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Pregnancy Rates in Beef Cattle Artificially Inseminated with Frozen-Thawed Aged Beef Semen

David Barry Carwell

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PREGNANCY RATES IN BEEF CATTLE ARTIFICALLY INSEMINATED WITH
FROZEN-THAWED AGED BEEF SEMEN

A Thesis

Submitted to the Graduate Faculty of the
Louisiana State University and
Agricultural and Mechanical College
in partial fulfillment of the
requirements for the degree of
Master of Science

In

The Interdepartmental Program of
Animal and Dairy Sciences

by
David B. Carwell
B.S., Arkansas State University, 2008
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ABSTRACT

The duration of frozen semen storage had no effect on pregnancy rates (60%, 61% and 61%, respectively) in Angus females inseminated with semen from 25 Angus bulls from Time Frames 1 (1960-1975), 2 (1976-1991) and 3 (1992-2006). Pregnancy rates were not different for beef females inseminated with semen from Time Frames 1, 2 and 3 (55%, 62% and 55%, respectively). There were no differences for total and progressive sperm motility across Time Frames 1 (42% and 29%), Time Frame 2 (51% and 38%) and Time Frame 3 (55% and 41%) and percentage of normal and abnormal sperm from bulls in Time Frames 1 (80% and 19%), Time Frame 2 (76% and 23%) and Time Frame 3 (71% and 28%).

No differences were detected between sperm concentrations/breeding unit for bulls within Time Frame 1 and Time Frame 2 or between Time Frame 1 and Time Frame 3. In contrast, a significant difference ($P < 0.05$) was detected for sperm concentration for bulls within Time Frame 2 ($59.9 \times 10^6/\text{mL}$) and Time Frame 3 ($37.1 \times 10^6/\text{mL}$). Within these bulls regardless of Time Frame, no differences were detected in total sperm and progressive sperm motility for bulls categorized as being high (54% and 41%), moderate (51% and 39%) or low (46% and 31%) fertility rates.

Brangus F_1 females had higher ($P < 0.05$) chute behavior scores when compared with Bons Mara, Romsinuano, Braford, Brangus, Beefmaster and Angus females. Furthermore, Brangus F_1 , Brangus and Beemaster females had higher ($P < 0.05$) mean chute exit velocities when compared with Angus, Romosinuano, Bons Mara and Braford females

Chute behavior scores were not significantly different for pregnant and nonpregnant females. A significantly higher chute exit velocity was noted for nonpregnant females when compared with pregnant females. Significant correlations ($r = 0.58$, $P < 0.05$) was detected for chute exit velocity on day 0 and day 7. Furthermore, a significant positive correlation ($r = 0.28$) was detected between mean subjective behavior chute scores and mean chute exit velocity.

CHAPTER I

INTRODUCTION

Textbooks now indicate that artificial insemination of cattle has made genetic improvement at a faster rate over time when compared with that of natural mating. In the beginning of field use, the storage of liquid semen would only allow bull semen viability to be extended up to 10 days (Salisbury et al., 1941). The discovery of glycerol as a cryoprotectant (Polge et al., 1949) subsequently allowed for bull gametes to be frozen and utilized for insemination at a later date.

There have been some concerns about the duration of semen storage time in liquid nitrogen and its effect on subsequent fertility rates (Haugan et al., 2007) and that disruption of sperm DNA would occur (Salisbury et al., 1961; Ackerman and Sod-Moriah, 1968), that would adversely effect post-thaw fertility. However, studies have suggested that frozen male gametes can be frozen for years and still remain viable (Nishikawa et al., 1976, Clarke et al., 2006).

Semen banking can provide current mammalian genetics to be preserved and utilized in the future. In the human infertility field, the ability to store frozen semen provides a means for reproducing later in life. In comparison, storage of frozen embryos from human females can also provide means of reproducing if subsequent female infertility occurs (Testart et al., 1987; Cohen et al., 1988; Go et al., 1998; Ben-Ozer and Vermesh, 1999; Quintans et al., 2002; Revel et al., 2004; Teijón et al., 2006).

The amount of time gametes can spend in liquid nitrogen in the frozen state is currently unknown. Some studies have reported that by subjecting mouse gametes to radiation, that a time period of up to 2,000 years could elapsed without any detrimental effect occurring or reduction in viability (Lyon et al., 1977; Glenister et al., 1984; Glenister and Lyon, 1986).

Since the beginning of commercial artificial insemination for cattle, appropriate semen packaging systems have been evaluated during various breeding programs. The glass ampule received much attention during the 1950s and into the 1970s, as well as, the semen pellet form of freezing, however, these were replaced in the early 1980s with the plastic semen straw (see review by Herman, 1981). All three methods of packaging have produced similar results in field cattle fertility trials (Adler et al., 1968; Alverson, 1972; Bean, 1972; Pickett and Berndtson, 1974; Mortimer et al., 1976; Coulter and Foote, 1977).

The genetic phenotype of Angus cattle has slowly changed in the past decades. Heavier, bigger frames calves are now being produced when compared with those from the 1950s, 1960s and 1970s (American Angus Association, 2010). Enns and Nicoll (2008) reported an average 0.43 kg increase in body weight per year for Angus cattle from the years 1973 to 1993. This value was lower (0.96 kg/year) than what was reported by Nadarajah et al. (1987) for 27,774 Angus calves born from the years 1953 to 1983.

More recently, animal temperament has received attention and there have been concerns that it would effect subsequent pregnancy rates. In any cattle operation, animal handling is required for herd management. Animals with excitable temperament can pose both a threat to the people handling them and to the animals around them.

We propose to utilized previously frozen semen from 25 Angus bulls processed from the 1960s through the early 21st Century and to artificially inseminate beef cattle in a controlled study to evaluate first service pregnancy rates across the three specific time periods. We also propose to evaluate the frozen bull sperm post-thaw in vitro to determine any differences that might have occurred during storage in liquid nitrogen. During this study, evaluations of cattle temperament during the estrous synchronization will be determined for its effect on subsequent pregnancy rates.

CHAPTER II

LITERATURE REVIEW

2.1. History of Artificial Insemination

Leeuwenhoek first described the spermatozoon in 1677. However, the earliest reported artificial insemination (AI) practice dates back to 1322 A.D. with Arabian horse breeders. The first documented successful insemination was in the bitch (Spallanzy, 1784). Just prior to Spallanzani's successful insemination, the first human artificial insemination (AI) was reported to occur in 1776 (see review by Foote, 1982).

Dog breeders in England would use Spallanzani's technique to inseminate bitches during the late 1800s. The first artificial vagina (AV) devised was used for the collection of dog semen in 1914 (see reviews by Herman, 1981 and by Foote, 1999). This would later lead to the development of artificial vaginas for larger species making it possible to subsequently use AI in all farm species.

This new technique of semen collection allowed insemination with fresh semen, this sparked interest in farmers and led to the first organized cooperative artificial breeding organization in 1937 in Denmark. In its first year there were 220 members and 1,070 cows were inseminated (see review by Herman, 1981; see review by Foote, 1999). In the United States, the first calf produce from AI was reported in 1907 (see review by Herman, 1981). However, it was not until 1938 that the first artificial breeding cooperative in the United States was formed in New Jersey (see review by Herman, 1981). The organization consisted of 102 members with 1,050 cows enrolled to be inseminated during the first year.

The first series of experiments utilizing AI were conducted in Russia, by researcher Ivanov in 1899 (see review by Foote, 1999). By the early 1900s, Ivanov had performed AI in a

variety of species including: cattle, sheep and horses (Ivanoff, 1922). By 1938, it was reported that 40,000 mares, 1.2 million cows and 15 million sheep in Soviet Union had been inseminated by AI (see review by Herman, 1981) . Since this time, artificial insemination has played a key role in the disbursement of male genetics across the female populations.

2.1.1 Semen Buffers

Generally semen is extended from its natural state to a more diluted form and females are inseminated with semen that is either fresh, cooled or in the frozen state. Development of a variety of buffers allowed the conservation of semen for several days at 5°C. Phillips and Lardy (1940) reported that with the addition of phosphate to the buffered medium, bull semen could be successfully extended for up to 5 days at 10°C, retaining motility and producing viable pregnancies in cattle. It was later shown that sodium citrate could be substituted for phosphate and extend the life of cooled bovine semen for up to 10 days in cattle (Salisbury et al., 1941).

However, this preservation technique did not allow the storage of bovine semen for longer than 10 days. The addition of glycerol would allow for semen to be cryopreserved and stored at -79°C (Polge et al., 1949). This first calf produced from frozen semen in the world was produced in 1951 in England (Stewart, 1951). In 1953, the first calf produced in the United States from frozen semen was reported from American Breeders Services in Wisconsin (see review by Herman, 1981).

2.2. Semen Packaging

To inseminate cows with collected and extended semen, a method of packaging needed to be developed for the commercial industry. Packaging considerations for the most efficient system had to provide easy handling, identification, most efficient storage and acceptable fertility results (see review by Pickett and Berndtson, 1974).

2.2.1. Pellets

The pellet form, invented by Japanese scientists, involved dispensing semen in a small depression in a dry ice block (see review by Herman, 1981). When comparing pellets (n = 2,136) to fresh semen (n = 2,818), Dawson (1968) reported a higher pregnancy rate in females inseminated with fresh bull semen (67%) when compared with bull semen that had been frozen in pellets (66%). However, when chilled 2 day-old fresh semen produced comparable pregnancy rates (67%) to that of semen frozen and packaged in glass ampules (65%). More inseminations were performed by Dawson (1968) utilizing fresh semen (n = 37,071) and semen pellets (n = 25,663). Pregnancy rates at 30 to 60 days for pellets and fresh semen were equal at 75.1%.

In contrast, Koskull and Hemnell (1968) reported a 0.8% higher pregnancy rate in cows inseminated with dry ice frozen pelleted semen (n = 4,856) when compared with fresh semen (n = 5,705). When dry ice frozen pelleted semen was compared with semen processed and frozen in plastic straws, a higher pregnancy rate was reported in the females that were inseminated with 0.5 mL plastic straws (68.5% vs. 59%, respectively) (Adler et al., 1968).

2.2.2. Ampules

The glass ampule was a common method of packaging semen for artificial insemination until the late 1970s. It was available in two different sizes, 0.5 mL or 1 mL. Berndtson and Foote (1975) reported similar post-thaw motility rates after semen was stored for up to 6 months for semen packaged in either pellet form (34%), glass ampules (33%) or plastic straws (30%).

Subsequently, Pickett et al. (1978) described results from breeding trials that compared different sizes of ampules containing different volumes of bull semen. A total of 9,219 dairy cattle inseminations were performed utilizing both 0.5 mL and 1.0 mL glass ampules. Pregnancy rates for 0.5 mL and 1.0 mL glass ampules were 73.2% and 76.2%, respectively.

Similar results were also found when dairy cows were inseminated with semen glass ampules containing 0.5 mL and 0.9 mL volumes, with pregnancy rates of 70.5% and 73.2%, respectively (Hafs et al., 1970).

2.2.3. Plastic Straws

The invention of the cellophane straw in 1940s would change the way producers and researchers utilized AI (see review by Foote, 2002), however, the plastic semen straw would not be introduced until the 1960s (see review by Foote, 2002). One benefit of the plastic straw was that it has the ability to store up to four times the number of semen units in one liquid nitrogen tank compared with that of glass ampules (Macpherson and King, 1966). The straw sizes used ranged from 0.25 to 0.5 mL in size with a surface area of 555 mm² and 1246 mm², and some straws available in 1.2 mL (Senger, 1980). The introduction of the 0.25 mL plastic straw was a way of reducing storage cost and improving storage efficiency. The surface to volume ratio is larger in the 0.25 mL French plastic straw than in the 0.5 mL French plastic straw (Senger, 1980).

However, freezing and thawing protocols reported for glass ampules and larger plastic straws had to be adapted in order to compensate for the surface area differences of the 0.25 mL plastic straw (Ennen et al., 1976). Unfortunately, in these studies fertility data were not reported to verify the results of these packaging systems.

One of the earliest reports indicates a 3% increase in pregnancy rates when dairy cows were inseminated with semen packaged in plastic straws (70%) compared with those packaged in glass ampules (67%) (Macpherson and King, 1966), while another study, reported similar differences in pregnancy rates for plastic straws (72%) and glass ampules (67%) (Aamdal and Andersen, 1968).

In their review, Pickett and Berndtson (1976) described a series of experiments involving over 400,000 dairy cattle inseminations comparing glass ampules to the plastic straw. It was concluded that the pregnancy rates achieved by plastic straws were comparable to that of frozen glass ampules (69.7% and 68.6%, respectively). It was also reported that semen frozen in plastic straws resulted in equal to or higher survival rates of glass ampules. However, Coulter and Foote (1977) reported a higher post-thaw motility on dairy bull semen that had been packaged in 0.5 mL plastic straws when compared with semen packaged in glass ampules (39.3% and 38.8%, respectively).

Bean (1972) reported that advantages of the plastic straw included: (1) higher pregnancy rates, (2) lower sperm mortality, (3) convenience of use in the field and (4) increased storage capacity when compared with the glass ampule semen packaging method. In a experiment designed to compare the two different packaging methods, semen was collected and processed from Holstein bulls ($n = 13$) to be used for AI (Bean, 1972). A total of 5,544 dairy cow inseminations were performed using semen packaged in plastic straws, with a total of 5,637 inseminations being performed with semen packaged in the glass ampule. Pregnancy rates were 71.5% and 70.3% for plastic straw and glass ampule, respectively.

Mortimer et al. (1976) reported a difference in pregnancy rates when bull semen was packaged in 0.25 mL Continental straws when compared with 1.0 mL glass ampules. Semen was collected and processed from Maine Anjou ($n = 1$) and Simmental ($n = 3$) bulls to be used for AI. In Trial 1, 294 cows were inseminated with semen packaged in plastic straws and a total of 280 cows were inseminated with semen packaged in glass ampules, with overall pregnancy rates of 70% for plastic straws and 50% for glass ampules, there was no difference in this study. To confirm these results, a second trial was conducted using only one of the bulls from the

previous study and pregnancy rates were 83% for plastic straws and 67% for glass ampules, these values were reported to be significantly different.

Macpherson et al. (1974) concluded that there were no differences when comparing pregnancy rates (71% and 71%, respectively) when dairy semen was packaged in the two different volumes (0.25 mL and 0.5 mL) of plastic straws. Johnson et al. (1995) also investigated the effect of packaging volume on pregnancy rates in Holstein dairy cattle and the results showed no difference (63% and 62%, respectively) between the two different volumes (0.25 mL and 0.5 mL) of plastic straws.

Further studies utilizing a larger number of dairy cows ($n = 515,943$) inseminated with 0.25 mL and 0.5 mL plastic straws and found no difference (67.6% and 66.4%, respectively) in pregnancy rates (see review by Pickett and Bernsdton, 1976). The use of meta-analysis confirms that the small difference between the two pregnancy rates does not provide enough evidence for a switch to the 0.25 mL plastic straw (Stevenson et al., 2009).

2.2.4. Alternative Packaging Methods

Alternative packing methods to that of the pellet, glass ampule, and plastic straw have been reported (Thanawala et al., 1988). Gelatin capsules containing frozen semen treated with various components were thawed and subjected to in vitro evaluation to determine semen viability. The capsules treatments included: (1) wax, (2) liquid paraffin and (3) no treatment (the plastic straw served as the control). No differences were found in the percentage of progressively motile sperm, percentage of sperm with undamaged plasma membranes or percentage of sperm consider morphologically abnormal. However, semen packaged in plastic straws did exhibit a higher percent of sperm with an intact acrosomes when compared with sperm in hard gelatin capsule (57.73% and 53.5%) (Thanawala et al., 1988).

2.3. Storage of Frozen Semen

2.3.1. Bull Semen Storage

The ability to store semen for periods of time allows the use of cryopreserved genetics. With the addition of glycerol (Polge et al., 1949), semen can be successfully frozen and thawed and still retain its viability. The ability to store frozen bull semen with the addition of glycerol for weeks without decreasing reproductive efficiency was then reported (Erickson and Graham, 1959). However, up until this point, no breeding trials had been conducted utilizing bull semen frozen stored further than 1 year.

Research suggests that semen stored in liquid nitrogen does have the ability to age. One study reported that bull semen collected from bulls and frozen stored for 2 years showed fluctuations in pregnancy rates, however, no differences were detected when semen from the same bulls was collected and frozen for 1 month (Foote 1972).

Bratton et al. (1955) compared fertility rates of cooled and frozen bull semen that had been stored (frozen and cooled) for different periods of time. Cooled semen was stored for 1 day at 5°C, while frozen semen was stored at -79°C for 1 week and 17 weeks. The control semen had an average 60 to 90 day pregnancy rate of 71.0%, while the pregnancy rates for semen stored for 1 and 17 weeks was 73.2% and 69.8%, respectively.

Some reports have indicated that bull semen frozen in liquid nitrogen exhibited a greater resistance to different freeze thaw treatments than that of semen frozen in an alcohol bath (Fowler et al., 1961; Pickett et al., 1961). In comparison, one study (Bean et al., 1963) reported a significantly higher post-thaw bull sperm motility for samples frozen in liquid nitrogen when compared with those frozen in dry ice. Bull semen samples that were initially frozen in dry ice and stored in either dry ice or liquid nitrogen after freezing had a post-thaw sperm motility of

28% and 34%, respectively. Furthermore, those samples frozen in liquid nitrogen initially and stored in either dry ice or liquid nitrogen have a post-thaw sperm motility of 21% and 26%, respectively.

A difference was reported between the two different refrigerants (liquid nitrogen and alcohol bath) for post-thaw sperm motility (6.1% and 5.8%, respectively) (Fowler et al., 1961). After 6 months of storage, a difference was noted in the number of progressively motile sperm with a higher proportion seen in the semen stored in liquid nitrogen than that frozen in the alcohol bath (2.36% and 0.58%, respectively).

Fertility results from semen frozen in liquid nitrogen and dry ice have reported to be superior for semen samples frozen in liquid nitrogen (Pickett et al., 1961). A variety of dairy and beef breed-based bulls (Holstein, Jersey, Guernsey, Angus, Ayrshire and Brown Swiss) were collected and processed in this study. A total of 3,214 inseminations were performed with bull semen frozen in liquid nitrogen. A total of 3,091 inseminations were performed with bull semen frozen in dry ice. The pregnancy rates were for semen frozen in liquid nitrogen and dry ice were 70% and 67%, respectively.

Miljkovic et al. (1972) reported no differences in pregnancies rates with frozen bull semen that had been stored in liquid nitrogen for 1 year. Macpherson (1954) reported that bull semen frozen and stored at -79°C could result in pregnancies when stored up to 15 months. It was later reported that storage time of frozen bull semen could be used for insemination successfully up to 24 months and still be capable of producing viable pregnancies (Macpherson 1955).

Further evaluation was later conducted on the viability of dairy bull semen frozen for 3 years (Macpherson, 1956). Semen from bulls was processed and frozen in dry ice (-79°C) until

evaluations were made. Microscopic evaluations were performed on all semen samples from bulls used in the trial for sperm motility estimation. Of the 12 bulls used in the trial, only 3 (25%) were considered to be not suitable for insemination due to nonacceptable post-thaw sperm motility. A total of 5 bulls were used for a breeding trial in which 40 cows were inseminated. In the breeding trial, a total of 15 cows were determined to be pregnant for a 37% pregnancy rate.

The pellet form of packaging has been more widely adapted in the Middle East and European countries. To determine viability of pellets stored over different periods of time, Lindstrom et al. (1972) compared the viability of frozen bull semen packaged in pelleted form that had been stored for 1 and 3 years. Two trials were conducted utilizing dairy breed bulls. In the first trial, 11 bulls were collected, packaged and stored for a period of 3 years. Pregnancy rates at 60 days for semen stored for 10 months and 3 years were 73% and 69%, respectively. In Trial 2, pregnancy rates at 60 days for semen stored for less than 1 year and 3 years were 69% and 72%, respectively.

Anderson and Pedersen (1976) demonstrated that semen could be used for insemination successfully after being stored for up to 5 years. Two different artificial insemination trials were conducted. Trial 1, utilized semen frozen in plastic straws from Holstein bulls, while Trial 2, utilized frozen stored pellets from the same Holstein bulls from Trial 1. A difference was found in pregnancy rates for frozen bull semen for 2 years when compared with semen that was frozen for 5 years.

