technology for hundreds of years. While anecdotal accounts of the use of AI date to the 1300's, most documented history begins with the work of Leeuwenhoek and his assistant Hamm, in 1678, who were the first to visualize spermatozoa (Foote, 2002). Over a century passed before the first successful insemination was performed. Spallanzani successfully inseminated a dog in 1784 (Foote, 2002). After another century, in 1899 a Russian scientist Ivanow published a paper in the Journal of Animal Science on his AI work in domestic farm animals, dogs, foxes, rabbits and poultry. He later developed semen extenders and helped develop a market for the use of AI in the production of superior livestock (Foote, 2002). Milovanov took over Ivanow's work in 1938 and designed advanced reproductive tools such as artificial vaginas (Foote, 2002). As improvements in reproductive technologies have been made, major concerns have been semen quality, conception rates and pregnancy rates. The object of the present study was to perform a controlled breeding trial with a new semen collection device, the Device for Improved Semen Collection (DISC) and compare results to more conventional means of semen collection.

CHAPTER II

LITERATURE REVIEW

In order for fertilization to occur sperm must swim to the egg, recognize the egg, penetrate the egg, fuse with the egg's membranes, and merge its DNA with that of the egg. Fertilization requires all the sequences to occur in order to be successful. The sequence begins with a group of changes affecting the sperm, which prepares them for the task ahead (Bowen, 2000). Without the act of fertilization, reproduction does not occur and ultimately causes producers' to lose profits. Therefore, understanding the processes of fertilization is vital to beef cattle reproduction.

Sperm Morphology

There are five domains involved with the makeup of spermatozoa. These consist of anterior head, posterior head, equatorial segment, midpiece, and principal tail piece (Taylor and Field, 2001; Bearden et al., 2004). Each of these five domains changes during maturation, capacitation and acrosome reaction in order for fertilization to occur. Two of the main functions of the anterior head are to bind to the zona pellucida and to fuse with the underlying acrosomal granule (E.L Bearer and D.S. Friend, 1990). During the later stages of spermatogenesis the acrosomal granule is shaped to become a large single granule. After sperm have matured they no longer have the ability to synthesize either secretory or membrane proteins (Fawcett, 1975).

The equatorial segment's major function is to provide a membrane capable of fusing with the external egg plasma membrane (E.L Bearer and D.S. Friend, 1990). However, it is not possible for the equatorial segment to fuse with the egg membrane prior to the acrosome reaction. The midpiece contains a single proximal centriole,

and the neck of the midpiece is rich in intermediate filaments, actin, and mitochondria (E.L Bearer and D.S. Friend, 1990). The principal piece or tail contains the elements that allow the sperm to be propelled through the cervical secretions, uterus, and oviduct (E.L Bearer and D.S. Friend, 1990). There is a requirement for ATP and calcium in order for the tail to move in a whip like fashion. Each of these five domains is crucial to the process of fertilization and the initiation of capacitation and the acrosome reaction.

Sperm Capacitation

Freshly ejaculated sperm must first undergo a series of changes known as capacitation. Capacitation is associated with removal of adherent seminal plasma proteins, and reorganization of plasma membrane lipids and proteins (Bowen, 2000). Normally, capacitation occurs while sperm reside in the female reproductive tract, specifically in the oviduct. Little is known about the factors that promote capacitation, but there is evidence that suggests the sperm surface proteins and female tract secretions react to cause capacitation. In most cases, capacitation media contain energy substrates such as pyruvate, lactate, glucose, a cholesterol acceptor, NaHCO3, Ca2+, low K+, and physiological Na+ concentrations (Salicioni, 2007). Cholesterol removal is found to be a major regulatory factor of intracellular signaling occurring during sperm capacitation (Visconti, 2002). Visconti et al. also have determined that serum albumin, specifically bovine serum albumin (BSA), can be used in the removal of cholesterol from sperm plasma. Once destabilization of sperm membrane has occurred, sperm become hyperactive, thus preparing sperm for the acrosome reaction and fertilization (Bowen, 2000).

Acrosome Reaction

Binding of sperm to the zona pellucida (ZP) and then penetration of the ZP is what defines the acrosome reaction (Bowen, 2000). Once sperm have bound to ZP, the difficult task is for the sperm to penetrate the zona pellucida to get to the oocyte and involves several protein kinases such as protein kinase A (PKA), C (PKC), and tyrosine kinase (PTK). The acrosome is the cap that covers the anterior end of the cell head and contains the golgi apparatus that is packed with enzymes. In mammals, the acrosome reaction is described as fusion with the outer acrosomal and plasma membranes. The fusion and vesiculation is followed by the release of acrosomal content and a display of an inner acrosome membrane (Jankovicova, 2006). One of the enzymes that assist with this reaction is hyaluronidase. For successful fertilization, a sperm must penetrate through the cumulus cells. Only if the acrosome reaction has occurred can sperm adhere to the oocyte (Austin, 1975). This happens due to the presence of hyaluronidase, which is attached to the plasma membrane on the head of the sperm by the lipid on glycosylphosphatidylinositol (GPI-anchored) (Lin, 1994). Then the sperm is propelled by its flagellum across the matrix to reach the zona pellucida where the acrosome fuses with the cytoplasmic membrane in the sperm head and releases its contents, including a soluble form of the hyaluronidase (Lin, 1994). Once a sperm has completely penetrated the ZP it travels through the previtelline space, the space between the ZP and oocyte plasma membrane. The vitelline membrane of the oocyte then fuses with the equatorial segment of the sperm. After this has occurred the sperm is engulfed by the oocyte (Hoodbhoy, 2004). There are three glycoproteins that encourage binding of the sperm to the egg coat these are ZP1, ZP2, and ZP3 (Oehninger, 2003). Currently the exact

mechanisms of the glycoproteins are unknown. There are studies that have been conducted that show ZP3 plays a major role in sperm binding, by adhering to proteins on the sperm plasma membrane. Further investigation of the glycoproteins may determine what role they play in fertilization.

Sperm-Egg Interaction

After a sperm penetrates the zona pellucida, it must bind and fuse with the plasma membrane or the vitelline membrane of the oocyte. Binding occurs at the posterior end of the sperm head. Once a single sperm has entered the oocyte a release of cortical granules is triggered. Cortical granules migrate to the cell surface to prevent other sperm from binding, thus preventing polyspermy. Following fertilization a one-cell zygote is formed and the completion of meiosis occurs (Hoodbhoy, 2004).

Semen Evaluation

In order to have successful AI one must properly evaluate semen because without high quality semen conception and pregnancy rates can significantly decline. There are four primary components to the semen analysis: (1) volume, (2) concentration, (3) motility, and (4) morphology (Bearden et al., 2004), although other parameters may be evaluated.

Volume is amount of ejaculate that a male produces and is evaluated based on the capacity to produce sperm per gram of testicular tissue and is highly correlated to scrotal circumference (Baracaldo, 2007). In 2004, DeJarnette observed 5.8±0.33 mL of semen for Angus bulls. It was also noted that techniques which could improve scrotal

circumference and semen production is highly desired. Average gel free seminal volume for the stallion was observed by McKinnon, et al., 1993; overall volumes for Quarter Horses were 40-44mL. For Quarter Horse stallion's evaluated, total scrotal width was 93mm.

Concentration of sperm is classified as very good, good, fair, and poor. These criteria are based on density, whereas creamy semen has approximately 750 to 1 billion spermatozoa per mL and poor, translucent semen has less than 250 million spermatozoa per mL (Baracaldo, 2007). Angus bull semen concentrations of $1.17 \pm 0.06 * 10^9$/ mL were documented by DeJarnette in 2004. Likewise concentration for Quarter Horse stallions averages range from 126-243 million sperm/mL (McKinnon, 1993).

Sperm motility is measured by a 5mm drop of semen placed on a warm glass-slide and observed under 40 X magnification. Spermatozoa are observed for forward progression and rapid movement (Baracaldo, 2007). Samples were subjected to a post thaw evaluation of motile cells after 0 and 3 hours of incubation at 37 C (DeJarnette, 2004). Motility was significantly different ($P<0.05$) in samples at these time periods. Angus bull semen evaluated at 0 hours was 74.5 ± 0.63% motile and semen evaluated at 3 hours was 30.1 ± 0.96% motile (DeJarnette, 2004). Average Quarter Horse stallion motility is 56% (McKinnon, 1993).

Morphology is determined by observing the number of sperm that are normal functional spermatozoa. In the study mentioned previously conducted by DeJarnette normal morphology of Angus bulls was 64.3 ± 2.0%. Many factors can affect morphology of semen and bulls should not be sold or slaughtered based on a single morphology failure. In stallions it has been reported that there is an inverse relationship between

abnormalities of spermatozoa and fertility. Average normal morphology for Quarter Horse stallions is 53-57% (McKinnon, 1993).

It is crucial to the success of AI and other advanced reproductive technologies that semen be viable. If a producer chooses to utilize less than standard dosing of semen it could dramatically decrease the conception rates. However, a producer may feel that the value of the animal outweighs the risk of decreased conception rates. In species such as the bovine, industry standards normally use frozen semen for AI, but fresh collected semen is also an option in an attempt to improve fertility rates. .

Semen Collection

Once a producer has determined there is a viable reason to use an animal in an AI program, semen must be collected. Normally, semen is collected into a collecting device attached at the end of the artificial vagina. Traditional collection methods allow for the ejaculate to be collected into a dry container and have media added soon after collection. While this method is still a practiced method, recent research suggests that adding body temperature media to the collecting device prior to collection is superior in that it will help to prevent temperature and pH shock (Johnson, 2005). It is noted that changes in pH can have effects on the motility of spermatozoa (Phillips and Lardy, 1940). The ideal pH is 6.75, but pH ranges 6.5 to 7.8 are acceptable. A commercial device has been developed to increase the viability of semen for longer periods than traditional collection methods; this device is named the DISC (Johnson, 2005). This device is an insulated collection container that contains a desired amount of 37 C warmed extender. The DISC will maintain temperatures of 30 to 37 C. By maintaining constant temperature semen samples are allowed to equilibrate (Johnson, 2005). The DISC also allows for improved

semen quality and preservation as compared to traditional methods due to the lessened cold and pH shock (Johnson, 2005). Previous research in the equine demonstrated that semen collected in the DISC, then fresh extended, remained fertile for extended periods as compared to samples collected using standard techniques. Similar results were observed in studies with the bull Langdon et al., 2007 found that when semen was collected in the DISC, and then fresh extended in both the DISC and TC as expected, all motilities decreased over time. Motility was maintained 178% longer in the two DISC treatments than in the TC (Control = 168.0 + 49.8 hrs, DISC+ = 297.0 + 34.5 hrs and DISC - = 295.3 + 35.9 hrs; P = 0.071). There was no time*treatment interaction (P = 0.83). It is an industry standard that straws of semen maintain a minimum of 20% motility to obtain suitable conception rates. It was also determined that the DISC maintained motilities over 20% for 176 hours.

Semen Extenders

In the United States most semen processed for AI is frozen prior to insemination, but in other countries such as New Zealand 95% of semen is used fresh (Verberckmoes, 2003). Extenders allow for cells to survive for longer periods of time due to their supply of nutrients. The media in which sperm are incubated play an integral role in many sperm processes. In 2005, Johnson et al. observed that the addition of 1mL of a commercial semen extender Biladyl lessened cold shock and pH shock to the spermatozoa. By utilizing the extender there was an improvement in motility and further a 16% increase of conception rates (Johnson, 2005). Given 2008 cattle prices, a 16% increase of conception could return producers significant profits.

