2002

The effect of nocturnal sampling on semen quality and the efficiency of collection in bovine species

Jennifer H. Yates
Louisiana State University and Agricultural and Mechanical College

THE EFFECT OF NOCTURNAL SAMPLING ON SEMEN QUALITY AND THE EFFICIENCY OF COLLECTION IN BOVINE SPECIES

A Thesis

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

in

The Interdepartmental Program in Animal and Dairy Sciences

by
Jennifer H. Yates
B.S., Louisiana State University, 2000
May 2002

ACKNOWLEDGMENTS

I would like to express my sincere thanks to my advisor Dr. John E. Chandler for his invaluable support, guidance and patience given to me during my program of study. I would also like to express my appreciation to the members of my committee, Dr. Cathleen C. Williams, and Dr. Donald E. Franke for their generous support and guidance.

I would also like to thank my coworkers; Anita Canal and J.B. Paul, without whom this project would have never been completed. Day and night they tolerated my project with very little complaint. I'll always remember the fun times at 1:00 AM.

Much appreciation goes to the people at Genex for all their time and assistance. The use of their bulls and collection facilities made this project a success. A special thanks goes to Mr. James Chenevert and Mr. Bobby Fair for always lending their help, time and advice during this project and throughout my time working at Genex.

A special thanks to my "top five" for all the love, support, crying, laughing, hugging, driving, and diet coke breaking! I love you all, and appreciate all the encouragement throughout this degree.

A special thank you is extended to my family. To my parents, Hal and Janice Long, and Dr. R.L. Horne, for all the love and support they have shown me. Also, to my brother Christopher, for all the encouragement and "fixing up" he has done for me. They have helped so much in making me the strong person I am today.

Lastly, I would like to thank my husband Clint for all his love and support throughout this degree. He always helped me through the tough times, believing in me and encouraging me to continue. I hope that I can return the favor someday. In addition, I would like to thank my two favorite girls, Riley and Abby, for all the “boxing” stress relief you both provided.

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ABSTRACT

The objective of this study was to evaluate two semen collection schedules utilizing the assumed behavioral differences between bovine species. The efficiency was measured by bull behavior and semen quality parameters. Four Holstein and four Brahman bulls were collected each during a morning and a night collection time weekly. Ejaculates (n=64) were obtained via artificial vagina over four-weeks. To avoid masking benefits of night collection, the first collection of the week was alternated between night and day. Two collection teams were employed to reduce the time needed for collection, and the variability in collection method. Sampling order and collection team were randomized throughout the study. Bull behavior parameters included reaction time to first mount, time to ejaculation, a refractory period test, and a thrust intensity test. As a managerial factor, the numbers of handler interruptions were counted. Pre-freeze semen parameters included total volume, initial motility and concentration. These were combined as total motile sperm harvested. Post-freeze semen viability parameters included post thaw motility, percent intact acrosomes, and 3-hour post thaw motility. Semen morphology parameters consisting of primary and secondary abnormalities were also measured. All data were analyzed by least squares methods. The bull within breed interaction was significant for all bull behavior parameters as well as the managerial parameter ($P < .05$). The bull within breed effect for total motile sperm harvested was not significant, but differed between breeds ($P < .05$). There was a mixed response due to bull within breed for the post freeze semen viability parameters. Bull within breed was not significant for the semen morphology parameters. The night versus day treatment was significant for the managerial parameter ($P = .002$). Consideration of a different collection schedule for *Bos* *indicus* cattle was not warranted. However, the efficiency of the collection process was impacted by extraneous environmental conditions.

Keywords: Bull behavior, Nocturnal collection, Semen characteristics

CHAPTER 1

INTRODUCTION

The primary goal of any Artificial Insemination Organization is to produce the largest quantity of the highest quality of semen possible in an efficient amount of time (Pennington, 1990). Meeting this objective in all breeds would be of great value to semen collection facilities that specialize in the custom collection of beef sires. Effective, reliable methods of semen collection would allow the product to go to the consumer faster, and promote advancements of the breed.

Louisiana's bull stud, Genex Inc., formerly Louisiana Animal Breeders Cooperative (LABC), previously collected approximately 98% dairy bulls with only a few custom collected beef bulls. Only since 1999 did custom collection of beef bulls begin full time at this site. Very few problems in regard to poor libido occurred when collecting aggressive dairy bulls, and their service times were kept to a minimum. However, problems have emerged with the collection of predominately Brahman influence beef bulls.

While Brahman bulls tend to be disease resistant and heat tolerant, they are also very "fragile". They are "highly intelligent" and shy animals when compared to other breeds, and thus can prove quite difficult in the collection arena (Cardwell, 1996). The biggest challenge to collection management when faced with Brahman bulls is how to get them to mount and properly serve an artificial vagina in an efficient amount of time, yielding a quality ejaculate.

In previous work by Mattner et al (1974), only 30% of the mounting chin ball marks on cows by Hereford bulls used could be attributed to mounts or services observed in daylight hours. This data suggests that beef bulls are naturally night breeders. Therefore, could collecting semen from Brahman bulls during nocturnal hours decrease the time to ejaculation of the bulls making the collection process more efficient while still providing a quality ejaculate?

Based on the current understanding of bull sexual behavior, and the limited knowledge on the use of nocturnal semen collections, the objectives of this research were 1.) To determine how semen collection during day versus night affects bull sexual behavior and libido, 2.) To improve the efficacy of collecting semen from Brahman bulls by decreasing the amount of time from visualizing the mount animal to ejaculation, 3.) To determine a relationship between Holstein versus Brahman breeds throughout the semen collection process, and 4.) To identify and evaluate management techniques that both aid and hinder the semen collection process.

CHAPTER 2

REVIEW OF LITERATURE

Nocturnal Sexual Behavior. Although it has not been proven scientifically, Brahman bulls are thought to be night breeders. While no research concerning the Brahman breed has been documented, Mattner et al (1974) observed the mating activity of Polled Hereford cattle throughout a 24-hour period. The cattle were observed from first light until darkness and the bulls were fastened with different colored chin ball markers to observe night activity. The researchers found that during daylight hours, overt estrus behavior (mainly group mounting by cows) was exhibited mainly in two time periods (5 to 11 am and 2 to 7 pm). The bulls were more sexually active at night than during daylight hours. Only 30% of the mounting marks on the cows could be attributed to mounts or services observed in daylight. They also reported greater sexual activity when using multiple rather than single sires.

Brahman Cattle History. Zebu cattle are classified as *Bos indicus*. There are over thirty distinct breeds in India, which are named from the areas from which they originated. The size and conformation of Indian cattle are as diverse as the geographical regions in which they are found. American Brahmans were primarily developed from four types of Indian cattle, the Guzerat, Gir, Nelore, and Krishna. According to Cardwell (1996), the cattle were ideal for the Southern Gulf Coastal Plains, primarily between the Mississippi and Rio Grand Rivers. This is because the Brahman had a natural resistance to the ticks that carried Spanish Fever, which had been a problem with U.S. cattle since 1814. It is generally accepted that the first importation of Indian cattle into the United States was in 1835 when Dr. Campbell R. Bryce of Columbia, South Carolina imported two bulls and four females from Egypt. The descendents of these early cattle were eventually far spread in the south. Fifty-one head of Indian cattle were imported in 1906, which is known as the Borden-O'Connor Importation (Cardwell, 1996).

Brahman Sexual Development. There is a common belief that *Bos indicus* bulls mature and reach puberty later than *Bos taurus* bulls. Fields et al (1982) studied the post weaning growth and reproductive traits in 10 Brahman and 12 Angus bulls from 8 through 20 months of age. It was found that Brahman bulls reached puberty at an average of 15.9 months. The breed by day interaction showed that initially, the Brahman scrotal circumference was smaller than the Angus scrotal circumference; however, by the end of the study, the Brahman scrotal circumference was larger than the Angus. Sexual development of the Brahman bull occurred at a later age and in a nonparallel pattern to that of the Angus.

In a previous study (Fields et al., 1979), Brahman bulls were found to have reached puberty at a later age than bulls of the Santa Gertrudis, Hereford and Angus breeds. Between 16 and 20 months of age, testes size, sperm motility and sperm cell concentration of the ejaculate increased markedly in Brahman and Santa Gertrudis bulls. Angus bulls exhibited stable to slight increases in testes size, semen volume and semen quality suggesting that this breed had reached its most rapid stage of development by 16 months of age.

Sexual Preparation. Sexual preparation is prolonging stimulation of the bull beyond that needed to induce mounting and ejaculation; this results in more, or enhanced contractions of the muscles involved in emission and ejaculation of semen. Sexual preparation will increase the number of sperm ejaculated (Amann, 1990). At A.I.

Organizations and to some extent on farms where a rigid routine of controlled mating is practiced, adequate sexual stimulation prior to ejaculation is not always provided. Compared to natural pasture matings, the preliminary courtship and displays of masculinity and libido are absent. Sex drive is consequently reduced as well as the vigor of the ejaculatory reflex. With lowered sex drive relaxation is prolonged and longer periods of sexual continence are required between collections (Kerruish, 1955). Kerruish (1955) found a significant improvement in sexual behavior and an 8.7% rise in conception rate when ten bulls were placed for five months on a regimen of intensive sexual stimulation prior to semen collection. Five months prior to the intensive sexual stimulation, the ten bulls had been on a sexual regimen of inadequate sexual stimulation.

A comparison of spermatological characteristics and fertility rates from semen collected after different lengths of sexual preparation time was performed by Kommisrud and Berg (1996). Semen volume was found to be significantly higher in the 12 Norwegian bulls used when sexual preparation time was less than 15 minutes compared to longer preparation.

