SEASONAL EFFECTS ON ESTROUS BEHAVIOR AND TIME OF OVULATION IN BEEF COWS

By

FRANKIE J. WHITE

Bachelor of Science

Texas Tech University

Lubbock, Texas

1998

Submitted to the Faculty of the
Graduate College of the
Oklahoma State University
in partial fulfillment of
the requirements for
the Degree of
MASTER OF SCIENCE
December, 2000

SEASONAL EFFECTS ON ESTROUS BEHAVIOR
AND TIME OF OVULATION
IN BEEF COWS

Thesis Approved:

Robert P. Wettemann

Thesis Adviser

Rodney D. Geisert

Gerald W. Horn

Alfred Carlozzi

Dean of the Graduate College

ACKNOWLEDGEMENTS

This thesis would not have been possible without the time, dedication, and hard work of others. I would like to first extend my sincere appreciation to my advisor, Dr. Robert P. Wettemann. Through his guidance, I have gained a wealth of knowledge. Dr. Wettemann always patiently answers my questions, yet continuously challenges me. He teaches by example, and I am grateful for the long hours and nights that he sacrificed for this research. Perhaps most importantly, Dr. Wettemann has not only taught me how to conduct research but also the importance of scientific integrity.

Dr. Rod Geisert also deserves gratitude for his guidance during my research. The teaching opportunities he made available to me were invaluable, and I hope to someday teach others with the same passion Dr. Geisert has exhibited. I also wish to thank Dr. Gerald Horn for his assistance and serving on my committee. True Red Raider fans are hard to find in Oklahoma!

Many people have contributed their time and assistance for this research. Mark Anderson, David Cox, Randy Jones, and Steve Welty worked around the clock assisting in data collection, and I am very grateful for their dedication and hard work. I appreciate Dr. Mike Looper's assistance in statistical analysis of the data, along with the late nights and endless hours that he gave during the project. LaRuth Mackey made a huge contribution through her expertise, guidance, and patience in the lab. She always greets

me with a smile and is a true friend to all students. I am grateful to Paula Cinnamon for her technical assistance in finishing this thesis. Her knowledge of Microsoft Word© is truly amazing. I have had the pleasure to work with and become friends with many graduate students. Specifically, I would like to thank Clay Lents and Norberto Ciccioli for their friendship and assistance with completing this thesis. I will never be able to repay "Ciccio" for being my chauffeur while I was on crutches following knee surgery.

I am eternally indebted to my parents for the sacrifices they made for my future. They are always ready to offer patience, encouragement, and knowledge. They have raised me to believe that I can accomplish anything, but without their steady hands guiding me I would have accomplished nothing. I thank my Father for instilling in me a love of science at young age. I also wish to give my love and thanks to my grandparents, Joe and Joyce Davis and Frank and Joyce White, who have shown me through example the value of a Christian life and hard work; they are my true heroes. To my brother who has always been my best friend, and I am grateful for your constant camaraderie and quiet support.

My wife made many sacrifices so that I could come to Oklahoma State University, none of which went unnoticed. Her love, encouragement, and friendship help me in everything I do. Because of her, I am a better man. Stephanie, you are my soul mate and I love you.

However, none of this would have been possible without my Heavenly Farther. His Word provides the perfect sentiment, as always. "But thanks be to God! He gives us victory through our Lord Jesus Christ." 1 Corinthians 15:57

TABLE OF CONTENTS

Chapter Page

LIST OF TABLES

Chapter II

Chapter III

LIST OF FIGURES

Chapter III

CHAPTER I

INTRODUCTION

There are about 100 million cattle and calves in the United States with a total value of 60 billion dollars, and of these 5.2 million are located in Oklahoma (United States Department of Agriculture, 1999). The importance of the cattle industry in the United States and Oklahoma cannot be underestimated. In 1999, the gross income from sale of cattle and calves in the United States and Oklahoma were 36.4 and 2 billion dollars, respectively. Oklahoma ranks fourth in number of beef cows and third in number of beef operations in the United States (United States Department of Agriculture, 1999).

Artificial insemination (AI) allows producers to take advantage of superior genetics and to reduce the time needed for genetic progress for specific traits. However, only 6 % of beef operations in the United States take advantage of the benefits of AI (NAHMS, 1997). Twenty-one percent of beef operations are located in Texas and Oklahoma (United States Department of Agriculture, 1999), and only 8% of these operations use AI (NAHMS, 1997).

For successful AI, the technician must deposit semen at the correct time relative to ovulation. Estrous behavior is the best external sign as to the time of ovulation and when to inseminate cows. Greatest conception rates in dairy cows occur with inseminations 4 to 12 h after the onset of estrous behavior (Dransfield et al., 1998). This requires the correct identification of the onset of estrus. Between 25 % and 60 % of dairy cows are inseminated at the wrong time due to inaccurate estrous detection (Reimers et al., 1985; Nebel et al., 1987).

Thirty-nine percent of beef operations do not use AI because extra time and labor are required and 20 % because of difficulties in performing inseminations (NAHMS, 1997). Only 3 % of producers believe that AI does not work. Much of the frustration and difficulty associated with AI are due to a general lack of information about estrous behavior and detection. In the dairy industry, AI is used regularly; however, 41 % of managers do not use estrous detection aids and 63% do not check for estrus at regular intervals (Coleman et al, 1985). However if estrous detection is done accurately, it can greatly increase the efficiency and profitability of herds. Dairy herds that use AI and maintain an average calving interval of 12 mo have superior estrous detection efficiency when compared with herds with calving intervals greater than 405 d (Bozworth et al., 1972). An increase in estrous detection efficiency will decrease days open and calving interval (Rounsaville et al., 1979).

While estrous behavior in dairy cows has been extensively studied, limited research has been conducted on estrous behavior in beef cows. Visual observation of beef heifers twice daily failed to detect 27 % of the estrous animals (Stevenson et al., 1996). The relationship between the onset of estrous behavior and time of ovulation must be determined for optimal timing of AI. However, time of ovulation relative to estrus has not been well defined in beef cows. A better understanding of estrus and time of ovulation could reduce time, labor, and difficulties that beef operations currently experience, and allow more producers to take advantage of the benefits of AI.

CHAPTER II

REVIEW OF LITERATURE

Introduction

Hormones from the hypothalamus, pituitary, and ovary interact throughout the bovine estrous cycle to control female receptivity (estrus) and release of the ova (ovulation) for fertilization. The normal bovine estrous cycle ranges from 18-22 d with a mean of 20 d for heifers and 21 d for cows (Moeller and VanDemark, 1951; Olds and Seath, 1951; Trimberger, 1956). Heape (1900) first classified and defined the 4 stages of the estrous cycle: estrus, metestrus, diestrus, and proestrus. These stages of the cycle are characterized by different endocrine, physiological, and behavioral changes.

Female gametes or ova mature in follicles in the ovary, which provide the correct physiological environment to prepare the ova for ovulation. Follicles grow and mature in waves, in which a cohort of follicles are recruited and begin to grow together (Rajakoski, 1960; Ireland and Roche, 1983a; Pierson and Ginther, 1984). Two or 3 waves are observed per estrous cycle. In cows, usually only one of the follicles per wave will become dominant, suppress growth of the other follicles, and synthesize and secrete the steroid hormone estrogen (Kaneko et al., 1991). Hormones from the ovary and pituitary ultimately determine if the dominant follicle from each wave will ovulate or regress and allow a new cohort of follicles to grow.

Estrus occurs just prior to ovulation and is characterized by maximal concentrations of estradiol from the preovulatory dominant follicle (Shemesh et al., 1972; Wettemann et al., 1972; Echternkamp and Hansel., 1973). Numerous physiological and behavioral changes are associated with estrus; most significantly, the cow is receptive and will stand to be mounted by either males or females.

The dominant follicle usually ovulates the day after estrus, and the remaining cells in the follicle become luteal tissue and secrete progesterone. Metestrus last from the end of estrous behavior until concentrations of progesterone in plasma increases above 1 ng/mL, about 3 d after estrus (Mason et al., 1972; Wettemann et al., 1972). During diestrus, progesterone secretion becomes maximal and dominates the cycle until approximately 15 d after estrus, at which time the function of the corpus luteum decreases (Garverick et al., 1971; Wettemann et al., 1972, Christensen et al., 1974;). The time from the start of minimal concentrations of progesterone in plasma until the onset of estrous behavior is termed proestrus.

There is probably a specific interval during which cows can be inseminated to achieve maximal fertility. Approximately 8 h after insemination, fertile sperm reach the site of fertilization, and by 12 to 24 h after insemination, only a small percentage of sperm are still in the reproductive tract (reviewed by Hawk, 1987). Bovine ova are fertile for an estimated 18 h after ovulation but may begin to loose fertility after only 10 h or less (Brackett et al., 1980; reviewed by Hunter, 1989). For fertilization to occur, semen must be deposited by either a bull or by AI at the correct time relative to the time of ovulation. The best external signal as to the time of ovulation is estrous behavior. A variety of management and environmental factors may alter estrous behavior. To develop better strategies for estrous detection and to determine the optimum time to AI, it is essential to understand the relationship between estrus and the time of ovulation.

Estrous Behavior

Successful reproduction requires the joining of the male and female gamete within a small window of time. This requires communication between the male and female to allow deposition of semen at the optimum time. In bovine and other species that exhibit estrus, the female will stand and allow the male to mount for only a limited time, termed estrus. Estrous behavior is controlled through the interaction of a multitude of hormones from various endocrine glands throughout the body, and concentrations of hormones are dramatically altered near estrus (reviewed by Allrich, 1994).

Endocrine Function at Estrus

Estradiol-17β is secreted by the dominant follicle on the ovary (Staigmiller et al., 1982; Ireland et al., 1984; Kaneko et al., 1991). The dominant follicle produces a preovulatory increase in the concentrations of estradiol in plasma. Estradiol in plasma does not increase until progesterone concentrations have reached their nadir (Henricks et al., 1971; Ireland et al., 1984).

Maximum synthesis and secretion of progesterone by the corpus luteum occur on d 14 to 16 (d 0 = estrus; Henricks et al., 1970; Garverick et al., 1971) or 6 d preceding estrus (Chenault et al., 1975) reaching concentrations in plasma of approximately 7 ng/mL. About 3 to 4 d before estrus, concentrations of progesterone in plasma begin to decline, and basal concentrations of less than 1 ng/mL occur 1 to 3 d prior to estrus

(Garverick et al., 1971; Henricks et al., 1971; Swanson et al., 1972; Wettemann et al., 1972) and remain minimal throughout estrus.

Peripheral concentrations of estradiol begin to increase gradually 3 to 4 d before the onset of estrous behavior (Henricks et al., 1971; Wettemann et al., 1972; Chenault et al., 1975; Glencross et al., 1981) and continue to increase until the day of estrus. Time of the maximal preovulatory concentration of estradiol has varied in previous reports. Henricks et al. (1971) concluded that maximum estradiol was attained 1 d prior to onset of behavior. Others have reported maximal concentrations from 12 h preceding estrus to simultaneous with the onset of estrous behavior (Shemesh et al., 1972; Wettemann et al., 1972; Glencross et al., 1981; Stevenson et al., 1998). Variation in results can be contributed to insufficient methods to detect estrous behavior or infrequent sampling. Visual observations to detect estrus at intervals throughout the day do not allow a precise timing of the onset and end of estrous behavior. Stevenson et al. (1998) concluded that maximum concentrations of estradiol-17β occurred 1.2 h prior to onset of estrous behavior. Estrus was determined with a radiotelemetric device (HeatWatch ®) that allowed continuous monitoring of cows and the precise detection of the onset of behavior, and blood samples were collected every 2 h. Maximal concentrations of estradiol in cows between 7 and 25 pg/mL have been reported (Henricks et al., 1971; Wettemann et al., 1972; Stevenson et al., 1998).

The preovulatory increase in plasma estradiol is not initiated until concentrations of plasma progesterone begin to decrease (Ireland et al., 1984). Induction of luteolysis with prostaglandin $F_2\alpha$ results in decreased concentrations of progesterone in plasma with a nadir by 24 h post injection (Chenault et al., 1976), which is associated with an increase in concentrations of plasma estradiol. Maximal concentrations of estradiol occurred 52 (Chenault et al., 1976) to 58 h (Stevenson et al., 1998) following treatment with prostaglandin injection, depending on the frequency of sampling.

Elevated concentrations of estradiol in plasma during proestrus are associated with the preovulatory surge of luteinizing hormone (LH) and follicle-stimulating hormone (FSH). Concentrations of LH and progesterone in plasma are negatively correlated (Garverick et al., 1971; Christensen et al., 1974). Chenault et al. (1975) concluded that maximum estradiol concentrations and the LH surge occur simultaneous, but others found that a preovulatory LH surge proceeds maximum concentrations of estradiol by 3 to 4 h (Kaneko et al., 1991; Stevenson et al., 1998). LH and FSH surges occur concurrently (Dobson, 1978; Kaneko et al., 1991) with maximum concentrations 3 to 8 h after the onset of estrous behavior, which was determined by visual observation (Henricks et al., 1970; Christensen et al., 1974; Chenault et al., 1976). Stevenson et al. (1998) found maximum concentrations of LH at 2.3 h after the onset of estrus that was determined with a radiotelemetric device (HeatWatch ®), and 58.8 h after treatment with $PGF_2\alpha$. Peripheral concentrations of LH remain elevated after the ovulatory surge for 8 to 12 h (Henricks et al., 1970; Christensen et al., 1974; Chenault et al., 1975). Maximum estradiol concentrations in plasma decreased by 50 % within 5 h after concentrations of LH were maximal (Chenault et al., 1975). Henricks et al. (1971) found that estradiol in plasma began to decrease 2 to 5 h after the onset of estrus, and Glencross et al. (1981) found that concentrations of estradiol in plasma were minimal by 12 h after the end of estrus.

Effects of Estrogens on Estrous Behavior

Treatment of ovariectomized heifers (Ray, 1965; Rajamahendran et al., 1979; Cook et al., 1986) or cows (Nessan and King, 1981; Cook et al., 1986) with estradiol benzoate induces standing estrus. Estradiol treatment of ovariectomized cows also induces other signs of estrus including vulva sniffing, head-head contact with herd mates, and mounting other animals in (Katz et al., 1980). Synchro-Mate B (an estrous synchronization treatment that utilizes an estradiol valerate injection) induced standing behavior in 57% and 55 % of ovariectomized heifers and cows, respectively (McGuire et al., 1990). Immunization of normal heifers against estradiol-17β inhibits expression of estrus after the preovulatory increase in estradiol (Martin et. al., 1978).

Concentrations of estradiol in plasma must reach a threshold to initiate estrous behavior; however, higher concentrations do not result in greater expression of estrus. A minimal dose of 300 µg of estradiol benzoate was required to invoke estrous behavior in ovariectomized cows and heifers, but increasing doses to 2400 µg did not increase the duration of estrus or frequency of behavioral interactions (Cook et al., 1986). Superovulated heifers have greater than normal concentrations of estradiol in plasma; however, estrous behavior was similar between superovulated and non-superovulated cycles (Coe and Allrich, 1989). Therefore, estrogen concentrations above the threshold required to induce behavior do not increase the expression of estrus.

Estrogens stimulate estrous behavior via receptors in the brain. Electrolytic lesions of the ventral hypothalamus, above the median eminence, inhibit estrus in ewes (Clegg et al., 1958). Estrogen implants in the mediobasal hypothalamus induced estrous behavior in ovariectomized ewes; however, implants in the preoptic area of the hypothalamus did not cause estrous behavior (Blache et al., 1991). Therefore, estrogens act at a defined region of the hypothalamus to influence estrous behavior.

Other Hormones That Effect Estrous Behavior

Estrogens are the primary regulators of estrus; however, other hormones may interact with estrogen to alter the effects. Rajamahendran et al. (1979) concluded that progesterone has an inhibitory effect on estrous behavior when given concurrent with estradiol in ovariectomized heifers. In a similar study, Allrich et al. (1989) observed no effect of simultaneous injections of progesterone with estradiol. A priming period with progesterone increases the response of ewes to estrogens (Robinson, 1954; Fabre-Nys and Martin, 1991), but this is not true of the bovine. Pretreatment of ovariectomized heifers with progesterone actually inhibited estradiol induced behavior (Carrick and Shelton, 1969), and the inhibition of behavior was linear with increasing progesterone concentrations (Davidge et al., 1987). Treatment of cows with estradiol at doses greater than normal physiological amounts can induce a period of refractoriness. In heifers that are refractive, estradiol will not stimulate estrous behavior, and progesterone treatment for 5 d is required before estrogens will induce behavior (Carrick and Shelton, 1969).

While estrogen and progesterone are the major controllers of estrus, other hormones may also influence estrous behavior. Testosterone, but not androstenedione or dihydrotestosterone, induced estrus in ovariectomized heifers, but the frequency of estrous interactions was less than with estradiol (Katz et al., 1980). A greater than physiological dose of testosterone may be required to invoke behavior (Nessan and King, 1981), and the effect could be due to its metabolic conversion to estradiol. Recombinant bovine growth hormone or somatotropin (BST) may influences estrous behavior.

Intensity of estrus in ovariectomized heifers was reduced by treatment with BST (Lefebvre and Block, 1992), but the study was limited to a small number of animals.

Stress may negatively alter estrous behavior in cows. The pituitary adrenal axis is stimulated during stress resulting in the release of adrenocorticotropin (ACTH) from the pituitary, which stimulates the secretion of the glucocorticoid cortisol. ACTH and cortisol prevented the expression of estrus in cows (Stoebel and Moberg, 1982). Dissimilar results have been reported in ovariectomized cows and heifers. ACTH and dexamethasone, a synthetic glucocorticoid, decreased estrous behavior in estradiol treated ovariectomized animals, but cortisol did not influence estrus (Cook et al., 1987; Allrich et al., 1989; Hein and Allrich, 1992). Because dexamethasone is synthetic, it may alter estrus through a non-glucocorticoid mechanism, or metabolism of dexamethasone may be slower than cortisol. Turner et al. (1999) found that repeated acute cortisol treatments did not influence estrous behavior in gilts, but if cortisol concentrations were sustained estrous behavior was inhibited. Differences between studies could be due to the small number of cows treated, types of treatments, or possible differences between intact and ovariectomized animals.

Estrous Behavior in Cows

Due to limited use of AI in the beef industry, research regarding estrous behavior is limited, but behavior of dairy cows has been studied extensively. Estrous behavior is associated with numerous physiological and behavioral changes. Standing while mounted by either a bull or herd mate is the best external sign of estrus (Esslemont et al., 1980; Hurnik and King, 1987) and is the most common factor used to quantify estrous behavior. The onset of estrus is typically defined as the first time a cow stands while

Table 1. Estrous behavior of beef and dairy cows determined with different methods of detection

Type	Method of detection	Duration of standing estrus, h	Mounts/ estrus, no	Reference
Dairy	HeatWatch®	7 – 10	8 - 14	Walker et al., 1996; Dransfield et al., 1998; Xu et al., 1998
Dairy	Continuous observation	8 – 13	14 - 33	Pennington et al., 1985; Walton et al., 1987
Beef (Bos Taurus)[a]	HeatWatch®	4 – 14	9 – 50	Hurnik and King, 1987; Stevenson et al., 1996; Mathew et al., 1999; Rae et al., 1999; Yelich et al., 1999
Beef (Bos Indicus)	Continuous observation	7.7	9.2	Mattoni et al., 1988
Beef (Bos Indicus)	HeatWatch®	6.7 – 8.3	22 - 25	Rae et al., 1999; Yelich et al., 1999

[a] Bos Taurus or Bos Indicus x Bos Taurus

mounted, and the end of estrus is the last mount received by the cow. A summary of the duration of estrous behavior and the number of mounts in cows is presented in Table 1. The average duration of estrus for beef cows ranged between 4 and 14 h with between 9 and 50 mounts per estrus (determined by continuous observation of cows; Hurnik and King, 1987; Mattoni et al., 1988; Stevenson et al., 1996). Breeds, environment, and management differed between herds and could explain differences observed. Angus x Hereford x Brahman beef heifers had a duration of estrus of 14 h with 50 mounts when determined with a rump mounted electronic sensor (HeatWatch®; Stevenson et al., 1996). Similarly, Angus cows were estrus for 14 hours and received 35 mounts (determined with HeatWatch®; Yelich et al., 1999).

