

**EFFECT OF ONCE-DAILY SUCKLING AND PARITY ON
FOLLICULAR DYNAMICS IN POSTPARTUM BRAHMAN COWS**

A Thesis

by

JAVIER RAMIREZ III

Submitted to the Office of Graduate Studies of
Texas A&M University
in partial fulfillment of the requirements for the degree of

MASTER OF SCIENCE

May 2006

Major Subject: Physiology of Reproduction

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ABSTRACT

Effect of Once-daily Suckling and Parity on Follicular Dynamics in Postpartum

Brahman Cows.

(May 2006)

Javier Ramirez III, B.S., Texas A&M University-Kingsville

Chair of Advisory Committee: Dr. Ronald D. Randel

Changes in follicular dynamics were monitored in forty-four Brahman multiparous (n=30) and primiparous (n=14) cows randomly assigned to control (n=22) and once-daily suckling (n=22) treatments. Daily transrectal ultrasonography images were taken from d 21 through 88 post-calving or detection of first estrus. Suckling treatment was introduced on d 28 post-calving. Follicular waves were profiled and characterized by development and regression of a variable number of small and medium sized (2-9 mm) follicles before one of the follicles differentiated and became the dominant follicle. Primiparous cows had 34% more ($P < 0.04$) follicular waves (4.8 ± 0.6) than did the multiparous cows (3.1 ± 0.4) prior to first ovulation (FO). Once-daily suckled multiparous cows had 37% fewer ($P < 0.03$) follicular waves (2.4 ± 0.5) prior to FO than controls. Interval from calving to FO was reduced ($P < 0.05$) by an average of 12 d by once-daily suckling in multiparous cows. Primiparous cows developed 30% more ($P < 0.02$) dominant follicles (6.3 ± 0.6) from d 21 to estrus than did the multiparous cows (4.3 ± 0.4). Ovulation before d 88 occurred in 42 of 44 (95%) cows. Behavioral estrus was not detected in 40 of 42 (95%) cows at FO. The length of the subsequent

estrous cycle was short (<17 days) in 39 of 42 (93%) cows. We can conclude from these data that ovarian function is resumed shortly after parturition and ovulation of a dominant follicle ends postpartum anestrous. Primiparous cows experienced more follicular waves and therefore grew more dominant follicles prior to first estrus. Once-daily suckling hastened return to estrus and reduced the number of follicular waves prior to first post-partum estrus in multiparous cows. The incidence of first ovulation being associated with behavioral estrus was low. Short cycles followed first ovulation. It appears obligatory that postpartum Brahman cows experience a silent ovulation with formation of functional luteal tissue prior to resumption of normal estrous cyclicity.

DEDICATION

This thesis is dedicated to my parents:

My father Javier... A man of integrity, dedication and determination.

And

My mother Elsa... My source of love, strength and, above all, faith.

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INTRODUCTION

Beef cows must maintain a yearly calving interval in order to sustain economic viability. Length of the postpartum interval has a direct effect on attainment of a yearly calving schedule. Postpartum cows undergo a period of anestrus after calving due to anovulation of a dominant follicle rather than lack of development of a dominant follicle (Murphy et al., 1990). Primiparous cows have a significantly large interval from calving to estrus compared to multiparous cows (Wiltbank, 1970). Suckling by the newborn calf suppresses return to estrous cyclicity which reduces opportunity for conception to occur earlier in the postpartum period (Margerison et al., 2002). Once-daily suckling has been proven to shorten the postpartum interval in first calf beef heifers (Randel, 1981). Despite the fact that suckling frequency and parity affect postpartum reproductive processes, the effect on follicular dynamics requires further clarification. Ultrasonography is a reliable method for monitoring follicular development (Pierson and Ginther, 1988). A daily record of follicular observations must be maintained to accurately describe differences in follicular dynamics. A method of ultrasonography was developed for ultrasonically characterizing follicular waves without the necessity of maintaining day-to-day identities of individual follicles. The “nonidentity” method is appropriate for profiling waves, detecting ovulation, and corpus luteum formation (Ginther, 1993). The proposed project was designed to determine differences in follicular dynamics between suckling treatments and parity by utilizing the

This thesis follows the style of the Journal of Animal Science.

ultrasonography based “nonidentity” method of observing follicles. Thus the objectives of this research were to examine the effects of suckling treatment and parity on:

- 1) postpartum follicular dynamics;
- 2) return to estrus;
- 3) postpartum reproduction;
- 4) calf weight gain;
- 5) cow body weight and body condition score.

LITERATURE REVIEW

Follicular Development

Folliculogenesis is a complex process involving pituitary gonadotropins, ovarian steroids, and other non-steroidal factors (Garcia et al., 1999). Ovarian follicular development in cattle occurs in waves (Sirois and Fortune, 1988). Follicular waves are the synchronous development of several follicles (5 to 10) on each ovary (Kastelic, 1994). Follicular development essentially involves three stages: follicular growth wave, maturation, and atresia. The dynamics of follicular waves include follicular recruitment, selection, and dominance. Follicular wave emergence is defined as occurring when the future dominant follicle first reaches a diameter of 4 mm (Ginther et al., 1998). Several follicles are recruited from a gonadotropin sensitive follicular pool, and subsequently, a few of the recruited follicles are selected for continued development. There is a relationship between the circulating concentrations of gonadotropins and the follicular concentrations of FSH receptors in determining the number of follicles recruited from the early antral pool (Adams et al., 1992). An even smaller proportion of the selected follicles move on to become dominant. The dominant follicle then inhibits growth of the subordinate follicles, which do not advance to the next stage of the wave and undergo atresia (Kastelic, 1994). Dominant follicles then compete for deviation. Deviation is defined as the beginning of a change in growth rates between the two largest follicles (Ginther et al., 1998). This takes place at around d 3 of the follicular wave, when the future dominant and the largest subordinate follicles are about 8 and 7 mm in diameter, respectively (Ginther et al., 1996). The importance of LH in the final stages of

differentiation of the follicle prior to the LH surge is emphasized by the results of experiments in which plasma progesterone was artificially maintained at concentrations that were between luteal and basal for prolonged periods of time (Roberson et al., 1989). Under those conditions, LH pulse frequency increased to follicular phase levels and growth of the dominant follicle continued in a linear fashion. The follicle attaining the greater rate of growth proceeds to maturation and possibly ovulation. Only those follicles recruited during or after CL regression are eligible for ovulation. The mechanisms involved in the selection of the dominant follicle have not yet been determined, but dominance appears to be maintained by negative feedback effects of products of the dominant follicle on circulating FSH (Fortune, 1994). An FSH surge is necessary for the emergence of a follicular wave and, therefore, the process of follicular growth is halted (Adams et al., 1992). Duration of dominance seems to have an effect on reproductive performance. Restricting the duration of dominance of the preovulatory follicle to ≤ 4 d at estrus, results in a precise onset of estrus and a high pregnancy rate following a single AI at a detected estrus (Austin et al., 1999).