Several studies have confirmed that the sexual performance of bulls was enhanced by allowing them to view their cohorts engaged in copulatory behavior (Blockey, 1981). A study by Mader and Price (1984) also demonstrated that in the context of controlled mating, sexual performance is slightly improved by a bull being restrained in close proximity to a stimulus female, and by being watched by another male while engaged in sexual interactions.

Two trials were conducted by Almquist (1973) using Angus and Hereford bulls to determine the effects of sexual preparation by false mounting on sperm output, semen traits and sexual activity. The data were compared to data for Holstein bulls collected twice in succession on 1 day per week after either three false mounts or one false mount. In this study it required nearly 10 times longer to stimulate beef than dairy bulls, based on time to first mount, and about three times longer to collect two successive ejaculates with 3 false mounts from beef than dairy bulls. However, giving 3 false mounts rather than no false mounts before semen collection increased sperm output by about 50% in first ejaculates for both beef and dairy bulls. Almquist (1973) found that changes of stimulus animal and semen collection location were commonly required to stimulate many of the beef bulls and to maintain their sexual interest during sexual preparation.

Reaction Time. The period of time during which the bull approaches the mount prior to copulation has been termed the reaction time (Kerruish, 1955; Kushwaha et al., 1955). In a semen collection environment, this time should be kept to a minimum for efficiency. Various researchers have recorded many factors influencing reaction time in past years. Kushwaha et al (1955) have observed that season may be a factor. These researchers reported significant differences between seasons in India for reaction time in bulls. Certain breeds are believed to have a long reaction time as a general rule. Couttie and Hunter (1956) claim that fat bulls, particularly the Aberdeen Angus breed, have a lack of libido and are slow to serve. Lagerlof (1954) has shown that breed type has an influence on the willingness of the animal to copulate. Almquist and Hale (1956) have observed that the frequency of copulation will ultimately increase the reaction time of a bull. A number of extraneous factors such as attendants and nearby structures can affect the bull's reaction time. (Kerruish, 1955).

With the objective of seeking further information on reaction times, Fraser (1960) observed bulls at service over a period of 4 years. The breeds observed included Holstein, Ayrshire, Jersey, Shorthorn, Brahman, Hereford, and Angus. The average reaction time found for all the subjects was 12.5 minutes. Fifty percent of the bulls commenced copulation or a mounting attempt within 2 minutes of encountering the teaser animal. Fraser found that for bulls 4 years of age and over, the reaction time of beef breeds was significantly greater than that of dairy breeds.

Collection Interval. Shorter intervals between collection days caused ejaculate volumes to decrease slightly, sperm concentration to decrease markedly, and sperm output per unit of time to increase considerably (Hafs, 1959). Similar trends were observed by other researchers (Amann, 1961; Bratton, 1954) when successive ejaculates are collected on the same day, especially if sexual preparation is intense for each ejaculate.

Almquist and Amann (1976) reported that 3.5 times more motile spermatozoa could be collected from 1 to 2 year old Holstein bulls when six ejaculates per week were collected as compared with one ejaculate per week. However, 40% more time was required to obtain an ejaculate on the six ejaculates per week schedule. These researchers also reported that maximum spermatozoal output could be achieved on a schedule of one ejaculate collected daily. With appropriate sexual preparation, these authors concluded that similar spermatozoal harvests could be achieved by collecting two to three ejaculates every 3 to 4 days.

Semen Collection Management. The collection of semen from bulls is the business for A.I. Organizations, and therefore proper management of the entire collection process is critical. There are several non-negotiable responsibilities that must be adhered to by any

A.I. Organization if it is going to accomplish the goal of obtaining a high quality ejaculate, thus maximizing the sperm harvested from the bull. These responsibilities include:

1. Employee Safety: Bulls are inherently dangerous. Employees can never exercise too much caution when handling bulls.

2. Bull Safety: A healthy bull population is the heart of the AI Organization. Protection from injury during the collection process is of the utmost importance. A collection area with non-slip flooring is needed for safe movement of bulls during teasing and semen collection (Pennington, 1990). Ejaculate quality and quantity may be negatively affected by improper footing.

3. Disease Transmission: Bulls should be health tested before entering isolation facilities, during the isolation period, and during residency at the AI center. Precautionary care minimizing the exchange of bodily fluids between bulls must be exercised. Mounting of bulls on a common teaser is a source of contamination. Wash the back and rear quarter of the teaser with a disinfectant between bulls. Brushing the contaminated area with ample disinfectant is essential (Schenk, 1998).

4. Proper Identification of Each Ejaculate: Accurate ejaculate identification can be achieved only if the bull is properly identified. Any ejaculate leaving the semen collection arena without unequivocally identifying the source should be discarded immediately (Schenk, 1998).

5. Mount Animal: Steers are commonly used in collection facilities for sexual stimulation. To achieve sexual stimulation in the shortest amount of time one can use a combination of mounts, familiar mounts in different location, or new mounts in different locations. Mounts should be selected based on size, temperament, and disease status (Miller, 1992).

Libido Testing. Libido has been defined as the willingness and eagerness of a bull to mount and to complete service of a cow or heifer. Mating technique has been defined as the ability to perform complete service conditioned by the anatomical structure of the bull and his copulatory organs (Hultnas, 1959). Therefore, mating ability presupposes a certain amount of libido. Reports indicate that dairy breeds may be more sexually active than beef breeds (Amann and Almquist, 1976) and that *Bos indicus* bulls generally show lower, and more variable, levels of libido than do *Bos taurus* bulls (Chenoweth et al., 1996; Chenoweth and Osborne, 1975; Vale-Filho et al., 1986). In several studies completed in tropical Australia, Brahman and Brahman-crossbred bulls obtained the lowest libido scores, Africander bulls and their crosses achieved the highest, while European bred bulls were intermediate (Chenoweth and Osborne, 1975). In studies performed in the United States, higher sex-drive scores were also obtained for *Bos taurus* bulls than for *Bos indicus* bulls (Chenoweth, et al., 1996; Randel, 1994).

Despite these results, a comparison of trials in which bulls were placed with estrus-synchronized females indicated that *Bos indicus* derived bulls were as efficient as European-breed bulls in detecting, serving, and impregnating estrous females, despite a lower service rate (Chenoweth, 1994). This discrepancy may be partly explained by observations that *Bos indicus* bulls tend to be selective and shy breeders, and that they generally do not perform well in pen tests to measure sex drive (Chenoweth, et al., 1996), even though they can be very active and efficient detectors of estrus in pasture situations.

In Florida, *Bos taurus* (Angus, Hereford) bulls obtained superior results in sex-drive tests compared to tropicalized *Bos taurus* (Senepol, Romosinuano) bulls, with *Bos indicus* (Brahman, Nellore X Brahman) bulls generally obtaining the poorest results (Chenoweth, et al., 1996). Commonly used testing procedures for sex drive may disadvantage *Bos indicus* bulls. Modifications that have been suggested by Chenoweth (1996) to improve the performance of *Bos indicus* bulls include the use of unrestrained estrous females and the avoidance of distractions during the test.

Chenoweth et al (1979) compared three methods of assessing sex drive in 113 yearling beef bulls. These were the serving capacity score, the libido score, and the reaction time to first service. Ovariectomized heifers restrained in service crates were the stimulus for all tests. Of the three scoring procedures compared, libido score appeared to have the most advantage in assessing sex drive in yearling beef bulls. It was concluded that a 10 minute libido test provided as much comparative information on the sex drive of yearling beef bulls as longer tests did. Further, the use of females in estrus appeared unnecessary to satisfactorily assess bull sex drive provided that proper restraint and presentation of stimulus was employed.

Several studies of libido-serving ability in bulls have been carried out. Fraser (1960) allowed bulls up to one hour in the presence of a cow and reported that 50% of the bulls examined undertook copulation or a copulating attempt within two minutes of encountering a cow. Wolf et al (1965) observed the sexual behavior of groups of bulls for a period of one hour, and bull calves were tested for 10 minutes or until they had three complete mounts. Bellows et al (1964) exposed bulls to estrogen-treated heifers for 10 minutes. A simple 5-minute test was devised to examine the libido and serving ability of unhandled beef bulls by Osborne et al (1971). Angus bulls, 12 to 15 months of age, were exposed to a cow in estrus, and their behavior was recorded. The bulls were given a score ranging from 0 to 5 based on their interest, mounting and servicing attempts throughout the 5 minutes. The results suggest that an exposure time of 5 minutes to a cow in estrus is adequate to determine the sexual behavior of a young bull.

Prior to 1994, most libido work had been completed using bulls in a natural mating situation. Sahoo and Pan (1994) completed a comprehensive study on the libido of bulls in stud and its impact on semen quality. The work was carried out on 6 Fresian X Hariana and 6 Jersey X Hariana bulls. At the time of semen collection, libido was assessed by reaction time: Delay before ejaculation, the time interval between presentation of bull to dummy and ejaculation. Libido was also assessed by intensity of thrust. It was quantified visually and scored as 1.) mild, 2.) moderate, and 3.) vigorous. The results showed that stronger intensity of thrust produced better quality semen. Though apparently a shorter reaction time was found to yield better quality semen, such influences were significant only for volume and individual motility.

Libido in bulls is thought to have a strong genetic component (Chenoweth, 1983). It is best assessed in young bulls as older bulls can have superimposed learning patterns, musculoskeletal problems, and inhibitions that adversely affect libido. However, it has been found that some young bulls raised in all male groups may show temporary deficiencies in libido and or mating ability (Chenoweth, 1981). Common causes of low libido in bulls include: over conditioned bulls that have been on high feeding levels, diseased bulls, stressed bulls either from handling or environment, or bulls satiated to their particular environment. The limiting factor to the reproductive performance of high libido bulls is their capability to produce sufficient numbers of viable spermatozoa to sustain fertility through multiple ejaculations at short intervals (Chenoweth, 1983).