Nishikawa et al. (1976) from Japan reported no decrease in semen post-thaw motility with frozen bull semen stored for 13 years when compared with semen frozen and stored for either 11 or 12 years (43%, 41% and 42%, respectively). Pregnancy rates for frozen bull semen stored for 10 and 12 years were 45% and 53%, respectively. Results also showed no differences in gestation periods and no abnormalities were noted in the calves born. In

comparison, Mixner (1968) reported viable pregnancies could also be achieved with frozen bull semen stored for up to 12 years. These two reports (Mixner, 1968; Nishikawa et al., 1976) utilize frozen bull semen stored for breeding trial purposes for the longest duration reported in the literature.

2.3.2. Mouse Semen Storage

Successful cryopreservation of mouse sperm is critical in the advancement of science. Sperm of any species are typically frozen in a medium containing a cryoprotectant. However, recent research involving freeze drying (Ward et al., 2003; Kawase et al., 2005) and evaporative drying (Li et al., 2007) of mouse semen have been reported.

Kawase et al. (2005) proposed that freeze dried mouse sperm could possibly be stored up to 100 years if maintained at temperatures below -80°C . This hypothesis was based on utilizing current blastocyst development rates with a Arrhenius plot. This hypothesis was verified by performing intracytoplasmic sperm injection with the freeze dried mouse sperm that had been frozen stored for a period of 6 months in which a 59% blastocyst rate was achieved (Kawase et al., 2005).

Ward et al. (2003) reported the successful cryopreservation of mouse sperm without a cryoprotectant. The mouse sperm was collected from the epididymides of male mice and suspended in a HEPES-buffered medium prior to freezing. The semen was frozen in liquid nitrogen and stored for different periods of time up to 1.5 years to determine viability. Viability was determined by the number of 2-cell embryos after intracytoplasmic Injection was performed. No differences were found between the two treatments (freeze drying and freezing without a cryoprotectant) when compared with fresh semen for 2-cell stage embryo development (87%, 87% and 88%, respectively). It was concluded that mouse sperm could be freeze dried or

frozen without a cryoprotectant and stored for a period up to 1.5 years without compromising DNA integrity or viability. A more recent study reported that mouse sperm could be successfully frozen utilizing a skim milk based extender and stored for 10 years without reduced fertility rates (Kaneko et al., 2006).

2.3.3. Ram Semen Storage

Sheep and goats provide an abundant source of food and fiber for many countries throughout the world. Recent trends of importations of high quality genetics into the United States have demanded the need for reproductive technologies in these species. It is also imperative that as time progresses, these initial genetics be preserved.

The scientific literature has reported that ram semen stored for 3 years in liquid nitrogen could still maintain viability and produce pregnancies in ewes (Salamon, 1972). However, when frozen semen was compared with fresh semen, a lower number of lambs were born in ewes that were inseminated with frozen stored semen (76.5% and 52.9 %).

Salamon and Visser (1974) later reported that ram semen could be successfully stored for a period up to 5 years without resulting in a decline in lambing rates. No differences were found in membrane integrity between both control semen (2 week of storage) and frozen stored semen (5 year storage) when analyzing the semen in vitro. Lambing results for control and treatment semen were 54.4% and 52.9%, respectively. Salamon (1976) later reported successful storage of ram semen for up to 7 years. In his study, frozen stored ram semen was frozen at two different temperatures (-79°C and -140°C). Lambing rates (51.4% and 52.1%) confirm no differences between the two frozen temperatures (-79°C and -140°C) during this trial.

2.3.4. Boar Semen Storage

Unlike bull semen, boar semen is usually collected and processed for immediate use,

usually within 7 days in the liquid form. However, some success in freezing of boar semen has been documented (Pursel and Johnson, 1975; Johnson et al., 2000). Salamon (1976) reported a 60% pregnancy rate in sows (n = 10) inseminated with frozen boar semen stored for 3 years. One study reported frozen stored boar semen stored for up to 19 years could be successfully used for in vitro fertilization. In this study, a 22% morula rate and 16% blastocyst rate was reported from frozen stored boar semen (Stroble et al., 2002).

2.3.5. Human Semen Storage

The first successful insemination with frozen human semen was reported by Bunge and Sherman (1953), just two years after the first calf was produced (Stewart, 1951). Therefore, with the successful semen freezing practice in humans being applied, semen banking would become an important factor for couples who result in subsequent infertility when trying to conceive naturally. Treatment for testicular cancer such as chemotherapy has been reported to result in infertility in the human (Horne et al., 2004). It is imperative that humans cryopreserve semen prior to starting chemotherapy treatment to retain viable genetic germplasm.

Human semen banks have made a large impact on infertility in humans. However, one major concern is that storage of semen over long periods of time can result in significant alteration in the DNA content located within the nucleus or mitochondria of the spermatozoa, thus resulting in lower fertility rates or complete infertility. Salisbury et al. (1961) reported a decline in sperm DNA content when cooled bull semen was stored at 5°C for 10 days. When fresh, refrigerated and frozen human semen stored up to 80 weeks was examined for DNA content, no differences were detected across all treatments (Ackerman and Sod-Moriah, 1968).

In a case report, Horne et al. (2004) described a human male, aged 17, who was diagnosed with testicular cancer. His semen was collected and frozen prior to cancer treatment.

A total of 5 ejaculates were collected and frozen. It was not until 1995; the male now 36 years of age, requested infertility assistance after 3 years of trying to naturally conceive. Once the male was consistently verified to be azoospermic (few sperm in ejaculate), the couple was accepted into the vitro fertilization (IVF) program. Semen quality post-thaw was considered usable on 4 of 5 ejaculates. Fertilization was achieved and fresh embryo transfer performed 3 of the 4 treatment cycles. The final result (cycle number 4) resulted in a healthy baby boy. This child represented successful storage of frozen human semen for 21 years. Feldschuh et al. (2005) reported successful intrauterine insemination with a pregnancy and live birth achieved with human semen stored for 28 years. This report (Feldschuh et al., 2005) is the longest storage time of frozen human semen to date resulting in a live birth.

2.4. Frozen Embryo Storage

Embryo transfer has made a tremendous impact on the disbursement of female genetics in the livestock industry. The ability to cryopreserve embryos allows genetics to be retained even after the animal is deceased. This technology also allows the importation and exportation of genetic stock worldwide. The first report on long term storage of frozen embryos was reported in the mouse (Whittingham and Whitten, 1974).

The success of embryo cryopreservation has allowed successful human embryo transfers and live births to be produced with human embryos cryopreserved and stored for years at a time. Ben-Ozer and Vermesh (1999) reported a successful human birth with an human embryo frozen and stored for 7.5 years. Other studies have reported human births with human embryos cryopreserved for 8 years (Go et al., 1998), 8.9 years (Quintans et al., 2002), 12 years (Revel et al., 2004) and 13 years (Teijón et al., 2006).

This technique of embryo cryopreservation also serves as a valuable tool in the human infertility field. Couples are able to cryopreserve their genetics at the present time with the ability to conceive and give birth at any given time. One study reported no effects on embryo quality in human embryos that had been stored for up to 16 months (Cohen et al., 1988), however, another study reported a decrease in embryonic survival rates for embryos stored for 1 month when compared to those stored for 6 to 15 months (71% vs. 53%) (Testart et al., 1987).

In livestock, successful transfer of embryos cryopreserved for 13 years was reported in sheep (Fogarty et al., 2000). A significant difference was detected in the number of ewes pregnant at 18 days that received frozen embryos (49%) and those artificially inseminated (74%). A significant difference was also detected in the number of lambs born per ewe (1.32 and 1.68, respectively), percentage of lambs born (41% and 82%, respectively) and the number of lambs weaned (34% and 58%, respectively) for ewes receiving frozen embryos or that were artificially inseminated. However, no difference was detected in the number of ewes lambing (31% and 49%, respectively) for ewes receiving frozen embryo or that were artificially inseminated.

2.4.1 Embryo Banking and Effects of Radiation

Benefits for embryo banking include: increase range of genetic stocks, alleles of known loci can be stored for future study, storage of unique chromosomal mutations, abnormalities can be preserved and provide ease of transport and safety from destruction by fire or diseases (Glenister and Lyon, 1986). In order to progress aging of cells, cells are subjected to radiation (Lyon et al., 1977; Ashwood-Smith and Friedmann, 1979; Glenister and Lyon, 1986).

Depending on the level of radiation exposure, cells can be aged from 2,000 years to 30,000 years. The typical amount of radiation specimens are subjected to in a given year is ~ 0.1 centi-Gray unit per year (cGy/year) (Glenister et al., 1984). Ashwood-Smith and Friedmann (1979) successfully demonstrated that ovarian fibroblast cells could be subjected to radiation equal to that of frozen storage up to 30,000 years.

Glenister et al. (1984) observed that no detrimental effect was found when 8-cell mouse embryos were subjected up to 200 cGy of radiation, the equivalent of 2,000 years of aging. In his study, mouse embryos were collected and frozen in dimethylsulphoxide (DMSO) and stored for a period from 5 to 8 months. During storage, the embryos were subjected to a gamma source radiation of 5-14 cGy per day. Five different total doses of radiation (0, 10, 50, 100, and 200 cGy γ -rays) were used. A total of 8 ampules containing embryos were thawed and evaluated to the blastocyst stage. No differences were detected in the number of embryos recovered, number of morphologically normal embryos at recovery and number of morulae and blastocyst after 24 hours in culture. Radiation also had no effects on the number of recipients becoming pregnant, number of implantations and the number of live fetuses born.

In another report, Glenister and Lyon (1986), confirmed results from that previously described (Glenister et al., 1984) in that no differences were detected in mouse embryos subjected to low and high levels of radiation. In this study, embryos were collected from superovulated female mice and frozen using a slow cool freezing protocol. The embryos were stored in three different nitrogen tanks placed at specific distances from the radiation source. The distances were arranged to make each tank receive different amounts of radiation based on the amount of background radiation present within the room (closest = 100X, middle = 10X, furthest = 2X). Embryos were thawed and evaluated from each tank at different time intervals starting initially at 6 months and extending to 5 years. A higher number of fresh control embryos

(96%) reached the blastocyst stage after culture when compared with the frozen embryos (50%) subjected to radiation. A small amount of deterioration was detected in the embryos subjected to the highest amount (100X) of radiation after 6 months of storage when compared to the lowest (2X). The affect of deterioration, however, did not continue to decline throughout the course of the study. Additionally, no differences were found in the number of live fetuses or percent live born.

2.5. In Vitro Fertilization

In vitro fertilization has served as a great tool for infertility in vivo. Results are obtained quickly (7 to 9 days) regarding blastocyst development rate. Leibo et al. (1994) reported successful blastocyst development rates in bovine oocytes utilizing frozen semen stored for 37 years. In this experiment, both aged frozen semen (treatment) and freshly frozen semen (control) were utilized for in vitro fertilization (IVF). Ovaries from mixed breed dairy cattle were obtained via abattoir and their oocytes aspirated. Semen samples were thawed and subsequently placed with the oocytes in culture medium. No differences were detected for cleavage rate for oocytes exposed to fresh control semen and aged frozen semen (42.8% vs. 8.2%). No differences were detected in the number of oocytes developing to blastocyst stage (15.6% vs. 25.0%) for fresh control semen or aged frozen, respectively, in Trial 1. In Trial 2, no differences were detected in the number of cleaved oocytes for fresh control semen and aged frozen semen (50.7% vs. 36.7%). The number of oocytes developing to the blastocyst stage was also not different (25.4% vs. 20.7%). These comparisons in the literature utilize frozen bull semen stored for the longest period of time, resulting in acceptable blastocyst development rates.

2.6. Angus Genetic/Growth Patterns

Advancements made in reproductive technologies have allowed for producers to multiply the number of quality animals entering the food production chain. However, through these changes, genetic changes over time have also made a substantial difference in the animals we now raise. Driven by consumer demand, genetic and growth patterns can be observed throughout different breeds of cattle. Since 1972, Angus cattle have increased in birth weight, weaning weight and yearling weight for calves born. Maternal characteristics that have shown increases include calving ease and higher milk production (American Angus Association, 2010a).

Meat quality and carcass production characteristics have also made improvements over the past few decades. In 1972, the average birth weight for Angus bulls calves were 31 kg with heifer calves averaging 29 kg. These values have since increased, bull calves in 2008 averaged 36 kg and heifer calves averaged 34 kg. The averaging weaning weights for bull calves in 1972 was 216 kg and for heifer calves was 192 kg. In 2008, these values also increased to 274 kg and 246 kg for bull and heifer calves (American Angus Association, 2010b).

Nadarajah et al. (1987) reported data on 13 different herds of Angus females consisting of 27,774 calves over a 30-year period. It was reported that weaning weights would increase at about 2 kg/year for Angus cattle. In contrast with these data, a marked decrease was noticed in maternal characteristics of Angus females. These differences may be contributed to the genetics used at the current time that were implemented into each breeding program.

Enns and Nicoll (2008) reported that the average weight change for a select group of Angus cattle was 0.43 kg/year. This data was collected from a herd database of 16,189 animals

from the years 1976 through 1993. Changes noted in birth weight yearling weight, harvest weight and mature weight were 0.29, 0.72, 1.7 and 0.13 kg/year, respectively.

2.7. Factors Affecting Fertility in Cattle

2.7.1 Heat Stress

Infertility in cattle can be affected by many environmental factors. These factors include: temperature (cold and heat), wind and humidity. Additional environmental factors that have been contributed to a decrease in pregnancy rates include: (1) maximum temperature the day of insemination, (2) the amount of rainfall on the day of insemination, (3) the amount of solar radiation the day of insemination and (4) the minimum temperature the day after insemination (Gwazdauskas et al., 1975).

The scientific literature has reported that a general decrease in pregnancy rates can occur during the warmer months of the year in cattle (Gwazdauskas et al., 1975; Badinga et al., 1985; Cavestany et al., 1985; see review by De Rensis and Scaramuzzi, 2003). A decrease in ovulation and implantation have been widely associated with heat stress in the female (Moberg, 1976). In cattle, the female has also been attributed to the significant factor in pregnancy rates between seasons (Stott, 1961).

Heat stress can cause embryonic mortality to occur between 35 to 41 days post-insemination with a delayed return to estrus in cattle (Stott and Williams, 1962; see review by De Rensis and Scaramuzzi, 2003). A decrease from 61% to 45% in pregnancy rates has been correlated to a 1°C increase in rectal temperature in dairy cattle. As external temperatures increase, a decrease is observed in the percentage of females exhibiting estrus, this in turn results in higher proportions of silent ovulations (see review by De Rensis and Scaramuzzi, 2003).

Badinga et al. (1985) reported a decrease in pregnancy rates when air temperatures increase from 23.9°C to 32.2°C. Cavestany et al. (1985) reported similar results when an increase in air temperature (29.7°C to 33.9°C) occurred in the month of April. Other research (Dunlap and Vincent, 1971) has reported that when heifers were exposed to high temperatures 72 hours following insemination a 0% pregnancy rate resulted when compared to 48% for heifers exposed to 21°C. For dairy cows, a critical time period has been extended to 4 to 6 days post-insemination (see review by Ayalon, 1978).

2.7.2. Management

Improper cow management can result in lower fertility rates (Roche et al., 2000). For a female to exhibit normal estrous cycles, a disease and reduced stress environment must be present (Moberg, 1976; Roche et al., 2000). Other studies have also reported that a decrease in reproductive efficiency can occur with an increase in size of the herd (Christian, 1971; Moberg, 1976), suggesting that herds divided up into small breeding groups could be beneficial.

2.7.3. Nutrition

Nutrition plays an important role in any production animal system. In order for animals to grow and reproduce to their fullest potential, a positive plane of nutrition must be provided. Postpartum intervals have been reported to decrease with increasing levels of nutrient intake (see review by Randel, 1990). Along with nutrient intake, inadequate energy sources during late pregnancy can lower subsequent pregnancy rates post-calving (see review by Randel, 1990).

Nutrient intake can be divided into two main categories of feedstuffs, fats or energy sources and proteins, both of which can affect pregnancy rates. Duration to first estrus in postpartum beef females has been correlated with levels of total digestible nutrients (TDN)

within the diet. On average, females receiving recommended levels (100%) of TDN came to estrus by 49 days postpartum, compared to those being fed 75% and 150% TDN levels of which did not display estrus until 73 days and 72 days postpartum, respectively (Wiltbank et al., 1964).

Dunn et al. (1969) reported that a higher percentage of females (87%) became pregnant when fed high energy diets compared with those fed moderate (72%) and low (64%) energy diets. In contrast, another report concluded that postpartum nutritional management had no effect on the interval to estrus or interval to pregnancy in cattle (Richards et al., 1986).

Pregnancy rates can be affected by both pre-calving and post-calving protein levels. One study (Sasser et al., 1988) reported that 89% of females exhibited estrus when fed adequate amounts of protein, whereas, only 63% exhibited estrus when fed restricted amounts of protein within their diets. This same group of females was subsequently bred and those fed adequate amounts of protein within their diet achieved a higher pregnancy rate (71%) than those fed a restricted amount (25%). It was also reported that those females fed restricted protein diets had longer intervals to first estrus, first service, and to pregnancy (Sasser et al., 1988).

2.7.4. Postpartum Interval

The postpartum interval is the time period from parturition to the first estrus (Dunn and Kaltenbach, 1980). This interval can vary depending on cow nutritional status (Dunn and Kaltenbach, 1980). Body weight and body condition scores are both common ways of assessing a female's nutritional status. Females with a body condition score of 7 to 9 were reported to return to estrus within 60 days (see review by Randel, 1990). Research has also shown that cows calving at a higher body condition score have a shorter calving interval than those in a lower body condition (Osoro and Wright, 1992).

In order for a cow to calve on a yearly basis, the female must conceive by 80 days postpartum (Dunn and Kaltenbach, 1980). Several factors can attribute to the length of this interval including: (1) body condition (2) protein intake (3) dietary energy intake and (4) body weight (Ferguson and Chalupa, 1989; Randel, 1990). The age of the cow and parity number can also affect the length of the postpartum interval. Heifers and first parity cows have been reported to have a longer postpartum interval than older cows (Randel, 1990).

2.7.5. Calf Removal

Using calf removal in an estrous synchronization protocol can prove to be difficult. It requires a well maintained facility and additional labor to separate the herd from one another. Research suggests that this method could provide additional pregnancies when implemented into an artificial insemination program.

Cows that are nursing have also been reported to have an increased postpartum interval. However, through research, it was reported that partial or temporary calf removal can shorten this interval (Edgerton, 1980). Browning et al. (1994) reported that by restricting calves from suckling, these females had a shorter (42 days) interval to estrus when compared with females whose calves were allowed to suckle (65 days). Higher pregnancy rates have been reported when calves were temporarily removed from cows (~ 48 hours) during the estrous synchronization protocols (Smith et al., 1979; Odde et al., 1986; Geary et al., 2001).

Higher pregnancy rates have been reported in cattle when calves were allowed to suckle once and twice daily (93% and 93%) when compared to 48 hour calf removal (91%) and no calf restriction (82%) (Odde et al., 1986). Kelser et al. (2009) reported higher pregnancy rates when calves were removed (48 hours) from the cows during the estrous synchronization process when compared with those whose calves were not removed (57% vs. 47%). In contrast, Smith

et al. (1979) reported no differences in pregnancy rates when calves were removed for 48 to 60 hours during the estrous synchronization process.

2.7.6 Cattle Temperament

Handling systems used in the cattle industry are designed to provide both an efficient and safe work environment for both cattle and man alike. Some reports indicate that repeated handling of livestock can cause the animal to become more stressed (Grandin, 1997). Chute score and exit velocity are coming ways of assessing cattle temperament (Grandin, 1997; Curley et al., 2006; Behrends et al., 2009; Cooke et al., 2009a; Rumph et al., 2009).

Chute scores are assigned to cattle while being restrained in a working chute. The score can range from 1 to 5, with 1 being calm to 5 being excitable (Curley et al., 2006). Exit velocity is measured by the amount of time it takes for cattle to transverse a distance of 1.86 meters. Several studies have reported a correlation between chute scores and exit velocity (Curley et al., 2006; Rumph et al., 2009). One study indicates that differences in temperament can be associated with the breed of cattle (Humes et al., 1987).