Estrous Cycle

Bovine are non-seasonal polyestrous species. For normal, pubertal cows and heifers the estrous cycle is reoccurring approximately every twenty-one days (Bearden, 2000). Day 0 of the estrous cycle is considered to be when the female exhibits estrus or heat (Williams, 2004). Estrus lasts twelve to eighteen hours, followed by ovulation, which occurs twenty-five to thirty-five hours after onset of estrus (Roberts, 1956). The bovine estrous cycle is broken into four phases of the cycle, proestrus, estrus, metestrus, and diestrus.

Proestrus

During the late luteal phase, progesterone is a dominant controller of the estrous cycle (Bearden, 2000). At this time progesterone inhibits the release of follicle stimulating hormone (FSH) and luteinizing hormone (LH; Bearden, 2000). If pregnancy has not occurred by day 16-17, prostaglandin is released. This causes luteal regression, until the destruction of the corpus luteum (CL) (Schams et al., 1977; Cupp et al., 1995). Once progesterone is inhibited, GnRH rises and stimulates LH and FSH. Pulsations of LH are needed to mature preovulatory follicles (Roberson et al., 1989; Stock and Fortune, 1993). FSH is required for the recruitment and maturation of follicles (Ireland, 1987). The dominant follicle establishes itself and is the main follicle that grows. The growing follicle produces estrogen in the granulosa cells. Estrogen signals initial signs of estrus in the cow. Prior to “heat”, estrogen peaks and causes the final LH release (Hafez, 1987).

Estrus

During estrus both estrogen and FSH are declining. The LH peaks during standing estrus. Once estrogen is released into the bloodstream at the onset of standing heat the

estrogen then triggers the central nervous system that causes the female begin to show signs of behavioral estrus. Behavioral estrus is generally associated with the animal mounting other animals, nervousness, a swollen vulva, mucus discharge and congregating around other animals. This behavior generally starts 4-48hrs before standing heat. Standing heat is determined by the female standing while another animal mounts her. Cows generally only spend four to six minutes each cycle in active standing heat (Williams, 2004). During estrus or "heat" cows and heifers stand to be mounted by another cow. Following "heat," approximately 12-18 hours, ovulation will occur by releasing the egg (Perry, 2004). During estrus thecal cells, which have been exposed to the LH surge and are beginning conversion to luteal cells, start producing progesterone, which inhibits LH and FSH release (Hafez, 1987).

Metestrus

Metestrus is the time of luteal development and lasts approximately 3-5 days. During metestrus the CL begins to form from the corpus hemorrhagicum, which is the follicle after ovulation (Hafez, 1987). At this time of the estrous cycle the CL will not be mature. As the CL matures progesterone continues to rise. Due to the lack of maturity, which involves the complete conversion and growth and duplication of theca and granulose cells to luteal cells, the CL injections of PGF2α will not work and should not be administered.

Diestrus

Diestrus occurs during days 5-16 of the estrous cycle. A mature CL begins to produce progesterone during this time. A fully functional CL allows for injections of

PGF2α to work properly and should be administered during this phase of the estrous cycle.

Estrous Synchronization

Basic protocols used for beef cattle reproduction consist of but are not limited to Prostaglandin F2α, Melengestrol Acetate (MGA), Gonadotropin Releasing Hormone (GnRH), and Controlled Intravaginal Device (CIDR). All of these drugs should be used in accordance with the manufacture's label and dosages should not be adjusted. Any adjustments could affect the outcome of the success of the AI program. The use of prostaglandin's (PG) in beef cattle began in 1971(Lauderdale, 2004). In 2004, Lauderdale also cited that many studies had demonstrated PGF2α can be released from the lungs, brain, spinal cord, kidney, iris, umbilical cord, fat, adrenals, stomach, intestines, nerves, menstrual fluid, amniotic fluid, seminal plasma, blood, skeletal muscle, cardiac muscle, salivary glands, thyroid, pancreas, and uterus. Prostaglandins induce the destruction of the Corpus Luteum (CL; Perry, 2004). However, a fully functional CL must be present to have successful synchronization with this compound. Therefore if the animal is not in the correct stage of the estrous cycle a shot of PGF2α will not affect the animal by returning them to estrus in a predictable fashion. Prostaglandins cannot induce estrus in beef cows or peripubertal beef heifers. Prostaglandins will not affect the cycle of cows with an immature CL. Generally this occurs between day one and day five of the estrous cycle. Nor will prostaglandin affect cows after the CL has already started to regress, about day 17 or 18 of the cycle.

Two-Injection Prostaglandin

Availability of PGF2α products are numerous and include Lutalyse/ Dinolytic Pronalgon F (Pfizer, New London, Connecticut), Estrumate/ Planate, Prosolvin (Intervet The Netherlands), Bovilene (Fort Dodge, Madison, New Jersey), and many generic products worldwide. A number of studies have been conducted to compare these products and no statistical differences have been reported (Lauderdale, 2004). In 2001, Lucy et al., documented a 5% increase in females pregnant after 31 days after injections of PGF2α versus animals that were not synchronized. Two injections of PG is a common estrus synchronization method utilized by commercial beef cattle producers. Two shots of prostaglandin are injected 11 to 14 days apart and females are observed for heat and bred for 5 days following the second injection (Stevenson et al., 2000).

CIDR Protocol

Controlled Internal Drug Release (CIDR) is an effective method of estrus synchronization that yields noticeably higher pregnancy rates (Merrel, 2003). The CIDR method allows for a more controlled synchronization period, but implementation of a CIDR protocol is very costly. CIDRS generally cost $10-$12 for the device plus an additional $2.50 for the injection. The device can be reused in order to reduce the cost, but the effectiveness also will decrease and a higher risk of venereal disease is also associated with its reuse (Merrel, 2003). The CIDR protocol consists of insertion of the device on day 0, injection of PGF2α on day 6, removal of the device on day 7, and heat detection and AI for 96 hours. This protocol can induce estrus, therefore working on both cyclic and anestrous cows.

Estrus Detection

Estrus detection is crucial in the success of any AI program. A producer's inability to detect estrus can be costly and highly ineffective. Downing et al., 1998 detected 39% more females in estrus using the HeatWatch (CowChips, LLC, Denver, CO) method versus visual detection. Pregnancy rates are highly correlated to estrus detection rates (DeJarnette et al., 2001). Current computerized estrus detection methods give producers 24hr surveillance of the animals. Animals are fitted with the device and observed until onset of estrus is determined by the number of mounts the animal receives (Cowchips, 2007). HeatWatch employs radio frequency data communication in order to determine onset of estrus. This is a more efficient way of estrus detection, but it is also more costly than visual estrus detection.

Conception Rates

Conception rates in beef cattle are determined by (no. pregnant / no. detected in estrus) *100. Conception rates are important when evaluating the success of any AI program. These rates can be determined by ultrasound, rectal palpation or the lack of return to estrus.

This review of literature that shows successful pregnancies are the key in any breeding program. In order to be successful, one must implement proper breeding techniques. The objective of the following study is to assess the improvement of conception rates of beef heifers and mares using the DISC versus a TC. Positive results in conception by use of the DISC could result in more field trials to further examine the value of DISC to bovine and equine industries.

CHAPTER III

BOVINE STUDY I

Introduction

Previous research has been conducted using artificial insemination and semen collected in the Device for Improved Semen Collection (DISC) to improve conception rates in beef females. The DISC device has been shown to provide a superior collection environment by: 1) providing temperature control, 2) decreasing the exposed surface area of the collected sample, 3) buffering changes in pH and 4) preventing osmotic shock, Further, preliminary breeding research has shown improvements in the overall conception rates; however, breeding data to date has been anecdotal and not evaluated in a large sample size. The objective of the present study was to retrospectively evaluate the effectiveness of the DISC in a non-controlled breeding trial of beef females in a production herd in Texas.

Materials and Methods

In this experiment, the DISC was used to evaluate conception rates in beef females versus a traditional control. Semen was collected from bulls using electro-ejaculation by a trained professional using the DISC or a traditional control (TC). Each collection of semen in the DISC had an additional, predetermined amount of warm extension media. Females were bred either by semen collected in the DISC, TC, or frozen semen from a proven sire. Conception rates were then determined by a trained ultrasound technician following insemination. As the original intent was solely to evaluate semen parameters (Johnson, 2005), no attempt was made to insure equal numbers of breedings per treatment.

Selection of Animals

This study was conducted on a large production cattle ranch in West Texas. Fresh-extended experiments were conducted using samples from three bulls collected in the DISC and TC simultaneously using a splitter developed in this laboratory. An additional bull was used for the frozen portion of the experiment. One hundred-thirty beef females were observed and randomly assigned to treatment when estrus was observed. Once estrus was determined, trained AI professionals inseminated the animals with semen collected in the DISC, traditional device or with frozen semen. In addition, data such as age, breed, diet, and AI technician were recorded for each individual female used in the experiment.

Semen Parameters

Semen samples were evaluated upon collection and at zero and one hour post collection. After the one-hour evaluation, semen was chilled and stored at 5C. The analysis consisted of measurement of volume, concentration, motility, forward progression, and viability. All parameters were evaluated manually using an Acuscope 3040 microscope equipped with phase optics (Acuscope; NY, NY) at 200X magnification. After a twelve-hour equilibration period semen was placed in standard 0 .5 mL straws and used for fresh insemination into commercial heifers.

Experimental Design

All data collected in this study were analyzed using the Statistical Program for the Social Sciences (SPSS ver 12, SPSS, Inc; Chicago, IL). Differences in estrous synchronization rate, overall conception rates, conception rate by bull, conception rate by diet and conception rate by breed of female were analyzed by chi-square analysis using the frequency procedure of SPSS.

Results

Synchronization Rate

One hundred-thirty beef females were observed for estrus by trained professionals. As expected, there was a lower overall synchronization rate in females due to estrus detection ability. Eighty-four females exhibited estrus (64.6%). As these animals were part of a production beef herd, all animals were artificially inseminated due to the ranch schedule. However, only animals with detected estrus were included in the present study.

Conception Rate

Eighty four animals met inclusion criteria. Overall, 39.3% [33/84], of synchronized animals conceived by artificial insemination there were no significant differences among treatments (P=.38; Fig. 3.1). Females that were bred with DISC collected semen conceived at a rate of 43% [16/37], females bred with TC semen conceived at a rate of 35% [7/20], and females bred with frozen semen conceived at a rate of 37% [10/27]. There were no differences among all three treatments (P=0.60). An

interaction occurred between conception rate and estrus response. Technician conception rates were not significant and conception rates were 16/42 [38.1%], technician 1; 17/42 [40.5%], technician 2.