The mean number of days from parturition to detection of the first postpartum dominant follicle has been reported to be 10.2 ± 0.5 (Murphy et al., 1990). However, the incidence of ovulation of the first dominant follicle was low. In fact, an average of three dominant follicles was observed for each animal before ovulation occurred. Signs of estrus are less obvious or absent preceding first ovulation, and first ovulation is followed by a short luteal phase. The shorter luteal phase is associated with less progesterone than subsequent luteal phases (Perry et al., 1991a). These abnormalities may be due to a

lack of synchrony between the preovulatory follicle and the LH surge that results in ovulation of a pre- or post-mature follicle (Breuel et al., 1993)

Follicular dynamics are altered by negative energy balance and lactation (Lucy et al., 1992). Perry et al. (1991b) reported that the prepartum level of dietary energy influenced birth weight and weight gain of calves, milk production, concentrations and pulse frequency of LH in serum, appearance rate of large follicles, and the interval to first ovulation. Low levels of energy after parturition decreased the percentage of cows that ovulated. Nutritional deprivation seems to affect ovarian follicular growth via a luteinizing hormone-independent mechanism, probably involving intraovarian growth factors, when the level of nutrition falls below a critical threshold (Mackey et al., 2000a).

Ovulation

The successful development of an ovulatory follicle depends upon an integrated chain of events involving the ovary, corpus luteum, uterus and hypothalamic-pituitary-ovarian axis. This chain of events begins with the development of a preovulatory follicle, the activation and continued development of the oocyte after the preovulatory surge of LH (Garcia et al., 1999). These events are set in motion by LH released from the pituitary gland (LeMaire, 1989). There is considerable evidence to suggest that FSH alone can also induce ovulation, but may not be required under physiological conditions (Schwartz, 1974). The preovulatory surge of LH is induced by a surge in the secretion of GnRH into the hypophyseal portal blood. The GnRH surge is massive and sustained, and is triggered by the increase in circulating estradiol secreted from the developing

preovulatory ovarian follicle (Karsch et al., 1992). The follicular reaction to the LH-receptor complex has a minimum of two discrete steps: 1) the stimulation of steroid synthesis by the LH-receptor complex, and 2) the activation of an ovulatory enzyme by the steroid released (Rondell, 1974). Furthermore, Rondell (1974) noted that the sequence of events leading to steroid synthesis involves the activation of adenylyl cyclase by the LH-receptor complex, which results in increased tissue levels of cyclic AMP, inducing protein synthetic processes essential for increased synthesis of progesterone. The progesterone then stimulates protein synthetic activity, essential to the elaboration of an enzyme which weakens the collagen framework of the theca externa. It is apparent that there is no significant change in intrafollicular pressure in ovulation but, that there is a marked increase in distensibility of wall tissue probably associated with a decrease in breaking strength (Rondell, 1970). Moreover, analyses of the mechanics of rupture indicate that a relatively small change in distensibility can produce an unstable follicle which would rupture at normal intrafollicular pressures.

Corpus Luteum Development

When the follicle ruptures at ovulation, blood vessels within the follicular wall also rupture, and produce a bloody clot-like structure called the corpus hemorrhagicum. At 3 to 5 d the CL begins to increase in size and lose its hemorrhagic appearance (Senger, 2003). The CL is orange to yellow in color. Its name is Latin for “yellow body.” Corpora lutea are a continuation of follicular maturation and form from granulosa and theca interna cells, which differentiate into large and small steroidogenic luteal cells, respectively (Smith et al., 1994). It appears that small and large luteal cells

synergize to increase secretion of progesterone (Grazul-Bilska et al., 1992). An essential component of luteal development is the recruitment of a blood supply. The proliferation of capillaries during angiogenesis has several aspects, including 1) basement membrane breakdown, 2) endothelial cell migration, 3) endothelial cell proliferation, and 4) development of capillary lumina (Smith et al., 1994). Proliferation of endothelial cells is requisite for the neovascularization during luteal development that results in the extensive capillary network within the corpus luteum (Niswender et al., 2000). The rate of tissue growth during this time is profound in that ovulatory follicular tissue in sheep weighing ~40 mg develops into a CL that weighs 600 to 700 mg in just a few days (Farin et al., 1986).

Postpartum Anestrus

Postpartum anestrus is of particular concern to beef cattle producers because a beef cow has only approximately 80 d from calving until conception if an annual calving interval is to be maintained (Dunn and Moss, 1992). Optimal reproductive performance in beef cows is often limited by prolonged postpartum anestrus intervals (Ciccioli et al., 2003). Anestrus is the major component of postpartum infertility and is affected by several minor factors, including parity, and by two major factors: suckling and nutrition (Short et al., 1990). The postpartum interval can vary considerably and is largely determined by those two factors. It is clear that postpartum intervals are significantly shorter in multiparous cows when compared to primiparous cows (Williams, 1989; Webb, 1999). Heifers bred to calve at 2 yr of age resume ovarian function 20 to 40 d later than mature cows (Wiltbank, 1970). An extended and variable period of

anovulation and anestrus occurs in suckled beef cows following parturition (Williams, 1990). Furthermore, underfeeding of the postpartum cow extends the period of ovarian inactivity (Randel, 1990). Thus, thin cows are less likely to reproduce than those in moderate or good body condition (Dunn and Moss, 1992).

The postpartum anestrous period allows time for uterine involution, regained competency by the hypothalamic-pituitary-ovarian axis, and resumption of follicular development. In general, complete uterine involution in cattle is normally achieved in 4 to 7 wk (Lindell et al., 1982). Functional competence of the hypothalamic-pituitary-ovarian axis is attained about 20 d post-calving (Short et al., 1990). Preceding hypothalamic-pituitary-ovarian axis competency is a period of hypersensitivity to the negative feedback of estrogen which is gradually overcome to allow preovulatory LH release. This is necessary for the cascade of events that must occur prior to ovulation. Graafian follicles secrete estrogen inducing a positive feedback effect on the secretion of LH followed by an LH surge and ultimately ovulation (Walters et al., 1982b).