Libido Measurement Factors. Many researchers have attempted to try and find a single factor that can accurately predict the libido of a bull. Bull production factors such as average daily gain as well as breeding soundness exam scores and blood testosterone levels have been used as potential indicators of fertility. In all probability, no one single parameter can be used to accurately correlate the measurement of that parameter with fertility (Morris et al., 1978). Breeding efficiency involves the interaction of several factors that influence the ability of a bull.

In a study by Ologun et al (1981), average daily gain and final test weight were compared with libido score, serving capacity score, reaction time to first mount, reaction time to first service and dominance value in 90 purebred yearling beef bulls. Although the data supported evidence for a strong genetic influence on bull sex-drive, findings suggested that libido and production traits such as average daily gain and final test weight were not favorably related in young beef bulls.

Forty Santa Gertrudis bulls were used to examine relationships among scrotal circumference, seminal quality, libido and fertility assessed as the percent pregnant of estrous females (PE rate) and the percent pregnant of females mated (PM rate) (Smith et al., 1981). A breeding soundness exam (BSE) was conducted on each bull approximately 45 days before the breeding period and immediately after the breeding period. The BSE score given to each bull was based on scrotal circumference, spermatozoal motility and morphology. The three components of the BSE score were not significantly correlated with PE rate or PM rate at either evaluation. Also there was no significant correlation between PM rate and scrotal circumference. The researcher thought that fluctuations in seminal quality between BSE evaluations were one possible explanation for low correlations between seminal parameters and PM rate. Although libido was positively correlated with PE rate, results of the study indicated that current methods of fertility evaluation did not accurately predict the fertility of the individual bulls as measured by PE rate and PM rate during the 4-day breeding period.

Prior to use in single-sire mating trials, Chenoweth et al (1988) evaluated 92 beef bulls to determine the relationships between BSE and sex drive. Based on the BSE, eighty bulls were classified as satisfactory potential breeders, while 12 were classified as questionable potential breeders. Sixty-nine bulls were classified as high libido and 23 were classified as medium libido bulls after completing two 10-minute libido/serving capacity tests. The researchers found that differences in scrotal circumference and spermatozoal motility did not influence the BSE classifications. However, differences in spermatozoal abnormalities, both primary and secondary, were the major factors affecting the BSE classification. Bulls classified as high libido serviced three times more during the two test periods than bulls classified as medium libido. High libido bulls were superior in all sex drive traits. Bulls in the two libido categories did not differ in semen quality or scrotal circumference. There was no relationship between BSE and sex drive traits found by Chenoweth (1988).

For years, poor libido was assumed to be related to a deficiency in circulating androgens. However, in 1976 Foote and his colleagues found that although there was a tendency for testosterone to increase with the age of the bull up to 6 or 7 years, the concentration of circulating testosterone was unrelated to libido or semen quality (Foote et al., 1976). Results of a study completed in 1979 by Chenoweth et al (1979) supported Foote's results finding that LH and testosterone values were not significantly correlated or were poorly correlated with sex-drive measurements.

CHAPTER 3

MATERIALS AND METHODS

Animals and Treatments. Four Holstein and four Brahman bulls were utilized in a 4-week experiment to determine the effects of nocturnal semen collection on bull sexual behavior, libido, and semen quality. The four Holstein bulls were obtained from the LSU Dairy Research and Teaching Unit, while the four Brahman bulls were obtained from private beef producers. All eight bulls were housed at Genex Inc. in the T.E. Patrick Dairy Herd Improvement Center for the duration of the study.

Semen was collected from each of the eight bulls eight times over the 4-week study period. The collections occurred 4 different times during a morning collection time beginning at 8 a.m., and 4 different times during a night collection time beginning at 11 p.m. To avoid masking the possible benefits of a nocturnal collection, the first collection of each week was rotated between night and day. All bulls were collected using an artificial vagina (AV).

Two semen collection teams were utilized to reduce both the time required for the semen collection process, and the variability in method of collection. Each team consisted of two people, a bull handler and a semen collector.

Sexual preparation of bulls was standardized. All bulls were brought directly from their pens to heater stalls just outside the semen collection arena at the same time. The order of semen collection and the team by which the bulls were collected were randomized weekly. After being led from the heater stall to the collection arena, the bulls were allowed to false mount the teaser as soon as they would respond. One minute later they were allowed to false mount again. After another minute of restraint, the collection was allowed to take place. The handler encouraged sexual interest during the entire time period, practicing only enough restraint of the bulls to prevent more than the two false mounts described. Mount animals were changed and an extra teaser was available for use if the assigned teaser did not arouse sufficient sexual interest. Also, a change of location was allowed if the bulls failed to mount or show any interest after 20 minutes.

Bull Behavior Parameters. In order to investigate and compare libido and sexual behavior of the two breeds, and to determine whether night collections are a viable option, a number of parameters were measured. Reaction time to first mount, time to first ejaculation, and refractory period were all recorded using a stopwatch. Intensity of thrust (Sahoo and Pan, 1994) was quantified visually by the collection team and scored as (1) mild, (2) moderate, or (3) vigorous. Also, a libido score was given every 2.5 minutes until service to the artificial vagina was achieved. This score was adapted from a scoring system described by Hultnas (1959).

0 = No interest in mount animal, although bull was led up and invited to mount.

1 = Little interest in mounting, despite sniffing at the rear end of mount and perhaps vague mounting attempts.

2 = Mounting after obvious repeated hesitation with weak clasping and seeking.

3 = Comparatively quick mounting without obvious eagerness. Satisfactory holding and seeking.

4 = Quick mounting with bulls attention focused on the mount with very good holding and seeking.

5 = Eager mounting with very good holding and seeking.

6 = Uncontrolled eager mounting with very good holding and intensive seeking.

Management Parameter. The number of team interruptions was counted and included all distractions for anyone involved in the semen collection process.

Semen Quality Parameters. A full semen analysis including initial motility, concentration, and volume along with a post-thaw motility, percent primary and percent secondary abnormalities, and percent intact acrosomes was completed on all samples. Ejaculates were stored at 37^{o}C in a water bath prior to initial evaluation. The initial concentration was determined using a spectrophotometer, and the volume was measured in a graduated test tube. Ejaculates were processed in 0.5 ml French straws and frozen using egg yolk citrate-glycerol extender method (Chandler, 1984) with modification. Three straws per bull were thawed at 37^{o}C for 30 seconds and were pooled for evaluation. All samples were examined in duplicate.

Percent progressive motility at 0 hours and again after a 3-hour incubation at 37^{o}C was evaluated from a live smear using phase contrast microscopy equipped with a warm stage at 40X magnification (Saacke, 1972). Primary and secondary abnormalities were determined by direct count of 100 cells from random fields of an immobilized smear using differential interference contrast (DIC) microscopy at 100X magnification (Mitchell, 1978). Acrosomal integrity, denoted by the presence of the apical ridge after 3 hours of incubation at 37^{o}C, was measured using differential interference contrast microscopy at 100X magnification counting 100 cells in an immobilized smear (Saacke, 1972). All immobilized smears were fixed with .02% glutaraldehyde (Johnson, 1976).

Statistical Methods and Calculations. The data were analyzed using the general linear model in the SAS program version 8 (SAS User's Guide: Statistics, 1999). Breed source of variation was tested with an adjusted error term. The other items in the model were tested with random error. Least squares means are presented where significance occurred.

The model included the following:

$$Y_{ijkl} = \mu + Breed_i + Bull(Breed)_{i(j)} + Treatment_k + Team_l + Team*Treatment_{lk} + Treatment*Breed_{ik} + Team*Breed_{il} + Team*Treatment*Breed_{ikl} + Collection_{ijkl} + random\ error_{ijkl}$$

where:

Y_{ijkl} = object

μ = overall mean

$Breed_i$ = fixed effect of breed i (Holstein or Brahman)

$Bull(Breed)_{i(j)}$ = random effect of the j^{th} Bull in the i^{th} Breed

$Treatment_k$ = fixed effect of Treatment k (day or night)

$Team_l$ = fixed effect of Team l (1 or 2)

$Team *Treatment_{lk}$ = interaction between Team l and Treatment k

$Treatment*Breed_{ik}$ = interaction between Treatment k and Breed i

$Team*Breed_{il}$ = interaction between Team l and Breed i

$Team*Treatment*Breed_{ikl}$ = interaction between Team l and Treatment k and Breed i

$Collection_{ijkl}$ = linear covariate to account for trends in learned animal behavior and technician knowledge

and

e_{ijkl} = random error

CHAPTER 4

RESULTS

Bull nested within breed was a significant (P<.05) source of variance in the model for time to first mount (Table 1).

Table 1: Mixed model analysis of variance for time to first mount.

Source	DF	Type III SS	Mean Square	F Value	Pr > F
Breed[1]	1	290.3432	290.3432	5.04	0.0655
BullID(Breed)	6	353.8835	58.9806	5.03	0.0005
Trmt	1	0.0413	0.0413	0.00	0.9529
Team	1	0.5717	0.5717	0.05	0.8262
Team*Trmt	1	64.5278	64.5278	5.51	0.0234
Trmt*Breed	1	0.0403	0.0403	0.00	0.9535
Team*Breed	1	1.0725	1.0725	0.09	0.7636
Team*Trmt*Breed	1	61.4665	61.4665	5.25	0.0267
Collection	1	3.8845	3.8845	0.33	0.5676
Error: MS(Error)	45	527.1975	11.7155		
Corrected Total	59				
R-Square (Percent)		62.9439			

[1]Breed tested with Error DF of 6.069. Error = 0.972*MS(BullID(breed)) + 0.028*MS(Error)

For the team by treatment interaction, Team 1 appeared to be more efficient (P<.02) at collecting the bulls during the day than at night with a significantly shorter time to first mount during the day. Team 2 was more efficient at collecting the bulls at night rather than during the day with a significantly shorter time to first mount at night. No logical explanation exists for why one team was more efficient than the other at collecting the bulls at night versus during the day (Figure 1).