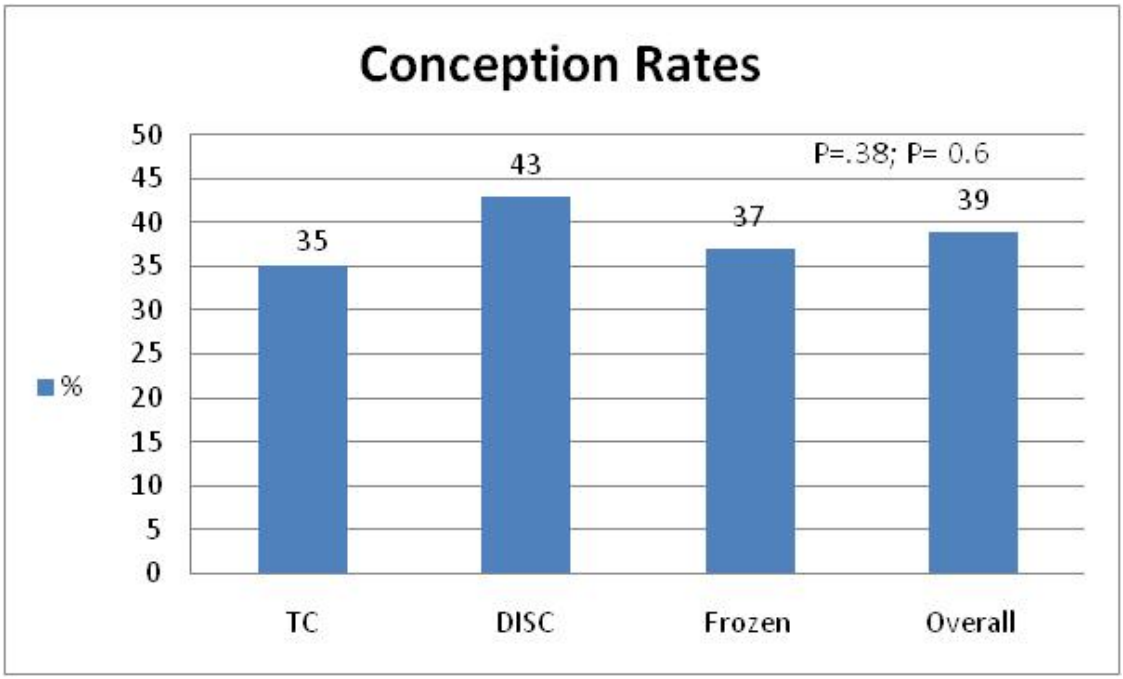

Figure 3.1. Differences in conception rates in an AI bred herd following semen collection in a novel collection device, verses a traditional control.

Conception Rates by Bull

The conception rates by bull for the control were as follows: 5/10 [50%], Bull A; 2 /10 [20%], Bull B; there were no females bred for Bull C (Fig. 3.2). Conception rates by bull for semen collected in the DISC were as follows: 0/8 [0%], Bull A; 8/13 [62%], Bull B; 8/16 [50%], Bull C. In both the TC and DISC there was a strong bull by treatment interaction ($P<0.002$).

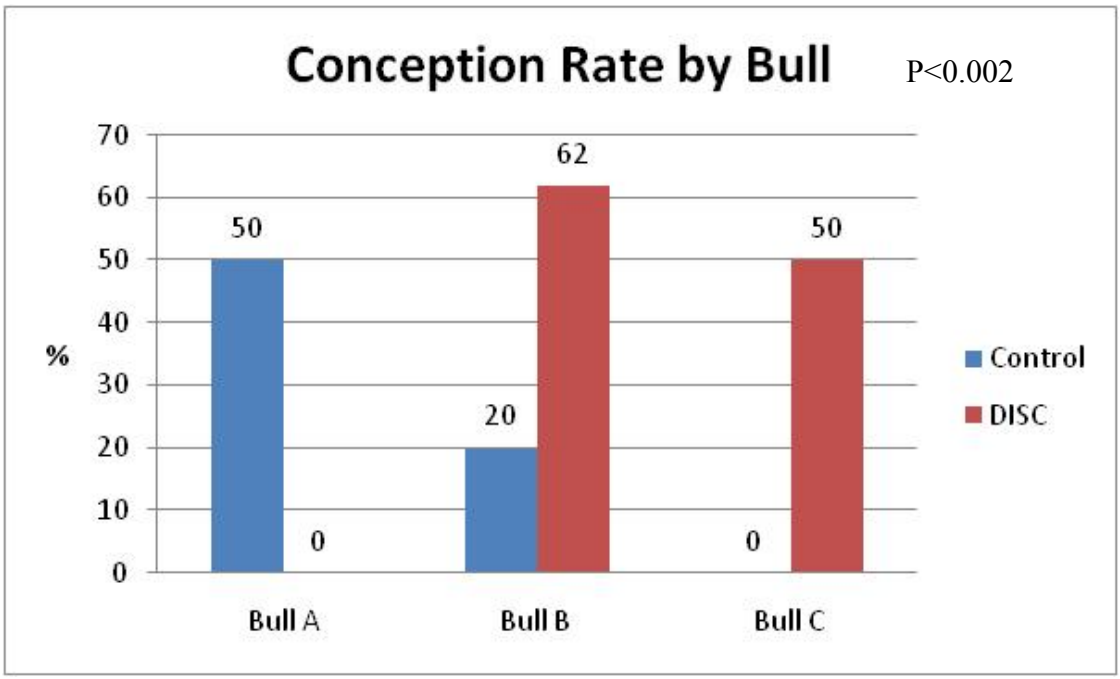

Figure 3.2. Differences in conception rates in an AI bred herd following semen collection in a novel collection device comparing the sires selected.

Conception Rates by Diet

Diet A consisted of wheat/rye hay and free choice Purina Accuration (Purina Mills LLC, St. Louis, MO). Diet B was triticale silage produced in accordance with the ranch protocol. The conception rates by diet for the control were as follows: 5/10 [50%], Diet A; 4 /13 [30.8%], Diet B. Conception rates by diet for females bred with semen collected in the DISC were as follows: 10/24 [41.7%], Diet A; 6/13 [46.2%], Diet B. Conception rates by diet for females bred with frozen bull semen were as follows: 5/12 [41.7%], Diet A; 5/18 [27.8%], Diet B, and these data indicate that conception rates by diet were significantly different (P<0.012; Fig. 3.3). There was no interaction of either

diet with treatments (P=.31). It was evident that females fed the silage diet gained more weight and had more fat, but lower conception rates were experienced. Further research should be conducted to determine the best diets to use when breeding females artificially.

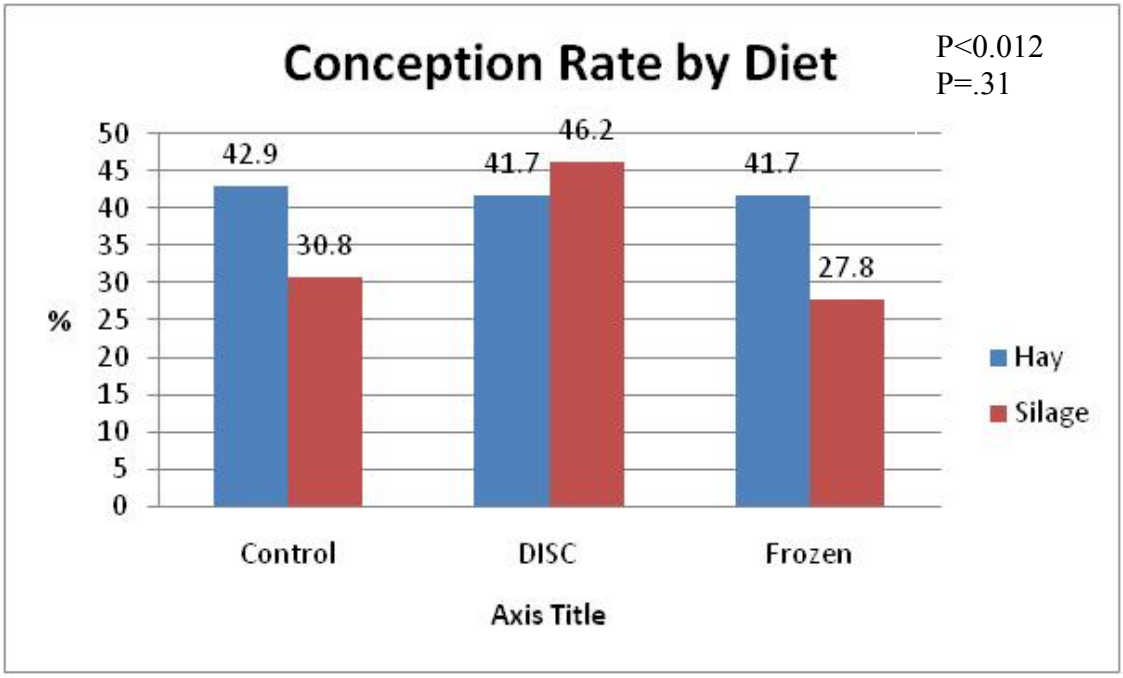

Figure 3.3. Differences in conception rates in an AI bred herd following semen collection in a novel collection device comparing diets of wheat hay versus triticale silage.

Conception Rate by Breed of Female

Females used for this experiment were composed of four different breed types. Conception rates for individual breeds were as follows: 18/59 [30.5%], Breed A; 6/6 [100%], Breed B; 7/17 [41.2%], Breed C; 2/2 [100%], Breed D (P<0.003; Fig. 3.4). Breed effect was significant, but further research with larger sample sizes needs to be conducted to determine if the significance is due to breed effect or low sample numbers.

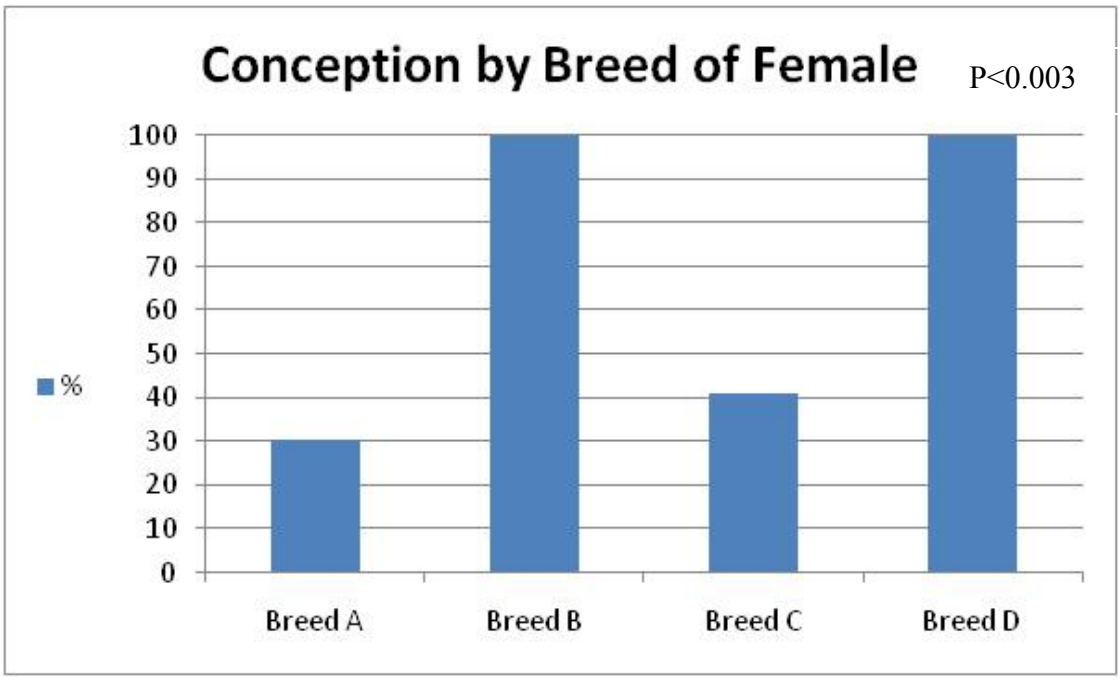

Figure 3.4. Differences in conception rates in an AI bred herd following semen collection in a novel collection device comparing the breeds of females selected.

Discussion

Females were obtained from a reputable ranch in Texas and were deemed suitable as breeding animals. Additionally, fresh-extended semen was supplied from three bulls at the same facility. Since frozen semen is the current standard practice in the bovine industry when artificially breeding beef females it was used in the study as a control. Semen collected in the DISC device and subsequently used to AI beef females was numerically more successful in conception than semen collected in the traditional device or for frozen semen (23% and 16%, respectively). Conception was affected significantly by diet, bull, and breed of female. Further research is needed to determine if these differences were attributed to low sample size or if they do impact AI conception rates of beef females.