Estrous behavior in beef cows is highly variable, and cows may be estrus for only .5 h or as long as 26.2 h with 3 to 154 mounts per estrus (Hurnik et al., 1987; Stevenson et al., 1996; Lorton et al., 1999). Intensity of estrous behavior may increase with age. Duration of estrus was 50 % longer in multiparous than primiparous dairy cows (determined with HeatWatch®; Walker et al., 1996), and the number of mounts per h of estrus was also greater in mature dairy cows as compared with heifers (Gwazdauskas et al., 1983). However Mathew et al. (1999) concluded that the length of estrus did not differ with age in beef cows, but the number of mounts per estrus increased with age (determined with HeatWatch®).

Dairy and Bos Indicus cows may exhibit a less intense estrus than Bos Taurus beef cows. Dairy cows had an estrus of 8 to 13 h with 14 to 33 mounts when estrus was evaluated using continuous visual observation (Pennington et al., 1985; Walton et al., 1987), and dairy cows were in estrus for 7 to 10 h with 8 to 14 mounts in recent studies using a rump electronic mounting sensor, HeatWatch® (DDx Inc.; Walker et al., 1996; Dransfield et al., 1998; Xu et al., 1998). Genetics, management, and environmental factors could result in differences between estrous behavior of dairy and beef cows. Beef cows housed in pens with cement flooring similar to those used in dairy herds were estrus for only 4.4 h (determined by continuous observation; Hurnik and King, 1987).

Bos Indicus cows were in estrus for only 6.7 to 8.3 h with 9 to 25 mounts (Plasse et al., 1970; Mattoni et al., 1988; Rae et al., 1999; Yelich et al., 1999). Rae et al. (1999) found breed differences in duration of estrus and number of mounts in Angus, Brahman, and crossbred cows. A less intense estrus in Bos Indicus cows could be due to a difference in endocrine function. Fewer ovariectomized Bos Indicus cows exhibited standing behavior after receiving the same concentration of estradiol as Bos Taurus cows (Rhodes and Randel, 1978).

Physiological and other behavioral changes, in addition to standing while mounted, are associated with the estrous period, and can be used to aid in identifying estrous cows. Secondary estrous characteristics including head butting, chin resting, sniffing, and licking the vulva occur with increased frequency near standing estrus (Esslemont et al., 1980; Hurnik and King, 1987; Vailes and Britt, 1990). Estradiol induces these secondary estrous characteristics as well as standing estrus in ovariectomized cows (Katz et al., 1980). Estrous characteristics are observed at a low frequency throughout the estrous cycle with an increase in intensity near estrus. An increase in secondary characteristics is observed shortly preceding and following standing estrus (Esslemont et al., 1980); therefore, secondary estrous characteristics should not be used alone to identify estrous cows.

Estrous cows often mount other estrous cows or attempted to mount non-estrus cows. Seventy nine percent of heifers mounting other animals were in estrus (Hurnik et al., 1975), and estrous heifers made 67 % of attempted mounts. Diestrus heifers initiated only 5 % of mounts (Helmer and Britt, 1985). While non-estrus cows can be observed mounting, the intensity of mounting is greater for estrous cows (Helmer and Britt, 1985). Although standing to be mounted by other cows is the best indicator of estrus, Stevenson et al. (1983) observed similar conception rates for 732 inseminations of dairy cows when standing to mount or mounting other cows was used to determine time of AI.

Physical activity of cows also increases near estrus. An increase in the amount of time dairy cows spent walking and a decrease in eating and resting were observed during estrus with a time-lapse video recorder (Hurnik et al., 1975). Confined beef cows had greater amounts of time standing and walking than lying down on the day of estrus compared with other days (Hurnik and King, 1987). Pedometers, instruments that attach to the legs of the cow and record motor activity, indicated increased activity readings during estrus of dairy cows (Kiddy, 1977; Pennington et al., 1986). Activity of estrous dairy cows housed in free stalls and comfort stalls increased 4 and 2.75 fold, respectively (Kiddy, 1977). Pedometer measurements on dairy cows were positively correlated with the number of mounts received, mounts made, chin resting on pelvis, times sniffed genitalia, head buttings, and times followed other cows (Pennington et al., 1986).

Physiological changes occur in the reproductive tract near estrus. Vaginal temperatures increase the day of estrus (Lewis and Newman, 1984), and standing estrus was correlated with maximum vaginal and rectal temperatures after norgestomet implant removal (Rajamahendran and Taylor, 1991). Vaginal pH reached a nadir the day of estrus (Lewis and Newman, 1984), and hydration of vaginal tissue was greatest at estrus (Lewis et al., 1989). Electronic resistance of vaginal tissue, measured via implanted electrodes, was least on the day of estrus and associated with alterations in ovarian steroids (Leidl and Stolla, 1976; Feldman et al., 1978; Lewis et al., 1989).

Vaginal mucus discharge was observed in 50% (Stevenson et al., 1983) and 60% (Hackett and McAllister, 1984) of estrous dairy cows, and conception rates were greater when cows had mucus discharge when inseminated (Stevenson et al., 1983). Cows with a discharge were estrus at insemination, but some of the cows without discharge were not estrus and therefore had reduced conception rates.

Pheromones, odors found in the mucus or urine of estrous cows, may serve as a stimulus for bulls and other cows to induce mounting or other sexual behavior. Mounting of diestrous cows can be induced when estrous mucus is applied to the hindquarters (Nishimura et al., 1991). Animal models have been developed to detect pheromones produced by estrous cows. Dogs (German Shepards and Labrador retrievers) were trained to detect differences in estrous and diestrus vaginal swabs (Kiddy et al., 1978). Trained rats (Ladewig and Hart, 1981) and an electronic polymer sensor (Lane and Wathes, 1998) have also been developed to distinguish between estrous and diestrous bovine odors.

Chemical compounds associated with estrous odors are distributed throughout the body of cows, and attempts have been made to characterize and identify the chemical structure of the odors. Nishimura et al. (1991) separated estrous mucus using dialysis and ion-exchange chromatography. When samples were applied to diestrous cows, only the dialyzable and neutral fractions would result in mounting. Using a rat bioassay, Dehnhard and Claus (1996) concluded that the mounting inducing pheromone is a polar, low molecular weight, neutral molecule which is sensitive to changes in pH.

Management

The onset of estrous behavior is hormonally controlled and as a result very precise compared to the end of estrus. The intensity and duration of estrus is altered by external factors that vary between management and environmental conditions between herds. Type of housing affects estrous behavior of dairy cows (De Silva et al., 1981; Gwazdauskas et al., 1983), and differences in the intensity of estrus may be due to differences in footing. Pennington et al. (1985) concluded that 80% of mounting occurred in areas with the best footing. Duration and intensity of estrus increased when dairy cows had access to dirt and cement surfaces rather than cement only (Rodtian et al., 1996). Estradiol treated ovariectomized cows had a longer duration of estrus and increase mounting and standing behavior on dirt compared with cement surfaces (Britt et. al., 1986). Dairy cows mounted estrous cows more times on dirt rather than concrete; however, secondary estrous signs did not differ between footing (Vailes and Britt, 1990). If standing estrous is used to identify cows for AI, cement flooring would reduce estrous detection rate; therefore, secondary signs of estrus should be used in addition to standing behavior when cows are housed on cement flooring. Milking, feeding, and moving cows may also influence estrous behavior (Pennington et al., 1985; Pennington et al., 1986; Hurnik and King, 1987), and detection should not be done at these times.

Management practices to synchronize estrus could alter estrous detection. Estrous behavior did not differ between cows treated with prostaglandin $F_{2\alpha}$ to synchronize estrus and cows with a natural estrus (Pennington et al., 1985; Walton et al., 1987; Walker et al., 1996). However, altering the number of sexually active cows in a herd may increase chances of estrous detection (Hurnick et al., 1975; Pennington et al., 1985; Walton et al, 1987). When only one cow was sexually active, estrous behavior was short with few mounts (Walton et al, 1987), and as the number of sexually active cows increased from 1 to 3, the number of mounts also increased from 11.2 to 52.6 (Hurnick et al., 1975).

Methods to Assist in Estrous Detection

Standing behavior is the best external sign of estrus, and visual observation is the most common method of estrous detection. However, visual observation is time and labor intensive, and estrous behavior is highly variable. Cows may not exhibit standing behavior or may have a short estrous with few mounts. Therefore, intensive or continuous observations would be necessary to identify all estrous cows. Visual observation twice daily for .5 h identified only 51 % of estrous dairy cows (Stevenson and Britt, 1977), and failed to detect 27% of estrous beef heifers (Stevenson et al., 1996). Only 19 % of first postpartum ovulations in dairy cows were identified by twice daily visual observation (Peter and Bosu, 1986). These difficulties have led to the development of methods to assist in identification of estrous cows (Table 2; reviewed by Lehrer et al., 1992, and Senger, 1994).

Due to the inconsistency and infrequency of mounting behavior, methods have been developed to either increase sexual interactions or identify mounts that are not visually observed. Surgically altered, infertile bulls increase the number of mounts cows receive during estrus (reviewed by Foote, 1974). Surgical alterations of bulls is expensive and handling bulls can be dangerous. Androgenized cows are also sexually aggressive and can be used to identify estrous cows. They marked 26 % of cows not

Table 2. Efficiency and accuracy of different methods of estrous detection

Method of detection	Efficiency [a]	Accuracy [b]	References
Visual observation (2x daily)	19 (postpartum cow) – 74 (non pregnant cow)	68 to 100	Stevenson and Britt, 1977; Williams et al., 1981; Peter et al., 1986; Redden et al., 1993; Stevenson et al., 1996; Van Vliet et al., 1996
Tail paint	98.4	97.6	Xu et al., 1998
Pedometers	55 – 92	21 – 83	Williams et al, 1981; Peter et al., 1986; Moore et al., 1991
Vaginal Temperature	81	71	Redden et al., 1993
Dogs trained to identify estrous odors	68 to 99		Kiddy et al., 1978, Kiddy et al., 1984
Androgenized females	47 – 74	80-100	Stevenson and Britt, 1977; Gwazdauskas et al, 1990,
Dye filled rump devices	70	29	Williams et al., 1981
HeatWatch®	91.7-100	100	Stevenson et al. 1996; Xu et al., 1998

[a] % estrous cows identified by method of estrous detection.

[b] % cows correctly identified as estrus by method of detection.

identified by visual observation, and were as efficient as infertile bulls (Kiser et al., 1977). Androgenized females detected between 47 and 74 % of estrus cows with high accuracy, but the ability to detect estrus varies greatly between individual cows (Stevenson and Britt, 1977; Gwazdauskas et al, 1990).

Estrous detection aids that attach to the tail head of cows are commercially available. Dye filled capsules (Kamar®; Steamboat Springs, Co) were highly efficient in identifying estrous cows (70 % efficiency; Williams et al., 1981), and they are activated by pressure from the brisket of the mounting cow. However, false activation of patches (44 to 49 %; Stevenson and Britt, 1977; Gwazdauskas et al., 1990) was observed, and accuracy of the devices was low (29 %; Williams et al., 1981). Dye filled rump mounted devices should be used in addition to visual observation or other estrous detection methods to reduce inaccuracy of the patches.

Tail paint or livestock markers are commonly used as estrous detection aids. A strip of paint is applied to the tail head and along the rump of the cow. When a cow is estrus, the brisket of the mounting cow disperses the paint. This method was used to detect 98.4 % of dairy cows in estrus with 97.6 % accuracy in two commercial herds (Xu et al., 1998); however, these herds were extremely well managed, and managers were highly skilled in estrous detection. Tail paint used in addition to visual observation is an effective method to detect estrus, but cows with few mounts or heifers that exhibit less mounting behavior are difficult to detect. An aerosol of contrasting color applied over a tail paint strip may increase the efficiency and accuracy of estrous detection. Animals are considered estrus if the tail paint color can be identified. This method detected 94.5 % of synchronized heifers (Macmillan et al., 1988); however, results may differ if heifers are not synchronized.

Motor activity of cows can be measured and used in estrous detection programs. Efficiency of pedometers attached to the rear leg of cows varied with between 55 – 92% of estruses identified, and an accuracy of 21 – 83 % (Williams et al, 1981; Peter and Bosu, 1986; Moore and Spahr, 1991). Peter and Bosu (1986) identified more first postpartum ovulations with pedometers than with visual observation (76 vs 35% of ovulations observed), and Williams et al. (1981) identified the greatest percent of estrous cows when both visual observation and pedometers were used (93%). Variation in efficiency and accuracy of pedometers could be due to type of mechanical equipment as well as differences in management practices. Pedometers should be used in conjunction with visual observation or other estrous detection methods to maximize the percentage of estrous cows identified and to decrease errors in detection.

Other technologies have been developed to identify physiological changes that occur during estrus. The onset of standing estrus after norgestomet implant removal was correlated with the hour of maximal vaginal and rectal temperatures ($r = .82$ and $.81$, respectively; Rajamahendran and Taylor, 1991), and temperature radio transmitters have been developed to identify temperature changes in the skin of the ear and vaginal wall. Eighty-one percent of the ovulations were detected with 69 % accuracy, if a cow was considered estrus if the temperature increased between .3 and 1 °C above the previous 4 d average (Redden et al., 1993). Probes have also been developed to measure the electronic resistance of the anterior vagina, and when probe measurements were taken every 3 d, 52% of cows inseminated with low measurements were pregnant (Foote et al., 1979).

German Shepherds and Labrador Retrievers, trained to identify estrous related odors, correctly identified between 68.1 to 81.6 % of vaginal swabs from estrous cows. Dogs accurately distinguished between estrus and diestrous urine (77.8 % efficiency) in the lab, and correctly identified 87.3 % of estrous cows on the farm (Kiddy et al., 1978). Dogs could discern between estrous and non-estrous milk and blood of cows with 99 and 94 % efficiency, respectively (Kiddy et al., 1984).

Enzyme-linked immunosorbent assays (ELISA) have been developed to quantify progesterone in milk and are commercially available to producers (reviewed by Nebel, 1988). These assays allow producers to distinguish between high and low progesterone concentrations in a quick and precise test, which can be done on the farm. Concentrations of progesterone in milk determined by ELISA assays on commercial dairy farms were highly correlated with values measured by radioimmunoassays in the laboratory (Nebel et al., 1987). Measuring milk progesterone is not a practical method to identify estrous cows, but it can be used as a tool to identify errors in estrous detection. Cows that are suspected to be estrus, but have high progesterone, should not be inseminated.

The previous methods of estrous detection are useful tools if used in conjunction with visual observation; however, due to low efficiency or accuracy, they are not adequate to replace visual observation. For optimal estrous detection, cows should either be visually observed or monitored continuously. Electronic mount sensors that attach to the rump of the cow have been developed to allow continuous monitoring of mounting behavior. The HeatWatch® (DDX, Denver CO) system uses a pressure sensor and radio transmitter to monitor behavior. The sensor and transmitter are battery operated and contained in a patch, which can be glued to the rump of the cow. When a cow is mounted, the transmitter is activated by pressure from the mounting herd mate, a radio signal is sent to a receiver. The cow is identified and the date and time are recorded in a computer database. This allows continuous monitoring and identification of estrous cows. The HeatWatch® system detected 91.7 % of estrous dairy cows in two commercial herds with 100 % accuracy (Xu et al., 1998). Estrous cows that were not detected had lost their transmitters. Stevenson et al. (1996) detected 27 % more synchronized beef heifers with HeatWatch® than with visual observation.

Follicular Growth and Ovulation

During prenatal development, the fetal bovine ovary develops, and in the heifer, the maximal number of ova in the ovary are present at birth. However, these ova are incapable of fertilization and must mature before ovulation. Only a small number of ova mature and ovulate, and most ova degenerate. The growth, maturation, and ovulation of ova are hormonally controlled by the precise interaction of the hypothalamus, pituitary, and the ovary.

Folliculogenesis

Meiotic divisions of oocytes occur prior to birth about 75 to 80 d of gestation, and maximal numbers of ova are found in the ovary (170, 000) at 150 to 170 d of fetal development in the bovine (Erickson, 1966a). After 170 d of prenatal development, ova are arrested in meiotic prophase and remain so until ovulation (Erickson, 1966a, reviewed by Richards, 1980). At 170 d of development, primordial follicles are formed, and consist of an oocytes surrounded by one layer of follicular cells (Erickson, 1966a). The function of the follicle is to provide the correct physiological environment for the ovum to grow and mature to prepare for ovulation. Approximately 133,000 primordial follicles are located on the bovine ovary at birth, but the number declines to only 3,000 by 15 to 20 years of age (Erickson, 1966b).

Folliculogenesis is the growth and maturation of primordial follicles and ova to mature follicles ready for ovulation and fertilization (Reviewed by Spicer and Echternkamp, 1986; Fortune, 1994). Majority of follicles on the ovary are primordial follicles (Erickson, 1966b). They are held in a resting state and serve as a pool from which follicles will begin growing and preparing for ovulation throughout the life of the cow. During folliculogenesis, a primordial follicle with a single layer of follicular cells grows and matures into a graafian follicle ready for ovulation. A graafian follicle consists of three cell types; the oocyte, granulosa, and theca cell. The graafian follicle has an antrum or fluid filled center. Growth of a primordial follicle to a graafian follicle takes approximately the length of two estrous cycles (Lussier et al., 1987).

An antrum is formed when bovine follicles reach .24 (Lussier et al., 1987) to .5 mm (Marion et al., 1968) in diameter. Follicles 1 to 2 mm in size have developed the three cell types of a graafian follicle (Marion et al., 1968). Follicles begin to grow prior to birth in the bovine, and follicles with an antrum develop in the fetal ovary by 250 d of gestation (Erickson, 1966a). Marion et al. (1968) concluded that follicles grow at a continuous rate that is independent of follicular development; however, the current hypothesis is that rate of follicular growth increases with the size of the follicle (reviewed by Fortune, 1994). During early follicular growth, granulosa cells are proliferating, and the rate of growth is slow; a follicle matures from .13 to .67 mm in diameter in 27 d (Lussier et al., 1987). However, large follicles mature at a much faster rate and growth occurs mainly through antrum fluid expansion rather than cell growth. Only 8 d are needed for an antral follicle to increase from 3.7 to 8.6 mm (Lussier et al., 1987). While all growing follicles are preparing for ovulation, very few actually ovulate. The fate of 99 % of growing follicles is regression of cell size and atresia (Ireland, 1987).

Follicular Waves

Early studies hypothesized that follicles mature and grow continuously on the bovine ovary independent of the estrous cycle (Choudary et al., 1968; Dufour et al., 1972). Development of sensitive techniques to evaluate follicular growth have allowed scientists to conclude that follicles grow in groups or waves. In the bovine, a cohort or group of follicles leaves the resting pool and simultaneously mature preparing for ovulation. A follicular wave describes the growth of a cohort and consists of 3 phases: recruitment, selection, and dominance. Initially, a group of follicles mature for ovulation, but only one follicle will be selected and continue growth while the rest of the cohort undergo atresia. The single healthy follicle establishes dominance over the rest of the cohort and prevents other follicles from maturing. Dominance is maintained until either the follicle ovulates or undergoes atresia itself allowing a new wave of follicles to grow. The stage of the estrous cycle and secretion of gonadotropins determine the fate of the dominant follicle.

Follicular waves are observed in both pregnant (Pierson and Ginther, 1986; Ginther et al., 1996) and cyclic cows, with a possibility of 2 to 4 waves occurring during an estrous cycle. Pierson and Ginther (1984, 1987) concluded that two waves occur during the estrous cycle of cows. However, Ginther et al. (1989) observed both 2 and 3 waves during estrous cycles with 2 waves occurring most frequently in heifers (Ginther et al., 1989). Other authors have concluded that 3 waves occur most predominantly during the bovine estrous cycle (Savio et al. 1988; Sirosis and Fortune, 1988).

In dairy heifers with 3 follicular waves, a cohort of follicles are recruited and begin to grow on d 1.9, 9.4, and 16.1 of the estrous cycle, respectively (d 0 = estrus; Sirois and Fortune, 1988). Dominant follicles of each wave reached maximum size on d 6, 16, and 21 of the cycle, respectivly. The ovulatory follicle is the dominant follicle of the third follicular wave and may be larger than the dominant follicles of the previous waves (Savio et al., 1988).