Bull nested within breed was the only significant (P<.05) source of variation in the model for the time to first ejaculation bull behavior parameter (Table 2). Since this measurement includes the previous measurement of time to first mount, and directly influences the efficiency of collection, it can be used as an indicator to compare bull within breed response. Figure 2 illustrates the bull within breed response of time to first ejaculation. Overall, Brahman bulls had a much longer time to first ejaculation and significantly greater variation between bulls within breed than the Holstein bulls. The treatment trend is similar within breed across bulls.

Table 2: Mixed model analysis of variance for time to first ejaculation.

Source	DF	Type III SS	Mean Square	F Value	Pr > F
Breed[1]	1	1,298.2482	1,298.2482	5.11	0.0642
BullID(Breed)	6	1,562.8387	260.4731	9.18	<.0001
Trmt	1	17.0084	17.0084	0.60	0.4429
Team	1	0.0185	0.0185	0.00	0.9797
Team*Trmt	1	32.4717	32.4717	1.14	0.2904
Trmt*Breed	1	26.6618	26.6618	0.94	0.3376
Team*Breed	1	3.1894	3.1894	0.11	0.7390
Team*Trmt*Breed	1	21.9365	21.9365	0.77	0.3839
Collection	1	0.8872	0.8872	0.03	0.8604
Error: MS(Error)	45	1,276.8902	28.3753		
Corrected Total	59				
R-Square (Percent)		68.1212			

[1]Breed tested with Error DF of 6.037. Error = 0.972*MS(BullID(breed)) + 0.028*MS(Error)

Breed and bull nested within breed were significant (P<.05) sources of variation in the model for the refractory period bull behavior parameter (Table 3). The individual bull nested within breed was the only significant (P<.05) source of variation in the model for the thrust intensity bull behavior parameter (Table 4).

Both the breed variation and individual bull nested within breed were significant (P<.05) for the bull behavior parameter of number of libido scores (Table 5). This was also true for the value of the libido score (Table 6). Also, treatment and the team by treatment interaction were significant (P<.05) sources of variation for the bull behavior parameter of libido score (Table 6). The graph of the least square means for the two above parameters demonstrate their relationship (Figure 3).

As shown in Figure 3, the Holstein bulls received significantly fewer libido scores than the Brahman bulls both during the day and at night, suggesting that they were much more efficient in mounting and servicing the artificial vagina.

Table 3: Mixed model analysis of variance for refractory period.

Source	DF	Type III SS	Mean Square	F Value	Pr > F
Breed[1]	1	243.1542	243.1542	12.85	0.0112
BullID(Breed)	6	115.7378	19.2896	3.17	0.0112
Trmt	1	0.0333	0.0333	0.01	0.9414
Team	1	4.5840	4.5840	0.75	0.3902
Team*Trmt	1	0.9167	0.9167	0.15	0.6999
Trmt*Breed	1	0.1023	0.1023	0.02	0.8975
Team*Breed	1	2.2168	2.2168	0.36	0.5493
Team*Trmt*Breed	1	0.0969	0.0969	0.02	0.9002
Collection	1	10.8415	10.8415	1.78	0.1889
Error: MS(Error)	45	274.0782	6.0906		
Corrected Total	59				
R-Square		61.0092			

[1]Breed tested with Error DF of 6.109. Error = 0.972*MS(BullID(breed)) + 0.028*MS(Error)

In regard to libido score, the Holsteins had a significantly greater libido score overall than the Brahman bulls did. Both the Holstein and Brahman bulls demonstrated significantly greater libido scores at night versus during the day.

Treatment was a significant ($P<.05$) source of variation for the managerial parameter of interruptions (Table 7). The least squares means graph demonstrates the treatment differences (Figure 4). Significantly more managerial interruptions occurred during the day semen collection than during the nocturnal semen collection.

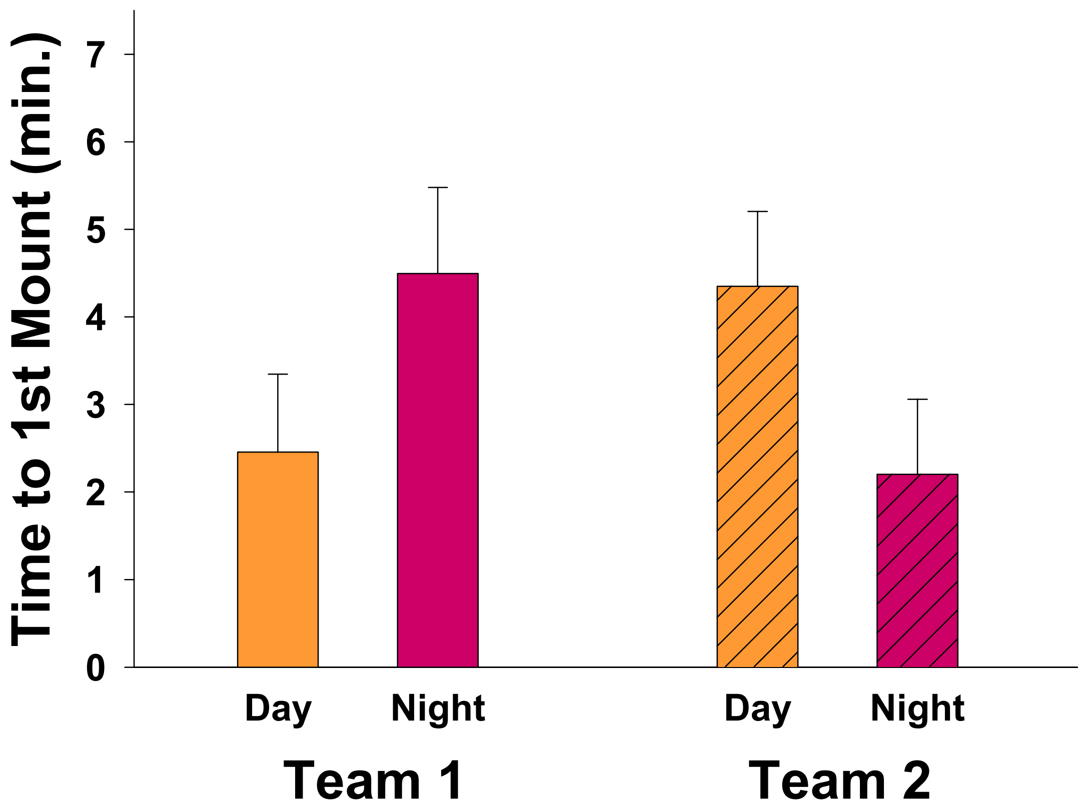

Figure 1. Least squares means graph for the team by treatment interaction for time to first mount.

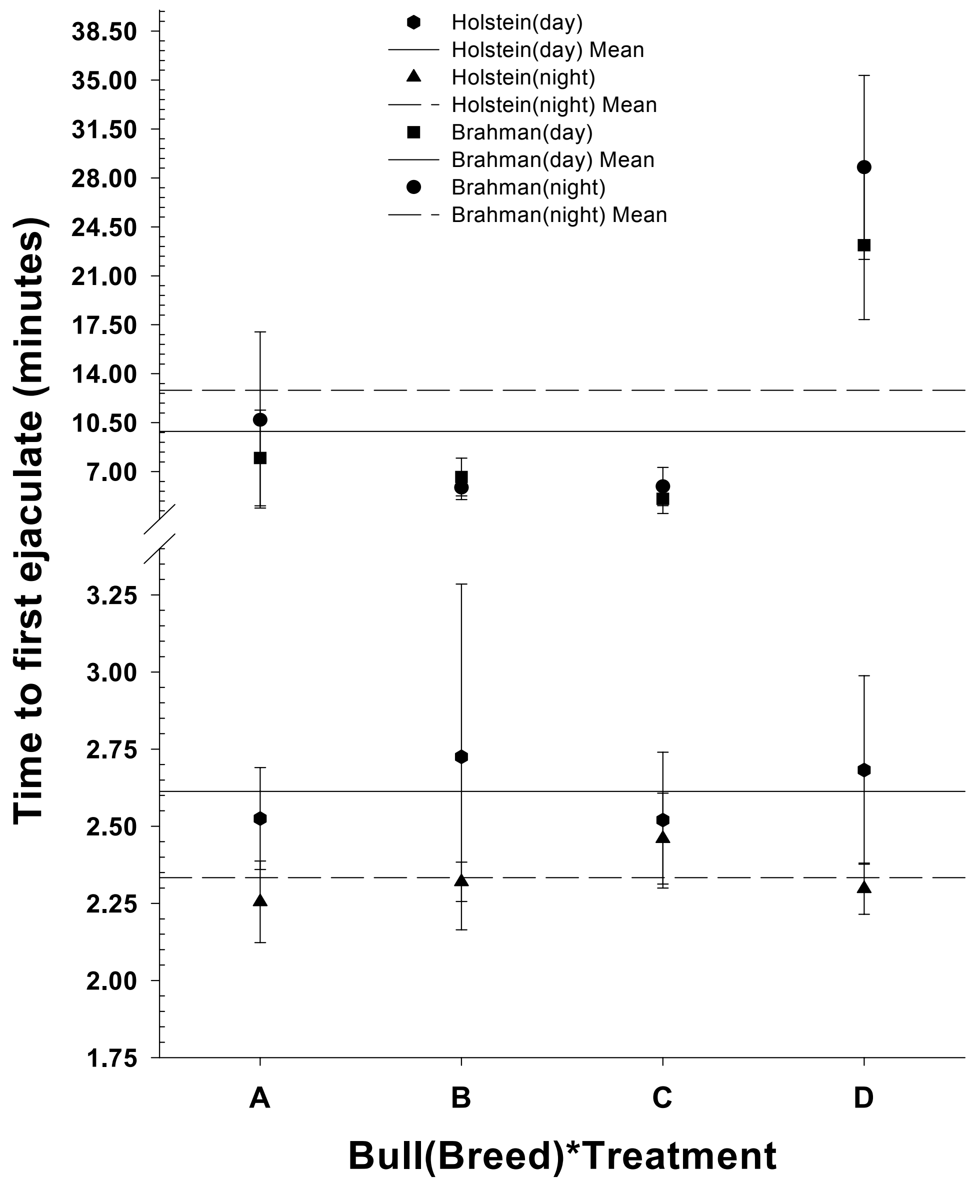

Figure 2. Average bull within breed by treatment interaction for the bull behavior parameter of time to first ejaculation

Table 4: Mixed model analysis of variance for thrust intensity.