Effects of Suckling

Suckling-mediated anovulation remains as one of the foremost problems associated with management of beef cattle throughout the world (Williams et al., 1996). Cows nursing their own calves have reduced reproductive performance (Margerison, 2002). Milking does not seem to prolong anovulation because it apparently does not mimic the perception of suckling (Lamb et al., 1999). Suckling delays estrual activity independent of nutrient intake (Short et al., 1972). Williams (1990) described how high concentrations of estrogen late in gestation inhibit the synthesis of LH, and reported that pituitary stores of LH are depleted at parturition. Restoration of maximum pituitary LH stores and releasable pools of LH, and the ability of the hypothalamus to respond the positive feedback of estrogen occur within 3 to 4 weeks after calving in suckled cows, but the required pulsatile LH secretion is not well established. The GnRH “pulse generator” must somehow be inhibited during this period (Short et al., 1990). Suckling also depresses the duration of an induced LH surge, preventing the reliability of exogenous hormonal treatments to counteract the effects of suckling (Forrest et al., 1980).

Suckling has also been reported to suppress follicular LH receptor populations (Walters et al., 1982a). Removal of the suckling stimulus increases pituitary responsiveness to GnRH, increases serum LH and FSH concentrations, and follicular fluid concentrations of prolactin which, together or separately, cause an increase in LH and FSH receptors in the largest follicle (Walters et al., 1982b). Decreased LH receptor

populations also decrease synthesis of androgens, which are the precursors for the pro-estrus estradiol rise (Murphy et al., 1990).

Control of the Suckling Stimulus

The mean natural suckling frequency was 8.4 ± 4.8 times per day (Vandenheede et al., 2001). Various methods of reducing the effect of suckling have been examined. Short et al. (1972) reported that removing calves at birth shortened postpartum intervals by about 40 d. Later it was shown that weaning calves at three days of age resulted in a 23 d reduction in postpartum interval (Bellows et al., 1974). Both of these methods, although effective, leave no alternative but to rear calves artificially. Consequently, artificial rearing may have a greater negative biological and economic impact on producers than lengthy postpartum intervals. Reducing the frequency of the suckling stimulus to once-daily or twice-daily suckling was found to hasten return to estrus by 99 d and 6 d, respectively (Randel, 1981; Odde et al., 1986). Cows that were once-daily suckled demonstrated a 22 d reduction in postpartum interval and increased in frequency of return to estrus 40 to 80 d after calving (Del Vecchio et al., 1988). Browning et al., (1994) found that once-daily suckling reduced the postpartum interval by 3 wk and that initiation of restricted suckling resulted in a cow body weight loss of 2.3 kg compared to controls. The body weight loss of cows was probably due to 1) the psychogenic stress of being separated from their calves for part of the day, 2) increased activity searching for “lost” calves, and 3) reduced feed consumption (Symington and Hale, 1967). However, overall cow and calf performance appears not to be negatively affected. Once-daily suckling of first-calf beef heifers, from 30 d after calving to first estrus, shortened the

postpartum interval without depressing cow-calf performance (Randel, 1981). In fact, the once-daily suckled heifers weighed more at weaning than did normal suckled heifers. In the same study, calf average daily gain from birth to weaning was similar, since weaning weights were similar.

Various experimental trials have provided evidence supporting the idea that control of the suckling stimulus is not entirely for neural endocrine control, but rather for breaking the maternal bond between cow and calf (Hoffman et al., 1996). The mother offspring bond is an important link in suckling-mediated inhibition of LH secretion and ovulation (Williams et al., 1996). Calf presence without suckling is one factor that delays the onset of first postpartum ovulation in beef cows (Hoffman et al., 1996). The ability of the suckling calf to suppress tonic LH secretion derives from unique cues that are not fully simulated by frequent milking and the presence of the nonsuckling calf (Williams et al., 1987). However, one or both of these factors may modulate behavioral estrus. If suckling by a cow's own calf is replaced under controlled conditions with that by an alien (unrelated) calf, the constraints normally imposed by suckling on LH secretion diminish over a 2 to 4 d period (Silveira et al., 1993). Stagg et al. (1998) described that cows once-daily suckled and calves kept in isolation away from their dams have shorter postpartum intervals than cows once-daily suckled and calves left penned adjacent to them with continued visual, audio, and olfactory exposure. Griffith and Williams (1996) confirmed that suckling-related events sustain the inhibited pattern of LH secretion in cows only if the offspring are positively identified as their own. Olfaction and vision are equally effective in permitting calf identification, but

elimination of both senses prevents calf identification and the negative effects of suckling on LH secretion.

Neural connections from the mammary gland have been suggested to be primary relays for signals that originate from the calf and that theoretically suppress function of the LH pulse generator (Short et al., 1972; Williams et al., 1987; Williams, 1990). However, more recently Williams et al. (1993) utilized an experimental animal model in which complete neural disconnection of the udder in beef cows was executed to study the role of mammary somatosensory pathways in suckling-mediated anovulation. The objective of the surgical denervation was to remove 1 to 4 cm sections of the lumbar nerves and the perineal nerves, which comprise all sensory innervations to the teat and udder. It was concluded that suckling-mediated anovulation is not dependent upon mammary somatosensory cues.

Ovarian Ultrasonography

Research has made it possible to clarify the nature of bovine folliculogenesis for follicles with antral diameters of 3 mm or more (Pierson and Ginther, 1984). Its application in monitoring ovarian function in mammals has advanced the understanding of follicular dynamics and regulation (Garcia et al., 1999). It is now feasible and practical to trace the development of individual follicles by ultrasonography (Sirois and Fortune, 1988). There was 100% agreement between ultrasound and ovarian slicing

results for identification of the CL and cavities within the luteal gland. Follicles as small as 2 to 3 mm can be visualized, quantitated, and sequentially monitored (Pierson and Ginther, 1987). Diagnostic ultrasonography was determined to be a reliable method of identifying and measuring follicles (Pierson and Ginther, 1988). A method was developed for ultrasonically characterizing follicular waves without the necessity of maintaining day-to-day identities of individual follicles (nonidentity method). Results were compared to a method in which follicular identity was maintained (identity method). The “nonidentity” method seemed an appropriate tool for characterizing follicular waves (Ginther, 1993). Data was collected more quickly and without requirement for advanced skill by the nonidentity method. When two successive dominant follicles developed on the same ovary, size information was obscured for a few days where the profiles for each follicle crossed. The crossing point was termed an “area of ambiguity.” The “nonidentity” method assumes the regression of a dominant follicle occurs before the regression of the subsequent dominant follicle.