As the present study was more observational than experimental, it had several potential flaws. Some animals did not show estrus but were bred 51/130 (39%) and were not included in the present study. Due to volume issues for the semen position of the study, not all bulls were represented in all breeding treatments. However, the increased pregnancy rates compared favorably to both a fresh and frozen control and while only numerical are suggestive and warrant further study. A follow-up breeding trial (to be discussed in the next chapter) controlling the issues limiting the current trial, was conducted to determine if there are true differences in success with semen collected in the DISC device.

CHAPTER IV

BOVINE STUDY II

Introduction

Previous observational research in a production herd has suggested an increase in conception rates of bovine using the Device for Improved Semen Collection (DISC) due to its improved environment for semen collection. However the nature of the previous study did not allow collection of data to evaluate ongoing pregnancy and calving data. Further, the observational nature of the study limited the direct comparison of the DISC with the traditional method of semen collection.

In the present study, five Angus bulls were collected alternating between the traditional method and the DISC. Following processing and being held for 24 hrs, the semen was used to breed forty-three Angus based females. The design not only allowed a direct comparison of conception rates between collection methods, but it also allowed observation of differences in pregnancy outcomes and calving.

Materials and Methods

In this experiment, semen was collected in the DISC or a traditional control, to evaluate the improvement of conception, pregnancy and overall calf viability. Following collection, all semen samples were processed using a standard extender and technique to produce a sample with an initial motile concentration of 40 million cells per mL for a planned breeding dose of 20 million motile cells.

Male Selection

Five Angus bulls, approximately 18 months of age, were selected for this study. The animals had previously been tested for breeding soundness and were demonstrated to provide sufficient volume and motile cell numbers for experimentation. The bulls were adjusted to a feed regimen in the same manner as the females (see below) and were housed and sheltered in a similar fashion. Bulls were collected in the DISC device containing a commercial media (not named, considered proprietary to device) and were warmed to approximately 37°C. All five bulls were collected using a standard electro-ejaculation collection technique for bulls, by a trained professional. Following collection, all semen samples were processed using a standard extender, non-glycerol Biladyl (Minitube of America, Inc.; Verona, WI) and technique to produce a sample with an initial motile concentration of 40 million cells per mL (a planned breeding dose of 20 million motile cells). Each sire was collected in both the DISC and traditional devices, using the standard 15mL conical tube. All collections were stored overnight at 5 C and the samples were used the next day to breed a maximum of five animals. The process was repeated until each bull had bred five females using sperm collected in a traditional fashion and five females using sperm collected in the DISC. A sample was also taken from each collection and preserved with gluteraldehyde (Sigma Chemical; St. Louis, MO) to allow for later testing of cells to determine if the cell's acrosomes reacted. After a twelve-hour equilibration period semen was loaded in 0.5 mL straws for fresh insemination into commercial Angus-based heifers.

Semen Parameters

Upon collection, semen samples were evaluated just after collection and prepared for storage using standard techniques. The samples underwent a second evaluation at one hour post collection. After the one-hour evaluation, semen was chilled and stored at 5C. Analyses were conducted for volume, concentration, motility, and forward progression. All parameters were evaluated manually with an Acuscope 3040 microscope equipped with phase optics (Acuscope; NY, NY) at 200X magnification by trained technicians. Details of the measurement of each parameter are provided below.

Volume

Initial total volume of semen was recorded at zero hours. For semen collected in the DISC, volume was determined by the total collection device minus the volume of extender added prior to collection.

Concentration

Exact concentrations of spermatozoa in a sample must be known in order to determine the number of doses that can be obtained from a single ejaculate. A specialized slide (Microcell; Conception Technologies; San Diego, CA) with a predetermined volume (3 uL) of semen was used to determine concentration of samples. Evaluation was conducted at a magnification of 200X. To complete the analysis, sperm were counted in ten blocks, selected at random in a 100 block micrometer. Using a manufacturer's predetermined equation for this magnification, the number of cells/mL were then calculated for each sample. Following completion of the original analysis, semen was initially extended to a 1:1 ratio of semen extender to semen and the

concentration was then recalculated. Sufficient extender was then added to the semen to dilute the cells to a final concentration 40 million motile cells per milliliter.

Motility

Sperm motility was determined by manually counting one hundred cells using the Microcell slide and a magnification of 200X. Spermatozoa were evaluated based on moving cells (motile) or non-moving cells (non-motile). Motility was expressed as a percentage.

Forward Progression

Spermatozoa were evaluated for forward progression after motility and based on a scale of 1 to 5. A score of 1 indicates sperm are moving with slight side to side movement, with little tail propulsion and no forward progression. A score of 2 indicates sperm cells are moving in circular or irregular patterns with no forward progression, 3 indicates rapid movement side-to-side, but slow forward progression. A score of 4 indicates cells moving in a slow but steady forward progression. Finally a 5 indicates rapidly moving cells in proper forward progression across the microscope field. With forward progression score of 5, cells should be moving across the field in less than one second.

Acrosome Reaction

Determinations of the acrosome reactions were made using the chlortetracycline technique of chlortetracycline fluorescence assay (Lee et al., 1987) with the samples fixed in gluteraldehyde. Chlortetracycline stain solution was prepared by filling a 50mL conical centrifuge tube with 5mL of powdered chlortetracycline and 35mL of water. The

contents were then mixed. Due to the light sensitive nature of the chemical, it was important to minimize contact with light throughout the remainder of the procedure.

To prepare the semen for further evaluation a new disposable pipette is used to extract a drop of semen and placed in a new micro-centrifuge tube with a single drop of chlortetracycline. The new mixture was then extracted using a disposable pipette, placed on a specimen slide and covered with a cover slip. The slide was then viewed under a ZEISS compound microscope (Carl Zeiss; New York, NY) equipped with epi-fluorescence using 630 X magnifications. A glowing fluorescent green sperm head indicates determination of an intact acrosomal cap. Lack of a glowing fluorescent green sperm cell indicates the acrosomal cap had either reacted, was damaged, or was no longer present. A total of 100 cells were counted and the data were expressed as a percentage of reacted cells.

Female Selection

Females were selected randomly by onset of estrus. All females were Angus-based commercial heifers approximately 14-15 months of age. Estrus was detected by the HeatWatch (CowChips, LLC, 2008) system and animals were bred approximately twelve hours after onset of estrus. Due to the animals being accustomed to range conditions, a two-week period was allowed for adjustment to dry lot conditions and to the feed ration to be administered during the experiment. The animals received four pounds per day of 20% CP range cubes, free choice Cargill mineral, and free choice high quality wheat hay. All animals were subjected to the same feeding, handling, and shelter conditions.

Breeding Procedures

As designed, semen from all five bulls was used in both treatments and five females were bred from each treatment for each bull with a maximum of 25 animals per treatment. Prior to breeding, all females were subjected to the two-shot prostaglandin estrus synchronization protocol. Animals received two 25 mg i.m. injections of PGF2α, first on day 0 and again on day 14. All animals were fitted with The HeatWatch estrus detection system from day 0 until day 45 to detect estrus. Each animal was fitted with a HeatWatch transmitter on day 0 that was placed on the tail head of the animal that recorded any mounting activity twenty-four hours a day. The system was checked twice daily, once in the morning at approximately 7:00 am and once at night at approximately 7:00pm. Artificial insemination was performed 12h after the first detection of the onset of estrus. Animals were inseminated using semen from one of five bulls, which were equally and randomly distributed throughout treatments. Estrus detection rate equaled (no. detected in estrus / no. assigned)* 100. Animals were evaluated for thirty days before they were delivered to their respective pastures with the bull. Following insemination and a 30-day period, all animals that were mated artificially were returned to their respective pastures with clean-up bulls of known fertility for 90 days. Overall conception rate equaled (no. pregnant / no. detected in estrus) *100. Artificial insemination rate was determined by date of birth of calf. Pregnancy rate was determined for artificial insemination rate and clean-up bull rate.

Statistical Analysis

Experimental Design

All data collected in this study were analyzed using the Statistical Program for the Social Sciences (SPSS ver 12, SPSS, Inc; Chicago, IL). Initial semen parameters for each treatment were analyzed using a one-way analysis of variance (ANOVA) with Tukey's mean separation. Comparisons between DISC and control devices for motility and viability were analyzed using the General Linear Model (GLM) and ANOVA. Chi-square analyses were used for forward progression data.

Differences in O/B interval (time between onset estrus and time of insemination) and mount total between treatments were analyzed by ANOVA. Estrous synchronization rate, overall conception rates, and pregnancy rates were analyzed by chi-square analysis using the frequency procedure of SPSS.

Results

One goal of the design was to inseminate cows with an equivalent (20 million/mL) dose of semen at 12 hrs post collection. Repeated collections were required from some animals (1-3 collections per animal/trt group). Resulting in 17 collections (9 vs 8 TC: DISC) respectively. There were no differences in the number of collections due to bull (P =.81) or treatment (P = .80) suggesting the collection process was equivalent for both treatments.

Motility

Initial motility was similar in both devices, 74.7 + 12.1% vs 73.1 + 15.4%, TC vs DISC respectively (P =.82). As expected, there were some instances were initial motility in the DISC was lower than TC, but this was due to the spermatozoa remaining stable in the new environment. Bull B had lower motility in the DISC device, but achieved a 100% conception rate. As in earlier experiments, motility in the DISC appeared to remain stable over time while the control decreased over the 12 hr holding period.

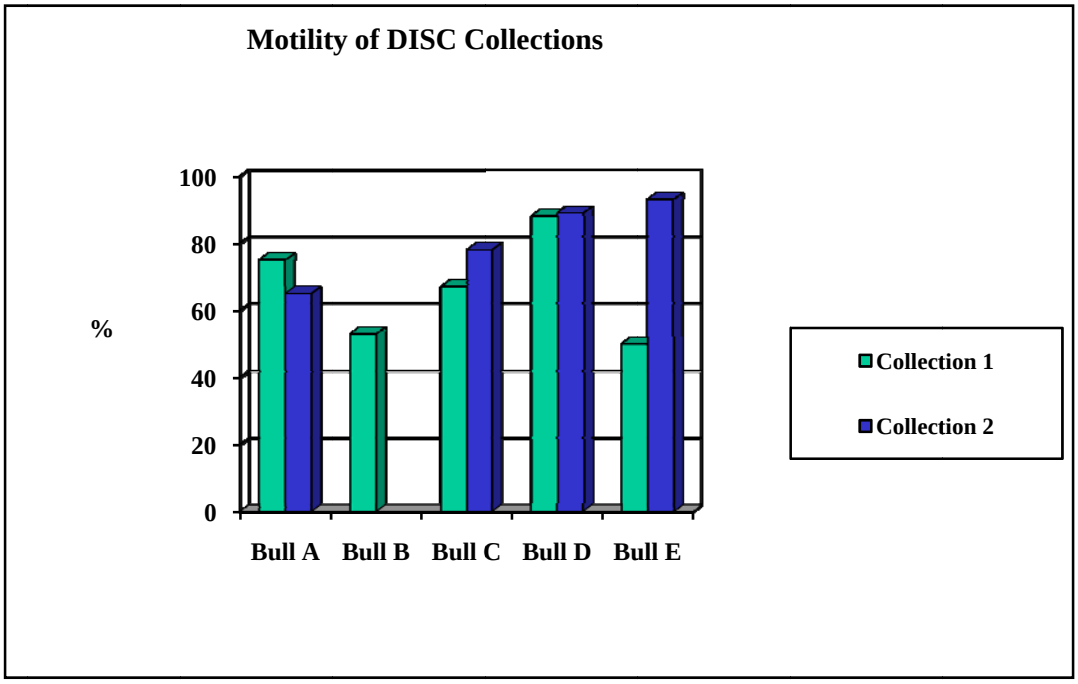

Fig. 4.1. Differences in motility comparing semen collections from individual bulls when samples were collected with a new semen collection device, the device for improved semen collection (DISC).