Source	DF	Type III SS	Mean Square	F Value	Pr > F
Breed[1]	1	2.4834	2.4834	1.45	0.2738
BullID(Breed)	6	10.5298	1.7550	4.45	0.0013
Trmt	1	0.0001	0.0001	0.00	0.9898
Team	1	0.0001	0.0001	0.00	0.9862
Team*Trmt	1	0.0538	0.0538	0.14	0.7135
Trmt*Breed	1	0.3180	0.3180	0.81	0.3739
Team*Breed	1	0.3057	0.3057	0.78	0.3831
Team*Trmt*Breed	1	0.0243	0.0243	0.06	0.8050
Collection	1	0.2047	0.2047	0.52	0.4748
Error: MS(Error)	45	17.7374	0.3942		
Corrected Total	59				
R-Square (Percent)		46.2001			

[1]Breed tested with Error DF of 6.078. Error = 0.972*MS(BullID(breed)) + 0.028*MS(Error)

Table 5: Mixed model analysis of variance for number of libido scores.

Source	DF	Type III SS	Mean Square	F Value	Pr > F
Breed[1]	1	167.5690	167.5690	7.18	0.0360
BullID(Breed)	6	143.0024	23.8337	4.21	0.0019
Trmt	1	2.5739	2.5739	0.45	0.5035
Team	1	4.2577	4.2577	0.75	0.3904
Team*Trmt	1	0.4153	0.4153	0.07	0.7877
Trmt*Breed	1	6.5827	6.5827	1.16	0.2866
Team*Breed	1	9.0627	9.0627	1.60	0.2122
Team*Trmt*Breed	1	0.0983	0.0983	0.02	0.8957
Collection	1	0.0509	0.0509	0.01	0.9249
Error: MS(Error)	45	254.6991	5.6600		
Corrected Total	59				
R-Square (Percent)		73.2376			

[1]Breed tested with Error DF of 6.082. Error = 0.972*MS(BullID(breed)) + 0.028*MS(Error)

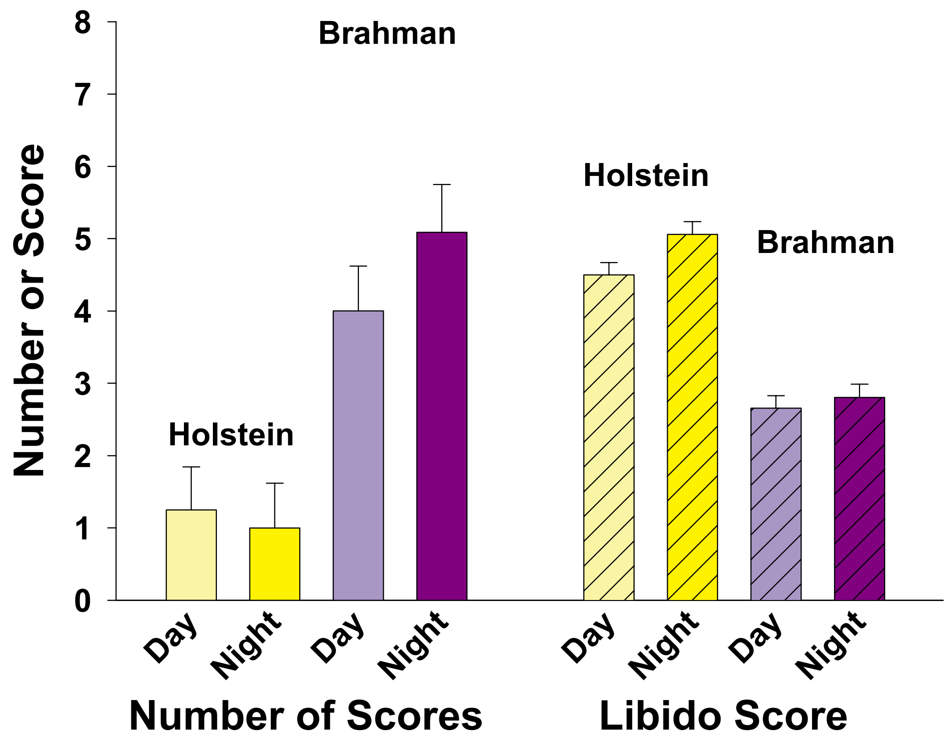

Figure 3. Least squares means graph of the treatment by breed interaction for the bull behavior parameters of number of libido scores and libido score

Table 6: Mixed model analysis of variance for libido score.

Source	DF	Type III SS	Mean Square	F Value	Pr > F
Breed[1]	1	60.3012	60.3012	33.22	0.0011
BullID(Breed)	6	11.1291	1.8549	4.12	0.0022
Trmt	1	1.8553	1.8553	4.12	0.0483
Team	1	0.2037	0.2037	0.45	0.5045
Team*Trmt	1	4.2834	4.2834	9.52	0.0035
Trmt*Breed	1	0.6219	0.6219	1.38	0.2460
Team*Breed	1	0.0714	0.0714	0.16	0.6922
Team*Trmt*Breed	1	1.7968	1.7968	3.99	0.0518
Collection	1	0.0053	0.0053	0.01	0.9140
Error: MS(Error)	45	20.2519	0.4500		
Corrected Total	59				
R-Square (Percent)		40.7875			

[1]Breed tested with Error DF of 6.043. Error = 0.972*MS(BullID(breed)) + 0.028*MS(Error)

Table 7: Mixed model analysis of variance for number of managerial interruptions.

Source	DF	Type III SS	Mean Square	F Value	Pr > F
Breed[1]	1	14.0464	14.0464	4.40	0.0793
BullID(Breed)	6	19.4024	3.2337	1.74	0.1347
Trmt	1	27.3663	27.3663	14.69	0.0004
Team	1	0.8674	0.8674	0.47	0.4986
Team*Trmt	1	0.1863	0.1863	0.10	0.7533
Trmt*Breed	1	1.9966	1.9966	1.07	0.3062
Team*Breed	1	5.4226	5.4226	2.91	0.0949
Team*Trmt*Breed	1	1.9966	1.9966	1.07	0.3062
Collection	1	0.0844	0.0844	0.05	0.8324
Error: MS(Error)	45	83.8597	1.8635		
Corrected Total	59				
R-Square (Percent)		47.0320			

[1]Breed tested with Error DF of 6.201. Error = 0.972*MS(BullID(breed)) + 0.028*MS(Error)

The bull nested within breed variation was the only variance source in the model that was significant ($P<.05$) for the initial semen quality parameters of volume, concentration, and initial motility (Table 8, 9, 10).

Table 8: Mixed model analysis of variance for ejaculate total volume.

Source	DF	Type III SS	Mean Square	F Value	Pr > F
Breed[1]	1	83.0973	83.0973	2.80	0.1451
BullID(Breed)	6	182.8026	30.4671	11.96	<.0001
Trmt	1	0.0002	0.0002	0.00	0.9939
Team	1	0.2927	0.2927	0.11	0.7362
Team*Trmt	1	2.4697	2.4697	0.97	0.3300
Trmt*Breed	1	1.8043	1.8043	0.71	0.4044
Team*Breed	1	0.9585	0.9585	0.38	0.5427
Team*Trmt*Breed	1	0.1471	0.1471	0.06	0.8112
Collection	1	0.0928	0.0928	0.04	0.8495
Error: MS(Error)	45	114.6088	2.5469		
Corrected Total	59				
R-Square (Percent)		71.4469			

[1]Breed tested with Error DF of 6.029. Error = 0.972*MS(BullID(breed)) + 0.028*MS(Error)

Table 9: Mixed model analysis of variance for ejaculate concentration.

Source	DF	Type III SS	Mean Square	F Value	Pr > F
Breed[1]	1	4,777.57	4,777.57	0.00	0.9618
BullID(Breed)	6	11,789,634.00	1,964,939.00	8.85	<.0001
Trmt	1	93,358.00	93,358.00	0.42	0.5199
Team	1	231,584.00	231,584.00	1.04	0.3125
Team*Trmt	1	82,351.00	82,351.00	0.37	0.5455
Trmt*Breed	1	4,306.15	4,306.15	0.02	0.8898
Team*Breed	1	1,640.20	1,640.20	0.01	0.9319
Team*Trmt*Breed	1	144,348.00	144,348.00	0.65	0.4242
Collection	1	614,913.00	614,913.00	2.77	0.1030
Error: MS(Error)	45	9,987,391.00	221,942.00		
Corrected Total	59				
R-Square (Percent)		57.39			

[1]Breed tested with Error DF of 6.039. Error = 0.972*MS(BullID(breed)) + 0.028*MS(Error)