Growth of follicular waves are similar for cycles with 2 or 3 waves until d 16 of the cycle (Savio et al., 1988; Ginther et al., 1989). In heifers with two follicular waves, the dominant follicle continues to grow after d 16. and becomes the ovulatory follicle, but if three follicular waves occur, the dominant follicle regresses and a new follicular wave begins. Because of a longer period of growth, dominant follicles of heifers with only two follicular waves may be larger than for heifers with three waves (Ginther et al., 1989). The length of the luteal phase may determine the number of follicular waves, because corpus luteum regression occurs later in cows with two follicular waves (Ginther et al., 1989).

Follicular Growth and Atresia

During recruitment, growth of a cohort of follicles is initiated by secretion of follicle stimulating hormone (FSH; reviewed by Fortune 1994). Increased concentrations of FSH in serum precedes the onset of a follicular wave throughout the bovine estrous cycle (Sunderland et al., 1994; Ginther et al. 1998), and cows with two or three waves have two and three surges of FSH, respectively (Adams et al., 1992). Increased concentrations of FSH in serum are detectable 2 to 4 d prior to the growth of a new cohort of follicles (Adams et al., 1992) and continue to increase from 8 h prior to until 8 h after wave emergence (Ginther et al. 1998). Follicular waves during pregnancy are also initiated by increased concentrations of FSH in serum (Ginther et al., 1996). Treating heifers with follicular fluid prohibits secretion of FSH and delays the recruitment of a new cohort of follicles (Turzillo and Fortune, 1990; Adams et al., 1992), and removal of the dominant follicle stimulates a new follicular wave which is preceded by an increase in FSH in serum (Adams et al., 1992). Injecting FSH after removing the dominant follicle results in recruitment of 4 to 7 times as many follicles compared with control animals (Gibbons et al., 1997).

After a new wave of follicles begin to grow, one follicle will establish dominance over the rest of the cohort, and the other follicles become atretic. Once the dominant follicle is selected, the rate of atresia in the rest of the follicles in the cohort increases (Lussier et al., 1987). The selected follicle suppresses the growth of the rest of the cohort by secreting factors that act either locally or systemically.

The dominant follicle causes atresia of other follicles in its cohort as well as prevents the emergence of another follicular wave by inhibiting FSH secretion. Follicles ≥ 5 mm may decrease FSH concentrations (Gibbons et al., 1997), and large dominant follicles have increased ability to suppress FSH concentrations (Ginther et al., 2000).

The dominant follicle suppresses FSH and growth of other follicles through secretion of estradiol (Ginther et al., 2000). Estradiol concentrations increase as FSH concentrations are suppressed (Evans et al., 1997). Follicles can be divided into estradiol active (greater concentrations of estradiol than either progesterone or androgens) and estradiol inactive follicles. Dominant follicles are estradiol active while subordinate follicles are estradiol inactive (Merz et al., 1981; Ireland and Roche, 1983b; Sunderland et al., 1994). The dominant follicle may continue to grow because it requires less FSH than the rest of the cohort of follicles. The future dominant follicle was the first follicle to grow to 4 mm, and maintained its size advantage over the next largest follicle until dominance (Kulick et al., 1999).

Deviation is the continued growth of the dominant follicle while the subordinate follicles cease growing. In cattle, deviation occurs 60 to 61 h after a wave emerges, at which time the follicle is approximately 8 to 8.5 mm in diameter (Ginther et al., 1998; Kulick et al., 1999). Deviation is associated with decreased concentrations of FSH and increasing LH concentrations in serum (Evans et al, 1997; Ginther et al., 1998; Kulick et al., 1999).

Gonadotropin concentrations and their receptors have a critical role in deviation of the dominant follicle (Boa and Garverick, 1998; Webb et al., 1999). Selection of the dominant follicle is associated with decreasing concentrations of FSH and increasing concentrations of LH in serum. Late in follicular growth, the dominant follicle becomes less dependent on FSH and has more LH receptors. This increase in response to LH allows the follicle to continue growth and steroid production in the presence of decreasing FSH concentrations. Subordinate and atretic follicles have few LH receptors and begin atresia.

Other molecules modulate selection and dominance of the follicle by either autocrine and paracrine pathways (at the ovary), or endocrine mechanisms (effecting gonadotropin secretion). Inhibin, activin, insulin like growth factor I , insulin like growth factor II, and insulin like growth factor binding proteins have been identified as possible modulators of the dominant follicle (reviewed by Roche, 1996; Boa and Garverick, 1998 Webb et al., 1999). Therefore, establishment of dominance is dependent on increasing LH receptors and modulation of growth and steroid synthesis by growth factors.

Ovulation

Progesterone concentrations in plasma determine the fate of a dominant follicle. If progesterone is synthesized and released from the corpus luteum, the dominant follicle will become atretic and a new wave of follicles will grow. During proestrus, progesterone concentrations decline allowing LH concentrations to increase.

Increasing concentrations of LH ultimately control growth, estradiol synthesis, and ovulation of the dominant follicle (Reviewed by Fortune, 1994; Bao and Garverick, 1998). Concentrations of LH increase after the decline in progesterone in plasma and continue to increase until the ovulatory surge of gonadotropins (Walters and Schallenberger, 1984). This preovulatory increase in LH is associated with an increase in steroid enzymes in the dominant follicle as well as estradiol synthesis and release (Tian et al., 1995). If LH concentrations are maintained but ovulation is prevented, the dominant follicles continue to grow and synthesize estradiol, which results in a larger ovulatory follicle (Sirois and Fortune, 1990; Stock and Fortune, 1993).

The ovulatory follicle can be identified on the ovary by d 16 of the estrous cycle (Savio et al., 1988). It is the largest follicle on the ovary 3 d prior to estrus, and continues to increase in size until estrus (Dufour et al., 1972; Pierson and Ginther, 1986; Savio et al., 1988). During final growth and maturation of the ovulatory follicle, estradiol synthesis increases to maximal concentrations and induce estrous behavior.

The preovulatory increase in plasma concentrations of estradiol stimulates the LH surge. Exogenous estradiol causes an increase in LH similar to the preovulatory surge in both ovariectomized heifers and cows (Short et al., 1973; Hausler and Malven, 1976), and immunization against estradiol inhibited the LH surge in ewes (Martin et al., 1978). The LH surge occurs 28 to 32 h prior to ovulation (Swanson and Hafs, 1971; Christenson et al., 1975). and stimulates the cellular mechansims responsible for the rupture of the ovulatory follicle.

Originally it was thought that increasing pressure from fluid in the atrum caused ovulation (reviewed by Espey, 1994). While this pressure may be important for final rupture of the follicle, other mechanisms are involved. Tissue breakdown has a critical role in ovulation of the mammalian follicle. Two hypotheses have been developed to explain this tissue breakdown, and evidence of both necrosis (cell death; reviewed by Espey, 1994) and apoptosis (programmed cell death; reviewed by Murdoch, 1999) can occur in the ovary near ovulation. Necrosis with inflammation and vascular injury occur at ovulation (Espey, 1994, Murdoch et al., 1999b), and some anti-inflammatory drugs inhibit ovulation.

Prior to ovulation, a stigma forms at the apex of the follicle and becomes the site of ovulation. Before the ova can be released, five layers of tissue at the stigma must be removed (reviewed by Espey, 1994). The outermost layer is the surface epithellium of the ovary; which surrounds the tunica albuginea or connective tissue of the ovary. Inside the tunica albuginea, the theca externa is the first layer of the follicle. The theca interna and granlulosa cells are the final follicular barriers to ovulation. During ovulation, these layers interact at the site of ovulation to allow apoptosis.

During apoptosis, the cytoplasm shrinks, and DNA fragments at the apex of the follicle, while the rest of follicle or ovary is not damaged (Murdoch, 1995; Murdoch et al., 1999b). Near ovulation, signs of apoptosis are observed in both the surface epithelium of the ovary and theca and granulosa cells of follicle (Murdoch, 1995). Cell apoptosis is regulated by tumor necrosis factor-α, which has a critical role in ovulation. Tumor necrosis factor-α induces ovarian apoptosis in vitro, and antibodies against tumor necrosis factor-α inhibit ovulation in the ewe (Murdoch et al., 1997).

The formation of a follicular stigma is regulated by plasmin, a protease (reviewed by Murdoch, 1999). The ovarian epithelium and follicular cells activate plasmin concentrations after gonadotropin secretion (Colgin and Murdoch, 1997). Plasmin stimulates collagenases that degrade connective tissue in the ovary and follicle and increase tumor necrosis factor-α activity (Murdoch et al., 1999a). The interaction of plasmin and collagenases allows the stigma to form and stimulates tumor necrosis factor-α to cause the release of ova. Once a stigma has formed in heifers, ovulation is completed quickly (1.2 h), and evacuation of follicular fluid occurred between 6 s and 14.5 min (Kot and Ginther, 1999).

Time of Ovulation

Estrous behavior is the best external sign of the time of ovulation and has been used to estimate when ovulation occurs. Time of the onset of estrus has been determined by visual observation at frequent intervals (Brewster and Cole, 1941; Nalbandov and Casida, 1942; Trimberger, 1948), continous visual observation (Christenson et al., 1975; Mattoni et al., 1988), or more recently with the HeatWatch® system (Walker et al., 1996). In the past, ovulation was evaluated using surgical procedures (Christenson et al., 1975) or rectal palpation of the ovary (Brewster and Cole, 1941; Nalbandov and Casida, 1942; Trimberger, 1948). Transrectal ultrasonography can be used to produce a visual image of the ovary and dominant follicle, to determine the occurrence of ovulation (Rajamahendran et al., 1989; Walker et al., 1996).

The time of ovulation has been determined relative to the onset and end of estrous behavior. Dairy cows ovulated 10 to 15 h after the end of estrous behavior (Brewster and Cole, 1941; Nalbandov and Casida, 1942; Trimberger, 1948). Eighty-two percent of dairy cows ovulated between 7 and 14 h after the end of estrus (Trimberger, 1948). Angus, Hereford, and Bos Indicus cows ovulated 13.6, 13.5, and 18.9 h, respectively, after the end of estrus (Brewster and Cole, 1941; Plasse et al., 1970). The end of estrus is variable and effected by management and other factors, so the beginning of estrus is now used to time ovulation. Dairy cows and heifers ovulated 28 to 29 h after the onset of estrous behavior, detected with visual observation and rectal palpation, (Hough et al., 1955; Swanson and Hafs, 1971) and 27.6 h after the onset detected with the HeatWatch® system and ultrasonography (Walker et al., 1996). Seventy-eight percent of dairy cows ovulated by 40 h after the onset of estrus (Walker et al., 1996). Beef heifers ovulated 31 h after the onset of estrus, and 71 % of heifers ovulated by 30 h, which was determined with surgical procedures (Christenson et al., 1975). Bos Indicus cows and heifers ovulated 26 h after the onset of estrus (Plasse et al., 1970; Mattoni et al., 1988). Time of ovulation in the beef cow has only been determined with visual observation for estrous detection and rectal palpation or surgical procedures.

Time of ovulation has been evaluated in other domestic livestock species. Ewes were continuously monitored for estrous behavior and time of ovulation was determined by repeated endoscopy (Whyman et al., 1979). Ewes ovulated 25.5 h (median) after the onset of estrous behavior. In a similar study, only 63 % of ewes had ovulated by 32 h after the onset of estrus (Bindon et al., 1984). Estrous behavior in ewes has also been evaluated with the HeatWatch® system, and ewes ovulated 34 h after the onset of estrus (Cardwell et al., 1998). Sows ovulated 41 h after the onset of estrus, or when 62 % of the estrus period had passed (Soede et al., 1997). Similarly, gilts ovulated 44 h after the onset of estrus with a range of 30 to 60 h (Almeida et al, 2000).

Effects of Heat Stress on Reproduction

Heat stress has detrimental effects on fertility of cows. Dairy cows have reduced conception rates in hotter months of the year (Gwazdauskas et al., 1975; Rosenberg et al., 1977; al-Katanani et al., 1999). Conception rates in a large Florida dairy herd decreased from 25 % in April to 7 % in July (Cavestany et al., 1985). Cows ovulated without estrous behavior with a greater frequency during the hotter months of the year (Rodtian et al., 1996), and acute heat stress may induce anovulation in beef cows (Bond and McDowell, 1972). Thermal stress may effect reproduction by altering the function of the pituitary and ovary.

Follicle Growth

Heat stress may reduce fertility by changing follicular growth. Dairy cows and heifers exposed to acute heat stress had more 3 wave cycles (82 %), while the majority of cows maintained in a thermal neutral environment ovulated the second wave dominant follicle (91 %; Wilson et al., 1998a; Wilson et al., 1998b). Thermal stress by heat chambers decreased the size of the dominant follicle of dairy heifers (Wilson et al., 1998a), and on d 8 of the estrous cycle, cows with access to shade had larger follicles that contained more fluid than cows without shade (Badinga et al., 1993). However, Wolfenson et al. (1995) observed similar size of dominant follicles in heat stressed cows with and without shade. Acute heat stress may alter the growth of the dominant follicle depending on the duration and severity of heat stress.

Chronic heat stress during hotter months of the year may also alter follicle growth in cows. During April, the dominant follicle of the first follicular wave had a larger diameter than in June, August, or November, and growth rate of this dominant follicle was slower in August (Badinga et al., 1994). In winter and autumn months, the dominant follicle of beef cows was larger and contained more granulosa cells than during the spring (McNatty et al., 1984). Conversely, Wolfenson et al. (1997) concluded that size of the dominant follicle was not altered by season or chronic heat stress, but acute heat stress did decrease the follicular fluid in the dominant follicle.

Thermal stress may alter the relationship between the dominant and smaller follicles, and the dominant follicle may partially loose its ability to suppress the growth of other subordinate follicles. Heat stress decreased the time of dominance of the largest follicle of the first wave and resulted in an earlier growth of the second wave dominant follicle (Wolfenson et al., 1995). The second largest follicle was larger in heat stressed than control heifers (Wilson et al., 1998a). Cows without access to shade had larger subordinate follicles containing more fluid (Badinga et al., 1993) and an increased number of large follicles on the ovary (Wolfenson et al., 1995). Growth of the dominant follicle was slower and a decrease ability of the dominant follicle to suppress the growth of the largest subordinate follicle was observed in August when compared with April, June, or November (Badinga et al., 1994).

An effect of heat stress on growth of small follicles is not clear. Cows acutely exposed to high temperatures had a greater number of small follicles (2-5 mm) on days 11 to 15 of the estrous cycle (Trout et al., 1998). However, other authors have observed a tendency for heat stress to decrease the number of small follicles present on the ovary (Wolfenson et al., 1995; Wilson et al., 1998a). These results indicate that heat stress inhibits the ability of the dominant follicle to suppress the growth of subordinate follicles, which may result in early regression and 3 follicular waves.

Hormone Concentrations

Thermal stress may compromise follicular growth in cows by altering secretion of hormones. The effects of heat stress on gonadotropin secretion are not established. Acute heat stress decreased the number of LH pulses during the early estrous cycle (Wise et al., 1988), but chronic or seasonal heat stress did not alter LH secretion (Rosenberg et al., 1982). Differences between studies could be due to small sample size (Wise et al., 1988), infrequent samples to measure LH (every 6 h; Rosenberg et al., 1982), or method of heat stress. Gilad et al. (1993) concluded that both chronic and acute heat stress altered gonadotropin secretion, but the effects were related to the concentrations of estradiol. Chronic heat stress decreased LH pulse amplitude in all cows, but thermal stress also decreased basal LH, mean LH, mean FSH, and maximum LH and FSH surges in cows classified as having low estradiol concentrations (Gilad et al., 1993). Acute heat stress depressed the maximum gonadotropin surge in cows with both high and low estradiol concentrations (Gilad et al., 1993). These data suggest that both chronic and acute heat stress alter gonadotropin secretion and estradiol has an important regulatory role. Few studies have adequately examined how heat stress effects secretion of gonadotropins.

Heat stress decreases dominance of the largest follicle over subordinates, and the mechanism may include altering estradiol synthesis. Previous studies have concluded that heat stress increases (Rosenberg et al., 1982), decreases (Wolfenson et al., 1995; Wolfenson et al., 1997; Wilson et al., 1998b), or does not alter (Wise et al., 1988) estradiol concentrations. In recent research, heifers and cows exposed to high temperatures had decreased estradiol on the day of estrus (Wilson et al., 1998a; Wilson et al., 1998b). During the summer, cows without access to shade also had decreased plasma estradiol. (Wolfenson et al., 1995). Wolfenson et al. (1997) concluded that both season and acute heat stress altered estradiol concentration in follicular fluid. The discrepancies between this research and earlier reports could be due to differences in the intensity of heat stress. Wise et al. (1988) reported that estradiol concentrations were similar in heat stress and control cows, but heat stress cows had access to shade. The degree of heat stress may have not been severe enough to decrease concentrations of estradiol. Thermal or seasonal stress may reduce estradiol concentrations, which is further evidence that dominance of the largest follicle is altered by heat stress.

Effects of thermal stress on progesterone concentrations are also unclear. In previous studies, heat stress increased (Wilson et al., 1998a; Wilson 1998b; Abilay et al., 1975), decreased (Rosenberg et al., 1977; McNatty et al., 1984; Howell et al., 1994), and had no effect (Wise et al., 1988; Wolfenson et al., 1995) on progesterone concentrations. Differences may be due to the length of exposure to heat stress. Cows acutely exposed to thermal stress via environmental chambers had increased progesterone secretion. Abilay et al. (1975) concluded that exposure to temperatures of 33.5 °C increased progesterone concentrations from d 2 to 19 of the estrous cycle.

In contrast to acute heat stress, chronic heat decreases progesterone concentrations in plasma. During summer months, concentrations of progesterone in plasma of dairy cows were less as compared with concentrations in winter (Rosenberg et al., 1977; Rosenberg et al., 1982) and spring months (Howell et al., 1994). Similarly, beef cows had increased progesterone concentrations in plasma during winter when compared to spring (McNatty et al., 1984). Abilay et al. (1975) demonstrated possible differences between acute and chronic heat stress. In this study, acute thermal stress increased progesterone concentrations from d 2 to 19 of the first cycle, but only from d 2 to 8 of the next cycle. After the initial exposure, animals may adjust to heat altering the effects of acute and chronic thermal stress.

Conclusions

In conclusion, increasing concentrations of estrogens induces estrous behavior, and other hormones may interact with estrogen to alter its effects. Standing while mounted is the best external sign of estrus, but numerous physiological and behavioral changes occur near estrus. External management and environmental factors influence the intensity and duration of estrus, and while estrous behavior in dairy cows has been extensively studied, research on estrus in the beef cow is limited.

Follicles mature and grow in waves in preparation for ovulation. Only one follicle will be selected and continue to grow while the rest of the cohort undergo atresia. The dominant follicle secretes estradiol, which inhibits the growth of other follicles and induces estrous behavior. Estrous behavior is the best external sign of when a cow ovulates and is used to time AI. The time of ovulation relative to the onset of estrus has not been determined in beef cows. Season and thermal stress decrease the reproductive efficiency of dairy cows, and alter endocrine function and follicular dynamics. However, seasonal effects on estrous behavior and time of ovulation relative to the onset of estrus have not been evaluated in beef cows.

CHAPTER III

SEASONAL EFFECTS ON ESTROUS BEHAVIOR AND TIME OF OVULATION IN BEEF COWS

ABSTRACT: Estrous behavior and time of ovulation relative to the onset of estrus were determined in mature Angus x Hereford cows (n=18 to 21 each season) during summer, winter, and spring for 2 yr. Estrous behavior was evaluated during the first of two consecutive estrous periods, and time of ovulation was determined during the second estrus. Concentrations of progesterone were quantified in twice weekly blood samples to ensure all cows had normal estrous cycles. The HeatWatch® system was used to measure the duration of estrus, number of mounts per estrus, and duration of the longest interval between mounts. Commencing 16 h after the onset of the second estrus, transrectal ultrasonography was performed every 4 h until the dominant follicle was no longer present on the ovary, and time of ovulation was defined as 2 h preceding the absence of the dominant follicle. There was a season x year effect ($P < .05$) for the duration of estrus. Cows were estrus longer in summer than winter in year 1, but duration of estrus did not differ during summer and winter of year 2. The duration of estrus did not differ during the winter and spring of year one, but in year 2, cows were estrus longer in winter than spring. Cows were mounted more times per estrus ($P < .05$) in winter (59 ± 5.3)

than in summer (43.6 ± 5.3) or spring (38.2 ± 5.8), and cows were mounted more times in year 1 (56.9 ± 4.5; $P < .05$) than in year 2 (36.9 ± 4.4).Cows had longer intervals between mounting activity in summer (4.1 ± .4 h; $P < .05$) than in the spring or winter (2.7 ± .4 h). During all seasons, mounting activity was greater between 0600 to 1200 (3.2 ± .2 mounts/ h of estrus) than during other times of the day (2.1 ± .2 mounts/h of estrus; $P < .01$). Cows ovulated 31.1 ± .6 h after the onset of estrus, and time of ovulation was not influenced by season or year. For individual cows, time of ovulation had a repeatability of .63 among seasons. We conclude that season influences estrous behavior of beef cows and cows are mounted more times in the morning. Time of ovulation relative to the onset of estrus is constant during all seasons and averages 31.1 h.