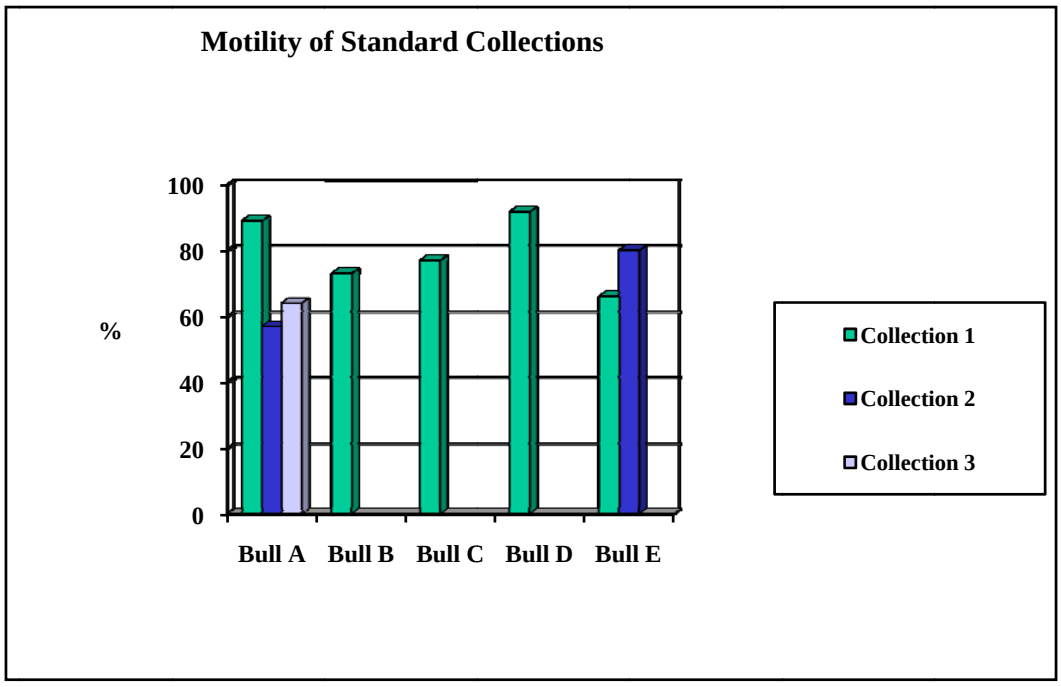

Fig. 4.2. Differences in motility comparing semen collections from individual bulls when samples were collected using a traditional collection (TC) device.

Concentrations

Overall concentrations for collections in the DISC device were higher than collections in the standard collection device (144 million vs 110 million respectively; $P < .01$). Bull D had lower concentrations in both the DISC and standard devices and two straws were used per breeding.

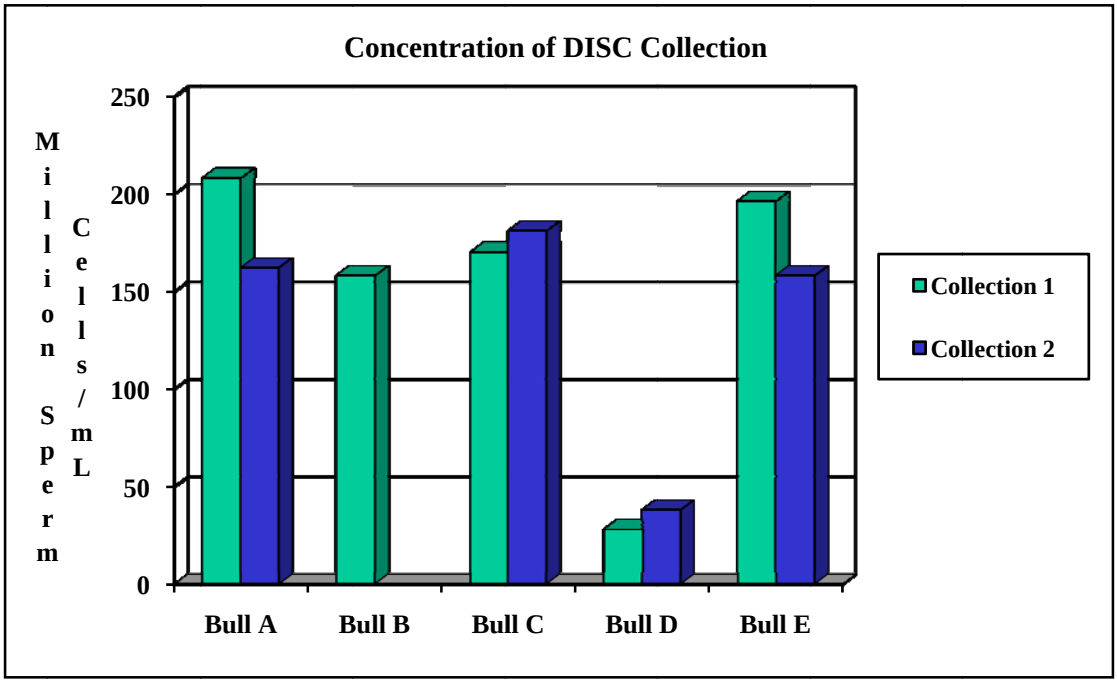

Fig. 4.3. Differences in concentration comparing semen collections from individual bulls when samples were collected with a new semen collection device, the device for improved semen collection (DISC).

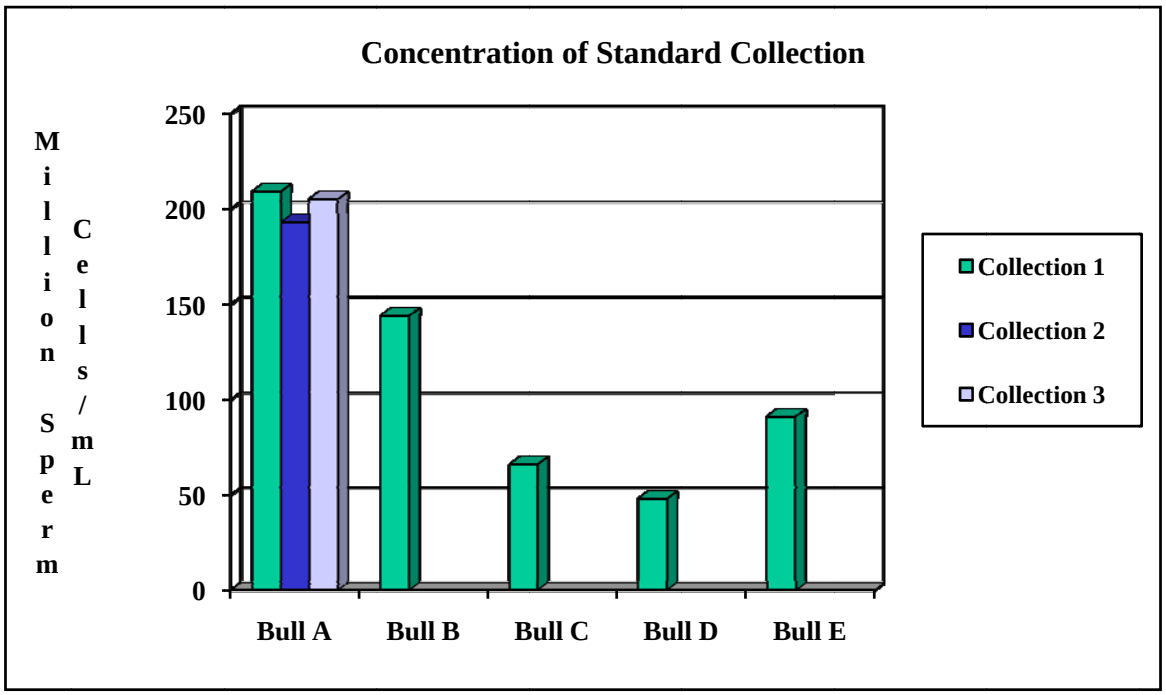

Fig. 4.4. Differences in concentration comparing semen collections from individual bulls when samples were collected using a traditional collection (TC) device.

These findings are not unusual given the normal distribution of sperm cells across males of any species (average concentration of 40 – 192 million/mL for each five animal set; $P < .02$), and the variability of sperm counts in a given male at different collection times. While one might have expected a normal distribution of cells/ejaculate across treatment, the collection device (TC vs DISC) should not have any effect on cell numbers unless cells are adherent to the side wall of the collection vessel. Moreover, as the final insemination dose was adjusted to 20 million cells/mL, the conception and pregnancy rates outlined below are still based on cell quality and not quantity.

Acrosome Reactions

Differences between TC and DISC of the semen and the acrosomal reaction rate are shown in Figure 4.5. There was a significant difference between treatments ($P<.012$). Mean acrosome reaction rates were approximately 35% for the TC and 52% for the DISC. In order for conception to occur the sperm cell must not have undergone the acrosome reaction. Therefore, animals collected with the DISC have a higher chance of conception because the cells have not undergone the acrosome reaction.

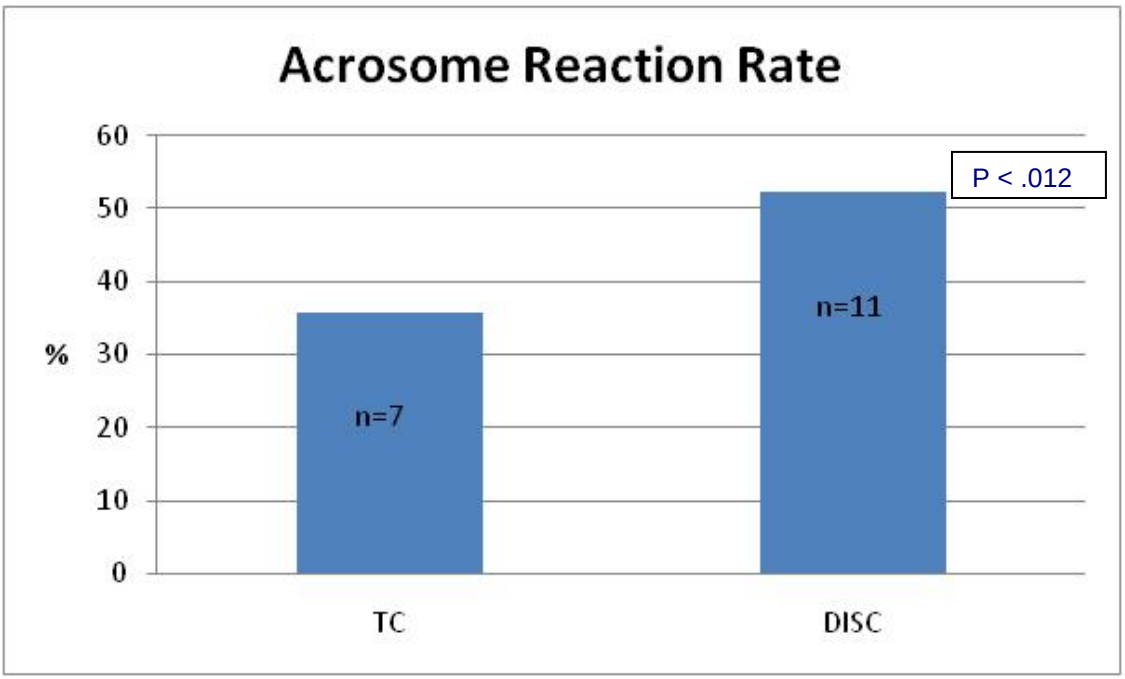

Fig. 4.5. Mean acrososome reaction rate comparing when samples were collected with a new semen collection device, the device for improved semen collection (DISC) or a device traditional collection (TC) device.