MATERIALS AND METHODS

Animals

Forty-four Brahman multiparous (n=30) and primiparous (n=14) cows were randomly assigned within parity, calving order, and sex of calf to either control (n=22) or once-daily suckled (n=22) treatments. Mean age for primiparous cows was $2.9 \text{ yr} \pm .1$ and $6.7 \text{ yr} \pm .2$ for multiparous cows and all were of similar weights and body condition score (Table 1). Calf sex was completely balanced within treatment and parity and all were of similar weights (Table 2). There were male (n=15) and female (n=15) calves in the multiparous group, and male (n=7) and female (n=7) calves in the primiparous group. Each suckling treatment group contained male (n=11) and female (n=11) calves. Cows were weighed and assigned a body condition score (BCS, 1 to 9, Mangione, 1998), and calves identified by ear tag and weighed within 24 h of parturition.

Cattle were maintained on rye and ryegrass pasture until calving. Post-calving cows were maintained on rye and ryegrass until that forage could no longer sustain nutritional requirements, and were placed on bermudagrass pasture through the end of the trial. Free choice water, salt, and minerals were made available. Coastal bermudagrass hay was fed as needed. Once-daily suckled calves were kept in a dry lot with access to free choice Coastal bermudagrass hay and supplemented with free choice corn/soybean meal (4:1 ration).

Table 1. Cow body weight (mean kg \pm SE) and body condition score (mean \pm SE) at treatment allotment (d 21 post-calving).

		Multiparous	Primiparous
Once Daily	Weight	511.3 \pm 17.3	459.9 \pm 15.9
	BCS	6.6 \pm 0.3	6.0 \pm 0.3
Control	Weight	543.7 \pm 13.5	490.2 \pm 15.5
	BCS	7.1 \pm 0.2	6.0 \pm 0.3

Table 2. Calf body weight (mean kg \pm SE) at treatment allotment (d 21 post-calving).

		Multiparous	Primiparous
Once Daily	Weight	36.5 \pm 1.4	37.9 \pm 1.4
Control	Weight	34.9 \pm 1.1	36.4 \pm 1.6

Data Collection

A Sonovet 600 (Universal Medical Systems) ultrasound equipped with a 7.5-MHz rectal probe was used to observe follicular size, ovulation, corpus luteum (CL) formation, and pregnancy. Follicular development on each ovary was monitored daily by transrectal ultrasonography from d 21 post-calving and continued through estrus detection or d 88 after calving. Two subsequent observations were made at d 7 and d 10 post-estrus for CL identification. Ovaries were scanned on multiple planes to ensure accurate follicle measurement. The internal ultrasound caliper was utilized to measure follicular diameters. The four largest follicles for each day on each ovary were measured. Images of the four follicles measured and the CL were saved on a 1.44-MB floppy disc by a Sony MaviCap (digital still image capture device). Images were transferred to a computer and a digital file was maintained for each individual cow.

Cows and calves were weighed and cow body condition score recorded weekly beginning at d 21 post-calving and continued through d 10 post-estrus. Further weight and BCS observations were recorded at 28 d intervals through weaning. Weaning weights were adjusted to 205 d for statistical analysis.

Blood samples were collected by venipuncture of a tail vessel of the cows into 10-mL evacuated blood collection tubes at weekly intervals to determine serum progesterone (P_4) concentration (d 21 through detection of estrus). Blood samples were also taken on d 7 and 10 post-estrus for corpus luteum confirmation. Samples were refrigerated (4°C) for 24 h followed by centrifugation (4°C, 2000x g) for 20 min. The collected serum was then stored at -20°C until hormone analysis. A radioimmunoassay

(RIA) procedure was utilized to determine blood progesterone content (Williams, 1989).

The antibody used was # 337 anti-progesterone-11-BSA serum at a 1:3300 dilution.

Assay constituents were as follows:

- 1) Inter-assay CV = 1.80%
- 2) Avg. Intra-assay CV = 13.15%
- 3) Avg. Max Binding (Bo/T) = 24.47%
- 4) Avg. Non-specific binding = 7.58%
- 5) Avg. Detection Limit = 0.063 ng/ml P₄

Suckling Treatment

Suckling treatments commenced on d 28 post-calving and continued through detection of estrus. Cows assigned to the control group had unlimited calf access and were exposed to *ad libitum* suckling. Once-daily suckled cows were limited to a suckling period of 45 to 60 min daily. However, once-daily cows did have visual, olfactory, and auditory exposure to their calves. Suckling was restricted by a physical barrier.

Artificial Insemination

Cows were artificially inseminated (AI) 12 h after detection of standing estrus. Semen from one bull and one single ejaculate was utilized for all cows. Vasectomized *Bos taurus x Bos indicus* bulls equipped with chin ball markers were introduced on d 1 post-calving for estrus detection. Bulls remained in the herd until every cow was detected in estrus. The cows were visually observed for estrus twice daily. Random allocation based on parity, treatment, and sex of calf was used to determine AI

technician (n=2) assignment. After first AI, cows were exposed to a fertile (intact) bull for the remainder of the breeding season. Pregnancy was established by uterine palpation per rectum and by ultrasonography 42 d after AI to determine first service conception rate.

Statistical Analysis

The effect of once-daily suckling, parity, and sex of calf on follicular dynamics, return to estrus, calf weight gains, cow weight and body condition score change, and progesterone concentrations were analyzed utilizing the general linear model (GLM) procedure of SAS with the cow as the experimental unit. Progesterone concentrations were also analyzed using the GLM repeated measure procedures of SAS. The calf served as the experimental unit for the calf weight analysis. Least square means (\pm standard error) are reported for all variables within main effect treatments. First service conception rates were analyzed by using the Chi Square procedures of SAS. All possible interactions were analyzed within the statistical model including 1) calf sex by parity, 2) calf sex by treatment, 3) parity by treatment, and 4) calf sex by parity by treatment.

RESULTS AND DISCUSSION

Effects of Parity

Cow body weight and body condition score. At the start of the suckling treatment (d 21), multiparous cows weighed (535.4 ± 10.3 kg) more ($P < 0.02$) than primiparous cows (480.4 ± 16.3 kg) (Table 3). Multiparous cows were numerically heavier at the end of the suckling treatment (Table 4). Cow body weights differed at weaning ($P < 0.02$) as well with primiparous weighing 482.8 ± 15.6 kg and multiparous cows weighing 545.8 ± 9.9 kg (Table 5). This can be attributed to the fact that primiparous cows were younger and had not reached mature weight.