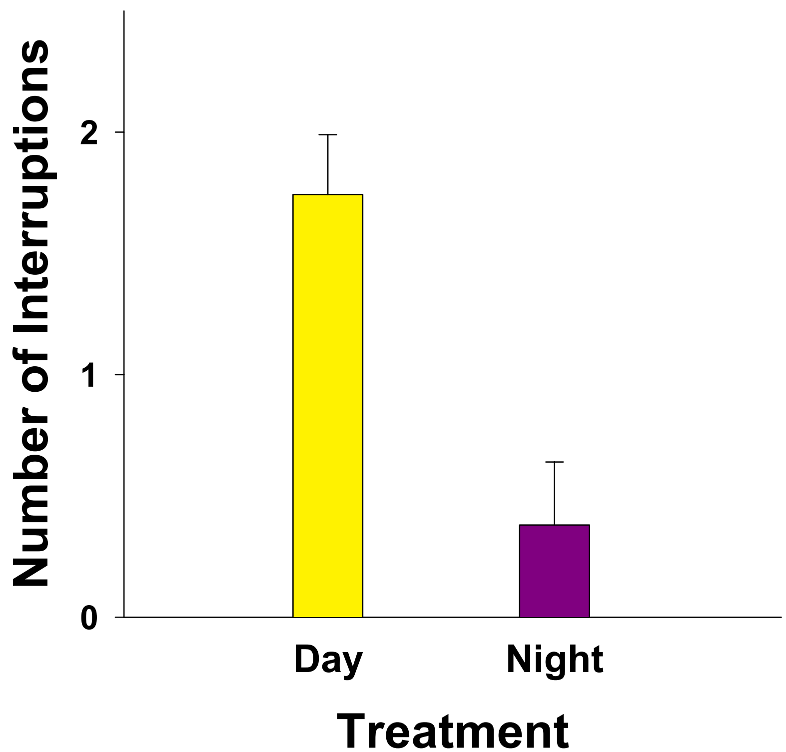

Figure 4. Least squares means graph of treatment differences for the managerial parameter of interruptions.

Table 10: Mixed model analysis of variance for ejaculate initial motility.

Source	DF	Type III SS	Mean Square	F Value	Pr > F
Breed[1]	1	2,200.5104	2,200.5104	3.31	0.1182
BullID(Breed)	6	4,083.2029	680.5338	8.09	<.0001
Trmt	1	153.3886	153.3886	1.82	0.1837
Team	1	22.7615	22.7615	0.27	0.6055
Team*Trmt	1	7.7605	7.7605	0.09	0.7628
Trmt*Breed	1	30.7910	30.7910	0.37	0.5483
Team*Breed	1	26.2943	26.2943	0.31	0.5789
Team*Trmt*Breed	1	107.0726	107.0726	1.27	0.2653
Collection	1	5.0279	5.0279	0.06	0.8080
Error: MS(Error)	45	3,786.4906	84.1442		
Corrected Total	59				
R-Square (Percent)		67.9557			

[1]Breed tested with Error DF of 6.042. Error = 0.972*MS(BullID(breed)) + 0.028*MS(Error)

When the above three semen quality parameters were combined, which is total motile sperm harvested the model did not fit the data (R^2, Table 11) sufficiently to identify any of its components as contributors to the variation. The graph of the least squares means demonstrates the breed differences (Figure 5). Brahman bulls had more total motile sperm cells harvested than Holstein bulls.

Breed and bull nested within breed were both significant (P<.05) sources of variation in the model for the semen quality parameter of post-thaw motility. The team by treatment by breed interaction was also mildly significant (P=.07) for post-thaw motility (Table 12). Figure 6 demonstrates the relationship of the treatment by breed interaction for post-thaw motility. For both day and night, Holstein bulls had a higher post-thaw motility than did Brahman bulls.

Table 11: Mixed model analysis of variance for total motile sperm harvested.

Source	DF	Type III SS	Mean Square	F Value	Pr > F
Breed[1]	1	26,808,904,000,000	26,808,904,000,000	3.29	0.1180
BullID(Breed)	6	49,425,767,000,000	8,237,627,800,000	1.60	0.1684
Trmt	1	4,105,135,900,000	4,105,135,900,000	0.80	0.3761
Team	1	1,576,183,900,000	1,576,183,900,000	0.31	0.5824
Team*Trmt	1	22,190,340,687	22,190,340,687	0.00	0.9479
Trmt*Breed	1	15,348,937,052	15,348,937,052	0.00	0.9567
Team*Breed	1	1,268,653,200,000	1,268,653,200,000	0.25	0.6217
Team*Trmt*Breed	1	120,613,750,098	120,613,750,098	0.02	0.8789
Collection	1	673,226,277,359	673,226,277,359	0.13	0.7191
Error: MS(Error)	45	231,199,620,000,000	5,137,769,200,000		
Corrected Total	59				
R-Square (Percent)		30.12			

[1]Breed tested with Error DF of 6.217. Error = 0.972*MS(BullID(breed)) + 0.028*MS(Error)

Table 12: Mixed model analysis of variance for semen initial post-thaw motility.

Source	DF	Type III SS	Mean Square	F Value	Pr > F
Breed[1]	1	7,076.0048	7,076.0048	21.04	0.0036
BullID(Breed)	6	2,130.2428	355.0405	8.05	<.0001
Trmt	1	72.5539	72.5539	1.64	0.2068
Team	1	16.9756	16.9756	0.38	0.5385
Team*Trmt	1	19.2119	19.2119	0.44	0.5130
Trmt*Breed	1	0.2875	0.2875	0.01	0.9360
Team*Breed	1	0.0308	0.0308	0.00	0.9790
Team*Trmt*Breed	1	152.5170	152.5170	3.46	0.0700
Collection	1	22.9092	22.9092	0.52	0.4752
Error: MS(Error)	42	1,853.4557	44.1299		
Corrected Total	56				
R-Square (Percent)		84.7505			

[1]Breed tested with Error DF of 6.095. Error = 0.94*MS(BullID(breed)) + 0.06*MS(Error)

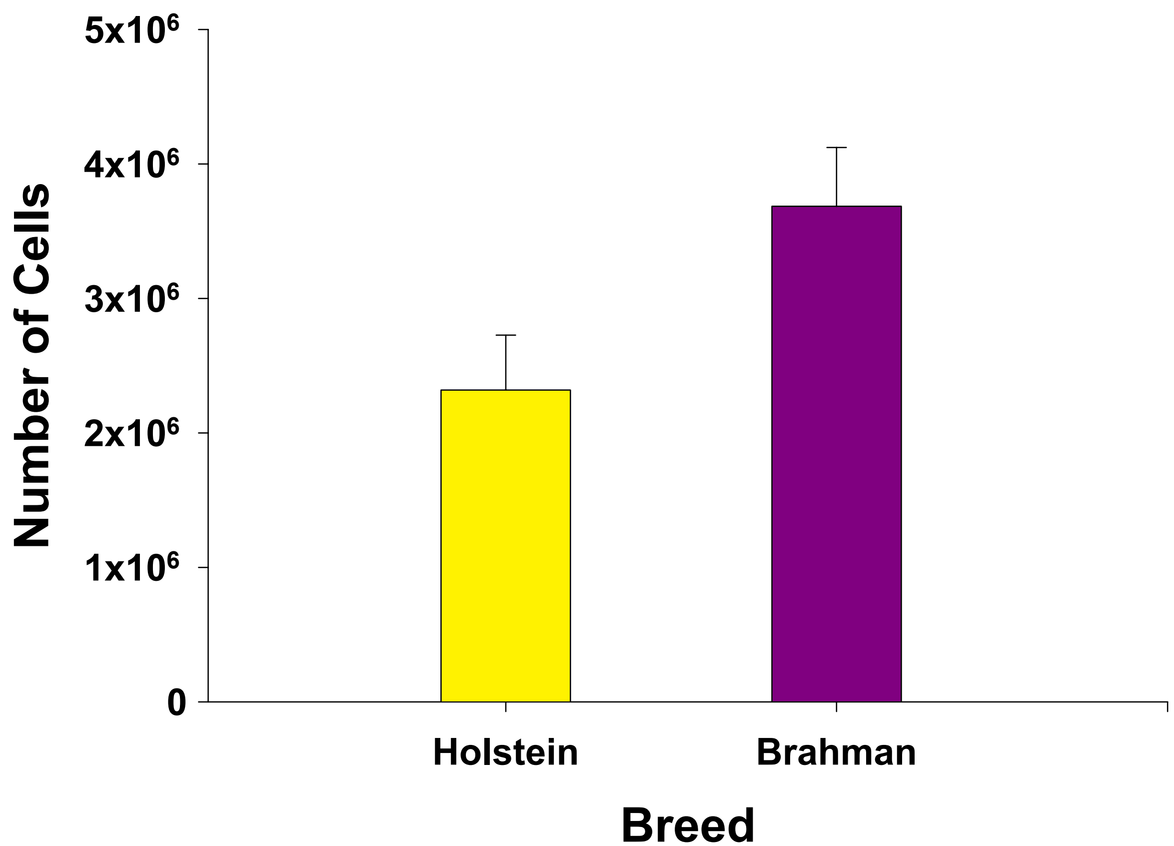

Figure 5. Least squares means graph of the breed variation for the semen quality parameter of total motile sperm harvested.