O/B Intervals and Mount Data

Mean O/B interval for heifers bred with the DISC device were 18.0 hours for average intervals of mounts and 16.94 for heifers bred with the TC (P = .33). Number of mounts was not significant for heifers bred with either device (P = .24). Average mounts for the heifers bred with the DISC were 56 and average mounts for heifers bred with the TC were 47. Mount intervals and O/B intervals were analyzed to determine if there was an interaction with conception. No differences were observed for mount interval (P=.56) or O/B interval (P=.52).

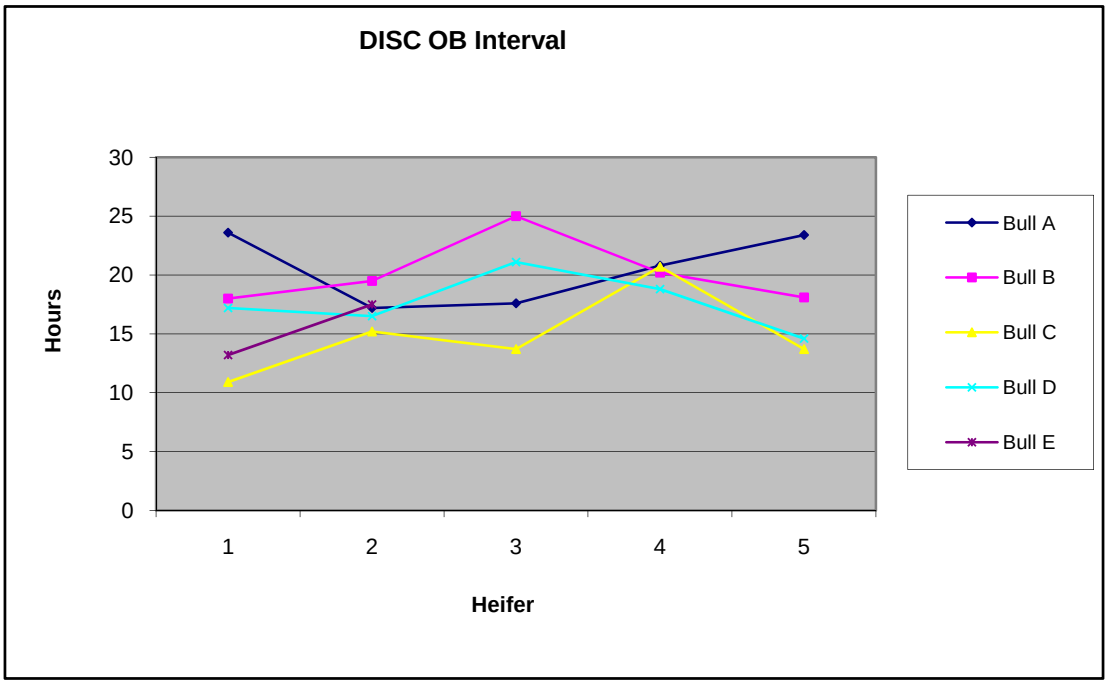

Fig. 4.6. Differences in O/B interval of heifers for individual semen collections from bulls when samples were collected using a device for improved semen collection (DISC).

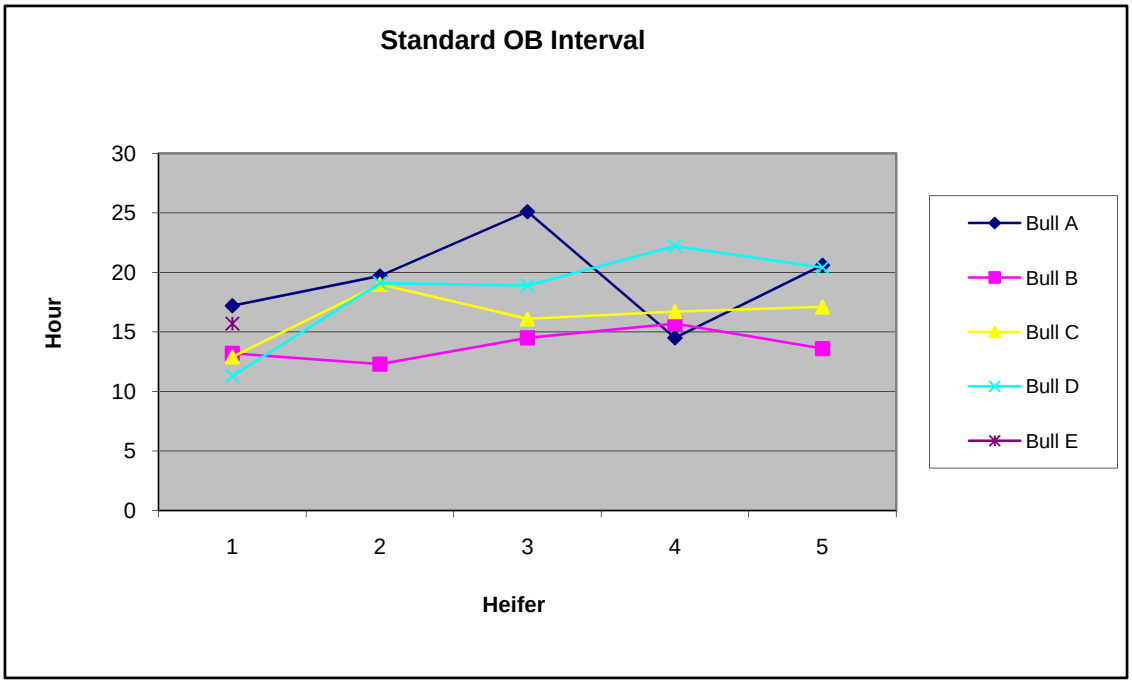

Fig. 4.7. Differences in O/B interval of heifers for individual semen collections from bulls when samples were collected using a device traditional collection (TC) device.

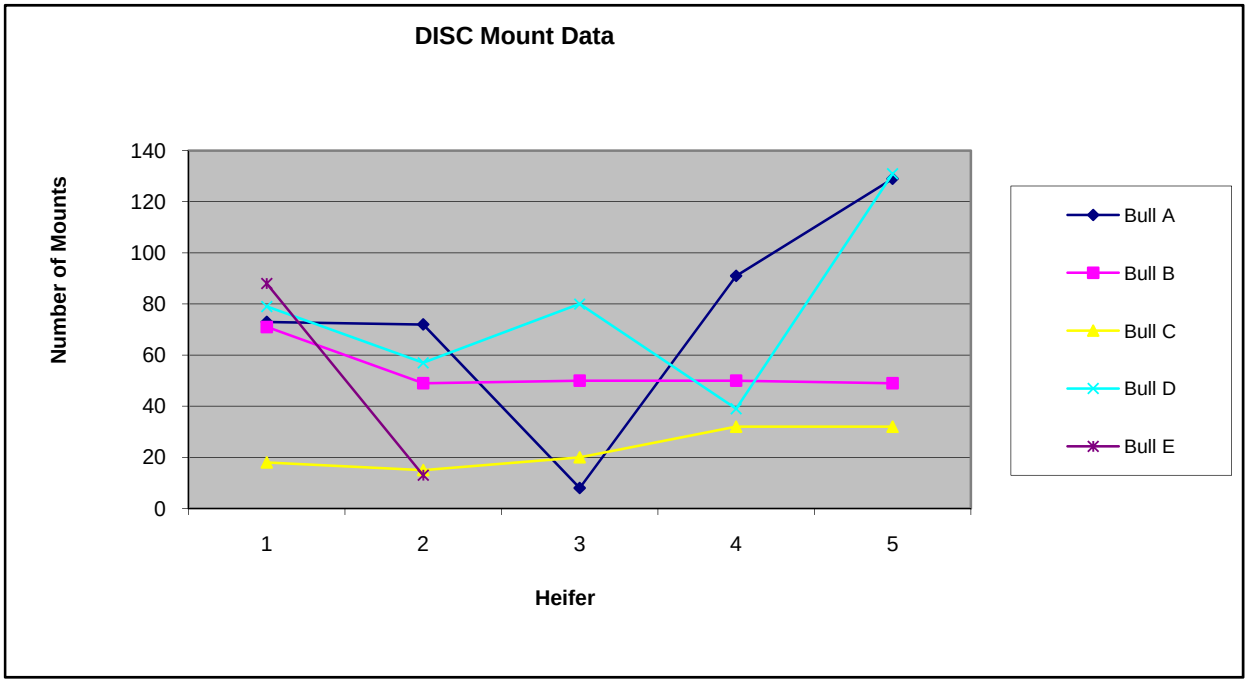

Fig. 4.8. Differences in mount data of heifers for individual semen collections from bulls when samples were collected using a device for improved semen collection (DISC).

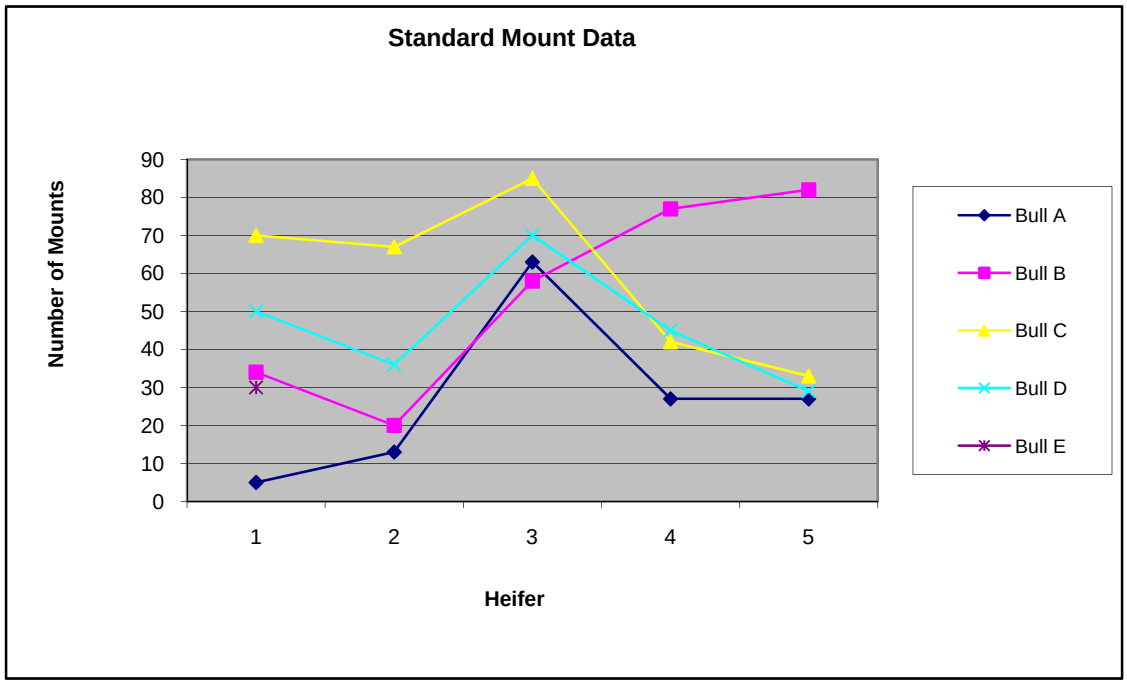

Fig. 4.9. Differences in mount data of heifers for individual semen collections from bulls when samples were collected using a device traditional collection (TC) device.