A BCS of 5 or greater during the postpartum period ensures that body stores of nutrients are adequate for postpartum reproductive performance (Dziuck and Bellows, 1983). Cow body condition scores did not differ ($P > 0.10$) early in the postpartum period, but were significantly different ($P < 0.001$) at weaning where primiparous BCS was 5.0 ± 0.3 and multiparous BCS was 6.6 ± 0.2 (Table 5). These results agree with previous research (Williams, 1989) in which primiparous and multiparous females calved in good body condition, with the primiparous heifers tending to lose condition during the postpartum period. Short et al. (1990) stated that the approximate order for partitioning of nutrients is as follows: 1) basal metabolism, 2) activity, 3) growth, 4) basic energy reserves, 5) pregnancy, 6) lactation, 7) additional energy reserves, 8) estrous cycles and initiation of pregnancy and 9) excess reserves.

Calf Performance. Calf body weight was not influenced ($P>0.10$) by parity at the start of the suckling treatment (Table 3). However, primiparous cows had heavier ($P<0.03$) calves (97.7 ± 6.0 kg) than did the multiparous cows (82.9 ± 3.8 kg) at the end of the suckling treatment (Table 4). There was no influence of parity on weaning weights (Table 5). Moreover, there was no difference ($P>0.10$) in calf growth rates from birth to weaning or during the suckling treatment due to parity (Table 6). Bellows and Short (1978) reported that calf body weight at weaning is affected by parity, with multiparous cows weaning the heavier calves. The current research did not indicate statistical significance between multiparous and primiparous calf weaning weights. Conversely, weaning weight (mean kg \pm SE) was numerically greater for the calves from primiparous cows.

Table 3. Effect of parity on cow body weight (mean kg \pm SE), cow BCS (mean \pm SE), and calf body weight (mean kg \pm SE) at the start of the suckling treatment (d 21 post-calving).

Parity	Start of the Suckling Treatment		
	Cow Wt.	Cow BCS	Calf Wt.
MULTIPAROUS	535.4 \pm 10.3 ^a	6.8 \pm 0.20	62.3 \pm 1.4
PRIMIPAROUS	480.4 \pm 16.3 ^b	6.1 \pm 0.30	67.7 \pm 2.2

^{ab}Means with different superscripts between parity differ (P<0.02)

Table 4. Effect of parity on cow body weight (mean kg \pm SE), cow BCS (mean \pm SE), and calf body weight (mean kg \pm SE) at the end of the suckling treatment.

Parity	End of the Suckling Treatment		
	Cow Wt.	Cow BCS	Calf Wt.
MULTIPAROUS	545.7 \pm 10.3	7.1 \pm 0.16	82.9 \pm 3.8 ^a
PRIMIPAROUS	507.1 \pm 16.2	6.5 \pm 0.25	97.7 \pm 6.0 ^b

^{ab}Means with different superscripts between parity differ (P<0.03)

Table 5. Effect of parity on cow body weight (mean kg \pm SE), cow BCS (mean \pm SE), and calf 205 d adjusted body weight (mean kg \pm SE) at weaning.

Parity	Weight and BCS at Weaning		
	Cow Wt.	Cow BCS	Calf Wt.
MULTIPAROUS	545.8 \pm 9.9 ^a	6.6 \pm 0.17 ^c	206.8 \pm 4.0
PRIMIPAROUS	482.8 \pm 15.6 ^b	5.0 \pm 0.27 ^d	210.8 \pm 5.7

^{ab}Means with different superscripts between parity differ (P<0.02)

^{cd}Means with different superscripts between parity differ (P<0.001)

Table 6. Effect of parity on calf ADG (mean kg \pm SE) from birth to weaning and during the suckling treatment.

Parity	Calf Average Daily Gain	
	ADG Birth-Weaning	ADG During Suckling Treatment
MULTIPAROUS	0.83 \pm 0.01	0.76 \pm 0.07
PRIMIPAROUS	0.84 \pm 0.02	0.75 \pm 0.08

Postpartum Follicular Data. Primiparous cows experienced more ($P < 0.04$) follicular waves (4.8 ± 0.6) from d 21 through first ovulation than multiparous cows (3.1 ± 0.4) (Table 7). The number of follicular waves after first ovulation, preceding second ovulation did not differ ($P > 0.10$) between the two groups (Table 7). The length of the follicular wave associated with first ovulation was not influenced by parity ($P > 0.10$), but wave length associated with second ovulation was longer ($P < 0.04$) in multiparous cows (7.6 ± 0.3 d) compared to primiparous cows (6.4 ± 0.7 d) (Table 8). Multiparous cows had a shorter interval from calving to first ($P < 0.07$) and second ($P < 0.05$) ovulations compared to primiparous cows (Table 9). There was no parity difference ($P > 0.10$) in interval from first ovulation to second ovulation. More dominant follicles were developed ($P < 0.02$) by primiparous cows than multiparous cows (Table 10). Diameters of the first and second follicles developed after d 21 (Table 11) and the diameter of the first ovulatory follicle was not different ($P > 0.10$) between parity groups (Table 12). This agrees with Perry et al. (1991a) observations which showed that the first postpartum estrous cycle was initiated by ovulation of a follicle that was similar in size to the follicle that resulted in the second ovulation. However, the second ovulatory follicle, which was associated with behavioral estrus in all but one cow, was significantly larger ($P < 0.002$) in multiparous cows (16.3 ± 0.4 mm) than in primiparous cows (13.9 ± 0.6 mm). This is in contrast to results reported by Webb (1999), in that during the follicular wave associated with estrus, the size of the largest follicle was not influenced by parity.

Table 7. Effect of parity on number of follicular waves (mean \pm SE) prior to (beginning d 21 post-calving) and following first ovulation (FO).

Parity	Waves Prior to FO	Waves Following FO
MULTIPAROUS	3.1 \pm 0.4 ^a	1.3 \pm 0.2
PRIMIPAROUS	4.8 \pm 0.6 ^b	1.7 \pm 0.2

^{ab}Means with different superscripts between parity differ (P<0.04)

Table 8. Effect of parity on length of follicular wave (mean $d \pm SE$) associated with first (FO) and second ovulation (SO).

Parity	Length of Wave Associated with FO	Length of Wave Associated with SO
MULTIPAROUS	9.1 ± 0.8	7.6 ± 0.3^a
PRIMIPAROUS	9.3 ± 1.2	6.4 ± 0.7^b

^{ab}Means with different superscripts between parity differ ($P < 0.04$)

Table 9. Effect of parity on interval from calving to first (FO) and second ovulation (SO; mean d \pm SE).