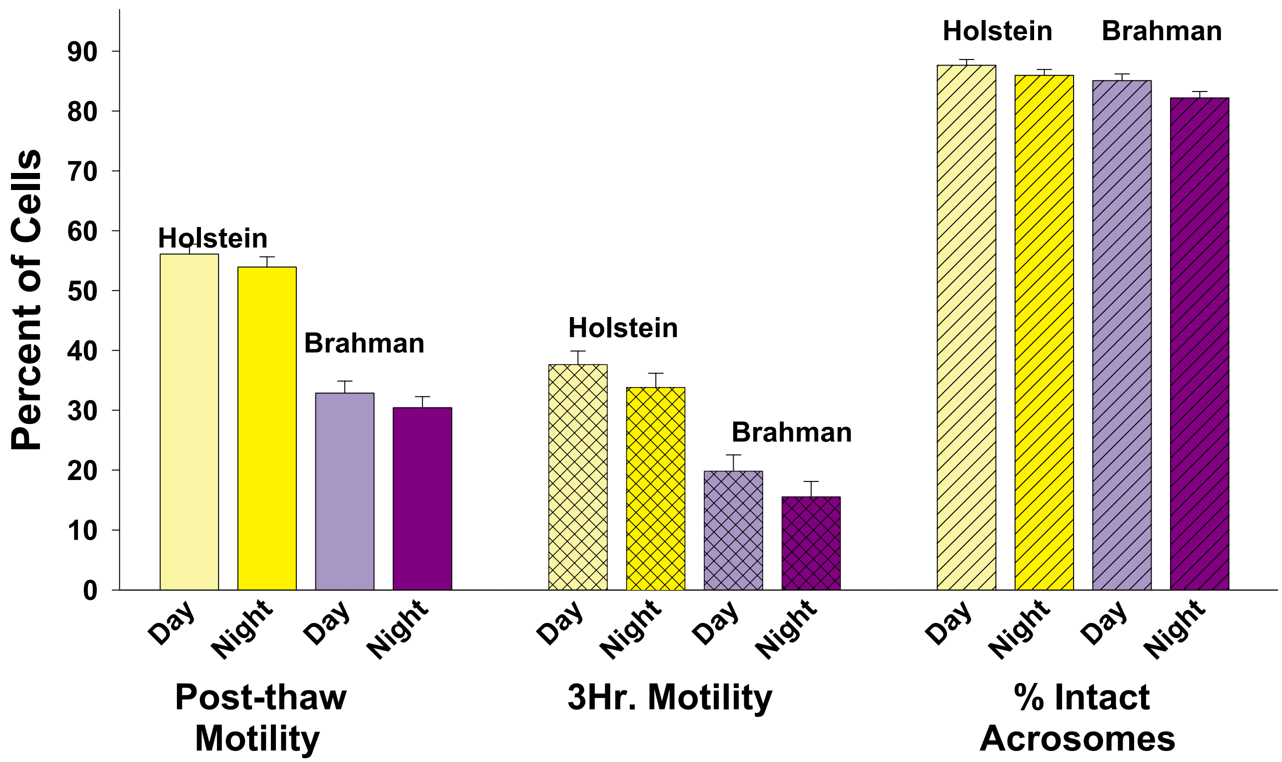

Figure 6. Least squares means graph of the team by treatment interaction for the semen quality parameters of post-thaw motility, 3-hour post thaw motility, and percent intact acrosomes (PIA).

Breed and bull nested within breed were both significant (P<.05) sources of variation in the model for the semen quality parameter of 3-hour post thaw motility (Table 13). The least squares means for the treatment by breed interaction for 3-hour post-thaw motility are graphed in Figure 6. The Holstein bulls demonstrated a significantly greater percentage of motile cells 3-hours post-thaw than Brahman bulls. Also, both the Holstein and Brahman bulls had a significantly greater percentage of motile cells from their day collection than from their night collection.

Table 13: Mixed model analysis of variance for semen 3-hour post thaw motility.

Source	DF	Type III SS	Mean Square	F Value	Pr > F
Breed[1]	1	4,224.1560	4,224.1560	9.57	0.0207
BullID(Breed)	6	2,786.8913	464.4819	5.62	0.0002
Trmt	1	225.6239	225.6239	2.73	0.1061
Team	1	25.3102	25.3102	0.31	0.5831
Team*Trmt	1	28.0414	28.0414	0.34	0.5635
Trmt*Breed	1	0.6974	0.6974	0.01	0.9273
Team*Breed	1	33.7185	33.7185	0.41	0.5266
Team*Trmt*Breed	1	128.6767	128.6767	1.56	0.2192
Collection	1	181.4249	181.4249	2.19	0.1461
Error: MS(Error)	42	3,473.7543	82.7084		
Corrected Total	56				
R-Square (Percent)		70.6006			

[1]Breed tested with Error DF of 6.137. Error = 0.94*MS(BullID(breed)) + 0.06*MS(Error)

The breed variation, treatment variation and collection variation were all significant (P<.05) sources in the model for percent intact acrosomes (Table 14). The least squares means for the treatment by breed interaction for percent intact acrosomes are graphed in Figure 6. Although small, there was a significant difference for both the Holstein and Brahman bulls between the night and day semen collections.

Table 14: Mixed model analysis of variance for percent intact acrosomes.

Source	DF	Type III SS	Mean Square	F Value	Pr > F
Breed[1]	1	130.1683	130.1683	13.87	0.0069
BullID(Breed)	6	54.3416	9.0569	0.62	0.7116
Trmt	1	71.4400	71.4400	4.91	0.0323
Team	1	15.1006	15.1006	1.04	0.3144
Team*Trmt	1	1.1054	1.1054	0.08	0.7843
Trmt*Breed	1	4.7764	4.7764	0.33	0.5699
Team*Breed	1	6.5366	6.5366	0.45	0.5066
Team*Trmt*Breed	1	3.0604	3.0604	0.21	0.6490
Collection	1	69.4210	69.4210	4.77	0.0347
Error: MS(Error)	42	611.6579	14.5633		
Corrected Total	56				
R-Square (Percent)		36.8579			

[1]Breed tested with Error DF of 7.284. Error = 0.94*MS(BullID(breed)) + 0.06*MS(Error)

Breed was a significant (P<.05) source of variation in the model for the semen quality parameter percent primary abnormalities (Table 15). Bull nested within breed and the team by breed interaction were also significant (P<.06) sources of variation in the model. The least squares means for the treatment by breed interaction are graphed in Figure 7. The Brahman bulls had significantly more abnormal primary cells both at night and during the day than did the Holstein bulls.

The treatment by breed interaction and the collection variance were both significant (P<.08) sources of variation in the model for the semen quality parameter percent secondary abnormalities (Table 16). The least squares means for the treatment by breed interaction are shown in Figure 7. The Holstein bulls had significantly more secondary abnormalities for the day semen collection than the night semen collection. In contrast, the Brahman bulls had significantly more secondary abnormalities for the night semen collection than the day semen collection.

Table 15: Mixed model analysis of variance for semen primary abnormalities.

Source	DF	Type III SS	Mean Square	F Value	Pr > F
Breed[1]	1	193.6819	193.6819	9.79	0.0190
BullID(Breed)	6	123.1560	20.5260	2.48	0.0384
Trmt	1	2.6553	2.6553	0.32	0.5743
Team	1	1.3703	1.3703	0.17	0.6863
Team*Trmt	1	2.3888	2.3888	0.29	0.5941
Trmt*Breed	1	0.3996	0.3996	0.05	0.8272
Team*Breed	1	31.8226	31.8226	3.84	0.0567
Team*Trmt*Breed	1	11.9669	11.9669	1.44	0.2361
Collection	1	24.2301	24.2301	2.93	0.0946
Error: MS(Error)	42	347.9161	8.2837		
Corrected Total	56				
R-Square (Percent)		50.1763			

[1]Breed tested with Error DF of 6.312. Error = 0.94*MS(BullID(breed)) + 0.06*MS(Error)

Table 16: Mixed model analysis of variance for semen secondary abnormalities.

Source	DF	Type III SS	Mean Square	F Value	Pr > F
Breed[1]	1	81.3104	81.3104	2.70	0.1454
BullID(Breed)	6	179.7065	29.9511	0.89	0.5092
Trmt	1	13.8508	13.8508	0.41	0.5241
Team	1	44.1001	44.1001	1.31	0.2582
Team*Trmt	1	0.0148	0.0148	0.00	0.9833
Trmt*Breed	1	111.0850	111.0850	3.31	0.0760
Team*Breed	1	7.7982	7.7982	0.23	0.6323
Team*Trmt*Breed	1	5.3353	5.3353	0.16	0.6921
Collection	1	297.9710	297.9710	8.88	0.0048
Error: MS(Error)	42	1,409.6276	33.5626		
Corrected Total	56				
R-Square (Percent)		38.8393			

[1]Breed tested with Error df of 6.884. Error = 0.94*MS(BullID(breed)) + 0.06*MS(Error)