Introduction

Artificial insemination (AI) allows the use of sires with superior performance; however, only 6 % of beef producers in the United States utilize AI (NAHMS, 1997). Two most common reasons that producers do not use AI are that additional time and labor are required (39%) and the procedures may be difficult (20 %; NAHMS, 1997). Initiation of estrous behavior is the best external sign of time of ensuing ovulation and is used to determine when to inseminate cows. Dairy cows were estrus for 7 to 10 h and received between 8 and 14 mounts per estrus when monitored continuously with the HeatWatch® system (Walker et al., 1996; Dransfield et al., 1998; Xu et al., 1998), and beef heifers were estrus for 14 h and were mounted 50 times (determined with the HeatWatch® system; Stevenson et al., 1996).

Dairy cows ovulated 10 to 15 h after the end of estrus (Brewster and Cole, 1941; Nalbandov and Casida, 1942; Trimberger, 1948), or 27.6 ± .6 h after the onset of estrous behavior (Walker et al., 1996), but time of ovulation in beef cows has not been well defined. Season and thermal stress have detrimental effects on reproductive efficiency of dairy cows (Rosenberg et al., 1977; De Silva et al., 1981; Cavestany et al., 1985) and alter endocrine function and follicular dynamics (Wolfenson et al., 1995; Wolfenson et al., 1997; Wilson et al., 1998a). However, the effects of season on estrous behavior and time of ovulation have not been determined for beef cows.

Characterization of the time of ovulation relative to the onset of estrus will facilitate development of recommendations for timing AI to achieve maximal fertility. Seasonal effects on estrous behavior and the time of ovulation must also be evaluated to maximize pregnancy rates with AI. The objectives of this study were to determine the time of ovulation relative to the onset of estrus in beef cows and to determine if season influences estrous behavior and time of ovulation.

Materials and Methods

Animals

Estrous behavior and time of ovulation relative to the onset of estrus were determined during summer (August and September), winter (December and January), and spring (April and May) of two consecutive years in Oklahoma. Mature (> 2.5 years of age) non-pregnant, non-lactating Hereford X Angus cows (n=18 to 21 each season) were managed in a 12 ha pasture with shade. Cows received water from metal tanks and did not have access to ponds for cooling. Cows weighed 522 ± 22 kg, had a body condition score of 5.5 ± .1 (1 = emaciated, 9 = obese; Wagner et al., 1988) and had access to native grass pasture and hay. A 20% CP supplement was fed to maintain body condition.

Estrus

The HeatWatch® system (DDx Inc., Denver, CO) is composed of a pressure sensor and radio transmitter that attach to the rump of a cow to continuously monitor when a cow is mounted. The sensor and transmitter are battery operated and contained in a patch. Hair on the rump anterior to the tail heads of cows was trimmed, and the patch was attached with industrial strength glue (OSI Quickbond®; Ohio sealants Inc., Mentor, Ohio). Estrous behavior of each cow was monitored for two consecutive estrous periods. The first estrus was induced with $PGF_{2\alpha}$ (Lutalyse®, 25 mg; Pharmacia & Upjohn, Kalamazoo, MI), and treatments were given so that between 1 and 4 cows were in estrus at any time. The second estrus was not induced and occurred 18 to 22 d after the first. The first estrus was used to quantify estrous behavior, and time of ovulation relative to the onset of estrus was determined during the second estrus. Two estrous periods were studied to avoid possible alterations in estrous behavior when cows were removed from the herd for ultrasonography to determine time of ovulation. In addition, during summer and winter of year 1, cows were observed twice daily (at 0700 and 1900 h in summer and 0730 and 1730 in winter) for 30 minutes to detect estrus.

Duration of estrus, number of mounts, the longest interval between subsequent mounts, and the number of mounts per h of estrus were evaluated. Onset of estrous behavior was defined as the first of three mounts within a 4 h period. The end of estrus was defined as the last mount without a subsequent mount for 12 h. However, to ensure the last mount was actual estrous behavior, at least one mount had to occur within 3 h before the last mount. Number of mounts was the number of times a cow was mounted by its herd mates. The 24 h distribution of mounting activity was determined by dividing the day into four 6 h periods. Data were analyzed as mounts per h of estrus to remove influences due to differences in the length of time a cow was in estrus during a period.

Ovulation

At the second estrus, time of ovulation relative to the onset of estrus was determined. Commencing 16 h after the onset of estrus (determined with HeatWatch®) transrectal ultrasonography (Aloka 500-V ultrasound and a 7.5-MHz transducer; Corometrics Medical Systems, Wallingford, CT) was performed every 4 h until the dominant follicle was not present on the ovary. Time of ovulation was defined as 2 h preceding the time that the dominant follicle was no longer present. Ultrasound images of the ovary were recorded at each evaluation, and the diameter of the follicle was the average of the length and the width (Pierson and Ginther 1988). Most cows were maintained in the herd throughout the experiment unless culled for common reasons. Time of ovulation for an individual cow was measured between 2 and 6 times, and the repeatability of the time of ovulation relative to the onset of estrus was determined.

Steroid Hormones

Blood samples were collected every 3 or 4 d by tail venipuncture. Frequent blood samples were collected from 12 cows during summer or winter of year 2 to quantify progesterone and estrogen at estrus and ovulation. Samples were collected daily commencing 5 d prior to estrus and every 4 h between 12 and 56 h after the initiation of estrus. Immediately after collection, samples were cooled in ice and centrifuged within 1 h at 2500 x g for 15 min. Plasma was removed and stored at -20° C until hormone analyses. Concentrations of plasma progesterone were measured by solid phase RIA (Coat-A-Count progesterone kit, Diagnostic Products Corp., Los Angeles, CA; Vizcarra et al., 1997), and estradiol was determined by RIA (Vizcarra et al., 1997). A normal luteal phase was defined as concentrations of progesterone greater than .5 ng/mL for 11 to 15 d.

Statistical Analysis

Seasonal effects on estrus and time of ovulation were determined with a completely randomized design using the PROC GLM procedure of SAS (1996). Data were arranged in a 2 x 3 factorial with year (1 and 2), season (summer, winter, and spring), and year x season in the model. The correlation between the duration of estrus and the number of mounts per estrus was quantified with PROC CORR (SAS, 1996). Treatment effects on the size of the dominant follicle were determined with PROC MIXED (SAS, 1996). Season, year, time, and interactions were included in the model, and cow(season) was a random effect. Significant main effects and interactions were separated with PDIFF (SAS, 1996). Concentrations of estrogen and progesterone in plasma at estrus and ovulation were analyzed using the PROC GLM procedure (SAS, 1996) with time relative to estrus and ovulation as the main effect. Significant time differences were compared with orthogonal contrast (Steel and Torrie, 1980). The repeatability of the time of ovulation for individual cows was determined according to Evans (1978) and Vizcarra and Wettemann (1996), and variances were estimated using the mean square error. Time of day when the greatest estrous activity occurred, effects of duration of estrous on the number of mounts, and influence of size of the dominant follicle on time of ovulation were determined with the PROC GLM procedure (SAS,

1996) with season, year, and the interaction included in the model. Significant treatment and interaction effects were separated with PDIFF (SAS, 1996)

Results

There was a season x year effect on daily temperature on the day of estrus (Table 1; $P < .01$). Mean ambient temperature was greater during the summer of year 2 than year 1 ($P < .01$). During the summer of year 2, there were 14 d when the maximum temperature was greater than 42 °C. However, the daily maximum temperature was less than 35 °C on all days in year 1. Average ambient temperatures were greater during the winter of year 1 than in year 2. During winter of year 2, the daily temperature was less than 0 °C on 14 d with an average daily minimum of –3.1 ± 2.9 °C, but during the winter of year 1, there were only 5 d when the temperature was less than 0 °C and the average minimum daily temperature was 1.7 ± 1.6 °C. Ambient temperatures during the spring of both years were similar.

Estrus

Twice daily visual observation failed to detect 29 % (5 of 17) of estrous cows that were identified by HeatWatch® during the first summer. Four of the five cows not observed standing were mounted but did not stand or mounted other cows during the observation periods. Visual observation failed to identify only 5 % (1 of 20) of estrous cows in the winter of year 1.

Season ($P < .05$; Table 2) and year ($P < .01$; Table 3) affected the number of times a cow was mounted by herd mates. Cows were mounted more times per estrus in the winter (59 ± 5.3) than summer (43.6 ± 5.3) or spring (38.2 ± 5.8). The number of mounts that cows received per estrus ranged from 3 to 182 (Table 2), and 71 % of cows were mounted between 10 and 70 times per estrus (Figure 1). Cows received more mounts in year 1 (56.9 ± 4.5) than in year 2 (36.9 ± 4.4). The longest interval between subsequent mounts was also influenced by season (Table 2; $P < .05$). Cows in estrus during summer had longer periods of inactivity between mounts (4.1 ± .4 h) than in spring (2.7 ± .4 h) or winter (2.7 ± .4 h). The maximum duration between mounts was 11 h.

There was a season x year effect ($P < .05$; Table 4) for the duration of estrus and the number of mounts per h of estrus. During year 1, cows were in estrus longer in summer (18.4 ± .8 h) than winter (14.7 ± .8 h), but the duration of estrus did not differ between summer and winter in year 2. In the first year, the duration of estrus did not differ during winter and spring; however, cows were estrus longer in winter (16.2 ± .8 h) than spring (11.6 ± .8 h) during year 2. During all seasons, the range for duration of estrus was .5 to 36.3 h (Table 4), and 70 % of cows were estrus for 11 to 20 h (Figure 2). The distribution throughout the day of the onset of estrus is illustrated in Figure 3. There was also, a season x year effect on the number of mounts per h of estrus ($P < .05$). Cows were mounted more times per h of estrus during the winter of year 1 (4.9 ± .4 mounts/h) compared with all other seasons (2.6 ± .5 mounts/h). During all seasons, mounting activity was greatest ($P < .01$) between 0601 and 1200 (3.2 ± .2 mounts/ h of estrus) than during other times of the day (2.1 ± .2 mounts/h of estrus; Figure 4). The duration of estrus and the number of mounts per estrus were correlated ($r = .32$; $P < .01$). Cows that were estrus longer than 15 h received an average of 58.4 ± 4 mounts, compared with 34.3 ± 4 mounts when cows were estrus less than 15 h ($P < .001$).

Ovulation

Time of ovulation after the onset of estrus was not influenced by season (Table 2; $P > .1$) or year (Table 3; $P > .1$). Cows ovulated $31.1 \pm .6$ h after the onset of estrus. Time of ovulation after the onset of estrus ranged from 21.5 to 42.8 h (Table 2), and 64 % of cows ovulated between 28 and 33 h after the onset of estrus (Figure 5). The distribution throughout the day of the time of ovulation is illustrated in Figure 6. There was a season x year effect on time of ovulation relative to the end of estrus ($P < .05$; Table 4), and cows ovulated longer after the end of estrus in the winter of year 1 (18 h) and spring of year 2 (19.8 h) than other seasons (14 h). Diameter of the ovulatory follicle did not change during the 12 h prior to ovulation ($P > .1$). There was a season x year effect ($P = .01$; Figure 7) on size of the ovulatory follicle. Cows had smaller follicles in summer ($12.4 \pm .2$ mm) than winter ($13.3 \pm .2$ mm) of year 1 ($P < .01$), but this effect was not observed during year 2 ($P > .1$). The ovulatory follicle was larger during the spring of year 2 than during all other seasons. During summer and spring of year 2, dominant follicles were larger than during the same seasons in year 1 ($P < .01$). When dominant follicles were divided into large (> 13.2 mm) or medium (< 13.2 mm), size did not influence time of ovulation. Time of ovulation relative to the onset of estrus for 25 cows was measured in different seasons between 2 and 6 times, and time of ovulation for individual cows was repeatable (0.63).

Steroid Hormones

Concentrations of progesterone in plasma of all cows during the two estrous cycles studied were typical for cows with normal cycles (Garverick et al., 1971; Swanson et al., 1972; Wettemann et al., 1972). Concentrations of progesterone in plasma during the periovulatory period were maximal (Figure 8; 7.4 ± .9 ng/mL) on d 4 prior to estrus and were minimal the day before estrus ($P < .01$). Concentrations of progesterone were < .5 ng/mL from d –1 through d 2 after the onset of estrus. Concentrations of estradiol in plasma increased ($P < .01$) from 1 d prior to estrus to a maximal concentration (3.6 ± .5 pg/mL) on the day of estrus. Concentrations of estradiol increased after concentrations of progesterone were less than 1 ng/mL. Plasma concentrations of estradiol decreased from 3.6 ± .5 pg/mL at 8 h after the onset of estrus to 1.7 pg/mL by 16 h after the onset of estrus ($P < .01$), approximately 16 h prior to ovulation (Figure 9). Estradiol concentrations in plasma were at the nadir at ovulation ($P > .1$).

Discussion

The HeatWatch® system identified 29 % more estrous cows in the summer and 5 % more in the winter than with twice daily visual observation for 30 min. Cows had more mounts per h of estrus in winter than in summer of year 1, which may have resulted in more estrous cows identified by visual observation. Similarly, Stevenson et al. (1996) found that HeatWatch® identified 27 % more estrous beef heifers than twice daily visual observations for 45 min. Geary et al. (1999) observed that visual observation for 2 h twice daily and HeatWatch® identified a similar number of estrous cows.

According to previous reports using visual observation to identify estrus, concentrations of progesterone begin to decrease approximately 3 d prior to estrus and were minimal 1 d before estrus (Henricks et al., 1971; Swanson et al., 1972; Wettemann et al., 1972). Similarly, we found that concentrations of progesterone in plasma began to decrease 4 d prior to estrus and concentrations were minimal on the day before estrus.

Using visual observation to define estrus, concentrations of estradiol increased 3 to 4 d prior to estrus and were maximal between 1 d before and the day of estrus (Henricks et al., 1971; Wettemann et al., 1972; Chenault et al., 1975). Variation in results can be due to insufficient methods to detect estrous behavior or infrequent sampling. In this study, concentrations of estradiol in plasma increased from 1 d prior to estrus and were maximal on the day of estrus. Using the HeatWatch® system, Stevenson et al. (1998) also observed maximal concentrations of estradiol on the day of estrus after treatment with $PGF2_{\alpha}$. Maximal concentrations of estradiol in cows between 7 and 25 pg/mL have been reported (Henricks et al., 1971; Wettemann et al., 1972; Stevenson et al., 1998). In this study, the maximal concentrations of estradiol in plasma were 3.6 pg/mL. Similarly, Mihm et al. (2000) found maximum concentrations of estradiol in serum of 5 pg/mL. Differences between studies are related to the sensitivity of assays used to measure estradiol.

Seasonal effects on estrous behavior of dairy cows are not consistent (Trimberger 1948; Pennington et al., 1985; Walker et al., 1996). Timberger (1948) observed cows twice daily and concluded that season did not alter estrous behavior. Walker et al. (1996) found that an increase in temperature did not influence the duration of estrus when estrus was monitored continuously with a HeatWatch® system. We found that beef cows were mounted more times during estrus in the winter, and the duration of estrus may be longer during the summer with longer intervals between mounts. Using continuous visual observation, Pennington et al. (1985) also found that dairy cows had a longer, less intense estrus in hot weather with longer intervals between mounts. Seasonal effects on estrous behavior may differ between locations because of breed of cow, variations in climate, environment, or management practices. Maximal ambient temperature was 24.4°C in the study by Walker et al. (1996), while the maximal temperature was 33.9°C in the study by Pennington et al. (1985). The maximal ambient temperature in our study averaged 30.8 °C in the first summer and 38.3 °C in the second summer. Management factors such as frequency of milking, movement of cows, feeding, and the types of surface alter estrous behavior and could contribute to differences between studies (Pennington et al., 1985; Britt et al., 1986; Pennington et al., 1986; Rodtian et al., 1996). Also, the number of cows in estrus and differences between age or stage of the estrous cycle of cows could influence estrous behavior (De Silva et al., 1981; Helmer and Britt, 1985; Walker et al., 1996; Floyd et al., 2001).

The season x year and year effects on estrous behavior in this study may be related to differences in ambient temperature. The mean daily temperatures during the summer and winter seasons differed between years 1 and 2. The second summer had 14 d when the temperature was greater than 38 °C with a maximal daily temperature of 42 °C. However, during the summer of year 1, the daily temperature was never greater than 35 °C. During the winter of year 2, the daily minimal temperature was less than 0 °C for 14 d compared with only 5 d less than 0 °C during the winter of year 1. Gwazdauskas et al., (1983) concluded that mounts per 0.5 h increased linearly in dairy cows with increasing temperature up to 25 °C, but decreased linearly after 30 °C. Holstein cows exhibit signs of estrus (rubbing and licking), other than mounting, with greater frequency in hot weather (Pennington et al., 1985). Seasonal effects on estrus could be due to climate effects on the willingness of herd mates to mount, rather than to a physiological effect of temperature on the cow in estrus.

A shorter duration of estrus with fewer mounts has been observed for dairy and *Bos Indicus* cows compared with an average estrus of 16 h with 47 mounts that we found in beef cows. In agreement with our study, Stevenson et al. (1996) observed beef heifers were estrus for 14 h with 50 mounts. Duration of estrus for dairy cows as determined with continuous visual observation (Pennington et al., 1985; Walton et al., 1987) or the HeatWatch® system (Walker et al., 1996; Dransfield et al., 1998; Xu et al., 1998) ranged from 7 to 13 h with 8.5 to 32.9 mounts. Similarly, the duration of estrus for Bos Indicus cows ranged between 6.7 h and 8.3 h with 9 to 25 mounts (Mattoni et al., 1988; Rae et al., 1999; Yelich et al., 1999). Rae et al. (1999) found breed effects for duration of estrus and the number of mounts in Angus, Brahman, and crossbred cows. In addition, the first estrus of lactating first-calf beef heifers and mature beef cows was shorter (4 and 5 h, respectively) and had fewer mounts (13 and 11mounts per estrus, respectively; Ciccioli and Wettemann, 2000; Lents et al., 2000) than the non-lactating, non-pregnant cows in this study.

Cows were mounted more times per h of estrus from 0600 until noon. Similarly, dairy cows had their greatest estrous activity during the morning hours (De Silva et al., 1981), and Bos Indicus cattle were mounted more frequently from 0600 - 0900 and 1600 – 1900 (Mattoni et al., 1988) than during other times of the day. Other studies with dairy cows have not found an effect of time of day on estrous activity, (Xu et al., 1998) or greater activity from midnight until 0600 was observed (Walton et al., 1987). Variation in times when cows were milked, moved, or fed (Pennington et al., 1985; Britt et al., 1986; Pennington et al., 1986) or ambient temperature could influence when the greatest estrous activity occurs.

Size of the ovulatory follicle differed with season. Ovulatory follicles were smallest in the summer of year 1 and largest in the spring of year 2. These differences were not related to minimum and maximum ambient temperatures on the day of estrus. The effect of heat stress on size of dominant follicles is inconsistent. Badinga et al. (1993) found that heat stress decreased size and fluid content of dominant follicles on d 8 of the cycle, and Mc Natty et al. (1984) and Badinga et al. (1994) observed that season influenced size of first wave dominant follicles and ovulatory follicles. Other studies determined that neither heat stress (Wolfenson et al., 1995; Trout et al., 1998) nor season (Wolfenson et al., 1997) influence size of dominant follicles. Inconsistent effects of heat stress on follicle growth could be due to differences in duration of exposure or maximal ambient temperatures.