Conception and Pregnancy Rates

As expected there were differences in both conception rates and pregnancy rates among the five bulls used in the study. Overall conception rates for the 43 animals bred were 74.4% (32/43) with a range of 33%-80% for individual bulls. Pregnancy rates followed a similar pattern, with 29/43 (67.4%) of the animals maintaining pregnancy to parturition. Both of these numbers are well within industry standards for cattle.

Conception rates for the DISC were as follows ([80%], Bull A; [100%], Bull B; [100%], Bull C; [80%], Bull D; [50%], Bull E). Conception rates for the control were ([60%], Bull A; [60%], Bull B; [40%], Bull C; [80%], Bull D; [0%], Bull E). These data suggest that there is a strong trend for the DISC device to improve conception rates (19/22, 86%) compared to the traditional collection technique (13/21, 62%; P=0.063).

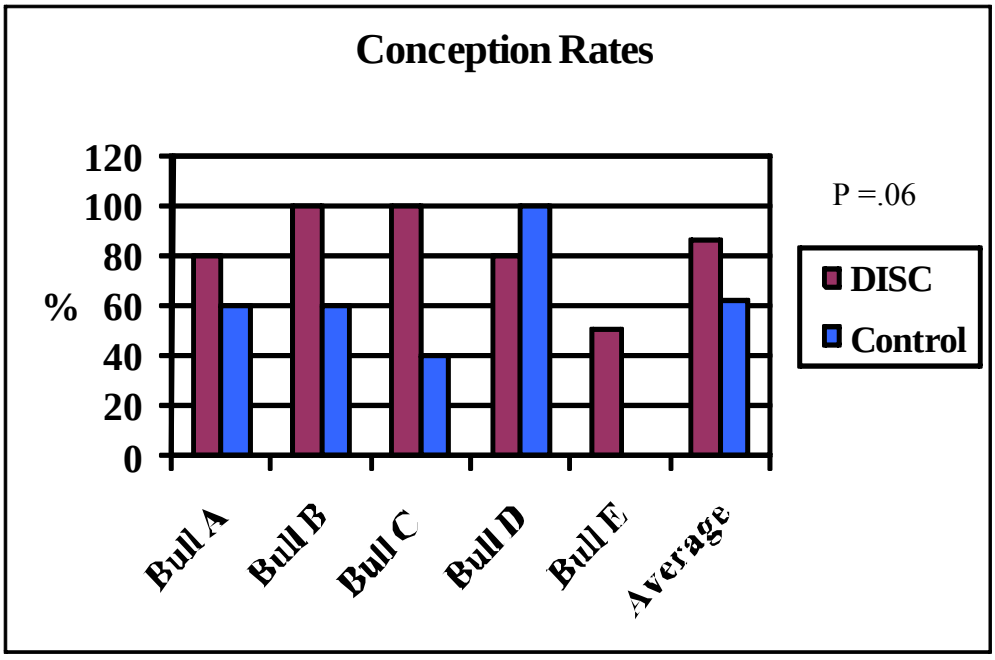

Fig. 4.10. Differences in conception comparing semen collections from individual bulls when samples were collected with a new semen collection device, the device for improved semen collection (DISC) or a device traditional collection (TC) device.

Pregnancy rates for the DISC were as follows: ([80%], Bull A; [80%], Bull B; [80%], Bull C; [60%], Bull D; [50%], Bull E). Pregnancy rates for the control were ([60%], Bull A; [60%], Bull B; [40%], Bull C; [80%], Bull D; [0%], Bull E). Pregnancy rates were 22% higher in animals inseminated with the DISC (16/22, 73%) compared to the traditional collection device (12/21, 57%; P=.22), suggesting a trend toward improved pregnancy rates using the DISC.

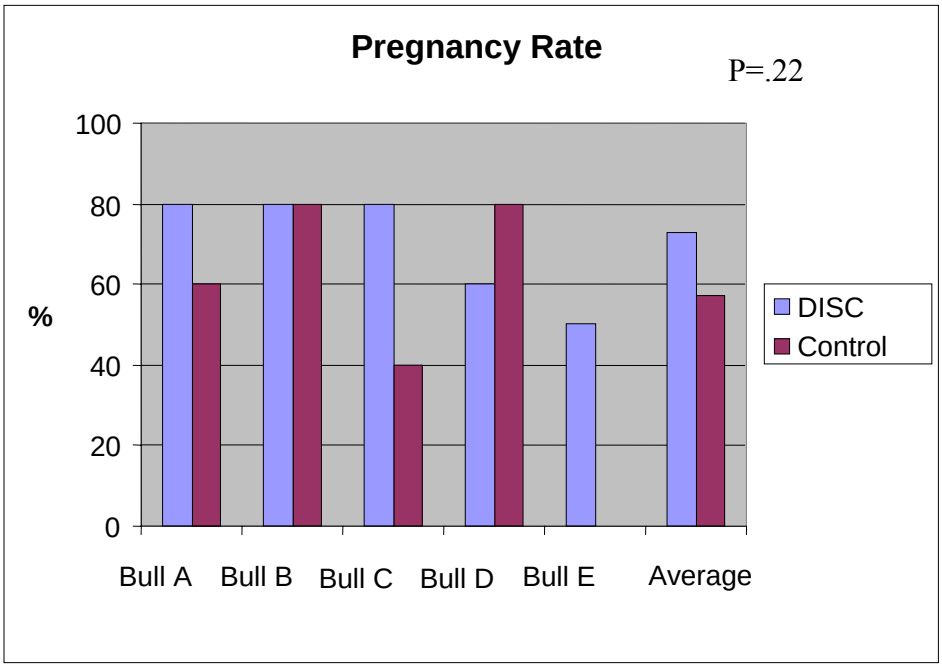

Fig. 4.11. Differences in conception comparing semen collections from individual bulls when samples were collected with a new semen collection device, the device for improved semen collection (DISC) or a device traditional collection (TC) device.

Discussion

Results obtained from this experiment demonstrated that collections from the DISC obtained higher conception and possibly pregnancy rates when compared to

collections from the TC. In 2005, Johnson demonstrated that semen fresh extended maintains acceptable semen parameters for longer periods of time when compared to TC. This study concludes that semen collected in the DISC maintain acceptable semen parameters compared to the TC. Importantly, all animals that were bred gave birth to healthy calves with no evidence of birth defects. Overall, this study indicates that the previous study was validated and results remained similar. The overall improvement of conception rates in the present study (74% vs 39% in the non-controlled study outlined in Chapter III) can be attributed to differences in cattle management and proper utilization of the technology. In a commercial production setting, AI is an effective technique to utilize current reproductive technologies. The data continue to suggest that the DISC provides a superior environment for the collection and storage of sperm and that use of these sperm in an AI program might improve productivity over more traditional methods. As so many factors can influence outcome of pregnancy between conception and parturition, a larger scale trial is needed to determine the role of the DISC in cattle. Further, past research suggested improvement of semen parameters in a number of species. Pregnancy studies will be needed in all to determine the role of the DISC in Assisted Reproductive Programs.

CHAPTER V

EQUINE STUDY

Introduction

The Device for Improved Semen Collection (DISC) has been described as a universal means for improving semen parameters and therefore conception potential in mammalian species. Research previously conducted using the DISC has shown to improve viability and motility in semen of stallions used for artificial insemination. Use of the DISC with addition of a predetermined media source prevents shifts in pH and regulates sperm cell osmoregulation, ultimately prolonging cell life and increasing semen parameters. While improvement in semen parameters has been reported in a number of species (Johnson 2005), conception data has only been reported for the bovine. The present study was conducted to not only evaluate improvement in semen parameters in a second species (the equine) but to provide conception data as well.

Materials and Methods

These studies were conducted at a commercial stallion station in western Colorado from May to July 2007. All semen used for these experiments was provided by repeated collection of the station's two stallions. In order to demonstrate differences between the control versus conventional collection methods, a modification of the Missouri model artificial vagina was used to produce a true split, equally fractioned collection. Both arms of the modified artificial vagina were lined with in-line nylon gel filters. Prior to collection, a traditional collection container (baby bottle with liner) was connected to one side of the artificial vagina. The DISC was connected to the other side of the artificial vagina which contained 37C media (a proprietary material) at

approximately 20% of the expected treatment sample volume (i.e. 6 mL of extender expecting 30 mL of sample). Collection procedures in the equine industry require stallions to be trained to mount a phantom mare which usually takes several sessions, but depends upon the trainability of the stallion. Using a phantom mare maximizes safety for both the collector and handler. At time of collection stallions were washed and the artificial vagina was placed on the penis. The handler manipulated the artificial vagina to ensure both treatment and control splits had equal amounts of semen. After ejaculation was complete, semen was evaluated for volume, concentration, motility, and forward progression (described below).

Once the initial analysis was completed, each fraction of the sample was extended with Inra-96 semen extender at a rate of 50 million cells/ml and packaged in 20 ml doses for a 1 billion total cell breeding dose. In order for the treatment sample to begin the process of osomoregulation the sample was allowed to equilibrate at a temperature of 22 C for 15 minutes. Commercial practices in the equine industry use of processed, cooled shipped semen. Therefore semen was collected, processed, packaged, and cooled according to commercial practices. The semen was then used for insemination at times of 24, 48, and/or 72 hours post collection. Semen collections were scheduled to provide semen of the proper age for scheduled inseminations.

Semen Parameters

Semen samples were evaluated at 0, 24, 48 and 72 hours post-collection. Both the treatment and control samples were processed in such a way to normalize each. Analyses were conducted based on volume, concentration, motility, forward progression, and viability. All parameters were evaluated with an Acuscope 3040 microscope equipped

with phase optics (Acuscope; NY, NY) at 200X magnification. After the appropriate equilibration period, semen was prepared for insemination into 20 mares during 45 estrous cycles.

Volume

Immediately following ejaculation total volume for both arms of the collection device was recorded. To achieve exact volumes in the DISC arm of the study, the total volume of the collection minus the volume of extender added was recorded.

Concentration

An Acuscope 3040 microscope equipped with phase optics (Acuscope, NY, NY) was used to evaluate semen concentration of both the control and treatment of the study. To prevent variability in samples a microcell (Microcell; Conception Technologies, San Diego, CA) slide was used at 100X magnification to count, at random, and calculate the number of sperm per milliliter of an undiluted sample. Concentrations were recorded for each sample as number of sperm per milliliter of ejaculate.

Motility

A major factor affecting conception rates in the equine is motility. Poor motility in spermatozoa can account for the lack of fertilization due to the lack in ability of the sperm cells to pass the zona pellucida. Evaluations of semen concentration used an Acuscope 3040 microscope with the conjunction of a microcell used to evaluate motility of both control and DISC treatments at 0, 24, 48 and 72 hours. One hundred sperm cells were counted and expressed as a percentage. Each count was made manually by a trained observer.

Forward Progression

Spermatozoa were evaluated for forward progression after motility and based on a scale of 1 to 5. A score of 1 indicates sperm are moving with slight side to side movement with little tail propulsion and no forward progression, 2 indicates sperm cells are moving with no forward progression in circular or irregular patterns, 3 indicates rapid movement side-to-side, but slow forward progression, 4 indicates cells moving in a slow but steady forward progression, and 5 indicates rapidly moving cells in proper forward progression across the microscope field. Forward progression of 5, cells should be moving across the field in less than a second.