Parity	Interval from Calving to FO	Interval from Calving to SO
MULTIPAROUS	41.8 \pm 3.5 ^a	51.8 \pm 3.4 ^c
PRIMIPAROUS	54.2 \pm 5.5 ^b	64.8 \pm 5.4 ^d

^{ab}Means with different superscripts between parity differ (P<0.07)

^{cd}Means with different superscripts between parity differ (P<0.05)

Table 10. Effect of parity on number of dominant follicles (mean \pm SE) from d 21 to estrus and interval from first to second ovulation (FO to SO; mean d \pm SE).

Parity	# of Dominant Follicles	Interval from FO to SO
MULTIPAROUS	4.3 \pm 0.4 ^a	9.4 \pm 0.9
PRIMIPAROUS	6.3 \pm 0.6 ^b	10.4 \pm 1.4

^{ab}Means with different superscripts between parity differ (P<0.02)

Table 11. Effect of parity on diameter (mean mm \pm SE) of the first and second dominant follicle detected after d 21.

Parity	1st Dominant Follicle Detected	2nd Dominant Follicle Detected
MULTIPAROUS	17.4 \pm 0.5	17.2 \pm 0.5
PRIMIPAROUS	15.1 \pm 0.9	15.7 \pm 0.8

Table 12. Effect of parity on diameter (mean mm \pm SE) of the first and second ovulatory follicle.

Parity	First Ovulatory Follicle	Second Ovulatory Follicle
MULTIPAROUS	17.7 \pm 0.4	16.3 \pm 0.4 ^a
PRIMIPAROUS	16.6 \pm 0.7	13.9 \pm 0.6 ^b

^{ab}Means with different superscripts between parity differ (P<0.002)

Progesterone concentrations associated with first and second luteal tissue did not differ ($P>0.10$) within parity (Table 13). Godfrey et al. (1989) reported similar results with no differences detected in post-estrus progesterone concentrations between multiparous and primiparous Brahman cows. Manns et al. (1983) indicated that the first increase in progesterone concentrations following parturition is typically of abnormal duration and low concentration when compared to females with normal luteal function. Similar observations were made in this study. Multiparous and primiparous cows showed a higher ($P<0.001$) serum progesterone concentration associated with the second luteal phase than with the first luteal phase (Table 13). The first period of elevated progesterone concentration has been described as a “priming event” and has been suggested to prime the hypothalamic-pituitary-ovarian axis for normal estrous cyclic activity. Mackey et al., (2000b) determined that progesterone treatment for 6 d preceding first ovulation brought about the desired effect of inducing a synchronous first ovulation, which was preceded by overt estrus activity and succeeded by an estrous cycle of normal duration. In addition, it was shown that LH increased more rapidly and serum estradiol concentrations were higher during the 3 d immediately before the preovulatory surge of LH in progesterone-treated postpartum cows (Breuel et al., 1993).

Table 13. Effect of parity on serum progesterone concentrations (mean ng/ml \pm SE) associated with first and second luteal tissue.

Parity	Serum P ₄ Conc. from First Luteal Phase	Serum P ₄ Conc. from Second Luteal Phase
MULTIPAROUS	1.35 \pm 0.24 ^a	5.57 \pm 0.59 ^b
PRIMIPAROUS	1.57 \pm 0.38 ^a	4.73 \pm 0.94 ^b

^{ab}Means with different superscripts differ (P<0.001)

Effects of Once-Daily Suckling

Cow body weight and body condition score. Cow body weights and body condition scores at the start and end of the suckling treatment were not statistically different between controls and once-daily suckling treatments ($P>0.10$) regardless of parity (Tables 14 and 15). Previous research showed that body weight and BCS and change thereof did not differ in multi- and primiparous cows among suckling treatments (Del Vecchio et al., 1988). However, control cows remained numerically heavier throughout the treatment period. Browning et al. (1994) reported that restricted suckling resulted in an average body weight decrease during the first week of calf separation, whereas unrestricted suckling maintained cow body weight during the same time period. The weight change became positive during the second week of calf separation. Cow body weight and body condition score at weaning were not affected ($P>0.10$) by suckling treatment in multiparous or primiparous cows (Table 16). This is in contrast to observations by Randel (1981) who reported that once-daily suckled primiparous cows weighed more at weaning than did the normal suckled primiparous cows, probably due to the short interval to return to estrus.

Table 14. Effect of once-daily suckling on cow body weight (mean kg \pm SE), cow BCS (mean \pm SE), and calf body weight (mean kg \pm SE) at the start of the suckling treatment (d 21 post-calving).

Start of the Suckling Treatment				
Parity	Treatment	Cow Wt.	Cow BCS	Calf Wt.
MULTIPAROUS	ONCE-DAILY	521.6 \pm 16.1	6.7 \pm 0.26	61.5 \pm 2.0
	CONTROL	549.3 \pm 16.1	7.0 \pm 0.26	63.2 \pm 2.0
PRIMIPAROUS	ONCE-DAILY	461.1 \pm 23.1	6.1 \pm 0.38	70.3 \pm 3.1
	CONTROL	499.7 \pm 23.1	6.0 \pm 0.38	65.0 \pm 3.1

Table 15. Effect of once-daily suckling on cow body weight (mean kg \pm SE), cow BCS (mean \pm SE), and calf body weight (mean kg \pm SE) at the end of the suckling treatment.

End of the Suckling Treatment				
Parity	Treatment	Cow Wt.	Cow BCS	Calf Wt.
MULTIPAROUS	ONCE-DAILY	534.3 \pm 15.6	6.9 \pm 0.25	73.9 \pm 5.3 ^a
	CONTROL	557.1 \pm 15.6	7.2 \pm 0.25	91.9 \pm 5.3 ^b
PRIMIPAROUS	ONCE-DAILY	498.5 \pm 22.9	6.4 \pm 0.35	95.9 \pm 8.4
	CONTROL	515.8 \pm 22.9	6.5 \pm 0.35	99.5 \pm 8.4

^{ab}Means with different superscripts between parity differ (P<0.03)

Table 16. Effect of once-daily suckling on cow body weight (mean kg \pm SE), cow BCS (mean \pm SE), and calf 205 d adjusted body weight (mean kg \pm SE) at weaning.