High temperament scores in feedlot calves have been reported to result in a lower average daily gain and higher yield grades (Falkenburg et al., 2007; Nkrumah et al., 2007; Behrends et al., 2009). Curley et al. (2006) reported that higher temperament scores in Brahman steers was significantly correlated with plasma cortisol levels.

Cooke et al. (2009) reported that when cattle were acclimated to human interaction, no decrease in chute scores or exit velocity was detected, however, an increase in pregnancy rates was noted. One study (Cooke, 2010) reported that cattle with excitable temperament could result in lower pregnancy rates when compared with those with adequate temperament at the end of the breeding season.

CHAPTER III

PREGNANCY RATES IN BEEF CATTLE ARTICIALLY INSEMINATED WITH AGED FROZEN-THAWED BEEF SEMEN

3.1 Introduction

Since the first calf was produced from frozen-thawed semen in 1951 (Stewart, 1951), artificial insemination has played a key role in cattle reproduction. This reproductive technique allows the disbursement of male genetics across a greater number of females when compared with that of natural breeding.

Prior to the use of frozen semen, semen was collected and used in its natural state. However, with the advancement science and the use of frozen semen, semen packaging would be necessary for semen storage purposes. The glass ampule was one of the first semen packaging methods used under field conditions (see review by Herman, 1981). This semen packaging method would be used extensively until the invention of the plastic straw (see review by Foote, 2002). This form of packaging was originally available in 0.5 mL, however, shortly thereafter, another version of the plastic straw was made with a reduced size of 0.25 mL (see review by Foote, 2002).

The effect of duration of storage time on post-thaw semen viability has not been reported in cattle. Some studies (Lyon et al., 1977; Glenister et al., 1984; Glenister and Lyon, 1986) utilizing radiation as an aging source have reported that frozen mouse embryos could retained viability after 2,000 years of storage. Furthermore, Ashwood-Smith and Friedmann (1979) proposed that mammalian tissue may remain viable after 30,000 years of storage in liquid nitrogen.

Several laboratories (Macpherson, 1954, 1956, 1955; Andersen and Pedersen, 1976) have reported no effect on pregnancy rates of short term storage (1 year to 5 years) on frozen

bull semen in liquid nitrogen. Two reports (Mixner, 1968; Nishikawa et al., 1976) have suggested that bovine semen frozen in liquid nitrogen can be stored for up to a period of 13 years. Mixner (1968) reported successful pregnancy rates (41%) with frozen bull semen stored in liquid nitrogen for up to 12 years, however only 24 females were inseminated in this trial. In comparison, Nishikawa et al. (1976) reported successful pregnancy rates (45%, n = 40) in beef cattle utilizing semen stored for up to 12 years when compared with frozen bull semen stored for 5 years (63%, n = 63), 6 years (55%, n = 27), 7 years (48%, n = 39), 8 years (49%, n = 77) and 10 years (50%, n = 58).

In a preliminary study conducted at this laboratory, crossbred beef females (n = 10) were estrous synchronized and subsequently inseminated from frozen-thawed aged Holstein bull semen in the month of December, 2009. The semen from bull number H69, H100, H120 and H131 was used for insemination. The frozen bull semen ranged in duration storage time from 43 to 47 years. Transrectal ultrasonography was performed at 45 to 50 days post-insemination to confirm pregnancy rates. A 60% (n = 6) pregnancy rate was achieved with a subsequent 50% calving rate occurring in January, 2010. These are the oldest frozen semen derived calves produced to date.

These previous studies (Mixner, 1968; Nishikawa et al., 1976) reported that after storage, the sperm was still able to produce viable pregnancies. While the two previous studies utilize frozen-thawed aged beef semen, no large scale controlled studies have been reported in the literature for pregnancy rates in cattle inseminated with frozen-thawed aged semen.

Leibo et al. (1994) reported that frozen bovine semen cryopreserved for 37 years in liquid nitrogen resulted in in-vitro produced blastocyst rates that were similar (16.5%) to the rates from semen cryopreserved for 2 years (29.2%). In this study, a total of 670 oocytes were exposed to the vintage frozen semen. A total of 149 of the oocytes cleaved for resulting in

a 22% cleavage rate. Furthermore, a total of 32 oocytes from the total number of oocytes reached the blastocyst stage for a blastocyst production rate of 5%. Unfortunately, in this study, no embryos were transferred resulting in no live calves being produced from the aged semen.

Studies utilizing frozen ram semen (Salamon, 1972,1976; Salamon and Visser, 1974), frozen boar semen (Salamon, 1976; Stroble et al., 2002) and frozen human semen (Horne et al., 2004; Feldschuh et al., 2005; Clarke et al., 2006) have suggested that long term storage of sperm in liquid nitrogen could still produce viable pregnancies. Salamon (1972, 1976) reported successful pregnancy rates in ewes when inseminated with ram semen frozen in liquid nitrogen stored for 3 years (52%) and for 7 years (51%). In comparison, Salamon and Visser (1974) reported a 52% pregnancy rate for ewes inseminated with frozen ram semen stored for 5 years.

In the sow, a successful pregnancy rate (60%, n = 10) and litter (39 piglets) was produced from boar semen frozen for 3 years (Salamon, 1976). Boar semen frozen for 19 years maintained a 30% sperm motility in-vitro and when exposed with pig oocytes produced a 22% morula and 16% blastocyst rate (Stroble et al., 2002). Unfortunately, in this study, no embryos were transferred resulting in no live births from the frozen-thawed aged boar semen.

A variety of factors can influence cow fertility including: environmental temperature and animal management (Gwazdauskas et al., 1975; Moberg, 1976), postpartum nutrition (Richards et al., 1986; see review by Randel, 1990;), lactational status (Edgerton, 1980), presence of a suckling calf (Odde et al., 1986; Geary et al., 2001).

Post-thaw semen evaluations are commonly performed on at least one unit of the semen from the batch that is used for cattle insemination. Evaluations typically include: total motility, progressive motility, semen concentration and sperm morphological abnormalities. These

factors have been previously evaluated for their affect on pregnancy rates in cattle (Foote, 1975; Saacke, 1982; Foote, 2003).

Post-thaw motility semen evaluations are generally observed visually by a trained technician. More recently, a CASA (Computer Assisted Sperm Analysis) system has been introduced into laboratories and in some cases has replaced visual appraisal (Farrell et al., 1998). Post-thaw sperm motility has been reported in various studies to be correlated with pregnancy rates in beef cattle (Linford et al., 1976; Wood et al., 1986; Januskauskas et al., 2000) and in other reports (den Daas, 1992; Stalhammar et al., 1994; Phillips et al., 2004) a poor correlation was noted.

To indentify bovine sperm abnormalities, a variety of semen fixatives and stains have been evaluated over the years (Barth and Oko, 1989b; Sprecher and Coe, 1996) . Semen abnormalities are classified into three categories: primary, secondary or tertiary (Saacke and White, 1972; Coulter, 1992;). Primary abnormalities are associated with defects of the sperm head. These defects can include: microcephalic, microcephalic, pyriform, tapered, ruffled or reacted acrosome and nuclear vacuoles. Secondary abnormalities are associated with disruption of normal midpieces or sperm that contain protoplasmic droplets (proximal and distal). Tertiary abnormalities are associated with abnormal sperm tails. These abnormalities are classified as being bent, coiled, stump-tailed and double-tailed sperm (Sullivan, 1978).

Martin (1990) reported that only primary and total bull sperm abnormalities were correlated with pregnancy rates, while secondary and tertiary abnormalities combined were not. In contrast, Woods et al. (1986) observed the exact opposite, in their study, where coiled tails and proximal droplets were found to be correlated to cattle pregnancy rates. Saacke and White (1972) found a significant correlation between primary, secondary and total abnormalities and pregnancy rates but not tertiary abnormalities in dairy cattle.

The concentration of sperm needed to fertilize cows with AI has been reported in detail in the literature (Bratton et al., 1954; Foote and Kaproth, 1997). Bull sperm concentrations as low as 5×10^6 can result in comparable pregnancy rates to that of bull sperm concentrations of 10×10^6 (Bratton et al., 1954). However, when sperm insemination doses were reduced to 2×10^6 , a significant reduction in cattle pregnancy rates have been reported to occur (Andersson et al., 2004).

The overall objective of this study was to compare the fertility rates of Angus and crossbred cattle inseminated with frozen-thawed aged Angus semen. The second objective was to compare birth weights and gestations lengths of calves born across three different Time Frames. The third objective of this study was to compare both fertility rates and post-thaw Angus semen parameters for the two different semen packaging methods utilized across the three different Time Frames. The fourth objective was to compare semen parameters of the frozen-thawed aged Angus semen under standard laboratory conditions.

3.2. Materials and Methods

Two experiments including Angus females (cows and heifers, Experiment I) and crossbred cows (Experiment II) were utilized for artificial insemination during the spring breeding season (April through May). The cows were maintained at both the LSU AgCenter Purebred Unit (Angus cows and heifers, Experiment I) and the Crossbred Beef Research Unit (Crossbred cows, Experiment II) of the Central Research Station of the LSU AgCenter, Baton Rouge, Louisiana. The females in each experiment were allowed free access to Bermudagrass and Ryegrass pastures and water *ad libitum*.

Semen from purebred Angus bulls ($n = 25$) representing three different Time Frames (Time Frame 1: 1960-1975, $n = 5$; Time Frame 2: 1976-1991, $n = 10$; Time Frame 3: 1991-

2009, n = 10) were utilized for artificial insemination in this study (Table 3.1). The frozen Angus bull semen used in this study was provided by the USDA-ARS National Animal Germplasm Program in Fort Collins, Colorado.

Prior to semen evaluation, all units of Angus bull semen samples were shipped overnight in a liquid nitrogen dry shipper from the National Animal Germ Plasm Program to the LSU Reproductive Biology Center, where they were allocated into different canes based on bull identification number (I.D.). They were then placed into a MVE Millennium 2000 XC 20 liquid nitrogen storage tank (Agtech, Inc., Manhattan, KS) until post-thaw evaluation was performed. All semen evaluations were conducted at the LSU School of Veterinary Medicine. On the day of evaluation, all semen samples were transported in their frozen state in a liquid nitrogen storage tank from the LSU Reproductive Biology Center to the LSU School of Veterinary Medicine.

3.2.3. Experimental Design

A stratified experimental design was utilized for this study. All females in each experiment were stratified across the three different Time Frames (Time Frames 1, 2 and 3) by breed type in Experiments 1 and 2. with the Angus bulls nested within each Time Frame.

3.2.2. Experiment I

Mature purebred Angus cows (n = 44) and heifers (n = 17), in good body condition, were estrous synchronized for artificial insemination. The average age for the Angus cows and the Angus heifers was 5.4 years (3 to 11 years) and 13.5 months (12 to 15 months), respectively. All mature females were required to be a minimum of 45 days postpartum before being enrolled into the estrous synchronization protocol. Prior to the estrous synchronization protocol, all

Table 3.1 Descriptive information for the Angus Bulls used in this study

Time Period	Bull No. ^a	NAGP I.D. ^b	Registration #	Name	Birth Year	Freeze Year	Package
1960-1975	1	2668	7036540	Charbow Chance 10 Y	1967	1973	Ampules
1960-1975	2	8580	5272722	Green Valley O B 67	1966	1969	Ampules
1960-1975	3	8590	6173161	Biffles Challenger 796	1968	1972	Ampules
1960-1975	4	8591	8086237	K A F Thunderbird 707	1973	1974	Ampules
1960-1975	5	8830	7101362	Puranbree Fabron 7156	1971	1973	Ampules
1976-1991	6	3116	10593697	Black Thunder of V A F	1984	1985	Straws
1976-1991	7	8563	7530832	Rito 149 of Ideal 632 72	1972	1987	Ampules
1976-1991	8	3091	9467046	Continental	1978	1980	Straws
1976-1991	9	3138	9934739	Eldorado 156 of Ideal	1981	1989	Straws
1976-1991	10	3396	10710606	Eldorado 5110 Ideal 2228 156	1985	1987	Straws
1976-1991	11	1054	9709378	C S U Shoshone 0128	1980	1981	Straws
1976-1991	12	2337	8679290	Lady Prides Duke	1967	1976	Ampules
1976-1991	13	3106	11199043	HW Power Play W238	1988	1990	Straws
1976-1991	14	3398	10710666	Landmark 5215 of Ideal 9100	1985	1986	Straws
1976-1991	25	3235	9034639	PJM Power Point	1977	1980	Straws
1992-2006	15	3375	11787983	Ideal 2292 of 1254	1992	1995	Straws
1992-2006	16	3050	11167039	Nichols Heavy Duty X100	1988	1997	Straws
1992-2006	17	3084	14215152	Jamesland ND 1407 70J	2002	2003	Straws
1992-2006	18	3293	14420104	Diamond Prime Cut 6763	2003	2004	Straws
1992-2006	19	3049	12234092	Nicols Performa D162	1994	2003	Straws
1992-2006	20	2989	13543367	Stassens Papa Universe 6	2000	2003	Straws
1992-2006	21	2993	13270868	Ankonian Victorio	1999	2000	Straws
1992-2006	22	3059	12215320	O G L Battle Cry 427 128	1994	1999	Straws
1992-2006	23	3086	13266172	R F A Generation Ext 881	1998	2002	Straws
1992-2006	24	3031	11447554	MS Mac Arnie	1990	1995	Straws

^aBull No. = Bull identification number used at LSU for the study.

^bNAGP = USDA-ARS National Animal GermPlasm Program official identification number.

females were assigned a body condition score and body weights recorded. The average body weights, days postpartum (DPP) and average body condition scores (1 = emaciated, 9 = obese) are presented in Table 3.2.

3.2.3. Experiment II

In Experiment II, mature crossbred females from six breed types (n = 196), in good body condition, were estrous synchronized for artificial insemination. The mean age of these cows was 6.5 years (3 to 8 years). All females were required to be a minimum of 45 days postpartum before being enrolled into the estrous synchronization protocol. During the estrous synchronization protocol all females were assigned a body condition score and weighed. All females were allowed access to Bermudagrass and Ryegrass pastures and water *ad libitum*. The average body weights, body condition scores (1 = emaciated, 9 = obese) and days postpartum are presented in Table 3.3.

3.2.4. Estrous Synchronization and Detecting Estrus (Experiments I and II)

The SelectSync Protocol was used for estrous synchronization (Leitman et al., 2008). Briefly, on day 0, all females received a Eazi-Breed[®] Controlled Internal Drug Releasing insert (CIDR; Pfizer Animal Health, New York, NY) and received a 200 µg injection (im) of a GnRH analogue (Factryl[®]; Fort Dodge, Overland Park, KS). Then on day 7 of treatment, the CIDR progesterone implants were removed and a 25 mg injection (im) of prostaglandin F_{2α}, (Lutalyse[®]; Pfizer Animal Health, New York, NY) were administered (im) to each female. All females not responding (no standing estrus) within 5 to 6 days to the synchronization protocol were again administered prostaglandin 8 to 10 days later.

The HeatWatch[®] (DDX, Inc., Denver, CO) system was utilized for detecting estrus. The detection system consisted of a computer, individual remote animal transmitters and nylon

Table 3.2. Angus cattle characteristics (Exp. I)

	Weight (kg) ^a	DDP ^b	BCS ^c
Cows	550 ± 10	77 ± 2	6.8 ± 0.09
Heifers	313 ± 6	n/a	6.2 ± 0.14

^a Mean body weight ± SE.

^b Days postpartum ± SE.

^c Mean body condition score ± SE.

Table 3.3. Mature postpartum crossbred cow characteristics (Exp. II)

Breed	Weight (kg) ^a	DPP ^b	BCS ^c
Beefmaster	529 ± 7	83 ± 3	5.5 ± 0.12
Bons Mara	530 ± 9	84 ± 3	6.1 ± 0.16
Brangus	531 ± 8	83 ± 4	5.9 ± 0.17
Romosinuano	503 ± 8	84 ± 3	6.0 ± 0.17
Brangus F ₁	515 ± 12	79 ± 3	6.0 ± 0.17
Braford	456 ± 19	86 ± 4	6.0 ± 0.08
Combined	511 ± 11	83 ± 1	5.9 ± 0.06

^a Mean body weight ± SE.

^b Days postpartum ± SE.

^c Mean body condition score ± SE.

patches. The individually numbered transmitters were placed into the nylon patches before being glued to the back of each female just cranial to the tailhead. The numbers of each transmitter were recorded for each female to be placed into the computer system. When a female was mounted, the pressure sensitive button located on the transmitter is pressed, sending information back to the computer system. The data sent back to the computer included: animal number, transmitter number, date, time of mount, number of mounts and duration of each mount.

For this study, a female was considered to be in standing estrus when she was mounted at least 3 times within a 4-hour period. To detect estrus for the second service, after females were inseminated at the first service, the HeatWatch[®] patch was removed and a scratch-off patch applied (Estrotest[™], Rockway, Inc., Spring Valley, WI). All females were artificially inseminated by the same experienced technician 12 to 14 hours after the onset of standing estrus.

For beef females in Experiment II., two different aids were used for detecting estrus. Estrotest[™] (Rockway, Inc., Green Valley, WI) patches and the HeatWatch[®] (DDX, Inc., Denver, CO) system were used in this experiment. During synchronization females were either fitted with Estrotest[™] patches or HeatWatch[®] patches. The Estrotest[™] patches consist of a scratch off side that reveals a color to notify that the animal has been mounted. The reverse side consists of an adhesive layer that allows the patch to stick to the animal and maintain its place during mounting activity. Females fitted with these patches were observed twice daily (A.M. and P.M.) for estrual behavior. Standing estrus was the criteria used for artificial insemination. Females whom were not observed in standing estrus and or had <50% of the Estrotest[™] patch scratched were not considered to be in standing estrus. All females were artificially inseminated once by the same experienced technician 12 to 14 hours after the onset of standing estrus.

3.2.5. Semen Thawing and Pregnancy Determination. Experiments I and II

For this study two different thawing methods were used depending on the packaging of the semen. Semen packaged in plastic straws from 18 bulls was thawed for 30 seconds in a 37°C water bath (Johnson et al., 2005). Once thawed, the plastic straw was wiped dry, loaded into the insemination gun (Agtech, Inc. Manhattan, KS) then the sealed end cut and an 18 inch plastic chemise (Agtech, Inc. Manhattan, KS) applied before being presented to the inseminator. Bulls (n = 7) packaged in 0.5 mL and 1.0 mL glass ampules were thawed for 80 seconds in a 37°C water bath (Chandler et al., 1983). Once thawed, the glass ampule was wiped dry, scribed and opened. The semen was then extracted with a plastic pipette insemination gun (IMV, Maple Grove, MN) before being presented to the inseminator. Vulva scores, the presence of mucus and semen deposition site recorded on each female. In Experiment I, a single Angus cleanup bull was placed with the females 2 weeks after the last insemination took place. In Experiment II, Charolais (n = 4) was placed with the inseminated cows 2 weeks after the last insemination took place.

Pregnancy diagnosis was determined at 45 to 50 days post-insemination via transrectal ultrasonography with a multi-range probe connected to a MicroMaxx ultrasound unit (Sonosite, Bothell, WA). Fetal measurements were also taken to aid in identifying the artificial inseminated pregnancies from those of the cleanup bull pregnancies. Final pregnancy rates based on calf phenotype were verified at birth. Shortly after birth, both calf weights and sex were recorded. Any calves that were considered either stillborn or died during the study was noted.

3.2.6. Motility Assessment

All semen samples were visualized for both total sperm motility (TM) and progressive sperm motility (PM) by two experienced technicians using a phase contrast microscope

(Olympus, Model BH-2, Japan) fitted with a 40X objective and a 10X eyepiece. A small droplet (5 μ l) of thawed semen was placed on a 37°C pre-warmed microscope slide and a cover slip attached. Total motility consisted of any movement noted by the sperm, progressive motility was assessed by noting any sperm movement moving in a linear direction. The average motility (total and progressive) consisted of looking at three different fields on the microscope slide and averaging the three. The final sperm motility (total and progressive) assessment consisted of the average of the two technicians (David Carwell and Dr. Jose Len) using the standard laboratory procedure for semen motility. Semen samples from the same bulls were also assessed utilizing a CASA unit attached to a trinocular phase microscope (Olympus, Model BX41, Center Valley, PA) with compatible software attached (Sperm Vision, Minitube, Verona, WI). Once the semen samples were thawed, a 20 μ l droplet was placed into the specialized slide and the sample then analyzed using the CASA Sperm Vision program.