Beef cows in the present study ovulated 31.1 ± .6 h after the onset of estrus, and season did not influence time of ovulation. Early studies determined that dairy cows ovulated 10.5 h after the end of estrus (Trimberger 1948) and beef cows ovulated 13 h after estrus (Brewster and Cole, 1941). In this study, the time of ovulation relative to the end of estrus was influenced by a season x year effect, and cows ovulated between 13.4 and 19.8 h after the end of estrus. Dairy and *Bos Indicus* cows may ovulate earlier after the onset of estrus than beef cows. Based on ultrasonography, dairy cows ovulate 27.6 ± .6 h after the onset of estrus (Walker et al., 1996), and as determined by rectal palpation, *Bos Indicus* cows ovulate 25.8 ± 5 h (Mattoni et al., 1988) after the onset of estrus. Yelich et al. (1999) found that Angus, Brahman, and Senepol breeds ovulated at 30.8 h after the onset of estrus. In agreement with our results, Trimberger (1948) concluded that season did not influence the time of ovulation in dairy cows.

Dransfield et al. (1998) concluded that the greatest conception rates for dairy cows occurred when cows were inseminated 4 to 12 h after the first mount identified by HeatWatch®; however, dairy cows may ovulate earlier than beef cows. The optimal time of insemination may also differ between the breeds. There is probably a specific interval after the onset of estrus during which cows can be inseminated to achieve maximal fertility. Approximately 8 h after insemination, fertile sperm reach the site of fertilization, and by 12 to 24 h after insemination, only a small percentage of sperm are still in the reproductive tract (reviewed by Hawk, 1987). Bovine ova are fertile for an estimated 18 h after ovulation but may begin to loose fertility after only 10 h or less (Brackett et al., 1980; reviewed by Hunter, 1989). Aged ova, capable of fertilization, may be incapable of producing a viable embryo. Trimberger (1948) determined that the optimum time of insemination in dairy cattle was between 6 and 14 h prior to ovulation, but conception rates were drastically decreased if cows were inseminated after ovulation. Fertilization rate was greater when dairy cows were inseminated 24 h compared with 0 and 12 h after the first mount, determined with the HeatWatch® system (Saacke et al., 2000). However, embryo quality decreased as cows were inseminated later after the onset of estrus, and cows inseminated 24 h after the onset of estrus had a greater percentage of degenerate embryos. In the current study, cows ovulated between 21 and 42 h after the onset of estrus. For maximal pregnancy rates, we recommend that beef cows should be inseminated 14 to 20 h after the onset of estrus. Using the HeatWatch® system, first-calf heifers inseminated 14 to 20 h after the onset of estrus had a 90 % pregnancy rate (Ciccioli and Wettemann, 2000).

We conclude that season alters estrous behavior of beef cows in Oklahoma. Cows received fewer mounts in the summer and spring than in winter, and cows had longer intervals between mounts during the summer when compared with winter and spring. Estrous cows were mounted more times between 0600 and noon than during other times of the day. Cows ovulated 31.1 ± .6 h after the onset of estrus, and time of ovulation after the onset of estrus is not influenced by season.

Implications

Intensity of estrous detection should be increased during the summer. Estrous detection aids should be used to identify cows with short estrus, few mounts, or long intervals between mounts. Cows should be inseminated 12 h after they are first observed in estrus, if visual observation is used to identify estrous cows. When beef cows are monitored continuously for estrus, cows should probably be inseminated 14 to 20 h after the onset of estrus. Additional studies under different environmental conditions should be conducted to determine when to inseminate beef cows after the onset of estrus for maximal pregnancy rates.

Table 1. Average ambient temperatures on the day of estrus

Season	Year	High, °C	Low, °C	Mean, °C [a]
Summer	1	30.8 ± 0.9	20.0 ± 1.1	25.2 ± 2.0^{b}
Summer	2	38.3 ± 1.2	22.9 ± 1.1	30.1 ± 1.8^{c}
Winter	1	16.8 ± 1.2	1.7 ± 1.6	8.3 ± 2.0^{d}
Winter	2	10.6 ± 3.7	-3.1 ± 2.9	3.4 ± 1.9^{e}
Spring	1	19.4 ± 2.0	6.9 ± 1.3	13.2 ± 1.9^{f}
Spring	2	20.3 ± 2.4	7.3 ± 2.4	14.4 ± 2.2^{f}

[a] Season x year effect on mean temperature.

[bcdef] Means in a column with different superscripts differ ($P<.01$).

Table 2. Seasonal effects on estrus and ovulation in beef cows

	Season		
Characteristics	Summer	Winter	Spring
Mounts received/estrus			
Means	43.6 ± 5.3[a]	59.0 ± 5.3[b]	38.2 ± 5.8[a]
Max	154	182	122
Min	11	6	3
Longest interval between mounts, h			
Means	4.1 ± .4[a]	2.7 ± .4[b]	2.7 ± .4[b]
Max	11.4	7.1	6.8
Onset of estrus to ovulation, h			
Means	31.2 ± .6[a]	31.6 ± .6[a]	30.6 ± .7[a]
Max	42.8	39.2	39.5
Min	21.8	21.5	22

[a,b] Least square means in a row with different superscripts differ ($P<.05$).

Table 3. Effect of year on estrus and time of ovulation relative to the onset of estrus

	Year	
Characteristics	1	2
Mounts received/estrus	56.9 ± 4.5 [a]	36.9 ± 4.4 [b]
Longest interval between mounts, h	3.0 ± .3	3.3 ± .3
Onset of estrus to ovulation, h	31.8 ± .5	30.6 ± .6

[a,b] Least square means in a row with different superscripts differ (*P*<.05).

Table 4. Season x year effects for duration of estrus, mounts per hour of estrus, and time of ovulation relative to the end of estrus

	Season						
	Summer		Winter		Spring		
Characteristics	Year 1	Year 2	Year 1	Year 2	Year 1	Year 2	MSE
No. of cows	18	21	20	19	18	20	
Duration of estrus, h							
Means	18.4 ^{a}	16.8 $^{a, b}$	14.7 $^{b, c}$	16.2 ^{a,b}	16.0 $^{a, b}$	11.6 ^{c}	24.4
Max	36.3	29.5	18.7	23.7	22.8	20.6	
Min	8.4	9.7	9.1	9.4	9	.5	
Mounts per h of estrus							
Means	2.6 ^{a}	2.6 ^{a}	4.9 ^{b}	2.7 ^{a}	3.1 ^{a}	1.9 ^{a}	3.5
Max	6.2	8.1	12	6.3	5.8	8.3	
Min	.6	.8	1.3	.6	1.4	.5	
End of estrus to ovulation, h							
Means	13.4 ^{a}	13.7 ^{a}	18.0 ^{b,c}	14.6 ^{a,c}	14.8 ^{a,c}	19.8 ^{b}	29.8
Max	24.4	18.8	30.1	22.8	21.5	26.1	
Min	-8.3	6.1	8.2	-2.2	8.8	9.6	

^{a,b,c} Least square means in a row with different superscripts differ ($P<.05$).

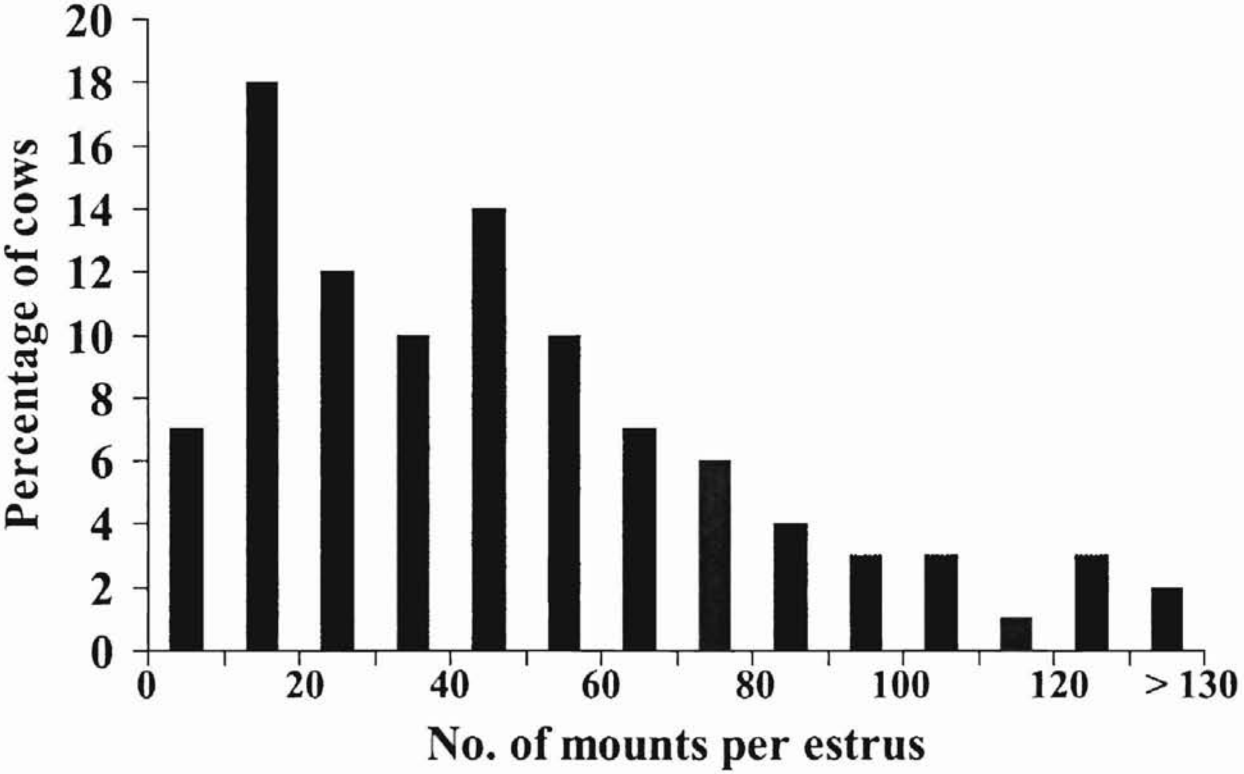

Figure 1. Frequency distribution of the number of mounts per estrus.

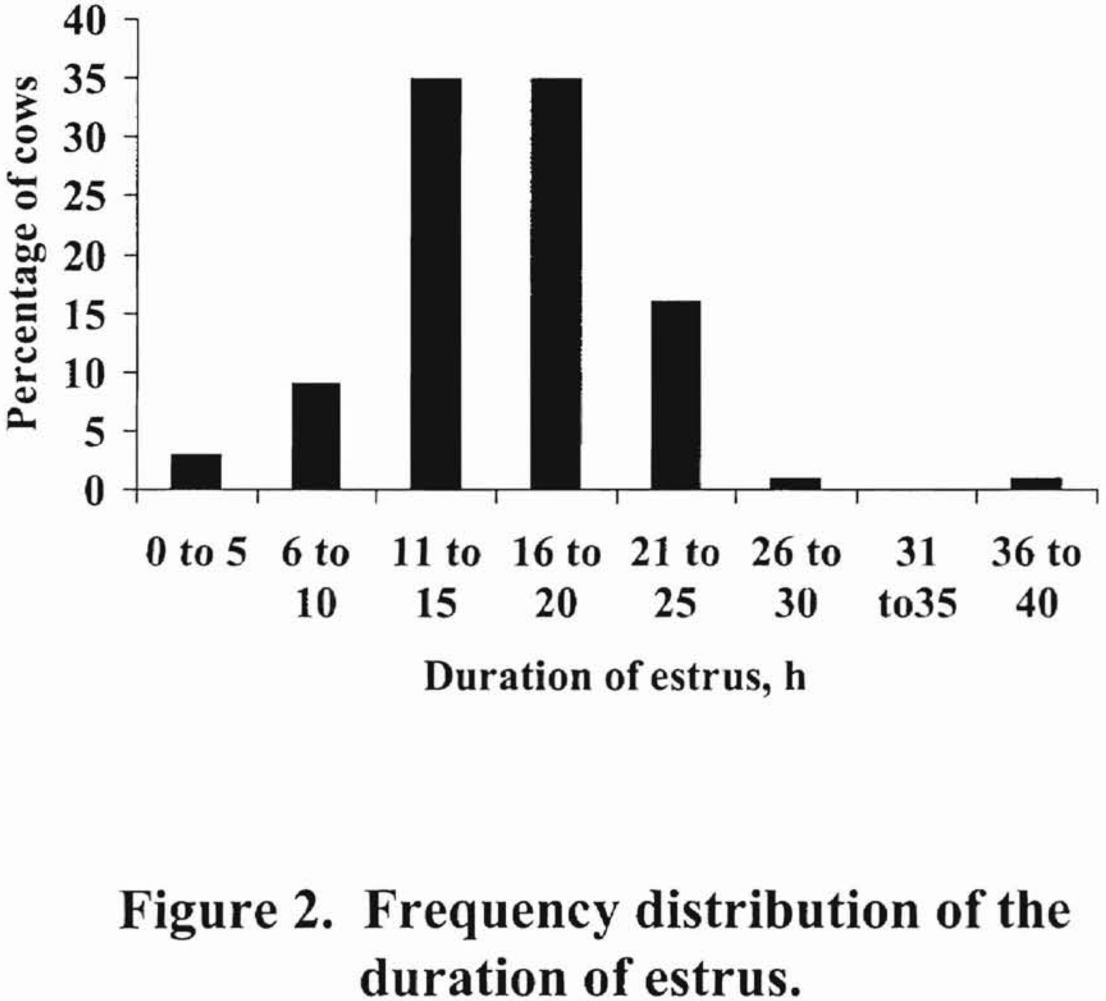

Figure 2. Frequency distribution of the duration of estrus.

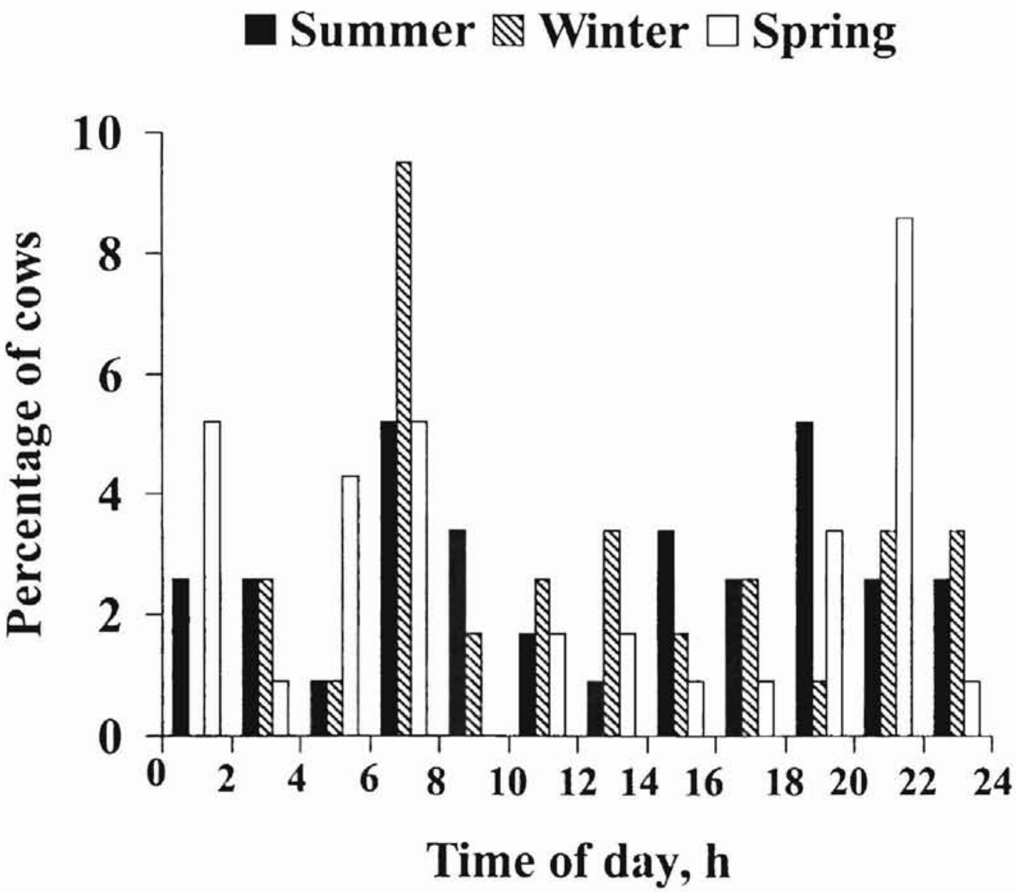

Figure 3. Distribution of the onset of estrus throughout the day (0 = midnight).

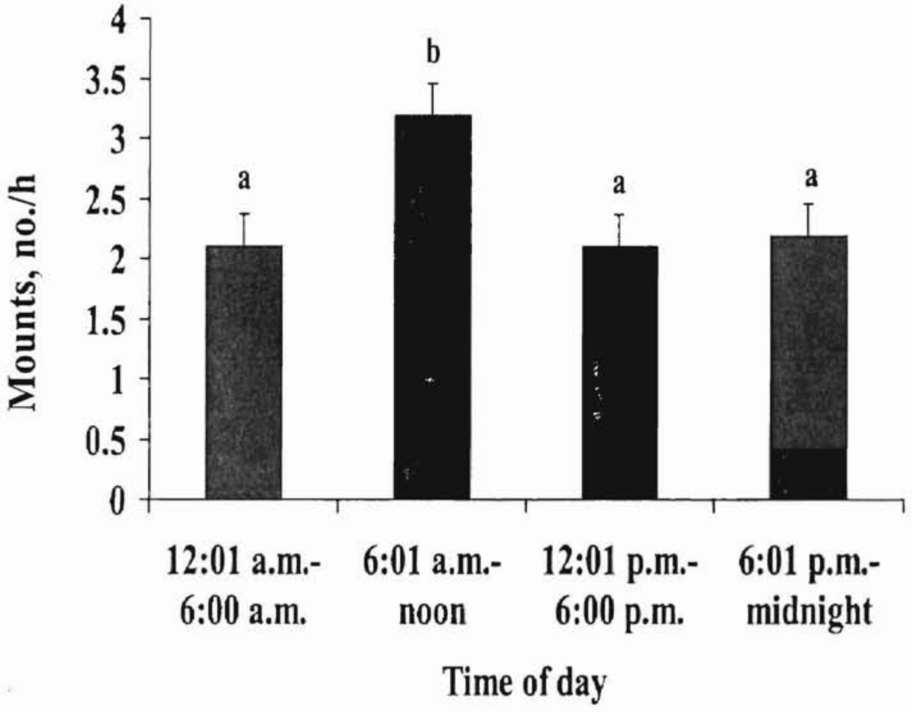

Figure 4. Number of mounts per hour of estrus during each 6 h period in beef cows . [a,b] Means with different superscripts differ ($P<.01$).