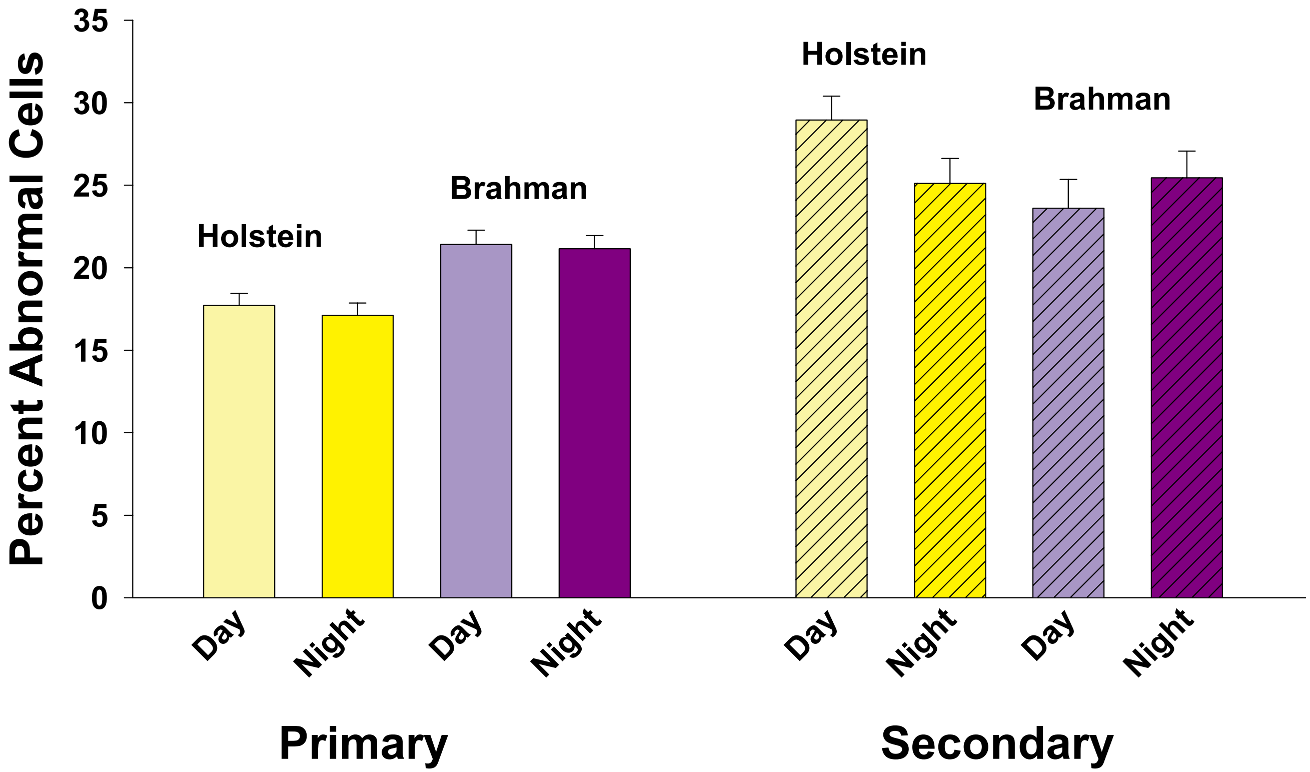

Figure 7. The least squares means for the treatment by breed interaction for the semen quality parameters of percent primary abnormal cells and percent secondary abnormal cells.

CHAPTER 5

DISCUSSION AND CONCLUSIONS

Discussion. Based on the current results, a night semen collection schedule does not seem to be warranted. Observing all the bull behavior parameters studied, there seemed to be no significant overall difference between the semen collection times of the two treatments; this includes time to first mount, time to first ejaculation, and refractory period. These findings were in contrast to those of Lagerlof (1954) who has shown that breed type has an influence on the willingness of the bull to copulate. However, there were significant differences of the recorded times of individual bulls nested within breed. This significance supports previous findings that it required nearly 10 times longer to stimulate beef bulls rather than dairy bulls (Almquist, 1973).

The number of libido scores received by the individual bull, with a higher number of scores suggesting inefficiency, was significantly different for both breed and bull nested within breed. The results suggest that the Holstein bulls were more efficient both at night and during the day as indicated by their receiving 1\6 the number of scores that the Brahman bulls received. This phenomenon could be explained by the fact that due to resource allocations, the Brahman bulls used for the present study were between 3 and 5 years of age, while the Holstein bulls were all 2 years of age. Fraser (1960) has shown that for bulls of 4 years of age and older, the reaction time for beef breeds is significantly greater than that of dairy breeds. However, it has been demonstrated by Fields (1982) that Brahman bulls do mature later than do *Bos taurus* breeds.

Both breed and treatment were significant sources of variation for libido score. This suggests that there exists a difference between the two breeds concerning libido, with Brahman bulls showing less libido. Both the Holstein and Brahman breeds demonstrated more libido at night. These findings agree with those of other researchers suggesting that dairy breeds may be more sexually active than beef breeds (Amann and Almquist, 1976), and that *Bos indicus* bulls generally show lower and more variable levels of libido than do *Bos taurus* bulls (Chenoweth, et al., 1996; Chenoweth and Osborne, 1975; Vale-Filho, et al., 1986).

Treatment variation was highly significant for managerial interruptions. Kerruish (1955) has also shown that a number of extraneous factors such as attendants and nearby structures can affect the bull's reaction. This research shows that there were far more interruptions for the semen collection team during the day than at night. Phone calls for collection team members, and co-worker questions from people not involved in the semen collection process were the most common disturbances. One advantage of the nocturnal semen collection was that the semen collection teams were the only people at the arena. Disturbances from outsiders were nonexistent because of the late hour.

While bull nested within breed was the only significant source of variation for the pre-freeze semen quality parameters of concentration, initial motility, and volume, the statistical model did not fit the combination of the parameters for total motile sperm harvested. Yet, more total motile sperm cells were harvested from the Brahman bulls than the Holstein bulls. This result was probably because the Brahman bulls were more mature than the Holstein bulls having the capacity to produce more cells.

Both day and night, regarding the post thaw motility and 3-hour post thaw motility, the Holstein bulls repeatedly produced significantly more motile cells than the Brahman bulls. Also, breed influenced percent intact acrosome and primary abnormality variation. Semen ejaculated over the course of the study was undergoing spermatogenesis before the bulls were put into the experiment. The Brahman bulls were in pastures with cows prior to the research, and subject to a number of environmental temperature stresses. As shown, ambient temperature is the one factor that affects scrotal surface temperature the most, and the scrotal surface temperature can have an affect on the primary abnormalities seen in an ejaculate (Coulter, 1988). Also, a mild thermal insult raising testicular temperature to approximately 35^{o}C can result in the production of abnormal spermatozoa without affecting sperm output (Vogler, 1993). Other possible stress indicators besides temperature is hormones. Adrenocorticotropic hormone (ACTH) is the major hormone associated with stress. A slight but significant increase in sperm head abnormalities and immature sperm has been observed in response to ACTH (O'Connor, 1985). Therefore, the higher primary abnormalities seen in the Brahman breed as compared to the Holstein breed could be a result of circumstances that occurred prior to the beginning of the research.

Secondary abnormalities seemed to decrease throughout the study. A negative time trend existed in the model for the collection variance indicating that the bulls were able to produce significantly better quality semen with less tail abnormalities as time progressed throughout the study. Since tail formation occurs later in the spermiogenesis process, thermal or other stresses were not a hindrance to the semen being produced and ejaculated at the end of the study, explaining how their were less secondary abnormalities as the research progressed. This agrees with the control used by Vogler (1993), and the Degelos (1995) post temperature insult trend.

Conclusions. Bull libido did seem to be affected by nocturnal collections. Both breeds used in this study demonstrated more libido at night versus during the day. However, even though both breeds of bulls showed more sexual activity and libido during the nocturnal semen collection, a faster, more efficient ejaculate with better semen quality than the day semen collection was not produced. This result suggests that a nocturnal semen collection schedule for A.I. organizations is neither necessary nor effective.

Extraneous environmental factors did prove to inhibit the semen collection process and should be kept to a minimum if day semen collections are to become more efficient. However, minimizing interruptions alone cannot be the only factor that will help produce the quality ejaculate for which A.I. organizations strive.

Future research is needed to investigate the bull within breed variation in the model that was common to all the bull behavior parameters in the current study. Perhaps more bulls within the two breeds would have shown a different outcome, eliminating some of the variation.

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APPENDIX

LEAST SQUARES MEANS (LSMEAN) AND STANDARD ERRORS (STD. ERROR).

Time to First Mount

Team	Treatment	LSMEAN	Std. Error
1	Day	2.4558	0.8908
1	Night	4.4950	0.9815
2	Day	4.3482	0.8558
2	Night	2.2032	0.8558

Number of Libido Scores

Treatment	Breed	LSMEAN	Std. Error
Day	Brahman	4.0013	0.6192
Day	Holstein	1.2492	0.5948
Night	Brahman	5.0874	0.6614
Night	Holstein	0.9986	0.6183

Libido Score

Treatment	Breed	LSMEAN	Std. Error
Day	Brahman	2.6538	0.1746
Day	Holstein	4.5003	0.1677
Night	Brahman	2.8031	0.1865
Night	Holstein	5.0604	0.1743

Managerial Interruptions

Treatment	LSMEAN	Std. Error
Day	1.7428	0.2463
Night	0.3804	0.2596

Total Motile Sperm Harvested

Breed	LSMEAN	Std. Error
Brahman	3685699.46	437053.98
Holstein	2317571.52	408778.99

Post-thaw Motility

Treatment	Breed	LSMEAN	Std.Error
Day	Brahman	32.8854	1.9964
Day	Holstein	56.0913	1.6608
Night	Brahman	30.4481	1.8636
Night	Holstein	53.9428	1.7260

3-Hour Post-thaw Motility

Treatment	Breed	LSMEAN	Std.Error
Day	Brahman	19.8330	2.7331
Day	Holstein	37.6494	2.2736
Night	Brahman	15.5646	2.5514
Night	Holstein	33.8308	2.3629

Percent Intact Acrosomes

Treatment	Breed	LSMEAN	Std.Error
Day	Brahman	85.0735	1.1469
Day	Holstein	87.6520	0.9540
Night	Brahman	82.2097	1.0706
Night	Holstein	85.9653	0.9915

Percent Primary Abnormalities

Treatment	Breed	LSMEAN	Std.Error
Day	Brahman	21.4092	0.8650
Day	Holstein	17.7163	0.7195
Night	Brahman	21.1408	0.8074
Night	Holstein	17.1074	0.7478

Percent Secondary Abnormalities

Treatment	Breed	LSMEAN	Std.Error
Day	Brahman	23.6049	1.7411
Day	Holstein	28.9462	1.4483
Night	Brahman	25.4413	1.6253
Night	Holstein	25.1061	1.5052

VITA

Jennifer H. Yates was born on October 12, 1977, in Philadelphia, Pennsylvania, to Dr. R. L. Horne and Mrs. Janice Long. She graduated high school from Louisiana School for Math, Science & Arts in 1995 and began her studies at Louisiana State University. She received her Bachelor of Science degree in Dairy Science in May, 2000.

Jennifer entered the Louisiana State University Graduate School to work toward her Master of Science Degree in Dairy Science in August of 2000. She studied reproductive physiology under the guidance of her major professor, Dr. John E. Chandler and will receive her degree at the May 2002 Commencement.

After graduation, Jennifer plans to attend the Louisiana State University School of Veterinary Medicine.