3.2.7. Sperm Concentration

Semen concentration was determined utilizing a standard red blood cell hemocytometer (Utech, Inc., Schenectady, NY). For initial dilution, 450 μ L of 10% formalin buffered saline (LSU School of Veterinary Medicine, Baton Rouge, LA) was placed into two 5 mL plastic tubes (Sarstedt, Germany). A 50 μ L semen sample was then placed into the first tube containing the formalin buffered saline for a 1:10 dilution ratio. A second 50 μ L sample was then taken from the first tube and placed into the second tube containing the formalin buffered saline for a 1:100 dilution ratio. A small sample was then taken from the 1:100 dilution with a pipette and placed into the hemocytometer. The samples were allowed to sit for 3 to 4 minutes prior to counting.

Sperm were counted using phase contrast microscope (Olympus, Model BH-2, Japan) fitted with a 40X objective. All 25 squares of the hemocytometer were counted. The average count of both chambers was recorded as the final sperm concentration/mL. If the two counts

were not within 10% of one another, the process was redone. Only sperm present within the hemocytometer cell and those located on the top and left outside line were counted towards the final semen concentration.

3.2.8. Sperm Morphology

Sperm morphology was assessed utilizing a standard laboratory wet-mount procedure. A raw semen sample (50 μ L) was first diluted to a 1:10 ratio with formal buffer saline. A small droplet (10 μ L) was placed on a microscope slide and a cover slip attached. Samples were observed with a differential interference contrast microscope (Olympus, Model HH-2, Japan) fitted with a 100X objective (oil) in combination with immersion microscope oil.

3.2.9. Morphology Classification

Sperm were classified as either being normal or abnormal. Normal sperm were considered to be free of any head abnormalities, protoplasmic droplets and tail abnormalities (Barth and Oko, 1989a; Sullivan, 1978). Abnormal sperm consisted of three different types of abnormalities (primary, secondary and tertiary). Those classified as being a primary abnormalities contained abnormal head shape, disrupted acrosomes or detached heads. Those classified as possessing secondary abnormalities contained bent or broken midpieces and protoplasmic droplets (proximal or distal). Those classified as possessing tertiary abnormalities contained any disruption of a normal sperm tail (coiled, kinked, stump or double tailed) (Barth and Oko, 1989a; Sullivan, 1978).

3.2.10. Bull Fertility Classification

In this study, all bulls (n = 25) were divided into three fertility groups (high, moderate or low) based on AI pregnancy rates from Experiments I and II. Post-thaw motility comparisons were then made across the three fertility levels.

3.3. Statistical Analysis Experiment I and II

For pregnancy rates across Time Frames in Experiments I and II, data were analyzed using the Chi-square procedure. A One-Way Random Analysis of Variance was utilized to determine difference in sperm sperm motility (total and progressive), sperm morphology (normal, abnormal, primary, secondary and tertiary) and pregnancy rates between the two different semen packaging methods (0.5 mL and 1.0 mL glass ampules and 0.5 mL plastic straws. A One-Way Random Analysis of Variance was utilized to test all semen parameters (total and progressive sperm motility, sperm concentration and sperm morphology).

A One-Way Random Analysis of Variance was also used for sperm motility comparisons (Laboratory v.s. CASA) and bull sperm motility (High, Moderate and Low). For values that failed normality, a transformation of log 10 was used for the data set. For significant values, a Duncan's Multiple Range Test was used for all pair-wise comparisons. All data was analyzed using the SigmaStat Statistical Software Version 3.5. The significance was set at the $P < 0.05$ level for all comparisons.

3.4. Results

3.4.1. Experiment I.

3.4.2. Synchronization Response

A total of 36 Angus cows (81%) responded to the estrous synchronization protocol; whereas, 9 (8%) of these females did not. Four additional females (44%) of the nonresponding females responded to the second round prostaglandin injection and were inseminated. A total of 13 Angus heifers (76%) responded to the estrous synchronization protocol; whereas, 3 (17%) of these females did not exhibit standing estrus. Two additional Angus heifers (66%) responded to the second round of prostaglandin injection and were inseminated.

A total of 49 of 61 Angus females (80%) responded to the estrous synchronization protocol; whereas, 12 (19%) of these females did not exhibit estrus. Six additional females of the 12 (50%) nonresponding females responded to the second round prostaglandin injection and were inseminated. A total of 55 Angus females (90%) were included in the first service data set in this study. Four Angus females were inseminated a second time at subsequent estrus resulting in a total of 59 females inseminated.

3.4.3. First Service Pregnancy Rates

First service pregnancy rates for Angus Cows for Time Frames 1, 2 and 3 were not significantly different (53%, 75% and 76%, respectively). For the Angus heifers, first service pregnancy rates for Time Frames 1, 2 and 3 were also not significantly different (80%, 40%, and 20%, respectively). No differences in pregnancy rates were detected within Time Frames between both Angus cows and heifers; therefore, the data was combined. First service combined pregnancy rates for Angus cows and heifers for Time Frames 1, 2, and 3 were 60%, 64%, and 61%, respectively (Table 3.4).

3.4.4. Second Service Pregnancy Rates

For second service, three cows were inseminated on a subsequent estrus to Angus bulls in Time Frame 2 and 3. Second service pregnancy rates for Time Frames 2 and 3 were 0% and 66%. No second service inseminations were performed for Time Frame 1.

3.4.5. Total AI Service Pregnancy Rates

The overall total service pregnancy rate for Angus cow and heifers for Time Frames 1, 2 and 3 was 60%, 61% and 61%, respectively (Table 3.5). The overall total pregnancy rate from AI for all Angus females was 61%.

Table 3.4. Combined Angus cow and heifer artificial insemination first service pregnancy rates across Time Frames 1, 2 and 3 (Exp. I)

Animals	Time Frame ¹	Pregnant Females (%) ²
Cows	1 (n = 15)	8 (53%)
	2 (n = 12)	9 (75%)
	3 (n = 13)	10 (76%)
Heifers	1 (n = 5)	4 (80%)
	2 (n = 5)	2 (40%)
	3 (n = 5)	1 (25%)
Combined	1 (n = 20)	12 (60%)
	2 (n = 17)	11 (64%)
	3 (n = 18)	11 (61%)

¹ Time Frame = (Time Frame 1 = 1960-1975; Time Frame 2 = 1976-1991; Time Frame 3 = 1992-2009.

² Pregnant Females = Number and percentage of females confirmed pregnant by calving.

Table 3.5. Combined Angus cow and heifer artificial insemination total service pregnancy rates across Time Frames 1, 2 and 3 (Exp. I)

Animals	Time Frame ¹	Pregnant Females (%) ²
Cows	1 (n = 15)	8 (53%)
	2 (n = 13)	9 (69%)
	3 (n = 16)	10 (62%)
Heifers	1 (n = 5)	4 (80%)
	2 (n = 5)	2 (40%)
	3 (n = 5)	1 (25%)
Combined	1 (n = 20)	12 (60%)
	2 (n = 18)	11 (61%)
	3 (n = 21)	13 (61%)

¹ Time Frame = (Time Frame 1 = 1960-1975; Time Frame 2 = 1976-1991; Time Frame 3 = 1992-2009.

² Pregnant Females = Number and percentage of females confirmed pregnant by calving.

3.4.6. Calving Data

The mean gestation lengths (\pm SE) were not significantly different for Angus calves born from bull semen from Time Frames 1, 2 and 3 for Angus cows (273 ± 1 , 278 ± 1 , and 283 ± 6 days, respectively). For Angus cows, the mean birth weights (\pm SE) were not significantly different of calves born across Time Frames 1, 2, and 3 (34 ± 2 , 36 ± 2 and 38 ± 1 kg, respectively). The average gestation lengths were not significantly different for Angus calves born from Time Frames 1, 2 and 3 for Angus heifers (280 ± 1 , 282 ± 1 and 277 days, respectively). For Angus heifers, the mean birth weights were not different of calves were not significantly different born across Time Frames 1, 2 and 3 was 36 ± 3 , 43 ± 3 and 29 kg, respectively. The overall mean birth weight for Angus calves born from the Angus bulls for Angus cows was 36 ± 1 kg and for Angus heifers the overall mean birth weight was 37 ± 3 kg. The overall mean gestation length for Angus calves born from the Angus bulls for Angus cows was 278 ± 3 days. For Angus heifers, the overall mean gestation length was 280 ± 1 day.

No differences were detected for mean birth weight or mean gestation length within Time Frames for both Angus cows and heifers; therefore the data were combined. Overall, the average birth weights were not different for Angus cows and heifers (Table 3.6) born across Time Frames 1, 2 and 3 was 35 ± 4 kg, 37 ± 5 kg and 38 ± 3 kg, respectively. The average gestation lengths were also not different for Angus cows and heifers for calves born across Time Frames 1, 2 and 3 was 275 ± 4 days, 279 ± 1 days and 281 ± 6 days, respectively. The number of male calves born across Time Frames 1, 2 and 3 was 8 (66%), 5 (45%) and 6 (46%), respectively. The number of females calves born across Time Frames 1, 2 and 3 was 4 (33%), 6 (54%) and 7 (53%), respectively. Overall a total of 19 (52%) male calves were born with a total of 17 (42%) female calves being born for an overall total of 36 calves born in this study.

Table 3.6. Combined Angus cow and heifer calving data across Time Frames 1, 2 and 3 (Exp. I)

Animals	Time Frame ¹	N ²	BW (kg) ³	GL (days) ⁴
Cows	1	8	34 ± 2	273 ± 7
	2	9	36 ± 2	278 ± 1
	3	10	38 ± 1	283 ± 6
Heifers	1	4	36 ± 3	280 ± 1
	2	2	43 ± 3	282 ± 1
	3	1	29 n/a	277 n/a
Combined	1	12	35 ± 5	275 ± 13
	2	11	37 ± 7	276 ± 7
	3	13	38 ± 4	276 ± 7

¹ Time Frame = (Time Frame 1 = 1960-1975; Time Frame 2 = 1976-1991; Time Frame 3 = 1992-2009).

² Number of females calving.

³ Mean birth weight ± SE.

⁴ Mean gestation length ± SE.

In this study, a total of 5 calves died either at birth or shortly after. A total of 3 of these 5 calves that died were represented from Time Frame 1. The additional two calves that died were represented from Time Frame 3. All deaths were considered to be a result of dystocia at parturition.

3.4.7. Noncalving females

At the end of this study a total of 12 Angus females were confirmed open after the cleanup bull had been removed after 50 days exposure. Two of these 12 females did not calve in the previous year.

3.4.8 Experiment II

3.4.9. Estrous Synchronization Response

A total of 197 mature crossbred females were estrous synchronized for AI. A total of 157 females (79%) responded to the estrous synchronization protocol and were inseminated; whereas, 40 females (21%) did not respond. A total of 32 females were resynchronized and of these 32, 6 females (18%) responded to the additional prostaglandin injection. A total of 6 (3%) females were artificially inseminated off of natural estrus occurring just prior to estrous synchronization. A total of 169 females were included in the first service data set in this study. Twenty additional females were inseminated a second at subsequent estrus resulting in a total of 189 females inseminated.

3.4.10. First Service Pregnancy Rates

First service pregnancy rates were not significantly different across Time Frames between breed types, therefore, these data were combined (Table 3.7). First service AI pregnancy rates for Time Frames 1, 2 and 3 for Beefmaster females were 40%, 70% and 45%,

Table 3.7. Crossbred cow total first service pregnancy rates across Time Frames 1, 2 and 3 (Exp. II)

Time Frame ¹	Pregnant Females ²
1 (n = 53)	30 (56%)
2 (n = 58)	36 (62%)
3 (n = 58)	32 (55%)

¹ Time Frame = (Time Frame 1 = 1960-1975; Time Frame 2 = 1976-1991; Time Frame 3 = 1992-2009).

² Pregnant Females = Number and percentage of females confirmed pregnant by calving.

respectively, resulting in an overall first service total AI pregnancy rate of 51%. First service AI pregnancy rates for Bons Mara females for Time Frames 1, 2 and 3 were 75%, 44% and 50%, respectively, for an overall first service total AI pregnancy rate of 58%.

First service AI pregnancy rates for Time Frames 1, 2 and 3 for Brangus females were 33%, 50% and 66%, respectively, for an overall first service total AI pregnancy rate of 53%. First service AI pregnancy rates for Romosinuano females were 66%, 88%, and 71%, respectively, for an overall first service total AI pregnancy rate of 75%. First service AI pregnancy rates for Time Frames 1, 2 and 3 for Brangus F₁ females were 50%, 70% and 66%, respectively, for an overall first service total AI pregnancy rate of 62%. First service AI pregnancy rates for Time Frames 1, 2 and 3 for Braford females were 66%, 50% and 36%, respectively, for an overall first service total AI pregnancy rate of 46%. First service AI pregnancy rates for Time Frames 1, 2, and 3 across all females were found to be not different (56%, 62% and 55%, respectively). The overall first service AI pregnancy rate for the crossbred females was 57%.

3.4.11. Second Service Pregnancy Rates

Second service AI pregnancy rates were not different across Time Frames 1, 2 and 3 (25%, 63% and 40%) and the overall second service AI total pregnancy rate was 50%.

3.4.12. Total Service Pregnancy Rates

Total service AI pregnancy rates were not different across Time Frames 1, 2 and 3 (54%, 62% and 53%, respectively) (Table 3.8). The overall total service AI pregnancy rate was 57%.

Table 3.8. Crossbred cow total service pregnancy rates across Time Frames 1, 2 and 3 (Exp. II)

Time Frame ¹	Pregnant Females ²
1 (n = 57)	31 (54%)
2 (n = 69)	43 (62%)
3 (n = 63)	34 (53%)

¹ Time Frame = (Time Frame 1 = 1960-1975; Time Frame 2 = 1976-1991; Time Frame 3 = 1992-2009).

² Pregnant Females = Number and percentage of females confirmed pregnant by calving.

3.4.13. Calving Results

The mean gestation length (\pm SE) for all crossbred females were not different across Time Frames 1, 2 and 3. The gestation lengths for calves born across Time Frames 1, 2 and 3 was 282 ± 1 days, 281 ± 1 days and 283 ± 1 days, respectively. Mean birth weights (\pm SE) for calves born across for Time Frames 1, 2 and 3 were also not different across all crossbred females. The mean birth weights for calves born across Time Frames 1, 2 and 3 was 37 ± 2 kg, 36 ± 2 kg and 36 ± 2 kg, respectively (Table 3.9). The number of male calves born across Time Frames 1, 2 and 3 was 15 (48%), 19 (44%), and 20 (58%), respectively. The number of female calves born across Time Frames 1, 2 and 3 was 16 (51%), 24 (55%) and 14 (41%), respectively. The overall total number of males calves born in this study was 54 (50%) and the total number of female calves born in this study was 54 (50%), resulting in a total of 108 calves born.

3.4.14. Noncalving Females

At the end of this study, a total of 25 crossbred females were confirmed to be open after the cleanup bulls were removed after a 50 day exposure. Of these 25, 6 were females (24%) that did not calve in the previous year.

During this study, a total of three calves crossbred calves died, either at birth or shortly after. One was sired from bulls in Time Frame 1, while the other two were sired from bulls in Time Frame 2. One of these calves sent to necropsy resulted in a diagnoses of having organ failure. The two deaths resulted directly or indirectly from dystocia. One additional calf was pulled from its dam due to the mother sustaining injuries.

Table 3.9. Mean calf birth weight and gestation length for crossbred females across Time Frames 1, 2 and 3 (Exp. II)

Breed	Time Frame ¹	BW (kg) ²	GL (days) ³
Beefmaster	1	36 ± 2	281 ± 3
	2	35 ± 2	279 ± 3
	3	36 ± 3	283 ± 3
Bons Mara	1	37 ± 2	280 ± 3
	2	33 ± 4	279 ± 1
	3	32 ± 2	284 ± 3
Brangus	1	38 ± 4	285 ± 6
	2	38 ± 3	282 ± 1
	3	36 ± 1	281 ± 1
Romo sinuano	1	39 ± 2	283 ± 1
	2	38 ± 1	278 ± 5
	3	37 ± 2	284 ± 1
Brangus F ₁	1	31 ± 1	285 ± 1
	2	39 ± 1	285 ± 2
	3	34 ± 1	285 ± 1
Braford	1	39 ± 1	286 ± 1
	2	34 ± 3	283 ± 3
	3	40 ± 2	283 ± 5
Combined	1	37 ± 1	282 ± 1
	2	36 ± 1	281 ± 1
	3	36 ± 1	283 ± 1

¹ Time Frame = (Time Frame 1 = 1960-1976; Time Frame 2 = 1976-1991; Time Frame 3 = 1992-2009).

² Mean birth weight ± SE.

³ Mean gestation length ± SE.

3.4.15. Angus Sired Calves Born

Typical calves born from purebred Angus females from bulls from Time Frames 1, 2 and 3 are shown in Figures 3.1, 3.2 and 3.3. Examples of calves born from crossbred females from bulls from Time Frames 1, 2 and 3 are shown in Figures 3.4, 3.5 and 3.6.

3.4.16. Results Experiments I and II

3.4.18. Pregnancy Rates

First service AI pregnancy rates for all females (Angus and crossbred) were not different across Time Frames 1, 2 and 3 (57%, 62% and 56%, respectively). The overall total first service AI pregnancy rate was 58%.

Second AI service pregnancy rates for all females (Angus and crossbred) were not different across Time Frames 1, 2 and 3 (25%, 58% and 50%, respectively). The total second service AI pregnancy rate for Angus and crossbred females was 42%.

Total service (first and second service) AI pregnancy rates across all females (Angus and Crossbred) were not different across Time Frames 1, 2 and 3 (55%, 61% and 55%, respectively). The overall Angus and crossbred female pregnancy rate was 58% (Table 3.10).

3.4.19. Noncalving Females

At the end of the project a total of 37 artificially inseminated females (Angus and crossbred) were confirmed to be open after the cleanup bulls were removed after a 50-day exposure. For 8 of these females (Angus and crossbred), this was their second year not to produce an offspring. Throughout this study (Experiments I and II), only 2 females did not calve that were confirmed pregnant via ultrasonography at 50 days post-insemination.

Table 3.10. Angus and crossbred total service pregnancy rates across Time Frames 1, 2 and 3 (Exp. I and II)

Time Frame ¹	Pregnant Females ²
1 (n = 77)	43 (55%)
2 (n = 88)	54 (61%)
3 (n = 84)	47 (55%)

¹ Time Frame = (Time Frame 1 = 1960-1975; Time Frame 2 = 1976-1991; Time Frame 3 = 1992-2009).

² Pregnant Females = Number and percentage of females confirmed pregnant by calving.



Figure 3.1. Purebred Angus calf sired from bull Number 1 from Time Frame 1.



Figure 3.2. Purebred Angus calf sired from bull Number 6 from Time Frame 2.



Figure 3.3. Purebred Angus sired from bull Number 21 from Time Frame 3.



Figure 3.4 Crossbred Angus calf sired from bull Number 2 from Time Frame 1.



Figure 3.5 Crossbred Angus calf sired from bull Number 7 from Time Frame 2.



Figure 3.6 Crossbred Angus calf sired from bull Number 17 from Time Frame 3.

3.4.20. Calving Results

Mean gestation lengths (\pm SE) for all calves (Angus and crossbred females) were not different for Time Frames 1, 2 and 3 (280 ± 1 days, 280 ± 1 days and 282 ± 1 days, respectively.) (Table 3.11). The mean birth weights (\pm SE) for all calves (Angus and crossbred females) were not different for Time Frames 1, 2 and 3 (36 ± 2 kg, 36 ± 2 kg and 36 ± 1 kg, respectively) (Appendix A).

3.4.21. Sex of Calves Born

The number of male calves born from all females (Angus and crossbred) born across Time Frames 1, 2 and 3 was 23 (53%), 24 (44%) and 26 (55%), respectively. The number of females calves born from all females (Angus and crossbred) across Time Frames 1, 2 and 3 was 20 (46%), 30 (55%) and 21 (44%), respectively.

3.5. COMPARISON OF GLASS AMPULES AND PLASTIC STRAWS

3.5.1 Sperm Motility

After post-thaw semen evaluation, no difference was detected in total sperm motility between the semen packaged in glass ampules or plastic straws (44% and 53%, respectively) (Appendix B). Furthermore, there were no differences detected in progressive sperm motility between both packaging systems (31% and 40%, respectively) (Figure 3.7).