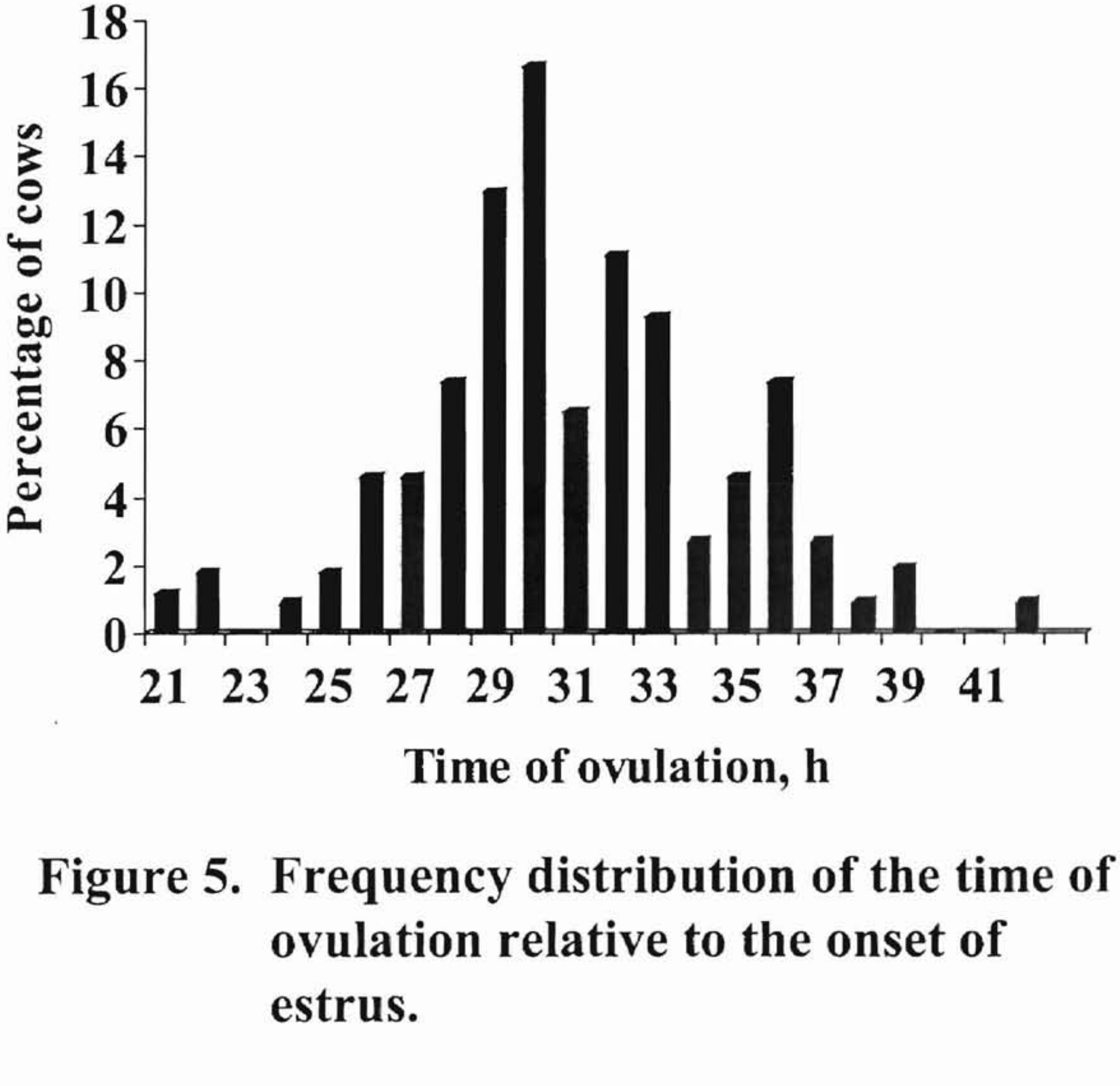

Figure 5. Frequency distribution of the time of ovulation relative to the onset of estrus.

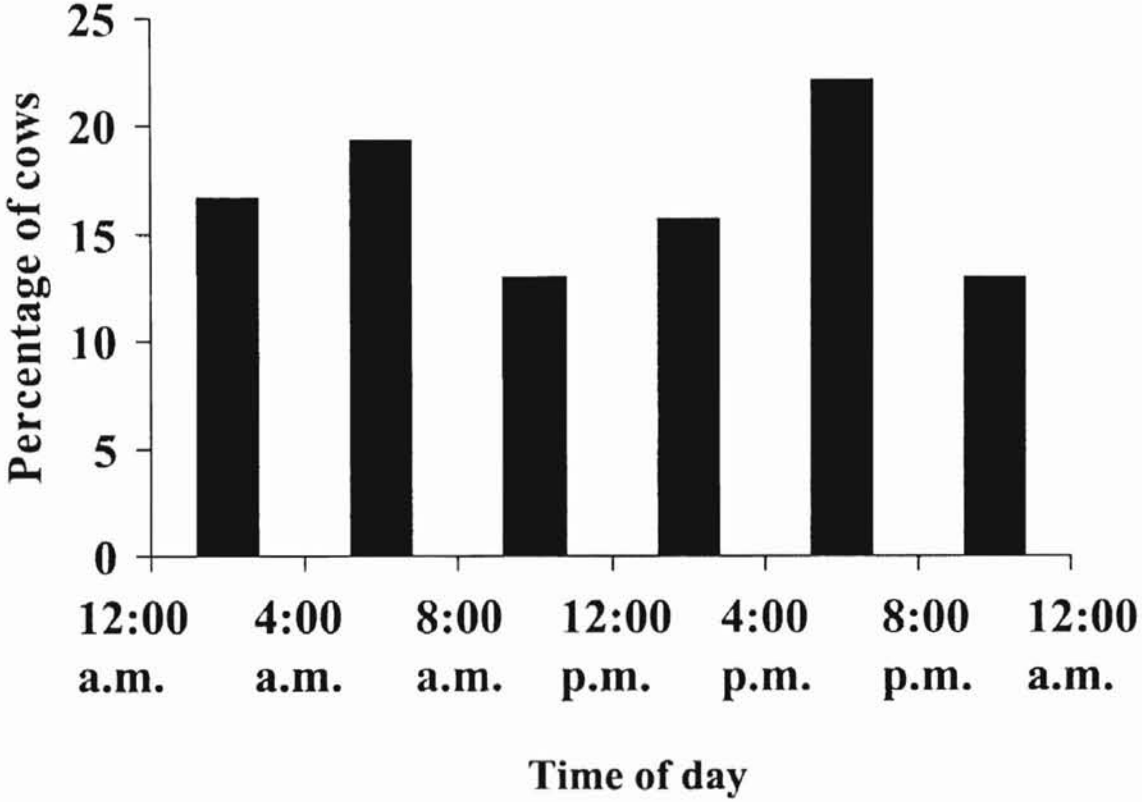

Figure 6. Distribution throughout the day of the time of ovulation relative to the onset of estrus.

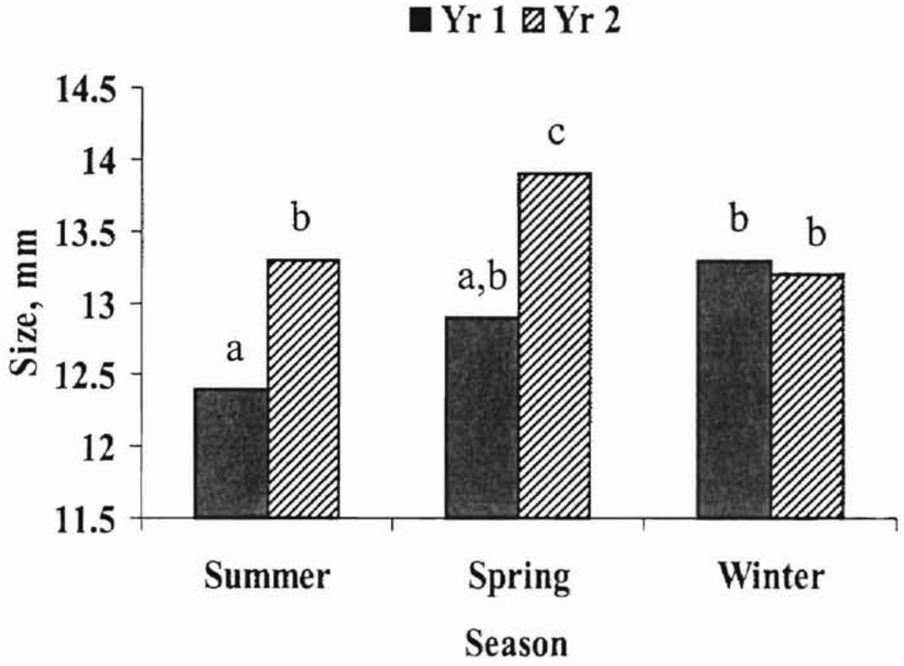

Figure 7. Least square means for diameter of the ovulatory follicle in beef cows at 2 to 4 h prior to ovulation during summer, winter, and spring in year 1 and year 2. Season x year (*P* = .01; MSE = 1.7; n = 15 - 21). ^{a,b,c} Means with different superscripts differ (*P*<.01).

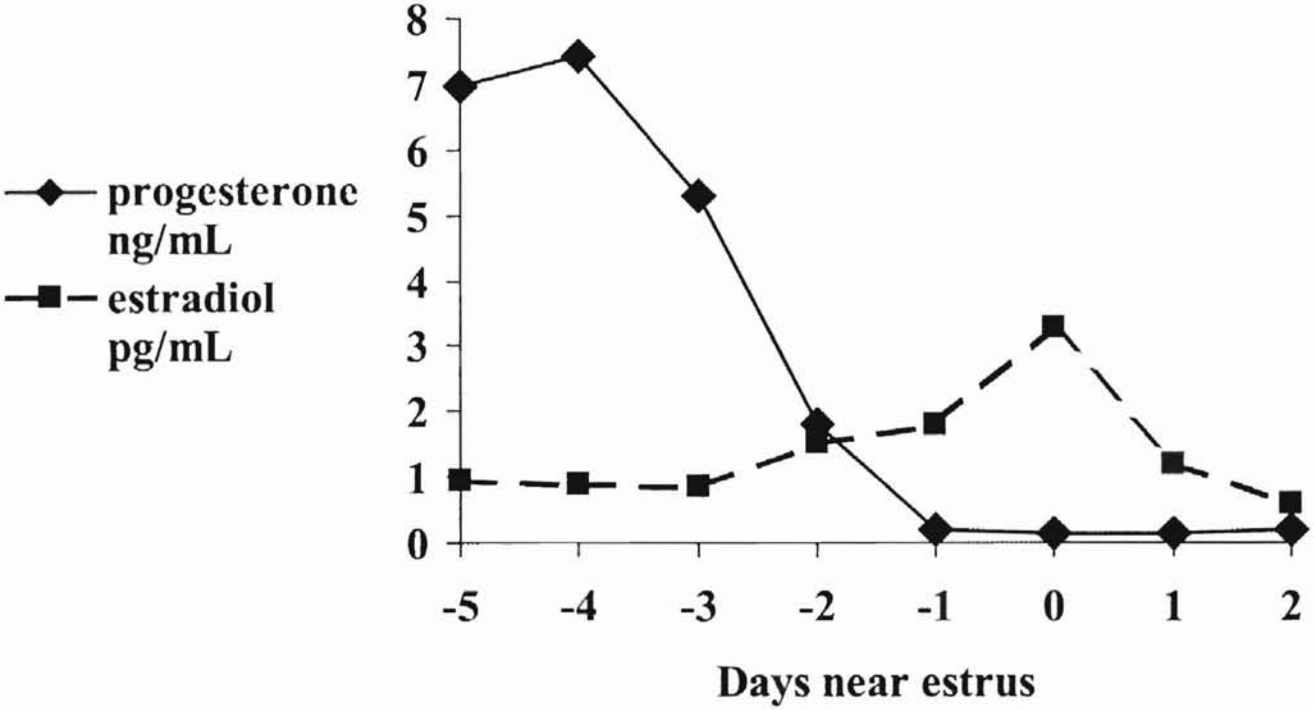

Figure 8. Least square mean concentrations of progesterone and estradiol in plasma before and after estrus in beef cows. 0 is the day of estrus. Day effect ($P < .01$; n = 12 cows per day) for progesterone (MSE = 3) and estradiol (MSE = .4).

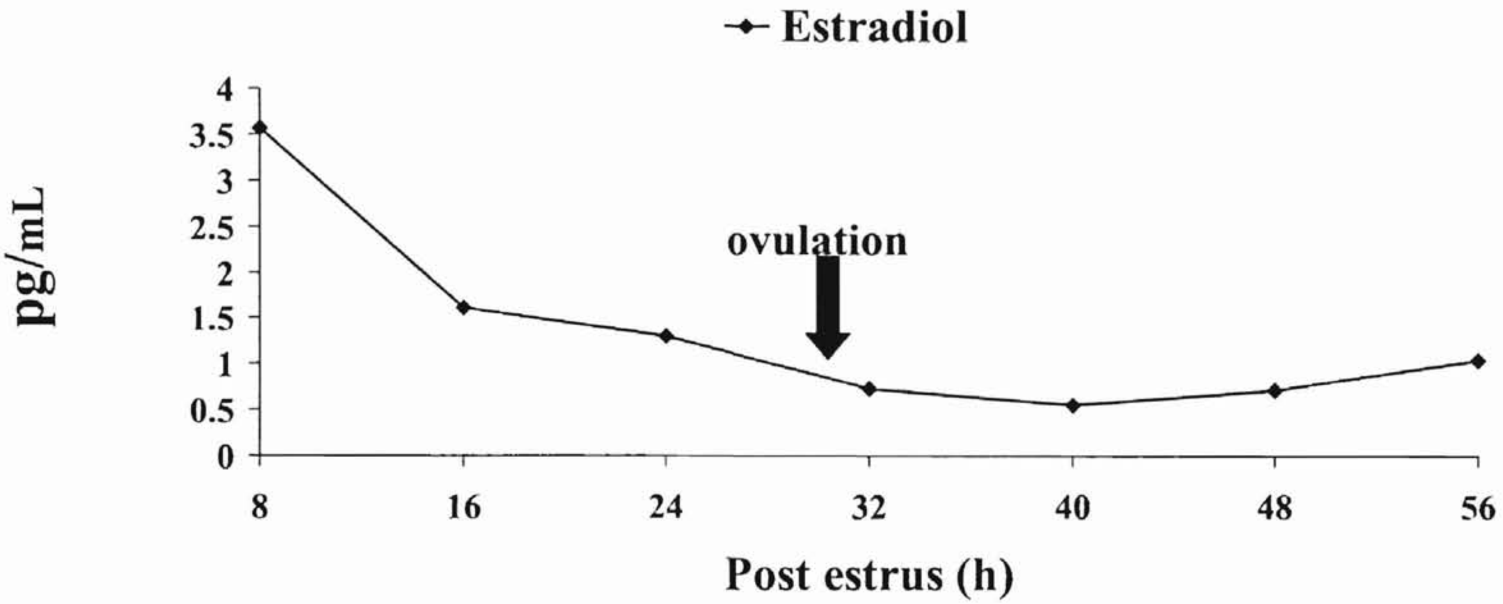

Figure 9. Least square mean concentrations of estradiol in plasma of beef cows after the onset of estrus (day effect; *P* < .01).

CHAPTER IV

SUMMARY AND CONCLUSIONS

Artificial insemination (AI) allows beef producers to mate cows with genetically superior bulls. Use of AI allows producers to improve the performance of calves, herd replacements, and ultimately profitability. Management constraints result in limited use of AI in the beef industry. For maximal pregnancy rates, it is essential that semen is deposited at the correct time relative to when a cow ovulates. Estrous behavior is the only reliable external sign to estimate the time of ovulation and is used to determine when to inseminate cows. Accurate identification of estrous cows is essential for maximal fertility with AI. Estrous behavior and the time of ovulation in dairy cows have been extensively studied, but information about beef cows is limited. A lack of knowledge about estrous behavior and time of ovulation in beef cows have contributed to the limited use of AI.

Season and thermal stress decrease conception rates, may alter growth of the dominant follicle, and may affect steroid hormone synthesis in cows. While a seasonal influence on the ovary has been indicated, the effect of season on time of ovulation and estrous behavior in beef cows has not been determined. To maximize pregnancy rates with AI in beef cows, knowledge about estrous behavior, time of ovulation, and potential seasonal effects on estrous behavior and time of ovulation is essential for producers to take advantage of the benefits of AI. The objectives of this research were 1) to evaluate estrous behavior in beef cows, 2) to determine the time of ovulation relative to the onset of estrus in beef cows, and 3) to determine seasonal effects on estrous behavior and the time of ovulation in beef cows.

Estrous behavior and the time of ovulation were evaluated in Hereford X Angus cows during the summer, winter, and spring seasons of two consecutive years. The HeatWatch® system was used to continuously monitor when a cow was mounted during two subsequent estrous cycles. The system is composed of a patch containing a sensor that attaches anterior to the tail head on the rump of a cow and records when a cow is mounted. The first cycle was used to evaluate estrous behavior. The duration of estrus, number of mounts, and the longest intervals between subsequent mounts were measured for each cow. The second estrus was used to determine when a cow ovulated after the onset of estrus. The time of ovulation and size of the dominant follicle were observed with transrectal ultrasonography. Frequent blood samples were collected from 12 cows during the summer and winter to quantify concentrations of progesterone and estrogen in plasma at estrus and ovulation.

Season did not influence the time of ovulation, and cows ovulated 31 hours after the onset of estrous behavior. The time of ovulation after the onset of estrus for an individual cow was repeatable among seasons. Size of the ovulatory follicle did not change during the 12 h prior to ovulation and did not influence the interval from the onset of estrus to ovulation. Cows ovulated between 21 and 42 h after the onset of estrus, and we recommend that beef cows should be inseminated between 14 and 20 h after the onset of estrus.

Season altered estrous behavior of beef cows. Cows received more mounts by herd mates in the winter than in spring or summer seasons. Also, cows were mounted more times in year 1 than year 2. This could be related to a colder winter and hotter summer in the second year than in the first year. Cows had longer periods between consecutive mounts during estrus in summer than in spring and winter. The maximal interval between mounts was 11 h. There was a season x year effect on the duration of estrus, which could be related to temperature differences between years. Duration of estrus may be influenced by season but these results are inconclusive. The length of estrus was positively correlated with the number of times a cow was mounted.

Twice daily visual observation of cows failed to detect 29 % and 5 % of estrous cows identified by HeatWatch in the summer and winter of year 1, respectively. Fewer cows may have been detected in the summer because cows were mounted fewer times and had longer intervals between mounts than during the winter. During all seasons, cows were mounted more times per estrus between 0600 and noon than other times of the day. Visual observation of cows in the morning should detect more estrous cows. Estrous behavior was extremely variable, and some cows had a short estrus (minimum of .5 h) with few mounts (minimum of 3 mounts).

Concentrations of progesterone in plasma were maximal on d 4 prior to estrus and decreased to minimal concentrations 1 d before estrus. Concentrations of estradiol in plasma were maximal on the day of estrus. Concentrations of estradiol in plasma decreased from the maximum at 8 h after the onset of estrus and concentrations were minimal at 12 h prior to ovulation. Both estradiol and progesterone concentrations in plasma were at their nadir near ovulation.

In conclusion, season altered estrous behavior. Cows were mounted fewer times in the summer and spring than winter season. Longer intervals between mounts were observed during the summer when compared with winter and spring. Therefore, the intensity of estrous detection should be increased in summer months to ensure all estrous animals are identified. Estrous behavior was extremely variable, and estrous detection aids should be used to ensure animals with few mounts or long intervals between mounts are identified for insemination. More cows were mounted during morning hours than during other times of the day. Cows ovulated 31 h after the onset of estrus, and time of ovulation was not influenced by season. Beef cows should be inseminated 14 to 20 h after the first mount of estrus.

Literature Cited

Abilay, T. A., H. D. Johnson, and M. Madan. 1975. Influence of environmental heat on peripheral plasma progesterone and cortisol during the bovine estrous cycle. J. Dairy Sci. 58:1836-1840.

Adams, G. P., R. L. Matteri, J. P. Kastelic, J. C. H. Ko, and O. J. Ginther. 1992. Association between surges of follicle-stimulating hormone and the emergence of follicular waves in heifers. J. Reprod. Fertil. 94:177-188.

Allrich, R. D. 1994. Endocrine and neural control of estrus in dairy cows. J. Dairy Sci. 77:2738-2744.

Allrich, R. D., D. L. Cook, L. A. Horstman, R. J. Knutson, and T. A. Winters. 1989. Influence of dexamethasone, progesterone, gonadotropin-releasing hormone, and testosterone on estrous behavior of estradiol-treated ovariectomized heifers. J. Dairy Sci. 72:2707-2711.

Al-Katanani, Y M, D. W. Webb, and P. J. Hansen. 1999. Factors affecting seasonal variation in 90-day nonreturn rate to first service in lactating Holstein cows in a hot climate. J. Dairy Sci. 82: 2611-2616.

Almeida, F. R. C. L., S. Novak, and G. R. Foxcroft. 2000. The time of ovulation in relation to estrus duration in gilts. Theriogenology 53:1389-1396.

Badinga, L., W. W. Thatcher, T. Diaz, M. Drost, and D. Wolfenson. 1993. Effect of environmental heat stress on follicular development and steroidogenesis in lactating Holstein cows. Theriogenology 39:797-810.

Badinga, L., W. W. Thatcher, C. J. Wilcox, G. Morris, K. Entwistle, and D. Wolfenson. 1994. Effect of season on follicular dynamics and plasma concentrations of estradiol-17β, progesterone and luteinizing hormone in lactating Holstein cows. Theriogenology 42:1263-1274.

Bao, B., and Garverick, H. A. 1998. Expression of steroidogenic enzyme and gonadotropin receptor genes in bovine follicles during ovarian follicular waves: A review. J. Anim. Sci. 76:1903-1921.