Weight and BCS at Weaning				
Parity	Treatment	Cow Wt.	Cow BCS	Calf Wt.
MULTIPAROUS	ONCE-DAILY	540.2 \pm 15.1	6.5 \pm 0.26	194.8 \pm 5.5 ^a
	CONTROL	551.3 \pm 15.1	6.6 \pm 0.26	218.7 \pm 5.5 ^b
PRIMIPAROUS	ONCE-DAILY	472.3 \pm 22.1	5.4 \pm 0.38	200.5 \pm 5.9 ^c
	CONTROL	493.4 \pm 22.1	4.5 \pm 0.38	221.1 \pm 5.9 ^d

^{ab}Means with different superscripts between parity differ (P<0.01)

^{cd}Means with different superscripts between parity differ (P<0.04)

Calf Performance. Calves out of primiparous and multiparous cows had similar body weights ($P>0.10$) at the start of the suckling treatment (Table 14). Calves of once-daily suckled multiparous cows weighed less ($P<0.03$) at the end of the suckling treatment than their control counterparts (Table 15). Suckling treatment did not affect ($P>0.10$) calf weight at the end of the treatment period in primiparous cows (Table 15). Weaning weights were influenced by once-daily suckling ($P<0.01$) in calves out of multiparous cows (Table 16). Once-daily suckled calves weighed 190.2 ± 5.9 kg compared to control calves weighing 211.1 ± 5.9 kg. Browning et al. (1994) and Stagg et al. (1998) noted that weaning weights were not significantly altered by suckling treatment in multiparous cows, which is not what was observed in the current research. Weaning weights of calves out of primiparous cows were affected ($P<0.04$) by suckling treatment (Table 16). Control calves weighed an average of 20 kg more than the once-daily suckled calves.

Average daily gain from birth to weaning and during the suckling treatment was affected ($P < 0.02$) by suckling treatment (Table 17) in calves out of primiparous cows. Control calves had the higher rate of gain at both measurement points. Contrasting performance in primiparous cows was seen in that average daily gain from birth to weaning was similar, since weaning weights were similar (Randel, 1981).

Once-daily suckling did affect calf average daily gain from birth to weaning ($P < 0.001$) and during the suckling treatment ($P < 0.04$) in multiparous cows. Once-daily suckled calves had an ADG of 0.77 ± 0.02 kg from birth to weaning and 0.64 ± 0.08 kg during the suckling treatment (Table 17). Control calves had an ADG of 0.90 ± 0.02 kg from birth to weaning and 0.89 ± 0.08 kg during the suckling treatment. Observations reported by Odde et al. (1986) show that suckling did not affect weight gains through weaning. This was not the case in the current research. However, in agreement with Odde et al. (1986), calf ADG was suppressed during the suckling treatment. This may be caused by reduced milk intake because restrictively suckled calves need to consume between 2 and 4 L per day of milk in order to grow between 0.35 and 0.50 kg/d (Margerison et al., 2002).

Table 17. Effect of once-daily suckling on calf ADG (mean kg \pm SE) from birth to weaning and during the suckling treatment.

Parity	Treatment	ADG Birth-Weaning	ADG During Suckling Treatment
MULTIPAROUS	ONCE-DAILY	0.77 \pm 0.02 ^a	0.64 \pm 0.08 ^c
	CONTROL	0.90 \pm 0.02 ^b	0.89 \pm 0.08 ^d
PRIMIPAROUS	ONCE-DAILY	0.79 \pm 0.03 ^e	0.55 \pm 0.12 ^e
	CONTROL	0.89 \pm 0.03 ^f	0.93 \pm 0.12 ^f

^{ab}Means with different superscripts between parity differ (P<0.001)

^{cd}Means with different superscripts between parity differ (P<0.04)

^{ef}Means with different superscripts between parity differ (P<0.02)

Postpartum Follicular Data. Once-daily suckled multiparous cows had fewer follicular waves ($P < 0.03$) from d 21 through first ovulation than control cows (Table 18). The number of waves after first ovulation were similar ($P > 0.10$) between treatment groups. The number of follicular waves prior to or after first ovulation were not affected ($P > 0.10$) by suckling treatment in primiparous cows (Table 18). Suckling treatment had no influence on length of follicular waves associated with first or second ovulation regardless of parity (Table 19). Del Vecchio et al., (1988) reported that once-daily suckling decreased postpartum interval in cows and first-calf heifers. This study shows that interval from calving to first ovulation was decreased ($P < 0.03$) in multiparous cows by once-daily suckling (Table 20). Interval from calving to second ovulation was not affected ($P > 0.10$) by suckling treatment in multiparous cows statistically however, once-daily suckled cows had numerically shorter intervals. In contrast, those intervals did not differ in primiparous cows (Table 20). There was no effect of suckling treatment ($P > 0.10$) on interval from first ovulation to second ovulation and on number of dominant follicles developed from d 21 to estrus in both primiparous and multiparous cows (Table 21). Statistical differences were not seen ($P > 0.10$) in size of first and second follicle detected, and size first and second ovulatory follicle in any treatment group (Tables 22 and 23). On the contrary, Savio et al. (1988) determined that the ovulatory follicles were larger than the previous ones detected.

Table 18. Effect of once-daily suckling on number of follicular waves (mean \pm SE) prior to (beginning d 21 post-calving) and following first ovulation (FO).

Parity	Treatment	Waves Prior to FO	Waves Following FO
MULTIPAROUS	ONCE-DAILY	2.4 \pm 0.5 ^a	1.5 \pm 0.2
	CONTROL	3.9 \pm 0.5 ^b	1.0 \pm 0.2
PRIMIPAROUS	ONCE-DAILY	5.0 \pm 0.8	1.8 \pm 0.3
	CONTROL	4.5 \pm 0.8	1.5 \pm 0.3

^{ab}Means with different superscripts between parity differ (P<0.03)

Table 19. Effect of once-daily suckling on length of follicular wave (mean $d \pm SE$) associated with first (FO) and second ovulation (SO).

Parity	Treatment	Length of Wave Associated with FO	Length of Wave Associated with SO
MULTIPAROUS	ONCE-DAILY	9.3 \pm 1.2	7.6 \pm 0.4
	CONTROL	8.9 \pm 1.2	7.6 \pm 0.4
PRIMIPAROUS	ONCE-DAILY	8.8 \pm 1.8	7.0 \pm 0.7
	CONTROL	9.7 \pm 1.8	5.7 \pm 0.7

Table 20. Effect of once-daily suckling on interval from calving to first (FO) and second ovulation (SO; mean d \pm SE).