3.5.2. Normal and Abnormal Sperm

There was a tendency ($P = 0.09$) for a higher percentage (mean \pm SE) of normal sperm packaged in ampules ($82 \pm 3.0\%$) when compared with those packaged in 0.5 mL plastic straws ($72 \pm 3.2\%$) (Table 3.12). There was also a tendency ($P = 0.09$) for a higher percentage

Table 3.11. Angus and crossbred female total calving data across Time Frames 1, 2 and 3 (Exp. I and II)

	Time Frame ¹	BW (kgs) ²	GL (days) ³
Angus	1 (n = 12)	35 ± 5	275 ± 13
	2 (n = 11)	37 ± 7	276 ± 7
	3 (n = 13)	38 ± 4	276 ± 7
Crossbred	1 (n = 31)	37 ± 1	282 ± 1
	2 (n = 43)	36 ± 1	281 ± 1
	3 (n = 34)	36 ± 1	283 ± 1
Combined	1 (n = 43)	36 ± 2	280 ± 1
	2 (n = 54)	36 ± 2	280 ± 1
	3 (n = 47)	36 ± 1	282 ± 1

¹Time Frame = (Time Frame 1 = 1960-1976; Time Frame 2 = 1976-1991; Time Frame 3 = 1992-2009).

²Mean birth weight ± SE.

³Mean gestation length ± SE.

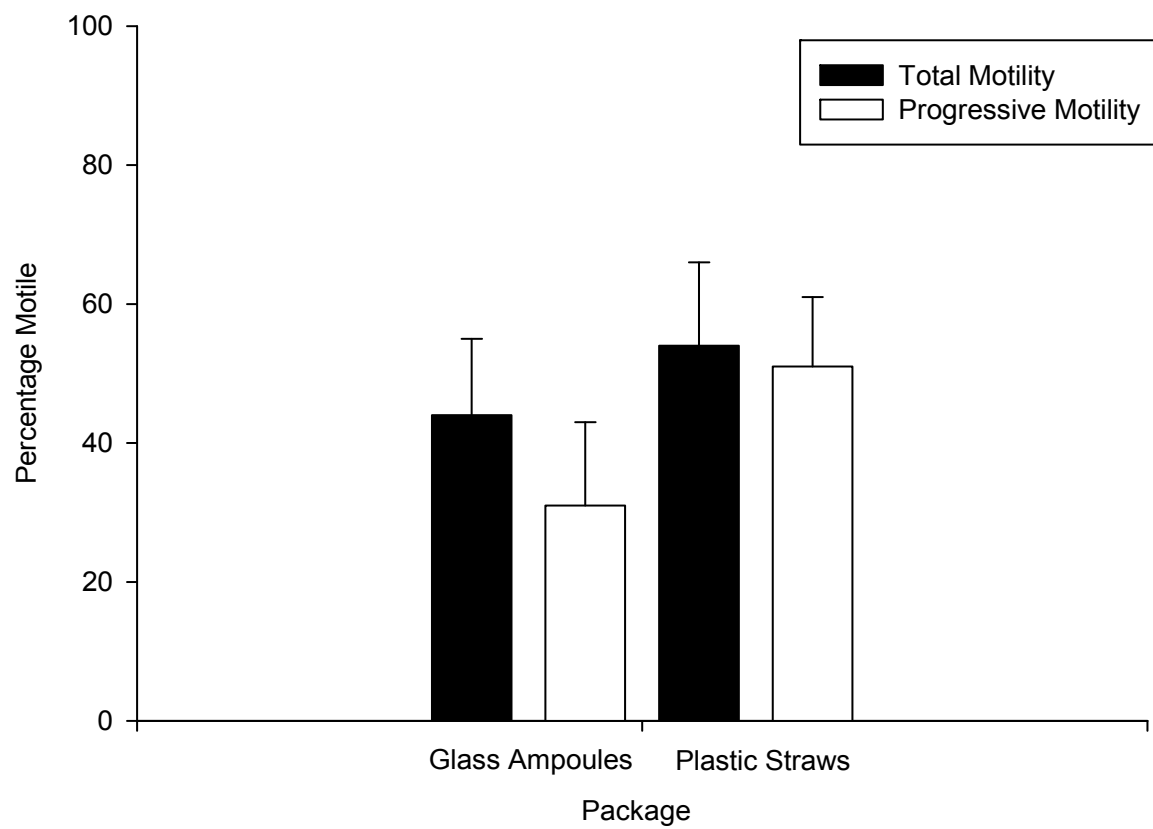


Figure 3.7. Mean \pm SE total and progressive motility of 25 Angus bull semen packaged in glass ampoules or plastic straws.

Table 3.12. Percentage of normal and abnormal sperm between different sperm packaging methods

Packaged	Normal ¹	Abnormal ¹	Primary ¹	Secondary ¹	Tertiary ¹
Glass Ampule	82 ± 3.0	17 ± 3.0	8 ± 2.0	1 ± 0.56 ^a	7 ± 1.73 ^a
Plastic Straw	72 ± 3.2	27 ± 3.2	6 ± 1.5	6 ± 1.28 ^b	14 ± 2.11 ^b

¹Mean percentage ± SE.

^{a,b}Values within columns are significantly different at the P<0.05 level.

(mean \pm SE) of abnormal sperm packaged in 0.5 mL plastic straws ($27 \pm 3.2\%$) when compared with those packaged in glass ampules ($17 \pm 3.0\%$). No difference was detected in the percentage of primary abnormalities for semen packaged in glass ampules ($8 \pm 2.0\%$) or 0.5 mL plastic straws ($7 \pm 1.5\%$). There was a higher ($P < 0.05$) percentage of secondary and tertiary abnormalities for semen packaged in glass ampules ($1 \pm 0.56\%$ and $6 \pm 1.28\%$) and 0.5 mL plastic straws ($7 \pm 1.73\%$ and $14 \pm 2.11\%$).

3.5.3. Pregnancy Rates

No difference in pregnancy rates was detected between Angus bulls packaged in glass ampules or packaged in 0.5 mL plastic straws (Figure 3.8). The pregnancy rate across Time Frames (1, 2 and 3) for Angus bulls packaged in glass ampules was 56%; whereas, bulls packaged in 0.5 mL plastic straws the pregnancy rate was 58%.

3.6. Post-Thaw Aged Semen Parameters

3.6.1. Sperm Motility

In this study, no differences were detected for total post-thaw sperm motility for Angus semen samples ($n = 25$) frozen during Time Frames 1, 2 and 3 (42%, 51% and 55%, respectively) (Appendix C). Additionally, no differences were detected for progressive post-thaw motility for Angus samples frozen in Time Frames 1, 2 and 3 (29%, 38% and 41%, respectively) (Figure 3.9.).

Total and progressive sperm motilities were not different across Time Frames 1, 2 and 3 when analyzed post-thaw by the CASA unit (Figure 3.10) (Appendix D). Total and progressive sperm motility for semen within Time Frame 1 was 35% and 28%, respectively. In Time Frame 2, total and progressive sperm motility was 40% and 30%, respectively. Total and progressive sperm motility within Time Frame 3 was 50% and 38%, respectively.

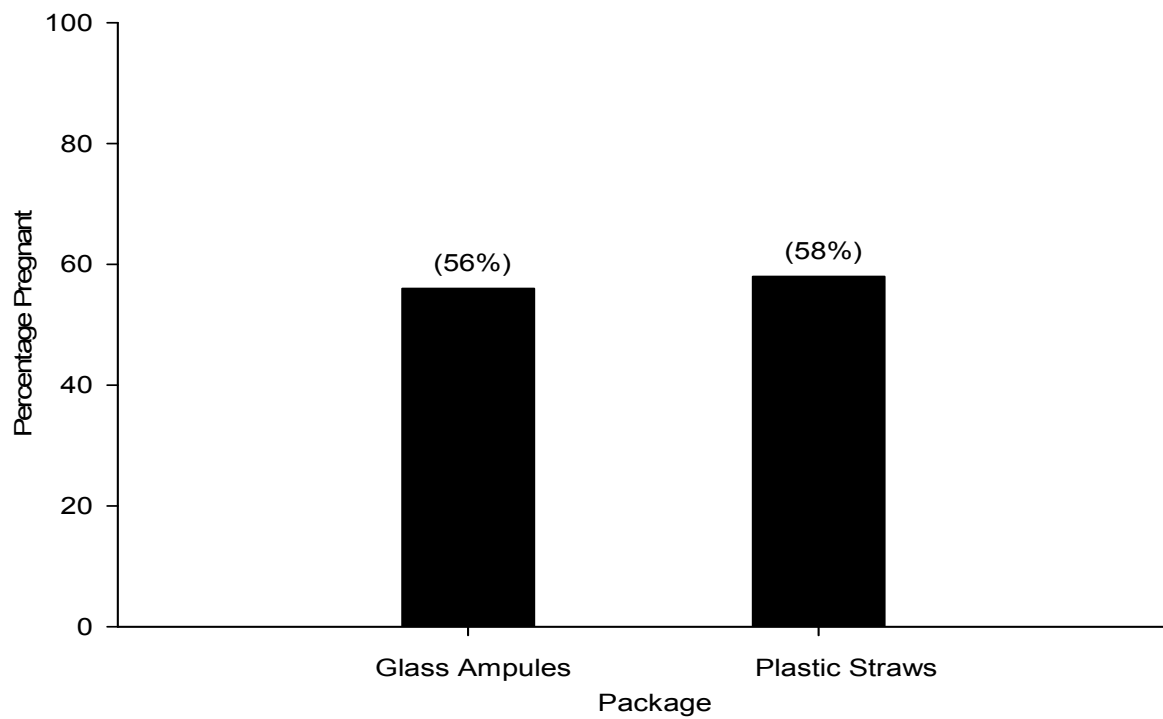


Figure 3.8. Pregnancy rates with semen packaged in either ampules or plastic straws. Angus bulls (n = 25) with total semen units (n = 248).

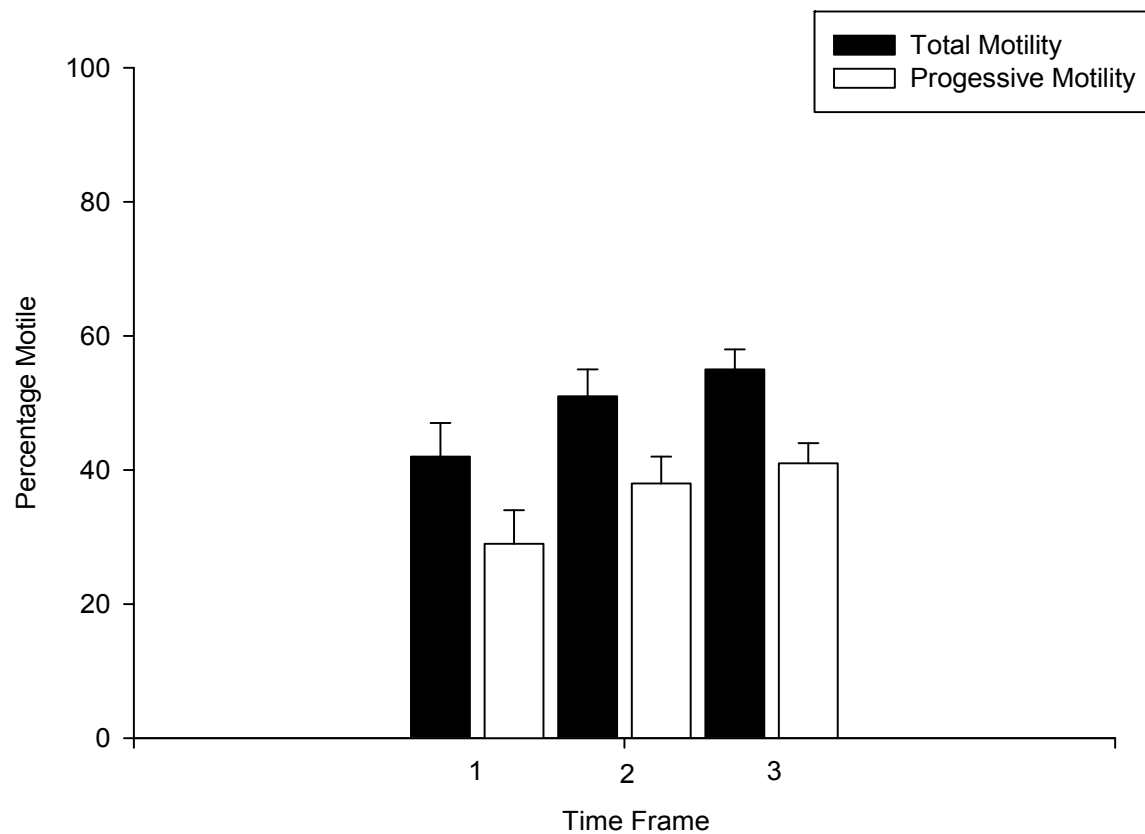


Figure 3.9. Mean \pm SE for total and progressive sperm motility for semen samples within Time Frames 1, 2 and 3 for 25 Angus bulls. Time Frame 1 = 1960-1976, Time Frame 2 = 1976-1991, Time Frame 3 = 1992-2006.

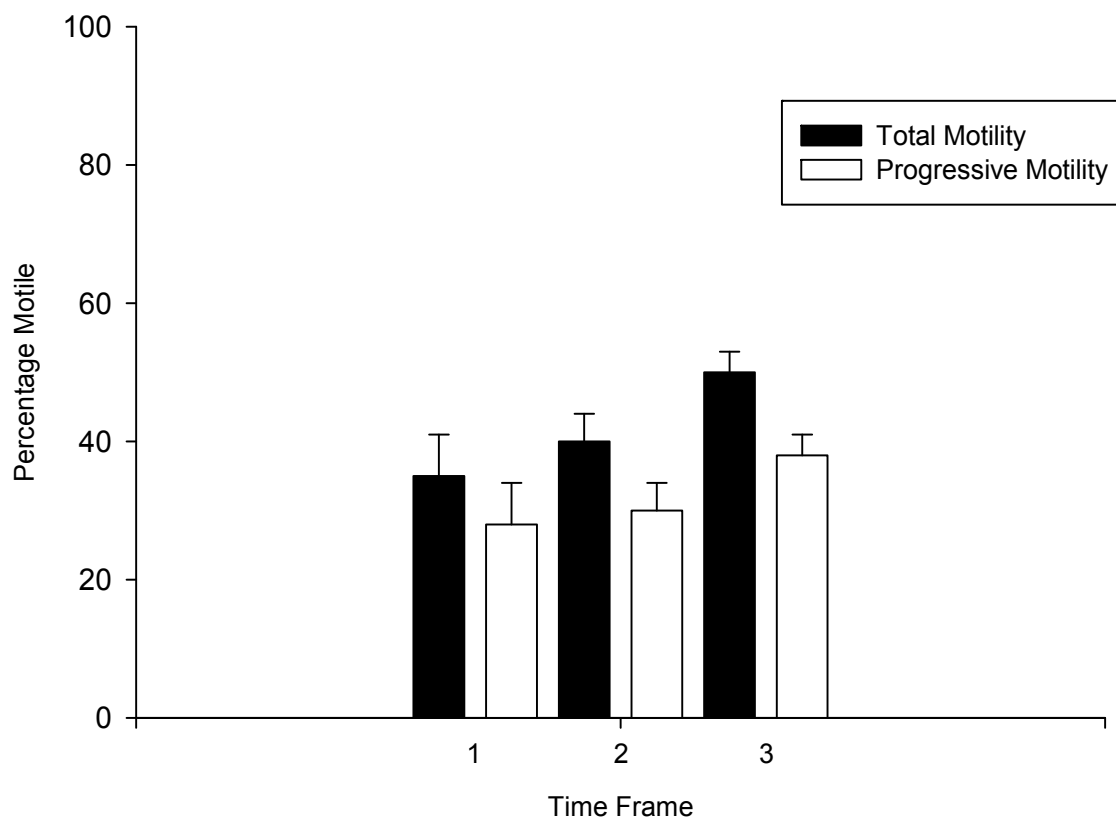


Figure 3.10. Mean \pm SE of total and progressive sperm motility of semen samples across Time Frames 1, 2 and 3 for 25 Angus bulls analyzed by the CASA unit. Time Frame 1 = 1960-1976, Time Frame 2 = 1976-1991, Time Frame 3 = 1992-2006.

Total sperm motility for bulls within Time Frames 1, 2 and 3 were 42%, 51% and 55% using standard laboratory procedure. These values were not different than those reported from the CASA unit for Time Frames 1, 2 and 3 (35%, 40% and 55%, respectively). Progressive sperm motility was also not different between Time Frames 1, 2 and 3 under standard laboratory observation (29%, 38% and 41%, respectively) and the CASA unit (29%, 30% and 38%, respectively). Overall the sperm motility values reported for the standard laboratory procedure were not different when compared with those reported by the CASA unit (Appendix E).

3.6.2. Sperm Concentration

No differences were found between Time Frames 1 and 2 (Figure 3.11.) for Angus bull semen concentration ($53 \times 10^6/\text{mL}$ and $59 \times 10^6/\text{mL}$, respectively). No differences were also detected between Time Frames 1 and 3 ($53 \times 10^6/\text{mL}$ and $37 \times 10^6/\text{mL}$, respectively). However, Time Frame 2 exhibited a higher ($P < 0.05$) semen concentration when compared with Time Frame 3 ($59.5 \times 10^6/\text{mL}$ and $37.1 \times 10^6/\text{mL}$, respectively).

3.6.3. Sperm Abnormalities

No differences were detected in the percentage of normal sperm for Angus bulls frozen in Time Frames 1, 2 and 3 (80.2%, 76.6% and 71.8%, respectively). Furthermore, there were no differences for the percentage of abnormal sperm for bulls frozen in Time Frames 1, 2 and 3 (19.8%, 23.4% and 28.2%, respectively). The percentage (mean \pm SE) of primary, secondary and tertiary abnormalities was not different between Time Frames 1 ($9 \pm 2\%$, $2 \pm 0.8\%$ and $7 \pm 2\%$, respectively), 2 ($7 \pm 2\%$, $5 \pm 1\%$ and $10 \pm 2\%$, respectively) and 3 ($6 \pm 0.9\%$, $6 \pm 2\%$ and $16 \pm 2\%$, respectively) (Table 3.13).

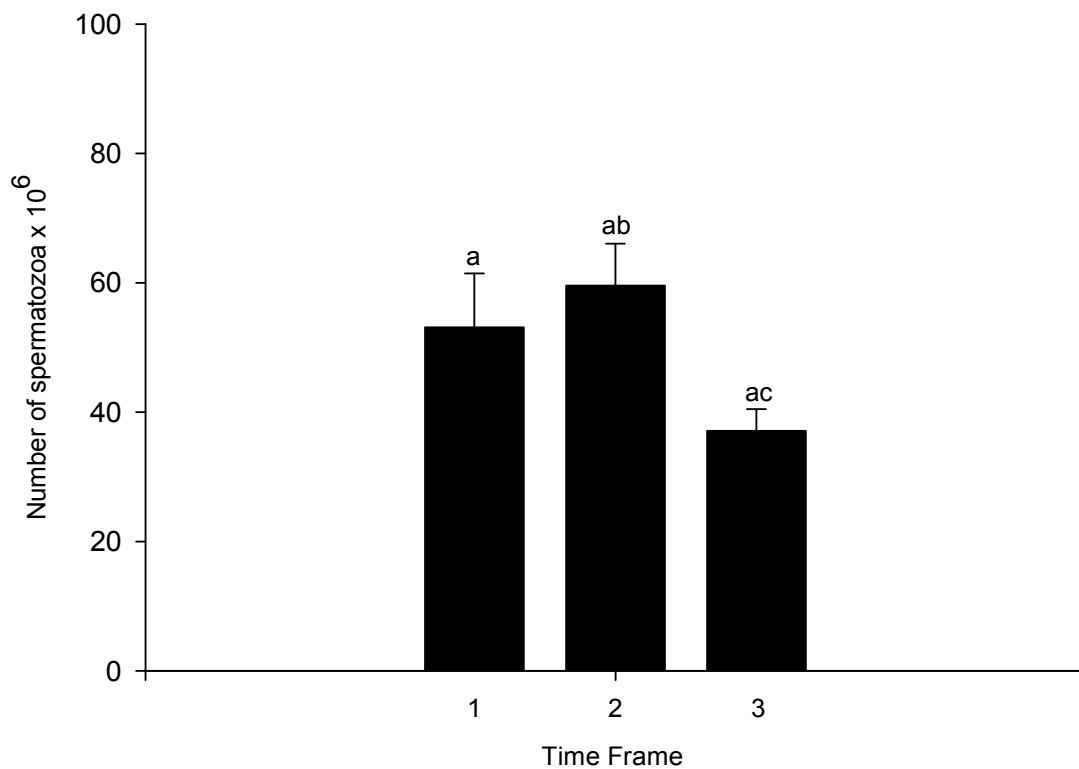


Figure 3.11. Mean \pm SE sperm concentration across Time Frames 1, 2 and 3 for 25 Angus bulls. Time Frame 1 = 1960-1976, Time Frame 2 = 1976-1991, Time Frame 3 = 1992-2006. ^{abc}Values differ at the ($P < 0.05$) level.