Bindon, B. M., L. R. Piper, and J. Timonier. 1984. Preovulatory LH characteristics and time of ovualtion in the prolific Booroola Merino ewe. J. Reprod. Fertil. 71:519-523.

Blache, D., C. J. Fabre-Nys, and G. Venier. 1991. Ventromedial hypothalamus as a target for oestradiol action on proceptivity, receptivity and luteinizing hormone surge of the ewe. Brain Research 546:241-249.

Bond, J., and R. E. McDowell. 1972. Reproductive performance and physiological responses of beef females as affected by a prolonged high environmental temperature. J. Anim. Sci. 35:820-829.

Bozworth, R.W., G. Ward, E.P. Call, and E.R. Bonewitz. 1972. Analysis of factors affecting calving intervals of dairy cows. J. Dairy Sci. 55:334-337.

Brackett, B. G., Y. K. Oh, J. F. Evans, and W. J. Donawick. 1980. Fertilization and early development of cow ova. Biol. Reprod. 23:189-205.

Brewster, J. E., and C. L. Cole. 1941. The time of ovulation in cattle. J. Dairy Sci. 24:111-115.

Britt, J. H., R. G. Scott, J. D. Armstrong, and M. D. Whitacre. 1986. Determinants of estrous behavior in lactating Holstein cows. J. Dairy Sci. 69:2195-2202.

Cardwell, B. E., G. Q. Fitch, and R. D. Geisert. 1998. Ultrasonic evaluation for the time of ovulation in ewes treated with norgestomet and norgestomet followed by pregnant mares serum gonadotropin. J. Anim. Sci. 76:2235-2238.

Carrick, M. J., and J. N. Shelton. 1969. Oestrogen-progesterone relationships in the induction of oestrous in spayed heifers. J. Endocrinol. 45:99-109.

Cavestany, D., A. B. El-Wishy, and R. H. Foote. 1985. Effect of season and high environmental temperature on fertility of Holstein cattle. J. Dairy Sci. 68:1471-1478.

Chenault, J.R., W. W. Thatcher, P. S. Kalra, R. M. Abrams, and C. J. Wilcox. 1976. Plasma progestins, estradiol, and luteinizing hormone following prostaglandin $F_{2\alpha}$ injection. J. Dairy Sci. 59:1342-1346.

Chenault, J. R., W. W. Thatcher, P. S. Kalra, R. M. Abrams, and C. J. Wilcox. 1975. Transitory changes in plasma progestins, estradiol, and luteinizing hormone approaching ovulation in the bovine. J. Dairy Sci. 58:709-717.

Choudary, J. B., H. T. Gier, and G. B. Marion. 1968. Cyclic changes in bovine vesicular follicles. J. Anim. Sci. 27:468-471.

Christenson, R. K., S. E. Echternkamp, and D. B. Laster. 1975. Oestrus, LH, ovulation and fertility in beef heifers. J. Reprod. Fertil. 43:543-546.

Christensen, D. S., M. L. Hopwood, and J. N. Wiltbank. 1974. Levels of hormones in the serum of cycling beef cows. J. Anim. Sci. 38:577-583.

Ciccioli, N. H., and R. P. Wettemann. 2000. Nutritional effects on estrus and ovarian activity of spring calving first-calf heifers. Oklahoma Agric. Exp. Stn. Res. Rep. P-980:160-163.

Clegg, M.T., J.A. Santolucito, J.D. Smith, and W.F. Ganong. 1958. The effect of hypothalamic lesions on sexual behavior and estrous cycles in the ewe. Endocrinology 62:790-797.

Coe, B. L., and R. D. Allrich. 1989. Relationships between endogenous estradiol-17β and estrous behavior in heifers. J. Anim. Sci. 67:1546-1551.

Coleman, D. A., W. V. Thayne, and R. A. Dailey. 1985. Factors affecting reproductive performance of dairy cows. J. Dairy Sci. 68:1793-1803.

Colgin, D. C., and W. J. Murdoch. 1997. Evidence for a role of the ovarian surface epithelium in the ovulatory mechanism of the sheep: Secretion of urokinase-type plasminogen activator. Anim. Reprod. Sci. 47:197-204.

Cook, D. L., T. A. Winters, L. A. Horstman, and R. D. Allrich. 1986. Induction of estrus in ovariectomized cows and heifers: Effects of estradiol benzoate and gonadotropin releasing hormone. J. Anim. Sci. 63:546-550.

Cook, D. L., T. A. Winters, L. A. Horstman, and R. D. Allrich. 1987. Influence of cortisol and dexamethasone on estrous behavior of estradiol-treated ovariectomized cows and heifers. J. Dairy Sci. 70:181-185.

Davidge, S. T., J. L. Wiebold, P. L. Senger, and J. K. Hillers. 1987. Influence of varying levels of blood progesterone upon estrous behavior in cattle. J. Anim. Sci. 64:126-132.

Dehnhard, M., and R. Claus. 1996. Attempts to purify and characterize the estrus-signalling pheromone from cow urine. Theriogenology 46:13-22.

De Silva, A. W. M. V., G. W. Anderson, F. C. Gwazdauskas, M. L. Mc Gilliard, and J. A. Lineweaver. 1981. Interrelationships with estrous behavior and conception in dairy cattle. J. Dairy Sci. 64:2409-2418.

Dobson, H. 1978. Plasma gonadotrophins and oestradiol during oestrus in the cow. J. Reprod. Fertil. 52:51-53.

Dransfield, M. B. G., R. L. Nebel, R. E. Pearson, and L. D. Warnick. 1998. Timing of insemination for dairy cows identified in estrus by a radiotelemetric estrus detection system. J. Dairy Sci. 81:1874-1882.

Dufour, J., H. L. Whitmore, O. J. Ginther, and L. E. Casida. 1972. Identification of the ovulating follicle by its size on different days of the estrous cycle in heifers. J. Anim. Sci. 34:85-87.

Echternkamp, S. E., and W. Hansel. 1973. Concurrent changes in bovine plasma hormone levels prior to and during the first postpartum estrous cycle. J. Anim. Sci. 37:1362-1370.

Erickson, B. H. 1966a. Development and radio-response of the prenatal bovine ovary. J. Reprod. Fertil. 10:97-105.

Erickson, B. H. 1966b. Development and senescence of the postnatal bovine ovary. J. Anim. Sci. 25:800-805.

Espy, L. L. 1994. Current status of the hypothesis that mammalian ovulation is comparable to an inflammatory reaction. Biol. Reprod. 50:233-238.

Esslemont, R. J., R. G. Glencross, M. J. Bryant, and G.S. Pope. 1980. A quantitative study of pre-ovulatory behaviour in cattle. Appl. Anim. Ethology 6:1-17.

Evans, D. G. 1978. The interpretation and analysis of subjective body condition scores. Anim. Prod. 26:119-125.

Evans, A. C., C. M. Komar, S. A. Wandji, and J. E. Fortune. 1997. Changes in androgen secretion and luteinizing hormone pulse amplitude are associated with the recruitment and growth of ovarian follicles during the luteal phase of the bovine estrous cycle. Biol. Reprod. 57:394-401.

Fabre-Nys, C., and G. B. Martin. 1991. Roles of progesterone and oestradiol in determining the temporal sequence and quantitative expression of sexual receptivity and the preovulatory LH surge in the ewe. J. Endocrinol. 130:367-379.

Feldmann, F., E. Aizinbud, H. Schindler, and H. Broda. 1978. The electrical conductivity inside the bovine vaginal wall. Anim. Prod. 26:61-65.

Floyd, L.N., C. A. Lents, F. J. White, and R. P. Wettemann. 2001. Effects of size of pen and number of cows in estrus on estrous behavior. J. Anim. Sci. 79:(in press).

Foote, R. H. 1974. Estrus detection and estrus detection aids. J. Dairy Sci. 58:248-256.

Foote, R. H., E. A. B. Oltenacu, J. Mellinger, N. R. Scott, and R. A. Marshall. 1979. Pregnancy rate in dairy cows inseminated on the basis of electronic probe measurements. J. Dairy Sci. 62:69-73.

Fortune, J. E. 1994. Ovarian follicular growth and development in mammals. Biol. Reprod. 50:225-232.

Garverick, H. A., R. E. Erb, G. D. Niswender, and C. J. Callahan. 1971. Reproductive steroids in the bovine. III. Changes during the estrous cycle. J. Anim. Sci. 32:946-956.

Geary, T. W., E. R. Downing, J. E. Bruemmer, and J. C. Whittier. 1999. Ovarian estrous response of suckled beef cows to the select synch estrous synchronization protocol. Prof. Anim. Sci. 16:1-5.

Gibbons, J. R., M. C. Wiltbank, and O. J. Ginther. 1997. Functional interrelationships between follicles greater than 4 mm and the follicle-stimulating hormone surge in heifers. Biol. Reprod. 57:1066-1073.

Gilad, E., R. Meidan, A. Berman, Y. Graber, and D. Wolfenson. 1993. Effect of heat stress on tonic and GnRH-induced gonadotrophin secretion in relation to concentration of oestradiol in plasma of cyclic cows. J. Reprod. Fertil. 99:315-321.

Ginther, O. J., D. R. Bergfelt, L. J. Kulick, and K. Kot. 1998. Pulsatility of systemic FSH and LH concentrations during follicular-wave development in cattle. Theriogenology 50:507-519.

Ginther, O. J., D. R. Bergfelt, L. J. Kulick, and K. Kot. 2000. Selection of the dominant follicle in cattle: Role of estradiol. Biol. Reprod. 63:383-389.

Ginther, O. J., L. Knopf, and J. P. Kastelic. 1989. Temporal associations among ovarian events in cattle during oestrous cycles with two and three follicular waves. J. Reprod. Fert. 87:223-230.

Ginther, O. J., K. Kot, L. J. Kulick, S. Martin, and M. C. Wiltbank. 1996. Relationships between FSH and ovarian follicular waves during the last six months of pregnancy in cattle. J. Reprod. Fertil. 108:271-279.

Glencross, R. G., R. J. Esslemont, M. J. Bryant, and G. S. Pope. 1981. Relationships between the incidence of pre-ovulatory behaviour and the concentrations of oestradiol-17β and progesterone in bovine plasma. Appl. Anim. Ethology. 7:141-148.

Gwazdauskas, F. C., J. A. Lineweaver, and M. L. McGilliard. 1983. Environment and management factors affecting estrous activity in dairy cattle. J. Dairy Sci. 66:1510-1514.

Gwazdauskas, F. C., R. L. Nebel, D. J. Sprecher, W. D. Whittier, and M. L. McGilliard. 1990. Effectiveness of rump-mounted devices and androgenized females for detection of estrus in dairy cattle. J. Dairy Sci. 73:2965-2970.

Gwazdauskas, F. C., C. J. Wilcox, and W. W. Thatcher. 1975. Environmental and managemental factors affecting conception rate in a subtropical climate. J. Dairy Sci. 58:88-92.

Hackett, A. J., and A. J. McAllister. 1984. Onset of estrus in dairy cows maintained indoors year-round. J. Dairy Sci. 67:1793-1797.

Hausler, C. L., and P. V. Malven. 1976. Interaction of progesterone, GnRH and estradiol in the control of LH release in castrate heifers. J. Anim. Sci. 42:1239-1243.

Hawk, H. W. 1987. Transport and fate of spermatozoa after insemination of cattle. J. Dairy Sci. 70:1487-1503.

Heape, W. 1900. The "sexual season" of mammals and the relation to the "pro-oestrum" to menstruation. Quart. J. Microscop. Sci. 44:1-70.

Hein, K. G., and R. D. Allrich. 1992. Influence of exogenous adrenocorticotropic hormone on estrous behavior in cattle. J. Anim. Sci. 70:243-247.

Helmer, S. D., and J. H. Britt. 1985. Mounting behavior as affected by stage of estrous cycle in Holstein heifers. J. Dairy Sci. 68:1290-1295.

Henricks, D. M., J. F. Dickey, J. R. Hill. 1971. Plasma estrogen and progesterone levels in cows prior to and during estrus. Endocrinology. 89:1350-1355.

Henricks, D.M., J. F. Dickey, and G. D. Niswender. 1970. Serum luteinizing hormone and plasma progesterone levels during the estrous cycle and early pregnancy in cows. Biol. Reprod. 2:346-351.

Hough, W. H., H. J. Bearden, and W. Hansel. 1955. Further studies on factors affecting ovulation in the cow. J. Anim. Sci. 14:739-745.

Howell, J. L., J. W. Fuquay, and A. E. Smith. 1994. Corpus luteum growth and function in lactating Holstein cows during spring and summer. J. Dairy Sci. 77:735-739.

Hunter, R. H. F. 1989. Ageing of the unfertilised cow egg in vivo: How soon is fertility compromised? Vet. Rec. 124:489-490.

Hurnik, J. F., and G. J. King. 1987. Estrous behavior in confined beef cows. J. Anim. Sci. 65:431-438.

Hurnik, J. F., G. J. King, and H. A. Robertson. 1975. Estrous and related behaviour in postpartum Holstein cows. Appl. Anim. Ethology 2:55-68.

Ireland, J. J. 1987. Control of follicular growth and development. J Reprod. Fertil. Suppl. 34:39-54.

Ireland, J. J., R. L. Fogwell, W. D. Oxender, K. Ames, and J. L. Cowley. 1984. Production of estradiol by each ovary during the estrous cycle of cows. J. Anim. Sci. 59:764-771.

Ireland, J. J., and J. F. Roche. 1983a. Development of nonovulatory antral follicles in heifers: Changes in steroids in follicular fluid and receptors for gonadotropins. Endocrinology 112:150-156.

Ireland, J. J., and J. F. Roche. 1983b. Growth and differentiation of large antral follicles after spontaneous luteolysis in heifers: Changes in concentration of hormones in follicular fluid and specific binding of gonadotropins to follicles. J. Anim. Sci. 57:157-167.

Kaneko, H., T. Terada, K. Taya, G. Watanabe, S. Sasamoto, Y. Hasegawa, and M. Igarashi. 1991. Ovarian follicular dynamics and concentrations of oestradiol-17β, progesterone, luteinizing hormone and follicle stimulating hormone during the periovulatory phase of the oestrous cycle in the cow. Reprod. Fertil. Dev. 3:529-535.

Katz, L. S., E. A. B. Oltenacu, and R. H. Foote. 1980. The behavioral responses in ovariectomized cattle to either estradiol, testosterone, androstenedione, or dihydrotestosterone. Hormones and Behavior. 14:224-235.

Kiddy, C. A. 1977. Variation in physical activity as an indication of estrus in dairy cows. J. Dairy Sci. 60:235-243.

Kiddy, C. A., D. S. Mitchell, D. J. Bolt, and H. W. Hawk. 1978. Detection of estrus-related odors in cows by trained dogs. Biol. Reprod. 19:389-395.

Kiddy, C. A., D. S. Mitchell, and H. W. Hawk. 1984. Estrus-related odors in body fluids of dairy cows. J. Dairy Sci. 67:388-391.

Kiser, T. E., J. H. Britt, H. D. Ritchie. 1977. Testosterone treatment of cows for use in detection of estrus. J. Anim. Sci. 44:1030-1035.

Kot, K., and O. J. Gintehr. 1999. Ultrasonic characterization of ovulatory follicular evacuation and luteal development in heifers. J. Reprod. Fertil. 115:39-43.

Kulick, L. J., K. Kot, M. C. Wiltbank, and O. J. Ginther. 1999. Follicular and hormonal dynamics during the first follicular wave in heifers. Theriogenology 52:913-921.

Ladewig, J., and B. Hart. 1981. Demonstration of estrus-related odors in cows urine by operant conditions of rats. Biol. Reprod. 24:1165-1169.

Lane, A. J. P., and D. C. Wathes. 1998. An electronic nose to detect changes in perineal odors associated with estrus in the cow. J. Dairy Sci. 81:2145-2150.

Lefebvre, D. M., and E. Block. 1992. Effect of recombinant bovine somatotropin on estradiol-induced estrous behavior in ovariectomized heifers. J. Dairy Sci. 75:1461-1464.

Lehrer, A. R., G. S. Lewis, and E. Aizinbud. 1992. Oestrus detection in cattle: Recent developments. Anim. Reprod. Sci. 28:355-361.

Leidl, W., and R. Stolla. 1976. Measurement of electric resistance of the vaginal mucus as an aid for heat detection. Theriogenology. 6:237-243.

Lents, C. A., F. J. White, D. L. Lalman, and R. P. Wettemann. 2000. Effects of body condition of beef cows at calving and protein supplementation on estrous behavior and follicle size. Oklahoma Agric. Exp. Stn. Res. Rep. P-980:164-168.

Lewis, G. S., E. Aizinbud, and A. R. Lehrer. 1989. Changes in electrical resistance of vulvar tissue in Holstein cows during ovarian cycles and after treatment with prostaglandin $F_{2\alpha}$. Anim. Reprod. Sci. 18:183-197.

Lewis, G. S., and S. K. Newman. 1984. Changes throughout the estrous cycles of variables that might indicate estrus in dairy cows. J. Dairy Sci. 67:146-152.

Lorton, L. L., T. D., C. F. Rosenkrans, and R. W. Rorie. 1999. Effect of estrous parameters and time of insemination on pregnancy rate in cattle. J. Anim. Sci. 77 (Suppl. 1):15 (Abs.).

Lussier, J.G., P. Matton, J. J. Dufour. 1987. Growth rates of follicles in the ovary of the cow. J. Reprod. Fertil. 81:301-307.

Macmillan, K. L., V. K. Taufa, D. R. Barnes, A. M. Day, and R. Henry. 1988. Detecting estrus in synchronized heifers using tailpaint and an aerosol raddle. Theriogenology 30:1099-1113.

Marion, G. B., H. T. Gier, and J. B. Choudary. 1968. Micromorphology of the bovine ovarian follicular system. J. Anim. Sci. 27:451-465.

Martin, T. E., D. M. Henricks, J. R. Hill, and N. C. Rawlings. 1978. Active immunization of the cow against oestradiol-17β. J. Reprod. Fertil. 53:173-178.

Mason, B. D., C. R. Krishnamurti, and W. D. Kitts. 1972. Oestrone and oestradiol in jugular vein plasma during the oestrous cycle of the cow. J. Endocrinology 55:141-146.

Mathew, S. R., W. P. McCaughey, A. D. Kennedy, N. J. Lewis, and G. H. Crow. 1999. Electronic monitoring of mounting behavior in beef cattle on pasture. Can. Vet. J. 40:796-798.

Mattoni, M., E. Mukasa-Mugerwa, G. Cecchini, and S. Sovani. 1988. The reproductive performance of east African (*Bos Indicus*) Zebu cattle in Ethiopia. 1. Estrous cycle length, duration, behavior and ovulation time. Theriogenology 30:961-971.

McGuire, W. J., R. L. Larson, and G. H. Kiracofe. 1990. Syncro-Mate B® induces estrus in ovariectomized cows and heifers. Theriogenology 34:33-37.

McNatty, K. P., N. Hudson, M. Gibb, K. M. Henderson, S. Lun, D. Heath, and G. W. Montgomery. 1984. Seasonal differences in ovarian activity in cows. J. Endocrinology 102:189-198.

Merz, E. A., E. R. Hauser, and B. G. England. 1981. Ovarian function in the cycling cow: Relationship between gonadotropin binding to theca and granulosa and steroidogenesis in individual follicles. J. Anim. Sci. 52:1457-1466.

Mihm, M., E. J. Austin, T. E. M. Good, J. L. H. Ireland, P. G. Knight, J. F. Roche, and J. J. Ireland. 2000. Identification of potential intrafollicular factors involved in selection of dominant follicles in heifers. Biol. Reprod. 63:811-819.

Moeller, A. N., and N. L. VanDemark. 1951. The relationship of the interval between inseminations to bovine fertility. J. Anim. Sci. 10:988-992.

Moore, A. S., and S. L. Spahr. 1991. Activity monitoring and an enzyme immunoassay for milk progesterone to aid in the detection of estrus. J. Dairy Sci. 74:3857-3862.

Murdoch, W. J. 1995. Endothelial cell death in preovulatory ovine follicles: Possible implication in the biomechanics of rupture. J. Reprod. Fertil. 105:161-164.

Murdoch, W. J. 1999. Plasmin-tumor necrosis factor interaction in the ovulatory process. J. Reprod. Fertil. Suppl. 54:353-358.