Mare Management

Mares were observed in groups of ten for a total of sixty scheduled insemination cycles. Each mare's estrous cycle was monitored by ultrasound. Once follicles had reached an appropriate size (40-42 mm), ovulation was induced by injecting Human Chorionic Gonadotropin (hCG). Once ovulation occurred mares were randomly assigned to 24hr control, 24hr treatment, 48hr control, or 48hr treatments. Stallions were continuously collected during the trial to insure semen of appropriate age. Due to needs of the ranch's production program, two mares were removed from the study to be used as recipients in the embryo transfer program. Further, only three control animals and five treatment animals were bred at 72 hrs due to the end of the breeding season.

Pregnancy was determined using ultrasound 14 days post-ovulation. If pregnancy occurred it was interrupted using PGF2α.to recycle the animal for further study. Excepted as noted for breedings at 72 hrs, each mare underwent an insemination trial with both treatment and control semen at each time point in the study. A single mare failed to cycle

initially due to infertility issues and was removed from the study and replaced with a second animal which assumed that animal's insemination schedule.

Statistical Analysis

Experimental Design

All data collected in this study were analyzed using the Statistical Program for the Social Sciences (SPSS ver 12, SPSS, Inc; Chicago, IL). Initial semen parameters for each treatment were analyzed using a one-way analysis of variance (ANOVA) with Tukey's mean separation. A Latin-square design was used to determine mare and semen sample rotations throughout the study.

Results

Semen Parameters

As in previous research (Johnson, 2005) the DISC improved overall semen parameters. Further, data confirmed that the processing procedures of control and treated semen samples removed any variation in initial concentration and volume for each breeding dose administered.

Motility

There was a trend toward increased motility rates in samples collected in the DISC device when compared to samples collected traditionally ($P=0.06$) up to 48 hrs (Figure 5.1). Progressive motility was more pronounced in samples as time was increased. Moreover, while not included in statistical analysis due to limited collections ($n=2$), at 72 hrs there were no motility cells in the control arm of the study while motile cells in the control arm remained above 50%.

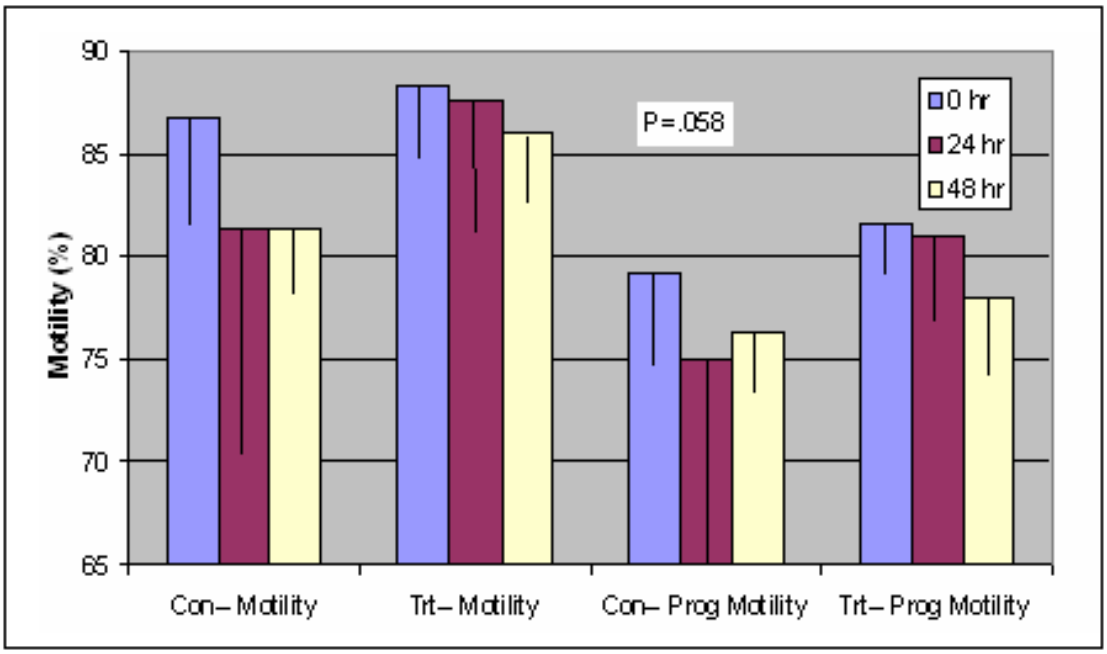

Figure 5.1. A comparison of motility and progressive motility rates of semen samples collected in the DISC (trt) vs a traditional collection method (con).

Conception Rates

Previous research has indicated that samples collected in the DISC have overall improved semen parameters over time, likewise, conception rates in mares after 24hrs did not differ because of semen collection method (P=.89). There was a trend toward increased conception rates in mares bred with semen that had been stored for 48hrs (P=.06) when semen was collected in the DISC device. Although semen collected with the traditional device had an overall decrease in conception rates by 25% at 24 and 48hrs of storage, the difference was not significant for 24hrs (P=.69). Furthermore, in the limited breedings conducted with semen stored 72 hrs, there was a significant increase (P < .001) in conception rates (100 % vs 0%) in animals bred with semen collected in the DISC as compared to the control.

Table 5. 1. A comparison of conception rate over time between animals bred with semen collected in the DISC vs standard collection techniques.

Time	Pregnancy Rate		P-Value
	Control	DISC	
24 hr	75% (6/8)	70% (7/10)	0.89
48 hr	50% (5/10)	80% (8/10)	0.06
72 hr	0% (0/3)	100% (5/5)	0.001

P-Values determined by Chi Square at specific time points

Discussion

Current equine industry standards are to collect and ship cooled semen, ultimately taking more than 24hrs post-collection before semen can be inseminated into the mare. Therefore a need exists in the equine industry for semen to maintain viability for longer periods of time. Semen collected in the DISC device and subsequently stored for 48hrs was 60% more successful in conception rates than semen collected in the traditional device. Preliminary results of semen collected and stored for 72hrs resulted in 0% conception of mares bred by semen collected in the traditional device versus 100% conception of mares bred by semen collected in the DISC. Semen collected in the DISC device and stored for more than 24hrs has shown greater conception rates and improved overall semen parameters when compared to traditional collection devices.

Data from the present appear to support earlier work in the bovine which show higher conception rates in artificial insemination (AI) programs utilizing semen collected in the DISC. These data would demonstrate that the specie specific DISC provides a

superior environment for semen collection as compared to traditional methods, yielding a superior sample for AI up to 72hrs post-collection. Further research is needed to determine at what point in time the semen parameters decline in semen that has been collected in the DISC device.

Chapter VI

CONCLUSIONS

Currently there are many biotechnologies available for the reproduction of domesticated animals. One of the most widely used is AI, a procedure generally thought to date to the 1300's when Arabian horse breeders would collect animals of rival tribes to win competitions (Bearden et al., 2004). In order to have successful AI one must properly evaluate semen. There are four parameters for which semen is evaluated: (1) volume, (2) concentration, (3) motility, and (4) morphology. Once a producer is certain his/her bull is a sound breeder and chooses to collect the bull for reproductive gain, semen must be collected. Semen is usually collected into a collecting device attached at the end of the artificial vagina. Traditional collection methods allow for the ejaculate to be collected into a dry container and have media added soon after collection. While this is still a practiced method, recent research suggests that adding body temperature media to the collecting device prior to collection is superior in that it will help to prevent temperature and pH shock. Based upon the concept of minimizing environmental change, the DISC allows for improved semen quality and preservation as compared to traditional methods due to: 1) decreasing the exposed surface area of the specimen, 2) insulating the sample from environmental temperature extremes, 3) providing buffers to prevent changes in pH and 4) inducing osmoregulation. Together, these attributes of the DISC are designed to lessen cold and pH shock and prolong cell function (Johnson, 2005).

Research using the DISC in the bovine proved to have overall better semen parameters and higher conception rates and, potentially, pregnancy rates when compared

to the TC. Moreover, there were no incidences of birth defects or other abnormalities observed in the calves born. There were no side effects noted in either research trial for cows or bulls used. Ultimately, cow-calf producer's primary goal is to have a healthy, live calf. Once they have achieved this the secondary goal is to produce a calf that meets their pre-determined target markets. Generally, producers want a group of calves that are the same age, certain breed or breeds, and weigh the most with the least amount of economic inputs. By utilizing semen collected in the DISC bovine producers can realize higher conception and pregnancy rates that will also be a return on their investment.

Research in the equine demonstrated that semen collected in the DISC, then fresh extended, remained fertile for extended periods of time as compared to samples collected using standard techniques. Commercially the equine industry standard is to collect and ship cooled semen, ultimately taking more than 24hrs post collection before semen can be inseminated into the mare. Therefore it is important to develop more efficient ways of prolonging the life span of semen. Semen collected in the DISC has shown the ability to have acceptable ranges of semen parameters at 72hrs post collection. Also, higher conception rates were observed at 72hrs when using the DISC collection device.

To briefly demonstrate the practical applications of the DISC device a model has been created. In theory a hundred beef females were to be bred by artificial insemination. Based upon Bovine Study II when the DISC was used pregnancy rates were 73%. Extrapolating the 73% for our model, 73 animals would become pregnant. By industry standards, 73% is an acceptable pregnancy rate. These animals would all be born within a shorter calving season, and with proper sire selection carry better genetics. Overall calves could be marketed as a more uniform group. Feed yards are an integral link in the

chain of beef production. Most calves that enter the feed yard weigh approximately 750lbs. The September fed cattle price was $1.07/ cwt. Resulting in the previously mentioned calves, weighing an average of 750lbs would have a purchase price of $802.50/head. When compared to the pregnancy rate of the Bovine Study II of 57% calves would still have the same purchase price, but the volume of calves would be less therefore resulting in less money obtained overall.

Webster's dictionary defines science as the "state of knowing." It also defines production as the "total output especially of a commodity or industry." In both the bovine and equine industry the overall end product is the important factor. However, do we know all there is to know about bovine and equine production? Some may conclude that in fact we do know everything, but the majority would most likely conclude there is always room for improvement. In retrospect of this research there are several areas that could be improved to further advance future research. The first, most obvious, is a larger sample size. As seen in the Bovine Study I variables such as bull, breed of dam, and diet impacted conception rates. It would be interesting to find out on a large scale if the differences found in the Bovine Study I would remain significant. Secondly, as seen in Bovine Study II factors were controlled more than the previous study, but comparison to frozen semen would be important to the industry. In addition to this freezing challenges of semen collected in the DISC would be important as well. In all three research trials standard commercial medias were used, changes in media to research the impact on conception based on variation in media would be important both as a reproduction stand point and as it would be to the success of the DISC device. In the equine industry, reproductive problems occur often in stallions. However, the importance of the stallion

outweighs his problems; therefore it would be research worthy to evaluate the benefit of collecting stallions with certain reproductive problems in the DISC device to determine if there are significant improvements both scientifically and productively.

In conclusion, based on the literature and present research it appears that the DISC is a superior method to the traditional methods that is predominately used. Economically, the use of the DISC for collection of bovine and equine can prove to be beneficial for producers. Producers of these species are in the business to create a profit and develop a superior product based on their target market. Webster's Dictionary defines success by "a favorable or desired outcome." By utilizing the DISC producers have a greater chance of being successful.

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