Table 3.13. Percentage of normal and abnormal sperm by three Time Frames for 25 Angus bulls

Time Frame ¹	Normal ²	Abnormal ²	Primary ²	Secondary ²	Tertiary ²
1	80 ± 3.6	19 ± 3.6	9 ± 2.5	2 ± 0.8	7 ± 2.4
2	76 ± 5.0	23 ± 5.0	7 ± 2.6	5 ± 1.4	10 ± 2.7
3	71 ± 4.0	28 ± 4.0	6 ± 1.0	6 ± 2.0	16 ± 2.7

¹ Time Frame 1 = 1960-1976; Time Frame 2 = 1976-1991; Time Frame 3 = 1992-2009.

² Mean ± SE.

3.6.4. Sperm Motility of High, Moderate and Low Fertile Bulls

In this study, there were no differences were detected in total motility (54%, 51% and 46%) or progressive motility (41%, 39% and 31%) for bulls categorized has having high (71%, n = 9), moderate (56%, n = 8) or low (45%, n = 8) pregnancy rates (Figure 3.12) (Appendix F).

3.7. Discussion

The use of cryopreserved semen generally results in lower fertility rates than that of natural breeding. Cyroinjury from the freezing process has been proposed to be the main limiting factor associated with decreased fertility rates when using frozen semen (see review by Holt, 2000). Other factors can be attributed to the reduced fertility of cryopreserved semen including: proportion of live sperm, cryoprotectant stress, decreased motility and receptor damage (see review by Watson, 2000).

In Experiment 1, no differences in pregnancy rates were detected between Time Frames 1, 2 and 3 for Angus cows (53%, 69%, and 75%, respectively) or for Angus heifers (80%, 40% and 20%, respectively). In addition, no differences were found in pregnancy rates across Time Frames 1, 2 and 3 when both Angus cows and Angus heifers were combined (60%, 61%, and 61%, respectively). In Experiment 2, no differences were detected between Time Frames 1, 2 and 3 for crossbred females (54%, 62% and 53%, respectively).

For Experiments 1 and 2, the pregnancy rates for Time Frames 1, 2 and 3 were 55%, 62% and 55%, respectively. In this study, semen that was allotted to Time Frame 1 was processed and frozen between the years 1960 to 1975. Pregnancy rates in our study were lower than those (69.5%) reported by Amadal and Anderson (1968), for frozen dairy bull semen packaged in glass ampules. Previously, Macpherson and King (1966) reported a 67%

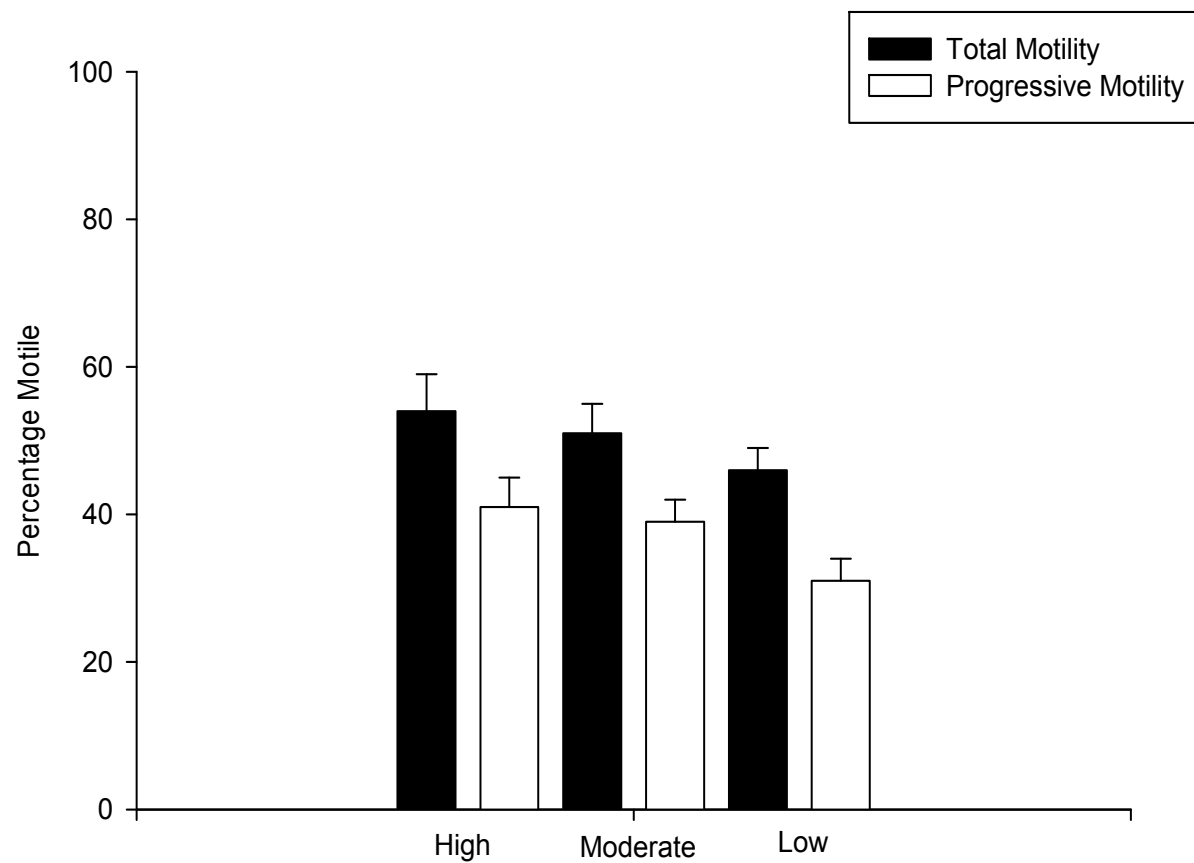


Figure 3.12. Mean \pm SE total and sperm progressive motility for bulls with high, moderate or low fertility pregnancy rates from artificial insemination.

non-return rate for frozen bull semen packaged in ampules utilizing 1,909 first AI services in Holstein females. Subsequently, Macpherson (1974) reported a 71.4% and 71.9% pregnancy rate with frozen bull semen using 0.5 mL and 0.25mL plastic straws and used over 560 and 705 inseminations, respectively.

The difference in pregnancy rates between frozen bull semen from this study compared with those of other studies in the literature from this Time Frame is unclear. In this study, a fewer number of beef females were inseminated than those reported in previous studies (Macpherson and King, 1966; Macpherson, 1974). Environmental factors such as increase heat and/or humidity could have played a role in the reduced pregnancy rates seen in this study (Stott 1961; Moberg, 1976). The temperature ranged from 23 to 37°C during this study for cows inseminated at the second insemination. Furthermore, some females used in this breeding trial in both herds (Angus and crossbred) did not calve in the previous year. This factor (yearly noncalving) could have also been a reason for a decrease in pregnancy rates for this study.

Frozen Semen in this study that was allotted to Time Frame 2 was processed and frozen between the years 1976 and 1991. Higher pregnancy rates in the present study were noted than those by Mortimer et al. (1976) who compared pregnancy rates between the 1.0 mL glass ampule (50%) and the 0.25 mL plastic straw (54%).

The third Time Frame of this study contained semen that had been processed and frozen from the years 1992-2009. Johnson et al. (1995) reported a higher pregnancy (62%) when compared with our study, when 681 Holstein dairy heifers were inseminated with 0.5 mL plastic straws. However, our results had comparable non-return rates to those (55%) reported by Haugan et al. (2007) whom inseminated 536,481 dairy cows.

Pregnancy rates in this study were higher than those reported by Nishikawa et al. (1976) for frozen bull semen stored for periods of 10 years and 12 years (50% and 45%) and higher than those (45%) reported by Mixner (1968) for bull semen frozen and stored frozen for 12 years. These comparisons, however, cannot be made entirely, due to the fact that the age of the semen in certain Time Frames (Time Frames 1 and 2) used in our study was stored for a greater period of time. In this study semen from Time Frame 1 was stored for a duration period of 35 to 50 years. Semen from Time Frame 2 was frozen and stored in liquid nitrogen for a duration period of 19 to 34 years.

Leibo et al. (1994) achieved a comparable blastocyst rates when oocytes were inseminated in vitro with 37 years old frozen bull semen when compared with bull semen frozen for only 2 years (22.8% and 20.5%). Stroble et al. (2002) reported successful morula (22%) and blastocyst (16%) development from pig oocytes inseminated with frozen boar semen stored for 19 years. While in this study beef cows were utilized as experimental units instead of cow or pig oocytes, these results agree that aged frozen stored semen can produce similar pregnancy rates to that of shorter term stored frozen semen.

Salamon, (1972) reported a reduced lambing rate (52.9%) with frozen ram semen that had been frozen stored in a dry ice container for 3 years when compared with fresh diluted ram semen (76.5%). Subsequently, Salamon and Visser (1973) reported a decrease in lambing rates when ewes were artificially inseminated with either frozen ram semen stored for 5 years (52.9%) when compared with frozen ram semen stored for 2 weeks (54.4%). In contrast, Salamon (1980) reported an increase in the percentage of ewes lambing inseminated with frozen ram semen stored for 11 years. In 2 trials utilizing frozen ram semen stored for 11 years, a total of 159 and 160 ewes were inseminated, resulting in lambing rates of 55.3% and 65.6%,

respectively. In three additional trials, ewes were inseminated with frozen ram semen stored for 3, 5 and 7 years resulting in lambing rates of 52.9%, 52.9% and 51.7%, respectively.

In dairy cattle, a reduce calving rate (54.1%) was found when cows were inseminated with frozen bull semen that had storage duration of 6.5 years when compared with frozen bull semen that had been frozen stored for only 3.5 years (55.3%), 4 years (55.7%), 4.5 years (55.8%), 4.9 years (55.8%) and 5.3 years (55.1%) (Haugan et al., 2007). In contrast, in our study, we found no significant decrease in calving rate with frozen Angus semen stored from 4 to 50 years. This be may be attributed to that in the previous study mentioned (Haugan et al., 2007) over 470,000 inseminations were performed and in this study on 248 inseminations were performed. Thus, with more inseminations a higher power is achieved and statistical differences are easier to detect.

In some of the studies reported previously, higher overall AI pregnancy rates were reported (Macpherson and King, 1966; Amadal and Anderson, 1968; Macpherson et al., 1975; Johnson et al., 1995) when compared with overall AI pregnancy rates in the present study. At the end of our study, a total of 37 females remained open after the cleanup bulls were removed after 50 days of exposure. These females, with unknown reproductive history, if removed from the data set would have improved the pregnancy rates in the present study.

In our study, no differences were found in the mean gestation lengths of calves (Nishikawa et al., 1976) between the three different Time Frames (Time Frame 1 = 280 ± 9 days; Time Frame 2 = 280 ± 9 days; 282 ± 10 days). A total of 144 calves were born in this study, although some calves ($n = 8$) were stillborn, no abnormalities were detected to suggest that the frozen aged bull semen might have been involved in premature death. The total number of calves born in this study was Horne et al. (2004) and Feldschuh et al. (2005) reported normal healthy human babies ($n = 3$) were born after frozen semen that was stored for

21 years and 28 years, respectively. These reports support our findings that duration of semen storage does not cause abnormal births. Healthy, normal human babies ($n = 6$) have also been produced from embryos stored frozen for 7.5 years (Ben-Ozer and Vermesh, 1999), 8 years (Go et al., 1998), 8.9 years (Quintans et al., 2002), 12 years (Revel et al., 2004) and 13 years (Teijón et al., 2006).

In our study, only a few cows received a second service. This may have been due to a decrease estrus detection after AI or possibly lower fertile females. One hypothesis is that when the cows came to ~21 days post-AI, they likely did not exhibit estrus or had a silent ovulation (see review by De Rensis and Scaramuzzi, 2003). The cattle used in this experiment were from a production herd, therefore, the bull was placed with the females shortly after one estrous cycle after artificial insemination.

The average birth weight for Angus calves born in Time Frame 1 was 36 kg and is higher (30 kg) than that was reported by the American Angus Association (2010b) for average birth weight of calves born during the 1970s. Birth weights were similar from calves born in Time Frame 2 (36.5 kg) to those (34 kg for heifers and 36 kg for steers) reported by the American Angus Association (2010b) for calves born in the 1980s and early 1990s. In the present study, higher, but comparable birth weights were also recorded in calves born in Time Frame 3 (37 kg) than those (35 kg) reported by the American Angus Association (2010b) for the average birth weight of calves born during the 2000s.

No clear explanation can be made on why higher birth weights were recorded in our study in Time Frames 1, 2 and 3 than those previously reported for Angus calves. Within time periods, the cattle market has demanded for different phenotypes of Angus cattle. In the 1960s and 1970s, the Angus cattle were smaller and more compact (Nadarajah et al., 1987; Enns and Nicoll, 2008). As time progressed, birth weight, frame and overall size of the Angus cattle have

increased (Enns and Nicoll, 2008; American Angus Association 2010b). Another possibility is that the birth weights reported by the American Angus Association (2010b) contain only genetics from that time period, while in the present study, only the bull represents that time period and not the females.

In this study, no differences were detected between pregnancy rates between semen packaged in glass ampules (56%) or plastic straws (58%). These pregnancy rates are comparable to that reported by Macpherson and King (1966), Bean (1972), Alverson (1972), and Pickett and Berndston (1974) for frozen bull semen packaged in 0.5 mL and 1.0 mL glass ampules and 0.5 mL plastic straws. Bean (1972) reported no differences in pregnancy rates in over 11,000 inseminations when dairy cows were inseminated with semen packaged in glass ampules or plastic straws. Aamdal and Anderson (1968) reported similar pregnancy rates with 457 inseminations in dairy cows inseminated with plastic straws (71.1%) and glass ampules (69.5%).

In contrast, Mortimer et al. (1976) reported in Trial 1 of his study, similar pregnancy rates in beef cows inseminated with frozen bull semen packaged in either 0.5 mL glass ampules (50%) or 0.25 mL plastic straws (70%). These pregnancy rates were reported to not be significantly different. Therefore, an additional trial was conducted. In Trial 2, a 83% pregnancy rate was reported for beef cows inseminated with frozen bull semen packaged 0.25 mL plastic straws and a 67% pregnancy rate for beef cows inseminated with bull semen frozen in 1.0 mL glass ampules. In this trial a significant difference was detected in pregnancy rates for semen packaged in plastic straws and glass ampules.

In this study, no difference was detected in total and progressive sperm motility for frozen bull semen packaged in 0.5 mL and 1.0 mL glass ampules (44% and 31%, respectively) and 0.5 mL plastic straws (53% and 40%, respectively).

Coulter and Foote (1977) reported a mean post-thaw sperm motility of 38.8% for frozen bull semen packaged in 0.5 mL plastic straws, which was comparable with sperm packaged in 1.0 mL glass ampules (39.3%). Alverson (1972) reported mean post-thaw bull sperm motilities of 45.1%, 38.5%, 41.6%, 41.6%, 50.0% and 42.7% for 0.5 mL plastic straws that were frozen 2, 6, 8, 10, 12, and 14 inches above the liquid nitrogen level. A lower post-thaw sperm motility (38.9%) was reported for the 1.0 mL glass ampule, which served as the control.

In contrast, Berndston and Foote (1972) reported higher post-thaw sperm motility for frozen bull semen packaged in pellet form (34%) and 1.0 mL glass ampules (33%) when compared with bull semen frozen in 0.5 mL plastic straws (30%).

A variety of new packaging techniques have been introduced into the AI industry. New packaging techniques are aimed at finding new packaging systems that could provide a more efficient storage options when compared with glass ampules or plastic straws and still maintain comparable pregnancy rates. However, through the year of evaluation, most systems have failed to produced pregnancy rates that were comparable with that produced by the standard packaging systems (Graham and Erickson, 1959).

In this study, the percentage of secondary and tertiary abnormalities for sperm packaged in both 0.5 mL and 1.0 mL glass ampules ($1 \pm 0.56\%$ and $6 \pm 1.28\%$) was lower when compared with 0.5 mL plastic straws ($7 \pm 1.73\%$ and $14 \pm 2.11\%$). However, no differences were detected in pregnancy rates between both bull semen packaging systems. It has been reported that abnormal sperm morphology are susceptible to barriers throughout the females reproductive tract, which can prohibit them from reaching the site of fertilization (Saacke, 1990).

The difference in abnormalities (secondary and tertiary) found in this study suggest that variability in bulls. Research suggests that secondary abnormalities occur during the sperm

maturation process (Barth and Oko, 1989a). The difference in tertiary abnormalities could be contributed to the initial processing of the sperm or improper semen handling procedures.

Martin (1990) reported that only total ($r = -0.67$) and primary abnormalities ($r = -0.74$) were significantly correlated with a decrease in pregnancy rates. In contrast, Saacke (1972) reported a significant correlation between pregnancy rates and percentage primary ($r = -0.34$), secondary ($r = -0.37$), and total abnormalities ($r = -0.27$) in bulls. Woods et al. (1986) reported significant correlations between pregnancy rates and sperm abnormalities in bulls. Sperm morphology was determined on both first and second ejaculates across 18 bulls. Significant correlations were detected in first and second ejaculates between bulls. However, only coiled tails (tertiary abnormality) and proximal protoplasmic droplets (secondary abnormality) were detected to have an effect on pregnancy rates in cows (Wood et al., 1986).

Data from this study suggests that glass ampules are just as an efficient packaging system as plastic straws. Though in this study no differences were detected between the different packaging systems, a less number of inseminations were performed in this study when compared to those previously reported in the literature.

Total and progressive motility for samples within Time Frames 1, 2 and 3 were 42%, 51%, 55% and 29%, 38% and 41%, respectively. Our Time Frame 1 motility values are comparable with that reported by Bratton et al. (1957), who reported a 41% post-thaw motility for frozen-thawed bull semen stored for 1 week. In the same study, these values dropped (38%) for the same frozen bull semen stored for 17 weeks. Macpherson (1956) reported a mean motility of 47% on 5 bulls whose semen was frozen and stored for 12 months. However, when the same semen was evaluated again at 24 months, the mean motility declined to 32%.

Our values reported are also comparable to that reported by Nishikawa et al. (1976) who reported a 41.9%, 42.9%, 41.3%, 48.7%, 46.9%, 41.7% and 42.7% motility for frozen-thawed bull semen stored for 13, 12, 11, 10, 9, 7 to 8, and 4 to 6 years. Stroble et al. (2002) reported a 30% motility rate on 47 boars whose semen that had been frozen and stored for 19 years.

Since the first successful artificial insemination was performed in the human from frozen semen (Bunge and Sherman, 1953), semen banking has become an important factor in subsequent couples with infertility. This process allows for couples to still have the potential to have children even if the male partner is unable to produce viable sperm.

Freund and Wiederman (1966) reported a mean motility of 53%, 34% and 37% for frozen human semen stored for 8 to 9, 14 to 15 and 23 to 24 months, respectively. In another study, post-thaw motility for frozen stored human semen was: 50%, 20%, 18.6%, 12.5% across four different specimens stored from 6 to 7 years. Another analysis of different semen samples was performed at 8 to 9 years of storage resulting in mean motilities of 20%, 50%, 40% and 50% (Smith and Steinberger, 1973). Overall, higher mean motilities were found in the frozen human semen stored for a longer period of time. This variability was contributed to those semen samples having higher mean pre-freeze motility.