Murdoch, W. J., D. C. Colgin, and J. A. Ellis. 1997. Role of tumor necrosis factor-α in the ovulatory mechanism of ewes. J. Anim. Sci. 75:1601-1605.

Murdoch, W. J., W. A. Van Kirk, and J. Murdoch. 1999a. Plasmin cleaves tumor necrosis factor-α exodomain from sheep follicular endothelium: Implication in the ovulatory process. Biol. Reprod. 60:1166-1171.

Murdoch, W. J., C. Wilken, and D. A. Young. 1999b. Sequence of apoptosis and inflammatory necrosis within the formative ovulatory site of sheep follicles. J. Reprod. Fertil. 117:325-329.

NAHMS. 1997. BEEF '97. USDA-APHIS Center for Epidemiology and Animal Health, Fort Collins, CO.

Nalbandov, A., and L. E. Casida. 1942. Ovulation and its relation to estrus in cows. J. Anim. Sci. 1:189-198.

Nebel, R. L., W. D. Whittier, B. G. Cassell, and J.H. Britt. 1987. Comparison of on-farm and laboratory milk progesterone assays for identifying errors in detection of estrus and diagnosis of pregnancy. J. Dairy Sci. 70:1471-1476.

Nebel, R. L. 1988. On-farm milk progesterone tests. J. Dairy Sci. 71:1682-1690.

Nessan, G. K., and G. J. King. 1981. Sexual behaviour in ovariectomized cows treated with oestradiol benzoate and testosterone propionate. J. Reprod. Fertil. 61:171-178.

Nishimura, K., K. Utsumi, T. Okano, and A. Iritani. 1991. Separation of mounting-inducing pheromones of vaginal mucus from estraul heifers. J. Anim. Sci. 69:3343-3347.

Olds, D., and D. M. Seath. 1951. Repeatability of the estrous cycle length in dairy cattle. J. Dairy Sci. 626-632.

Pennington, J. A., J. L. Albright, and C. J. Callahan. 1986. Relationships of sexual activities in estrous cows to different frequencies of observation and pedometer measurements. J. Dairy Sci. 69:2925-2934.

Pennington, J. A., J. L. Albright, and M. A. Diekman. 1985. Sexual activity of Holstein cows: Seasonal effects. J. Dairy Sci. 68:3023-3030.

Peter, A. T., and W. T. K. Bosu. 1986. Postpartum ovarian activity in dairy cows: Correlation between behavioral estrus, pedometer measurements, and ovulations. Theriogenology 26:111-115.

Pierson, R. A., and O. J. Ginther. 1987. Follicular populations during the estrous cycle in heifers. I. Influence of day. Anim. Reprod. Sci. 14:165-176.

Pierson, R. A., and O. J. Ginther. 1986. Ovarian follicular populations during early preganancy in heifers. Theriogenology 26:649-659.

Pierson, R. A., and O. J. Ginther. 1988. Ultrasonic imaging of the ovaries and uterus in cattle. Theriogenology 29:21-37.

Pierson, R. A., and O. J. Ginther. 1984. Ultrasonography of the bovine ovary. Theriogenology 21:495-505.

Plasse, D., A. C. Warnick, and M. Koger. 1970. Reproductive behavior of Bos Indicus females in a subtropical environment. IV. Length of estrous cycle, duration of estrus, time of ovulation, fertilization and embryo survival in grade Brahman heifers. J. Anim. Sci. 30:63-72.

Rae, D. O., P. J. Chenoweth, M. A. Giangreco, P. W. Dixon, and F. L. Bennett. 1999. Assessment of estrus detection by visual observation and electronic detection methods and characterization of factors associated with estrus and pregnancy in beef heifers. Theriogenology 51:1121-1132.

Rajakoski, E. 1960. The ovarian follicular system in sexually mature heifers with special reference to seasonal, cyclical, and left-right variations. Acta Endocrinol. (Suppl.) 52:1-68.

Rajamahendran, R., P.C. Lague, and R.D. Baker. 1979. Estrus and LH release in ovariectomized heifers following vaginal devices containing ovarian steroids. J. Anim. Sci. 49:554-559.

Rajamahendran, R., J. Robinson, S. Desbottes, and J. S. Walton. 1989. Temporal relationships among oestrus, body temperature, milk yield, progesterone, and luteinizing hormone levels, and ovulation in dairy cows. Theriogenology 31:1173-1182.

Rajamahendran, R., and C. Taylor. 1991. Follicular dynamics and temporal relationships among body temperature, oestrus, the surge of luteinizing hormone and ovulation in Holstein heifers treated with norgestomet. J. Reprod. Fertil. 92:461-467.

Ray, D. E. 1965. Oestrous response of ovariectomized beef heifers to oestradiol benzoate and human chorionic gonadotrophin. J. Reprod. Fertil. 10:329-335.

Redden, K. D., A. D. Kennedy, J. R. Ingalls, and T. L. Gilson. 1993. Detection of estrus by radiotelemetric monitoring of vaginal and ear skin temperature and pedometer measurements of activity. J. Dairy Sci. 76:713-721.

Reimers, T. J, R. D. Smith, and S. K. Newman. 1985. Management factors affecting reproductive performance of dairy cows in the northeastern United States. J. Dairy Sci. 68:963-972.

Rhodes, R. C., and R. D. Randel. 1978. Reproductive studies of Brahman Cattle I. Behavioral effect of various dose levels of estradiol-17β upon ovariectomized Brahman, Brahman x Hereford and Hereford cows. Theriogenology 9:429-435.

Richards, J. S. 1980. Maturation of ovarian follicles: Actions and interactions of pituitary and ovarian hormones on follicular cell differentiation. Physiol. Rev. 60:51-89.

Robinson, T. J. 1954. Necessity for progesterone with estrogen for the induction of recurrent estrus in the ovariectomized ewe. Endocrinology 55:403-408.

Roche, J. F. 1996. Control and regulation of folliculogenesis- a symposium in perspective. Reviews of Reproduction. 1:19-27.

Rodtian, P., G. King, S. Subrod, P. Pongpiachan. 1996. Oestrous behaviour of Holstein cows during cooler and hotter tropical seasons. Anim. Reprod. Sci. 45:47-58.

Rosenberg, M., Y. Folman, Z. Herz, I. Flamenbaum, A. Berman, and M. Kaim. 1982. Effect of climatic conditions on peripheral concentrations of LH, progesterone and oestradiol-17β in high milk-yielding cows. J. Reprod. Fertil. 66:139-146.

Rosenberg, M., Z. Herz, and M. Davidson, and Y. Folman. 1977. Seasonal variations in post-partum plasma progesterone levels and conception in primiparous and multiparous dairy cows. J. Reprod. Fertil. 51:363-367.

Rounsaville, T. R., P. A. Oltenacu, R. A. Milligan, and R. H. Foote. 1979. Effects of heat detection, conception rate, and culling policy on reproductive performance in airy herds. J. Dairy Sci. 62:1435-1442.

Saacke, R. G., J. C. Dalton, S. Nadir, R. L. Nebel, J. H. Bame. 2000. Relationship of seminal traits and insemination time to fertilization rate and embryo quality. Anim. Reprod. Sci. 60-61:663-677.

SAS. 1996. SAS/STAT® Software: Changes and Enhancements through Release 6.11. SAS Inst., Inc. Cary, NC.

Savio, J. D., L. Keenan, M. P. Boland, and J. F. Roche. 1988. Pattern of growth of dominant follicles during the oestrous cycle of heifers. J. Reprod. Fertil. 83:663-671.

Senger, P. L. 1994. The estrus detection problem: New concepts, technologies, and possibilities. J. Dairy Sci. 77:2745-2753.

Shemesh, M., N. Ayalon, and H. R. Lindner. 1972. Oestradiol levels in the peripheral blood of cows during the oestrous cycle. J. Endocrinol. 55:73-78.

Short, R. E., B. E. Howland, R. D. Randel, D. S. Christensen, and R. A. Bellows. 1973. Induced LH release in spayed cows. J. Anim. Sci. 37:551-557.

Sirois, J., and J. E. Fortune. 1990. Lengthening the bovine estrous cycle with low levels of exogenous progesterone: A model for studying ovarian follicular dominance. Endocrinology 127:916-925.

Sirois, J., and J. E. Fortune. 1988. Ovarian follicular dynamics during the estrous cycle in heifers monitored by real-time ultrasonography. Biol. Reprod. 39:308-317.

Soede, N. M., W. Hazeleger, J. Broos, and B. Kemp. 1997. Vaginal temperature is not related to the time of ovulation in sows. Anim. Reprod. Sci. 47:245-252.

Spicer, L. J., and S. E. Echternkamp. 1986. Ovarian follicular growth, function and turnover in cattle: A review. J. Anim. Sci. 62:428-451.

Staigmiller, R. B., B. G. England, R. Webb, R. E. Short, and A. Bellows. 1982. Estrogen secretion and gonadotrophin binding by individual bovine follicles during estrus. J. Anim. Sci. 55:1473-1482.

Steel, R. G. D., and J. H. Torrie. 1980. Principles and Procedures of Statistics: A Biometrical Approach. 2nd ed. McGraw-Hill Publishing Co., New York.

Stevenson, J. S., and J. H. Britt. 1977. Detection of estrus by three methods. J. Dairy Sci. 60:1994-1998.

Stevenson, J. S., G. C. Lamb, Y. Kobayashi, and D. P. Hoffman. 1998. Luteolysis during two stages of the estrous cycle: Subsequent endocrine profiles associated with radiotelemetrically detected estrus in heifers. J. Dairy Sci. 81:2897-2903.

Stevenson, J. S., M. K. Schmidt, and E. P. Call. 1983. Estrous intensity and conception rates in Holsteins. J. Dairy Sci. 66:275-280.

Stevenson, J. S., M. W. Smith, J. R. Jaeger, L. R. Corah, and D. G. LeFever. 1996. Detection of estrus by visual observation and radiotelemetry in peripubertal, estrus-synchronized beef heifers. J. Anim. Sci. 74:729-735.

Stock, A. E., and J. E. Fortune. 1993. Ovarian follicular dominance in cattle: relationship between prolonged growth of the ovulatory follicle and endocrine parameters. Endocrinology 132:1108-1114.

Stoebel, D. P., and G. P. Moberg. 1982. Effect of adrenocorticotropin and cortisol on luteinizing hormone surge and estrous behavior of cows. J. Dairy Sci. 65:1016-1024.

Sunderland, S. J., M. A. Crowe, M. P. Boland, J. F. Roche, and J. J. Ireland. 1994. Selection, dominance, and atresia of follicles during the oestrous cycle of heifers. J. Reprod. Fertil. 101:547-555.

Swanson, L. V., and H. D. Hafs. 1971. LH and prolactin in blood serum from estrus to ovulation in Holstein heifers. J. Anim. Sci. 33:1038-1041.

Swanson, L. V., H. D. Hafs, and D. A. Morrow. 1972. Ovarian characteristics and serum LH, prolactin, progesterone, and glucocorticoid from first estrus to breeding size in Holstein heifers. J. Anim. Sci. 34:284-293.

Tian, X. C., A. K. Berndtson, and J. E. Fortune. 1995. Differentiation of bovine preovulatory follicles during the follicular phase is associated with increases in messenger ribonucleic acid for cytochrome P450 side chain cleavage, 3β-hydroxysteroid dehydrogenase, and P450 17α-hydroxylase, but not P450 aromatase. Endocrinology 136:5102-5110.

Trimberger, G. W. 1948. Breeding efficiency in dairy cattle from artificial insemination at various intervals before and after ovulation. Nebraska Agric. Exp. Stn. Res. Bull. 153:1.

Trimberger, G. W. 1956. Ovarian functions, intervals between estrus, and conception rates in dairy cattle. J. Dairy Sci. 39:448-455.

Trout, J. P., L. R. McDowell, and P. J. Hansen. 1998. Characteristics of the estrous cycle and antioxidant status of lactating Holstein cows exposed to heat stress. J. Dairy Sci. 81:1244-1250.

Turner, A. I., P. H. Hemsworth, B. J. Canny, and A. J. Tilbrook. 1999. Sustained but not repeated acute elevation of cortisol impaired the luteinizing hormone surge, estrus, and ovulation in gilts. Biol. Reprod. 61:614-620.

Turzillo, A. M., and J. E. Fortune. 1990. Suppression of the secondary FSH surge with bovine follicular fluid is associated with delayed ovarian follicular development in heifers. J. Reprod. Fertil. 89:643-653.

United States Department of Agriculture. 1999. Agricultural Statistics. United States Government Printing Office. Washington. pp. VII 1-18.

Vailes, L. D., and J. H. Britt. 1990. Influence of footing surface on mounting and other sexual behaviors of estrual Holstein cows. J. Anim. Sci. 68:2333-2339.

Vizcarra, J. A., and R. P. Wettemann. 1996. Reproducibility, repeatability, and degree of expertise required to assess body condition score in beef cattle. Prof. Anim. Sci. 12:28-31.

Vizcarra, J. A., and R. P. Wettemann, T. D. Braden, A. M. Turzillo, and T. M. Nett. 1997. Effect of gonadotropin-releasing hormone (GnRH) pulse frequency on serum and pituitary concentrations of luteinizing hormone and follicle-stimulating hormone, GnRH receptors and messenger ribonucleic acid for gonadotrophin subunits in cows. Endocrinology 138:594-601.

Wagner, J. J., K. S. Lusby, J. W. Oltjen, J. Rakestraw, R. P. Wettemann, and L. E. Walters. 1988. Carcass composition in mature Hereford cows: Estimation and effect on daily metabolizable energy requirements during winter. J. Anim. Sci. 66:603-612.

Walker, W. L., R. L. Nebel, and M. L. McGilliard. 1996. Time of ovulation relative to mounting activity in dairy cattle. J. Dairy Sci. 79:1555-1561.

Walters, D. L., and E. Schallenberger. 1984. Pulsatile secretion of gonadotrophins, ovarian steroids, and ovarian oxytocin during the periovulatory phase of the oestrous cycle in the cow. J. Reprod. Fertil. 71:503-512.

Walton, J. S., L. P. Veenhuizen, and G. J. King. 1987. Relationships between time of day, estrous behavior, and the preovulatory luteinizing hormone surge in Holstein cows after treatment with Cloprostenol. J. Dairy Sci. 70:1652-1663.

Webb, R., B. K. Campbell, H. A. Garverick, J. G. Gong, C. G. Gutierrez, and D. G. Armstrng. 1999. Molecular mechanisms regulating follicular recruitment and selection. J. Reprod. Fertil. Suppl. 54:33-48.

Wettemann, R. P., H. D. Hafs, L. A. Edgerton, and L. V. Swanson. 1972. Estradiol and progesterone in blood serum during the bovine estrous cycle. J. Anim. Sci. 34:1020-1024.

Whyman, D., D. L. Johnson, T. W. Knight, and R. W. Moore. 1979. Intervals between multiple ovulations in PMSG-treated and untreated ewes and the relationship between ovulation and oestrus. J. Reprod. Fetril. 55:481-488.

Williams, W. F., D. R. Yver, and T. S. Gross. 1981. Comparison of estrus detection techniques in dairy heifers. J. Dairy Sci. 64:1738-1741.

Wilson, S. J., C. J. Kirby, A. T. Koenigsfeld, D. H. Keisler, and M. C. Lucy. 1998a. Effects of controlled heat stress on ovarian function of dairy cattle. 2. Heifers. J. Dairy Sci. 81:2132-2138.

Wilson, S. J., R. S. Marion, J. N. Spain, D. E. Spiers, D. H. Keisler, and M. C. Lucy. 1998b. Effects of controlled heat stress on ovarian function of dairy cattle. 1. Lactating cows. J. Dairy Sci. 81:2124-2131.

Wise, M. E., D. V. Armstrong, J. T. Huber, R. Hunter, and F. Wiersma. 1988. Hormonal alterations in the lactating dairy cow in response to thermal stress. J. Dairy Sci. 71:2480-2485.

Wolfenson, D., B. J. Lew, W. W. Thatcher, Y. Graber, and R. Meidan. 1997. Seasonal and acute heat stress effects on steroid production by dominant follicles in cows. Anim. Reprod. Sci. 47:9-19.

Wolfenson, D., W. W. Thatcher, L. Badinga, J. D. Savio, R. Meidan, B. J. Lew, R. Braw-Tal, and A. Berman. 1995. Effect of heat stress on follicular development during the estrous cycle in lactating dairy cattle. Biol. Reprod. 52:1106-1113.

Xu, Z. Z., D. J. Mcknight, R. Vishwanath, C. J. Pitt, and L. J. Burton. 1998. Estrus detection using radiotelemetry or visual observation and tail painting for dairy cows on pasture. J. Dairy Sci. 81:2890-2896.

Yelich, J. V., C. L. Barnett, J. K. Fullenwider, J. R. Kempfer, J. W. Lemaster, and C. C. Chase, Jr. 1999. Effect of season on behavioral estrus, ovulation, and estrous cycle length in Angus, Brahman and Senepol cows in a subtropical environment. J. Anim. Sci. 77 (Suppl. 1): 230 (Abs.).

APPENDIX

Time of ovulation after the onset of estrus for cows measured during multiple seasons

Cow ID	Season	Year	Time of ovulation after the onset of estrus
105	summer	1	35.4
105	spring	1	35
105	summer	2	34
140	summer	1	30.45
140	winter	1	36.43
140	spring	1	33.5
140	summer	2	36
162	winter	2	33
162	summer	2	30.25
177	summer	1	30.78
177	winter	1	34.9
177	spring	1	33
193	winter	2	33
193	spring	1	29.5
193	summer	2	28.25
335	summer	1	33.8
335	winter	1	36.98
335	spring	1	38
387	summer	1	29.57
387	winter	1	32.02

387	spring	1	32
387	summer	2	33.5
402	winter	2	31
402	spring	2	31
402	summer	2	29
501	winter	2	32
501	spring	2	28.5
501	summer	2	32.5
558	winter	2	28.5
558	spring	2	30.75
558	summer	1	30.75
558	winter	1	30.1
558	summer	2	26.5
597	summer	2	26.75
597	summer	1	35.62
597	winter	1	35.22
598	summer	1	36.25
598	winter	1	36.27
736	summer	1	21.82
736	winter	1	32.13
833	winter	2	37
833	winter	1	39.17
833	summer	2	29.5
878	winter	2	31.5
878	winter	1	26.65
878	spring	1	28.5
878	summer	2	29.75
882	winter	1	28.67

882	spring	1	29.5
882	summer	2	28.5
2600	winter	2	30
2600	winter	1	36.87
2600	spring	1	22
2600	summer	2	30.25
6301	winter	1	30.98
6301	spring	1	32.5
7381	winter	1	31.23
7381	spring	1	32.5
7841	winter	2	21.5
7841	spring	2	39.5
7841	summer	1	33.33
7841	winter	1	30.53
7841	spring	1	30
7841	summer	2	34.75
8632	winter	2	29
8632	spring	1	29
8632	summer	2	30
8901	summer	1	30.18
8901	spring	1	29.5
8901	summer	2	33.5
8911	winter	2	29.5
8911	spring	1	28.5
8911	summer	2	27
9220	summer	1	37.5
9220	winter	1	31
9220	spring	1	36

9402	winter	2	31
9402	winter	1	32.23
9402	spring	1	25
9402	summer	2	33.75

VITA

Frankie Joe White

Candidate for the Degree of

Master of Science

Thesis: SEASONAL EFFECTS ON ESTROUS BEHAVIOR AND TIME OF OVULATION IN BEEF COWS

Major Field: Animal Science

Biographical:

Personal Data: Born in Wichita Falls, Texas; November 22, 1975; the son of Frank and Diana White.

Education: Graduated from Jacksboro High School, Jacksboro, Texas in May 1994; Received Bachelor of Science in Animal Science from Texas Tech University, Lubbock, Texas in May 1998. Completed the requirements for the Master of Science Degree in Animal Science at Oklahoma State University, December 2000.

Professional Experience: Graduate research and teaching assistant, Oklahoma State University, June 1998 to December 2000.

Professional Organizations: American Society of Animal Science, American Registry of Professional Animal Scientists; Sigma Xi Scientific Research Society, Society for the Study of Reproduction, Society for Experimental Biology and Medicine, Phi Kappa Phi Honor Society, Gamma Sigma Delta Agricultural Honor Society