Only one study (Horne et al., 2004) has described the long term effect of storage of human post-thaw sperm motility of frozen semen. Horne et al. (2004) reported a mean motility of 33% across five ejaculates of frozen human semen that had been stored for 21 years.

In comparison with other studies (Macpherson, 1956; Bratton et al., 1957; Freund and Wiederman, 1966; Smith and Steinberger, 1973; Nishikawa et al., 1976; Stroble et al., 2002; Horne et al., 2004; Feldschuh et al., 2005), frozen semen in our study that was stored for a longer period of time (Time Frame 1 and Time Frame 2) was noted to have a lower post-thaw

total (42% and 51%, respectively) and progressive motility (51% and 38%, respectively). These decreasing values cannot be entirely contributed to the duration time the semen was stored. One possibility why semen motility did not decrease during storage is the quality of semen pre-freeze. We propose that if higher quality semen was processed and handled correctly prior to freezing, then these samples should maintain their viability, regardless of frozen storage time.

In this study we were only given minimal information about the initial semen analysis prior to freezing. The information provided included: bull registration number, initial semen processor and origin of semen. Depending on the bull, values could have already been low or at the minimum level for freezing. Another factor that could be considered is the variability between bulls (Al-Makhzoomi et al., 2008; Parkinson, 2004; Zhang et al., 1998). Some bulls, just like all males, can undergo the freezing and thawing process and retain a higher post-thaw motility than others (Fowler et al., 1961; Foote, 1970, 1975; Smith and Steinberger, 1973; Nishikawa et al., 1976; Harrison and Sheppard, 1980; Stroble et al., 2002).

Visual appraisal has and will likely remain the standard for frozen semen post-thaw evaluation. However, with the ever changing technology, computer systems can provide aid to assist in processing samples at a quicker pace. Total and progressive sperm motility for bulls within Time Frames 1,2 and 3 were not different when analyzed by either the standard laboratory procedure (42% and 29%, 51% and 38%, 55% and 41%, respectively) when compared with values reported by the CASA unit (35% and 28%, 40% and 30%, 50% and 38%, respectively). Our results indicate that a trained technician can correctly assess semen quality that is comparable to that made by a CASA unit, thus provides a more economical means of assessing sperm motility for laboratories.

The concentration of semen for Time Frames 1, 2 and 3 were $53.1 \times 10^6/\text{mL}$, $59.9 \times 10^6/\text{mL}$ and $37.1 \times 10^6/\text{mL}$. As reported in Chapter III, no differences were detected in

pregnancy rates across the three Time Frames (55%, 61% and 55%, respectively). Higher sperm concentrations were associated with bull semen within Time Frame 2 ($59.9 \times 10^6/\text{mL}$) when compared to Time Frame 3 ($37.1 \times 10^6/\text{mL}$). The overall AI pregnancy rates in the present study were 62% for Time Frame 2 compared with semen from Time Frame 1 with a 55% pregnancy rate and Time Frame 3 with a 55% pregnancy rate. The higher sperm concentration reported for Time Frame 2 could suggest why higher pregnancy rates were achieved. This statement, however, cannot be made entirely based on this suggestion alone due to Time Frame 2 sperm concentration not being significantly different from Time Frame 1 whose pregnancy rates were equal with that represented from Time Frame 3.

Over the years, through several cow breeding trials (Bratton et al., 1954; Pace et al., 1981; Schenk et al., 1987) bull sperm concentrations have been reduced for use in AI. Dairy bull sperm concentrations as low as $4 \times 10^6/\text{mL}$ have been reported to produce pregnancy rates comparable with sperm concentrations of $14.3 \times 10^6/\text{mL}$ and $9.5 \times 10^6/\text{mL}$ (Bratton et al., 1954). In contrast, one study reported when dairy bull sperm concentrations were reduced to $2 \times 10^6/\text{mL}$, a significantly lower pregnancy rate occurred (Andersson et al., 2004).

Foote and Kaproth (1997) reported a series of experiments artificially inseminated dairy cows with various semen concentrations. In the first experiment, no differences in pregnancy rates were detected when cows were inseminated with $20 \times 10^6/\text{mL}$ (69%), $25 \times 10^6/\text{mL}$ (70%), $30 \times 10^6/\text{mL}$ (70%) or $40 \times 10^6/\text{mL}$ (70%). In the second experiment, no difference in pregnancy rates was detected when dairy cows were inseminated with semen concentrations of $12 \times 10^6/\text{mL}$ (70%), $16 \times 10^6/\text{mL}$ (72%) and $20 \times 10^6/\text{mL}$ (70%). Almquist (1975) reported a significantly higher pregnancy rate with over 4,600 inseminations in dairy females when pre-freeze semen concentrations were $20 \times 10^6/\text{mL}$ compared with 10 and $15 \times 10^6/\text{mL}$. In

contrast, Hafs et al. (1970) reported no difference in pregnancy rates when dairy cows were inseminated with 12, 24 or 35 X 10⁶/mL.

Semen quality is one of the main factors affecting AI pregnancy rates in cattle. Good quality semen must be used in order to achieve acceptable pregnancy rates. Lower quality semen can result in early embryonic failure or no fertilization at all (Saacke, 1982). A variety of tests are generally performed on semen post-thaw in an effort to predict subsequent fertility rates (Buckner et al., 1954; Bratton et al., 1956; Saacke and White, 1972; Coulter, 1992; den Daas, 1992; Mocé and Graham, 2008)

When comparing motilities (total and progressive) between bulls with high (54% and 41%, respectively), middle (51% and 39%, respectively) and low (46% and 31%, respectively) pregnancy rates utilized in this study, no significant difference was detected. In contrast, some reports suggest that bulls with higher post-thaw concentration of motile sperm could have improved pregnancy rates (Bean et al., 1963; Centola et al., 1992; Zhang et al., 1998). One study also reported that dairy bull semen motility incubated for 10 hours at 37°C was significantly positively correlated with pregnancy rates (Saacke and White, 1972) .

Correa et al. (1997) reported a higher post-thaw motility when comparing Holstein bulls with high (72%) and low (67%) fertility levels. Within these same bulls, high fertility bulls also had a higher percentage of intact acrosomes (83%) and normal sperm (82%) post-thaw when compared with bulls with low fertility (78% and 75%, respectively). A significant correlation was found between post-thaw motility for both bulls with high fertility ($r = 0.61$) and low fertility ($r = 0.39$) (Correa et al., 1997). In comparison, Mocé and Graham (2008) reported a correlation values between total ($r^2 = 0.36$) and progressive ($r^2 = 0.35$) sperm motility in Holstein bulls and subsequent AI pregnancy rates. However, in this particular study (Mocé and Graham, 2008) when more sperm viability variables were added into the statistical model, a higher correlation

occurred ($r^2 = 0.62$), suggesting that other sperm factors in combination with sperm motility could provide a higher predictability in post-thaw pregnancy rates.

In conclusion, in this study, pregnancy rates from aged frozen Angus bull semen from Time Frames 1 and 2 (55% and 61%) were not different than frozen Angus bull semen from Time Frame 3 (55%). This study suggests that the duration of storage time does not appear to lower pregnancy rates in beef females. Furthermore, the duration of storage time from initial semen processing and freezing had no effect on post-thaw evaluation of Angus bull semen.

CHAPTER IV

EFFECT OF ANIMAL TEMPERAMENT ON PREGNANCY RATES

4.1. Introduction

Animal temperament has been reported in detail and its affect on beef cattle carcass characteristics (Vann, 2006; Nkrumah et al., 2007; Behrends et al., 2009). Two studies have reported that Brahman cattle with excitable temperament have higher plasma cortisol levels when compared with cattle assessed as having adequate temperament (Curley et al., 2006; Cooke et al., 2009a;).

Some reports have indicated that temperament can be associated with breed type. Humes et al. (1987) reported that Angus cattle had a more adequate temperament during the handling process when compared with Chianina breed females. Cooke et al. (2010) reported that females of Brahman influence had a higher temperament during handling procedures when compared with Angus and Simmental x Angus females.

The effect on animal temperament on cattle reproductive efficiency has not been reported in detail. Cooke et al. (2010) reported that Angus x Herford cattle with excitable temperament result in lower pregnancy rates at the end of the breeding season when the bulls were removed. In comparison, Cooke (2010) reported that beef females with a higher serum cortisol level were less likely to become pregnant at the end of the breeding season when compared with females who had a lower serum cortisol level. However, no controlled studies have been reported on the effect of animal temperament and its affect on subsequent AI pregnancy rates.

Therefore, the objective of this experiment was to evaluate cattle temperament during estrous synchronization for its effect on subsequent AI pregnancy rates.

4.2. Materials and Methods

4.2.1. Experimental Units

Angus and crossbred females (n = 212) utilized in this experiment were the same females from the previous experiments (Chapter III).

4.2.2. Estrous Synchronization, Detecting Estrus, Artificial Insemination and Pregnancy Determination

The estrous synchronization protocol, method for detecting estrus and artificial insemination procedure used in this experiment was the same as described in the previous experiments (Chapter III). Pregnancy determination was made as described in the previous experiments (Chapter III).

4.2.3. Temperament Scores (Experiments I and II)

All females were handled similar during estrous synchronization and artificial insemination. During both day 0 and day 7 of the estrous synchronization procedure, while restrained, each female was assigned a chute behavior score by the same technician. The chute behavior scores were categorized as follows: 1 = calm, no or little movement, 2 = moderate, slightly restless movement or 3 = excited, flighty into chute, extremely restless.

Exit velocity was measured on each females as they exited the working chute. To obtain exit velocity, an electronic timing system (Farmtek, Wylie, TX) equipped with a Polaris Multi-Event timer was utilized. This system contains a set of two laser detectors, one for starting the timer (Set 1) and one for stopping the timer (Set 2). Set 1 was placed 0.91 meters from the exit of the working chute. This was to prevent any female that may jump from the chute and result in a false reading. Set 2 was placed 1.86 meters from Set 1. Exit velocity was measured as the

amount of time it took for the females to transverse 1.86 meters. These times were then converted to meters/second for data analysis. Any female that diverted from timer or had incomplete temperament assessment was excluded from the data set. Others have used this system beef cattle to assess temperament (Curley et al., 2006, Curley, 2010).

4.3. Statistical Analysis

Differences in pregnancy rates based on animal temperament were assessed using the Logistic Regression procedure. A Pearson Correlation was utilized for all correlations between exit velocity and chute scores. Statistics were analyzed using SigmaStat statistical software No. 3.5. Significance was set at the $P < 0.05$ level for pregnancy rates and temperament scores.

4.4. Results

4.3.1. Comparison of Chute Scores and Exit Velocity

A breed effect was detected for mean chute scores and mean velocity exit scores (Table 4.1). A higher exit velocity score indicates that the female exited faster as she was released from the squeeze chute. Brangus F_1 females had a significantly ($P < 0.05$) higher mean chute score (2.0 ± 0.13) when compared with Romosinuano (1.2 ± 0.08), Braford (1.5 ± 0.13), Brangus (1.5 ± 0.12), Angus (1.3 ± 0.06), Beefmaster (1.6 ± 0.11) and Bons Mara (1.6 ± 0.11) females. In comparison, Brangus F_1 females had a significantly higher ($P < 0.05$) mean exit velocity (3.04 ± 0.22) when compared with Romosinuano (2.3 ± 0.20), Braford (2.1 ± 0.25), Bons Mara (2.2 ± 0.18) and Angus (2.0 ± 0.12) females. However, no differences were detected in the mean exit velocity between Brangus F_1 (3.0 ± 0.22) when compared with Brangus (2.4 ± 0.17) and Beefmaster (2.5 ± 0.13) females (Appendix G).

No significant differences were detected in the mean subjective behavior chute scores for all pregnant (1.5 ± 0.6) and all nonpregnant females (1.5 ± 0.62) (Figure 4.1). A higher

Table 4.1. Temperament characteristics of artificially inseminated crossbred and Angus beef females

Breed Type	Chute Score ¹	Exit Velocity ¹
Beefmaster	1.6 ± 0.11 ^a	2.5 ± 0.13 ^{ab}
Bons Mara	1.6 ± 0.11 ^a	2.2 ± 0.18 ^a
Brangus	1.5 ± 0.12 ^a	2.4 ± 0.17 ^{ab}
Romosinuano	1.2 ± 0.08 ^a	2.3 ± 0.20 ^a
Brangus F ₁	2.0 ± 0.13 ^b	3.0 ± 0.22 ^b
Braford	1.5 ± 0.13 ^a	2.1 ± 0.25 ^a
Angus	1.3 ± 0.06 ^a	2.0 ± 0.12 ^a

¹Mean ± SE.

^{ab}Values within columns are significantly different (P<0.05).

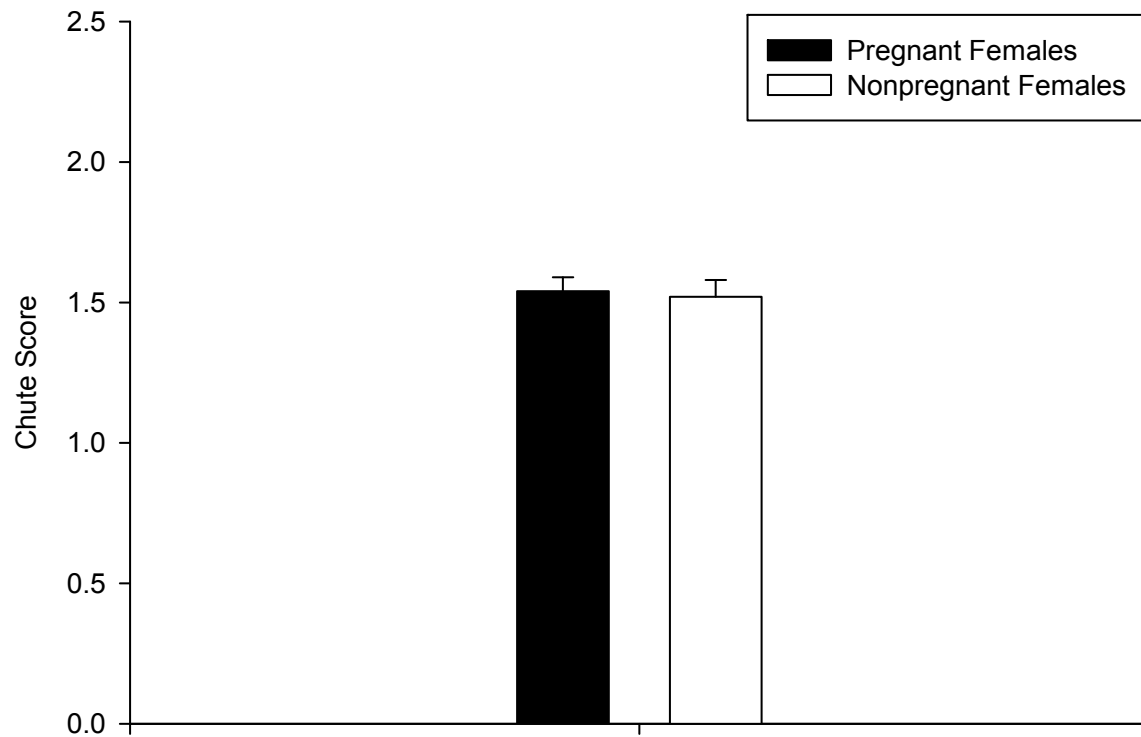


Figure 3.9. Mean \pm SE subjective behavior chute scores for first service artificially inseminated pregnant and nonpregnant beef females (n =212).

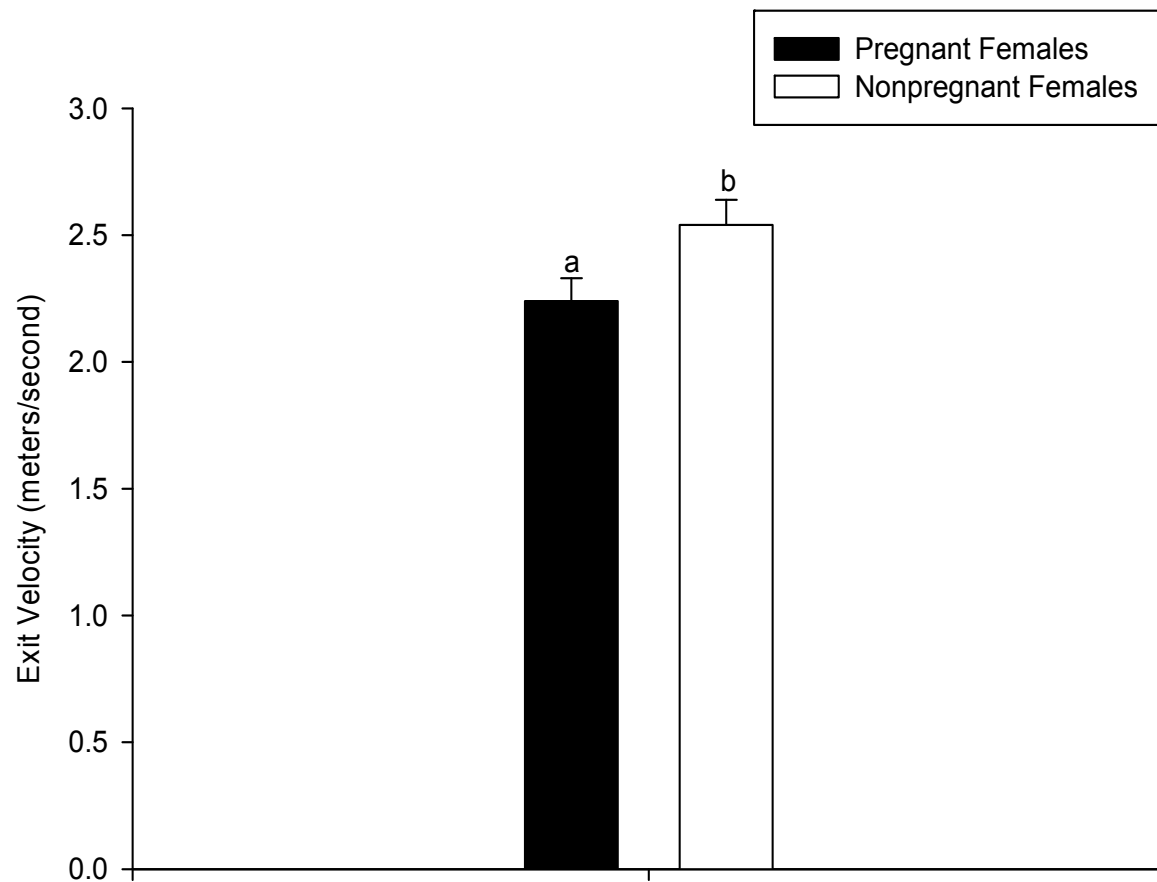


Figure 3.10. Mean \pm SE chute exit velocity for first service artificial insemination pregnant and nonpregnant beef females. ^{ab}Values are significantly different at the $P < 0.05$ level ($n = 212$).

($P < 0.05$) mean exit velocity was detected for inseminated nonpregnant females (2.54 ± 0.06) when compared with inseminated pregnant females (2.24 ± 0.06) (Figure 4.2.).

4.3.2. Temperament Correlations

A significant positive correlation was detected for females ($r = 0.52$, $P < 0.05$) for day 0 (1.5 ± 0.04) and day 7 (1.5 ± 0.05) subjective behavior chute scores. Furthermore, a significant positive correlation ($r = 0.58$, $P < 0.05$) was detected for females between both day 0 chute exit velocity (2.4 ± 0.07) and day 7 chute exit velocity (2.5 ± 0.08). A significant positive correlation ($r = 0.28$, $P < 0.05$) was detected in chute scores (1.5 ± 0.6) and chute exit velocity (2.3 ± 1.0) across all females (Figure 4.3).

4.5. Discussion

Human-animal interaction is required in almost any animal production system. It is imperative that a safe environment be presented for both the animal and handler. Recent studies have also reported that beef cattle with excitable temperament do not perform as well as cattle with a calmer temperament in the feedlot (Nkrumah et al., 2007; Behrends et al., 2009).

The positive correlations found between chute score and exit velocity indicate that as animal temperament chute scores increases, the chute exit velocity (meter/second) traveled increases. Positive correlations between these two variables have been previously reported in beef cattle (Curley et al., 2006; Vann, 2006). Research has reported that cattle with excitable temperament can result in higher plasma cortisol level (Curley et al., 2006; Vann, 2006; Cooke et al., 2009a; Cooke et al., 2010) decreasing subsequent pregnancy rates (Cooke, 2010). One study reported that by acclimating cattle to humans could lower animal temperament assessments (Cooke et al., 2009b).

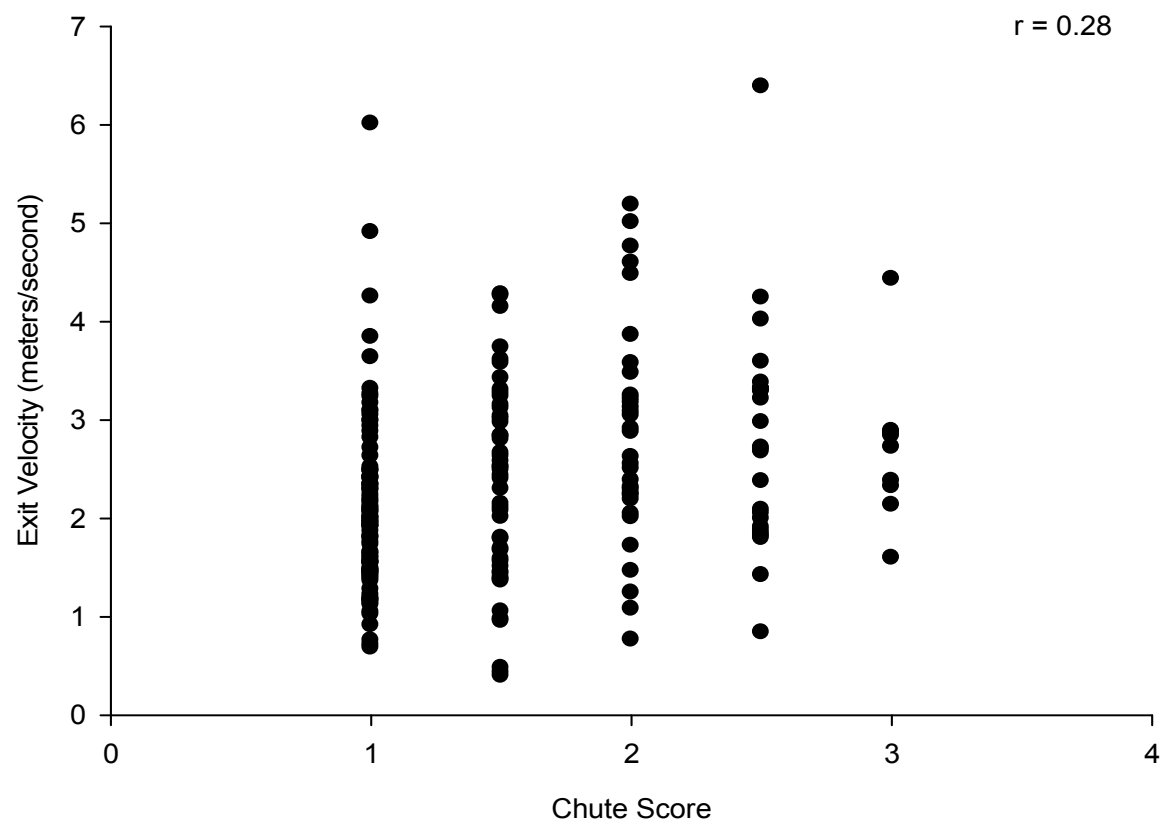


Figure 3.10. Positive correlation at the ($P < 0.05$) level between mean chute scores and mean exit velocity across all beef females ($n = 212$).

In this study, a breed difference for temperament was detected. This difference among breed types has been previously reported (Humes et al., 1987; Cooke, 2010). In this study, Brangus F₁ females has higher (2.0 ± 0.13) mean chute scores when compared with Beefmaster (1.6 ± 0.11), Bons Mara (1.6 ± 0.11), Brangus (1.5 ± 0.12), Romosinuano (1.2 ± 0.08), Braford (1.5 ± 0.13) and Angus (1.3 ± 0.06) females. Furthermore, Brangus F₁ (3.0 ± 0.22), Brangus (2.4 ± 0.17) and Beefmaster (2.5 ± 0.13) had a higher mean exit velocity when compared with Bons Mara (2.2 ± 0.18), Romosinuano (2.3 ± 0.20), Braford (2.1 ± 0.25) and Angus (2.0 ± 0.12) females. While the Brangus F₁ females were assessed as having a higher mean subjective chute score when compared with other breed types, they had a higher first service pregnancy (62%) when compared with Beefmaster (51%), Bons Mara (58%), Brangus (56%), Braford (46%) and Angus females (60%). Only one breed type (Romosinuano) had a higher first service pregnancy rate (75%) compared with the Brangus F₁ females (62%).

Humes et al. (1987) reported a difference in temperament scores when comparing embryo transfer donor cows of the Angus and Chianina breed. When comparing the two breeds, a higher mean temperament score was reported for Chianina when compared to Angus donors entering into the squeeze chute (3 ± 0.21 and 2 ± 0.28 , respectively) and while being restrained in the squeeze chute (5 ± 0.31 and 3 ± 0.41 , respectively), however, no differences were reported for both breeds of donors while cows were in the chute prior to being restrained (2 ± 0.19 and 2 ± 0.26) or while exiting the squeeze chute (3 ± 0.21 and 2 ± 0.28 , respectively). Furthermore, Cooke (2010) reported a higher mean temperament score between Brahman x Herford (3.6 ± 0.15) and Brahman x Angus (3.8 ± 0.22) females when compared with Angus (1.7 ± 0.19) and Simmental x Angus (1.8 ± 0.07) females.

The effect of cattle temperament on subsequent AI pregnancy rates was detected by mean exit velocity values and not by mean subjective behavior chute score values. Even

though these values were correlated to one another, unlike exit velocity, chute scores are a subjective measurement. The chute score values were taken on two separate days (day 0 and day 7), even though they were taken by the same technician, variability between the days could possible affect the outcome.

Cooke et al. (2010) reported a decrease in pregnancy rates occurred when comparing 435 Angus x Hereford cows with either excitable (89%) or adequate (94%) temperament. While this study reported a significant effect for chute scores, that study used only pasture breeding and not artificial insemination, as was used in this study. This is the only study compare first service pregnancy rates to the temperament prior to artificial insemination. In the previous study, the cows were allowed multiple services by natural mating and were evaluated for pregnancy status at the end of the breeding season (Cooke et al., 2010).

CHAPTER V

SUMMARY AND CONCLUSION

Artificial insemination has provided means of dispersing male genetics across a greater female population. In Experiments I and II, there were no differences in pregnancy rates across Time Frames 1, 2 and 3. This studies result suggests that no decrease in reproductive efficiency occur with gametes stored in liquid nitrogen for an extended period of time. Research has suggested that these findings merit a large scale controlled breeding trial, however, no controlled study has been reported in the scientific literature comparing pregnancy rates in beef cattle with frozen semen stored for years (Smith and Steinberger, 1973; Nishikawa et al., 1976; Salamon, 1976; Leibo et al., 1994; Stroble et al., 2002; Ward et al., 2003; Kaneko et al., 2006) . The exact number of years gametes can survive in a frozen state is still unknown at the present time. At our laboratory, we have found that the fertility of frozen bull semen in both dairy and beef cattle remains similar between the years 1967 to 2009. However, studies utilizing radiation as a source of time elapsing have suggested that gametes may remain viable when stored for up to 2,000 years (Glenister et al., 1984; Glenister and Lyon, 1986) and mammalian tissue for up to 20,000 years (Ashwood-Smith and Friedmann, 1979) ‘

The calves born in this Experiment I and II did not differ in gestation length or birth weight across Time Frames 1, 2 and 3. Across all calves born no abnormalities were observed for calves produced from semen stored from Time Frames 1, 2 and 3. These results provide some insight that frozen stored gametes maintain the ability to not only participate in fertilization, but also retain competent amounts of normal DNA to produce healthy viable offspring (Nishikawa et al., 1976;; Cohen et al., 1988; Go et al., 1998; Ben-Ozer and Vermesh, 1999; Quintans et al., 2002; Revel et al., 2004; Teijón et al., 2006).

Semen packaging has evolved since the first frozen calf was produced (Stewart, 1951). Different types of packages for cattle have been evaluated in ampules, plastic straws, pellets and. As reported in this study, the actual package of semen itself may not result in a lower post-thaw motility or lower pregnancy rates. It is more important to focus on the quality of semen placed into the package pre-freeze rather than the actual package itself.

Genetics in any farm animal rapidly changes from generation to generation. In this study, no differences were observed in birth weights for calves born across Time Frames 1, 2 and 3 for both Experiments I and II. These data are in contrast of what is reported by the American Angus Association (2010b) for average calf birth weights for each given time period. One might conclude that the bulls used in this study were higher birth weight producing bulls for certain time frames additionally, while those weights reported by the American Angus Association (2010b) for the given decades contain genetics from both male and females from that given time period. In this study, only the male from those distinct time periods contributed to the genetic pool. Another speculation is the limited amount of data in this study when compared with that collected by the American Angus Association (2010b).

The invitro analysis of semen parameters provided a deeper understanding to fertility results achieved in Experiments I and II. Though no differences were detected for motility or morphological abnormalities between semen stored between the three Time Frames, this suggests that the technicians responsible for the initial freezing of the semen were well trained in semen processing and freezing. The differences in sperm concentration within each time period could be attributed to the different bull studs in which they were initially processed. Scientist have found that by lowering the number of sperm per mL for cattle, acceptable pregnancy rates can be achieved. It is also more economical for bull studs to be able to lower the sperm concentration per mL. This allows for more units of semen to be produced per

ejaculate, thus, providing more income for the processor and more units available for the bull owner. The semen used in this study was from several different bull studs from three different time periods and each and every one maintained his/her own quality control standards at that time.

In this study, when comparing Angus bulls that had high, moderate and low fertility rates at AI, no differences were detected in post-thaw sperm motility among the three fertility levels. These data suggest that post-thaw motility (within an acceptable range) may not be an accurate predictor in subsequent AI pregnancy rates and that other post-thaw sperm factors (membrane integrity, intact acrosomes and morphology) may play a role in the variation in pregnancy rates. Additional research should be conducted on this topic to help provide a better understanding of the importance of post-thaw semen motility.

Recently, some attention has been given to animal temperament and its affect of reproductive efficiency (Cooke, 2010a; Cooke et al., 2010b). In this study, significant positive correlations between subjective behavior chute scores and chute exit velocity on both estrous synchronization days (day 0 and day 7) were detected. This data suggests that cattle behave similar and are not affected by handling. A correlation was also detected between chute scores and exit velocity values across all females.

Chutes scores in this study did not affect subsequent beef cattle AI pregnancy rates; however, exit velocity did affect subsequent cattle AI pregnancy rates. Furthermore, the high exit velocity score (exiting faster out of squeeze chute) correlates with a decrease in pregnancy rates in beef cattle. Previous data (Cooke, 2010a; Cooke et al., 2010b) has reported that animals with higher subjective behavior chute scores can result in lower pregnancy rates at the end of the breeding season. The significance found for chute exit velocity and not subjective behavior chute scores is hypothesized as human error. This is the first study known to this

author that has evaluated the effect of cattle temperament on subsequent AI pregnancy rates in beef cattle.

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APPENDIX A: CHAPTER III SUPPLEMENTARY CALVING DATA ACROSS TIME FRAMES
FOR ANGUS AND CROSSBRED FEMALES

	DF	MS
Angus Females (Gestation Length) Between Groups	2	133.103
Angus Females (Birth Weight) Between Groups	2	80.989
Crossbred Females (Gestation Length) Between Groups	2	35.492
Crossbred Females (Birth Weight) Between Groups	2	34.876

APPENDIX B: CHAPTER III SUPPLEMENTARY ANOVA DATA TABLE FOR POST-THAW
SPERM PARAMETERS ACROSS DIFFERENT PACKAGING METHODS

	DF	MS
Total Sperm Motility Between Groups	1	143.719
Progressive Sperm Motility Between Groups	1	210.746
Sperm Morphology (Normal) Between Groups	1	240.286
Sperm Morphology (Abnormal) Between Groups	1	480.286
Sperm Abnormalities (Primary) Between Groups	1	9.067
Sperm Abnormalities (Secondary) Between Groups	1	107.531 [*]
Sperm Abnormalities (Tertiary) Between Groups	1	281.105 [*]

^{*}Values significantly different at P<0.05 level.

APPENDIX C. SEMEN PARAMETERS UNDER STANDARD LABORATORY CONDITIONS OF THE ANGUS BULLS USED IN THIS STUDY

Time Period	Bull No. ¹	NAGP I.D. ²	Registration #	Name	TM ³	PM ⁴	Concentration ⁵
1960-1975	1	2668	7036540	Charbow Chance 10 Y	45	35	74
1960-1975	2	8580	5272722	Green Valley O B 67	35	20	53
1960-1975	3	8590	6173161	Biffles Challenger 796	30	15	25
1960-1975	4	8591	8086237	K A F Thunderbird 707	60	45	48
1960-1975	5	8830	7101362	Puranbree Fabron 7156	40	30	65
1976-1991	6	3116	10593697	Black Thunder of V A F	45	30	33
1976-1991	7	8563	7530832	Rito 149 of Ideal 632 72	60	50	84
1976-1991	8	3091	9467046	Continental	50	35	44
1976-1991	9	3138	9934739	Eldorado 156 of Ideal	50	40	42
1976-1991	10	3396	10710606	Eldorado 5110 Ideal 2228 156	45	30	81
1976-1991	11	1054	9709378	C S U Shoshone 0128	70	50	39
1976-1991	12	2337	8679290	Lady Prides Duke	40	25	92
1976-1991	13	3106	11199043	HW Power Play W238	80	65	114
1976-1991	14	3398	10710666	Landmark 5215 of Ideal 9100	30	25	58
1976-1991	25	3235	9034639	PJM Power Point	45	35	64
1992-2006	15	3375	11787983	Ideal 2292 of 1254	45	30	32
1992-2006	16	3050	11167039	Nichols Heavy Duty X100	65	50	31
1992-2006	17	3084	14215152	Jamesland ND 1407 70J	65	55	46
1992-2006	18	3293	14420104	Diamond Prime Cut 6763	50	35	35
1992-2006	19	3049	12234092	Nicols Performa D162	65	50	42
1992-2006	20	2989	13543367	Stassens Papa Universe 6	70	50	38
1992-2006	21	2993	13270868	Ankonian Victorio	45	35	23
1992-2006	22	3059	12215320	O G L Battle Cry 427 128	40	30	20
1992-2006	23	3086	13266172	R F A Generation Ext 881	50	35	50
1992-2006	24	3031	11447554	MS Mac Arnie	55	40	52

¹Bull No. = Bull identification number used at LSU for this study.

²NAGP = USAD-ARS National Animal GermPlasm Program official identification number.

³TM = Total motility.

⁴PM = Progressive motility.

⁵Million sperm/package.

APPENIDX D. SEMEN PARAMETERS USING THE CASA UNIT OF THE ANGUS BULLS USED IN THIS STUDY

Time Period	Bull No. ¹	NAGP I.D. ²	Registration #	Name	TM ³	PM ⁴
1960-1975	1	2668	7036540	Charbow Chance 10 Y	39	29
1960-1975	2	8580	5272722	Green Valley O B 67	14	8
1960-1975	3	8590	6173161	Biffles Challenger 796	27	22
1960-1975	4	8591	8086237	K A F Thunderbird 707	54	44
1960-1975	5	8830	7101362	Puranbree Fabron 7156	44	37
1976-1991	6	3116	10593697	Black Thunder of V A F	n/a ⁵	
1976-1991	7	8563	7530832	Rito 149 of Ideal 632 72	49	36
1976-1991	8	3091	9467046	Continental	46	32
1976-1991	9	3138	9934739	Eldorado 156 of Ideal	23	15
1976-1991	10	3396	10710606	Eldorado 5110 Ideal 2228 156	n/a ⁵	
1976-1991	11	1054	9709378	C S U Shoshone 0128	55	50
1976-1991	12	2337	8679290	Lady Prides Duke	34	27
1976-1991	13	3106	11199043	HW Power Play W238	36	23
1976-1991	14	3398	10710666	Landmark 5215 of Ideal 9100	n/a ⁵	
1976-1991	25	3235	9034639	PJM Power Point	39	28
1992-2006	15	3375	11787983	Ideal 2292 of 1254	49	38
1992-2006	16	3050	11167039	Nichols Heavy Duty X100	65	54
1992-2006	17	3084	14215152	Jamesland ND 1407 70J	55	45
1992-2006	18	3293	14420104	Diamond Prime Cut 6763	48	39
1992-2006	19	3049	12234092	Nicols Performa D162	54	44
1992-2006	20	2989	13543367	Stassens Papa Universe 6	52	35
1992-2006	21	2993	13270868	Ankonian Victorio	33	19
1992-2006	22	3059	12215320	O G L Battle Cry 427 128	36	27
1992-2006	23	3086	13266172	R F A Generation Ext 881	56	43
1992-2006	24	3031	11447554	MS Mac Arnle	54	44

¹Bull No. = Bull identification number used at LSU for this study.

²NAGP = USAD-ARS National Animal GermPlasm Program official identification number.

³TM = Total motility.

⁴PM = Progressive motility.

⁵n/a = Sample not processed.

APPENDIX E: CHAPTER III SUPPLEMENTARY ANOVA DATA TABLE FOR POST-THAW SPERM PARAMETERS ACROSS TIME FRAMES

	DF	MS
Sperm Total Motility (Visual Appraisal) Between Groups	2	283.750
Sperm Progressive Motility (Visual Appraisal) Between Groups	2	246.750
Sperm Total Motility (CASA) Between Groups	2	416.068
Sperm Progressive Motility (CASA) Between Groups	2	255.431
Sperm Morphology (Normal) Between Groups	2	129.600
Sperm Morphology (Abnormal) Between Groups	2	129.600
Sperm Morphology (Primary Abnormalities) Between Groups	2	40.417
Sperm Morphology (Secondary Abnormalities) Between Groups	2	13.292
Sperm Morphology (Tertiary Abnormalities) Between Groups	2	126.817
Sperm Concentration Between Groups	2	1305.607*

*Significantly different at P<0.05 level

APPENDIX F: CHAPTER III SUPPLEMENTARY ANOVA DATA TABLE FOR POST-THAW
SEMEN PARAMETERS FOR BULLS WITH HIGH, MODERATE OR LOW FERTILITY

	DF	MS
Total Sperm Motility Between Groups	2	146.701
Progressive Sperm Motility Between Groups	2	199.181

APPENDIX G: CHAPTER IV SUPPLEMENTARY DATA ANOVA TABLE FOR THE EFFECT OF ANIMAL TEMPERAMENT ON PREGNANCY RATES

	DF	MS
Breed Effect (Exit Velocity) Between Groups	6	1.578*
Breed Effect (Chute Score) Between Groups	6	2.246*

*Values significantly different at $P < 0.05$ level.

VITA

David Barry Carwell was born on April 9, 1987 in on the Fort Stewart Army Base in Hinesville, Georgia, to Darryl and Stephanie Carwell. In 1990, David moved to Cherry Valley, Arkansas, where he was then adopted by his grandparents, Barry and Linda Carwell. He spent most of his summers riding around with his grandfather on the family grain operation until he was old enough to work full time.

During the fall and winters months, you would catch him in the woods of Crowley's Ridge, hunting squirrels, doves and deer. David was also an avid baseball player, starting at 5 years of age and ending during his senior year of high school.

Though his family had own livestock previously, David's interest was sparked in 2001 when he first saw Boer goats. The first set of goats he owned were bought from a nearby farm. As the years past, David's family began getting more and more involved with the Boer goats and was running up to 300 goats at one time. His interest in reproductive physiology was also in part to raising Boer goats. At his ranch, two to three times yearly they would perform embryo collection and transfer on some of his higher quality animals.

In 2005, David graduated from high school and quickly earned at spot at the American Boer Goat Association Judges Certification Program. At the age of 18, David was the youngest person in history to ever become a certified Boer goat judge. In 2008, David graduated from Arkansas State University with his Bachelor's degree in Agriculture with an emphasis in Animal Science. He was then accepted in the program at Louisiana State University in Baton Rouge where he is currently finishing his master's degree in reproductive physiology.