Parity	Treatment	Interval from Calving to FO	Interval from Calving to SO
MULTIPAROUS	ONCE-DAILY	36.0 \pm 4.1 ^a	48.0 \pm 4.3
	CONTROL	48.2 \pm 4.1 ^b	56.3 \pm 4.3
PRIMIPAROUS	ONCE-DAILY	54.7 \pm 7.3	67.0 \pm 7.2
	CONTROL	53.7 \pm 7.3	62.5 \pm 7.2

^{ab}Means with different superscripts between parity differ (P<0.03)

Table 21. Effect of once-daily suckling on number of dominant follicles (mean \pm SE) from d 21 to estrus and interval from first-to-second ovulation (FO-to-SO; mean d \pm SE).

Parity	Treatment	# of Dominant Follicles	Interval from FO to SO
MULTIPAROUS	ONCE-DAILY	3.9 \pm 0.5	10.7 \pm 1.1
	CONTROL	4.8 \pm 0.5	8.1 \pm 1.1
PRIMIPAROUS	ONCE-DAILY	6.5 \pm 0.8	12.1 \pm 2.0
	CONTROL	6.0 \pm 0.8	8.7 \pm 2.0

Table 22. Effect of once-daily suckling on diameter (mean mm \pm SE) of the first and second dominant follicle detected after d 21.

Parity	Treatment	1st Dominant Follicle Detected	2nd Dominant Follicle Detected
MULTIPAROUS	ONCE-DAILY	17.4 \pm 0.8	16.9 \pm 0.7
	CONTROL	17.3 \pm 0.8	17.5 \pm 0.7
PRIMIPAROUS	ONCE-DAILY	14.3 \pm 1.2	15.7 \pm 1.1
	CONTROL	15.8 \pm 1.2	15.7 \pm 1.1

Table 23. Effect of once-daily suckling on diameter (mean mm \pm SE) of the first and second ovulatory follicle.

Parity	Treatment	First Ovulatory Follicle	Second Ovulatory Follicle
MULTIPAROUS	ONCE-DAILY	17.4 \pm 0.6	15.9 \pm 0.5
	CONTROL	17.9 \pm 0.6	16.7 \pm 0.5
PRIMIPAROUS	ONCE-DAILY	15.8 \pm 0.9	13.7 \pm 0.9
	CONTROL	17.3 \pm 0.9	14.2 \pm 0.8

Once-daily suckled, multiparous cows tended ($P < 0.06$) to have higher concentrations of progesterone associated with the first luteal tissue developed than controls, but not with the second luteal phase (Table 24). Differences within primiparous cow treatment groups were not significant ($P > 0.10$) (Table 24). However, progesterone concentration associated with first ovulation was numerically higher for the once-daily suckled primiparous cows. Higher progesterone concentrations during the luteal phase may contribute to frequency of cows experiencing more follicular waves (Diaz et al., 1998). This may explain why the effects of once-daily suckling on primiparous cows in this study did not agree with prior research. Both treatment groups demonstrated higher ($P < 0.001$) progesterone concentrations associated with the second than with the first corpus luteum (Table 24). This agrees with Perry et al. (1991a) who reported that the first postpartum ovulation was followed by a shorter luteal phase that was associated with less progesterone than was detected for the CL of the second ovulation.

Table 24. Effect of once-daily suckling on serum progesterone concentrations (mean ng/ml \pm SE) associated with first and second luteal phase.

Parity	Treatment	Serum P ₄ Conc. from First Luteal Phase	Serum P ₄ Conc. from Second Luteal Phase
MULTIPAROUS	ONCE-DAILY	1.81 \pm 0.32 ^{ac}	4.92 \pm 0.93 ^d
	CONTROL	0.89 \pm 0.32 ^{bc}	6.22 \pm 0.93 ^d
PRIMIPAROUS	ONCE-DAILY	2.13 \pm 0.54 ^c	5.53 \pm 1.33 ^d
	CONTROL	1.05 \pm 0.54 ^c	3.92 \pm 1.33 ^d

^{ab}Means with different superscripts differ (P<0.06)

^{cd}Means with different superscripts differ (P<0.001)

Sex of calf itself had no effect ($P>0.10$) on any variable regardless of suckling treatment or parity. However, there was a tendency ($P<0.06$) of a three-way interaction between sex of calf, parity, and treatment on interval from calving to first ovulation and interval from calving to second ovulation (Table 25). Data collected in the current research show that multiparous cows which gave birth to female calves and were once-daily suckled had the shortest intervals to first and second ovulation. This interaction also affected ($P<0.03$) the number of dominant follicles that developed from d 21 postpartum through estrus (Table 25). It is apparent that parity (Wiltbank, 1970) and restricted suckling (Browning et al., 1994) influence postpartum interval. Bellows et al. (1982) reported that dams nursing bull calves tended to return to estrus more “slowly” than dams nursing heifer calves. Furthermore, Lammoglia et al. (1995) reported that Brahman multiparous cows that gave birth to female calves had higher concentrations of prostaglandins. Velez et al. (1991) showed that by increasing peripheral prostaglandin concentrations through one uterine manipulation or exogenous administration, the length of time to resumption of estrous cycles and establishment of conception was reduced. The shorter period of time in which follicular development had the opportunity to occur may have resulted in fewer dominant follicles developed.

Table 25. Calf sex by parity by treatment interaction effect on interval from calving to FO and SO (mean d \pm SE), and number of dominant follicles developed d 21 through estrus (mean \pm SE).

Calf Sex	Parity	Treatment	Interval from Calving to FO (P<0.06)	Interval from Calving to SO (P<0.06)	# of Dominant Follicles (P<0.03)
FEMALE	MULTIPAROUS	ONCE-DAILY	32.3 \pm 6.3	43.9 \pm 6.3	3.4 \pm 0.7
		CONTROL	41.1 \pm 6.3	49.9 \pm 6.3	4.1 \pm 0.7
	PRIMIPAROUS	ONCE-DAILY	50.7 \pm 10.3	59.0 \pm 10.2	5.3 \pm 1.2
		CONTROL	69.3 \pm 10.3	78.3 \pm 10.2	8.0 \pm 1.2
MALE	MULTIPAROUS	ONCE-DAILY	39.6 \pm 6.7	51.9 \pm 6.7	4.4 \pm 0.8
		CONTROL	55.6 \pm 6.7	63.0 \pm 6.7	5.6 \pm 0.8
	PRIMIPAROUS	ONCE-DAILY	58.7 \pm 10.3	75.0 \pm 10.2	7.7 \pm 1.2
		CONTROL	38.0 \pm 10.3	46.7 \pm 10.2	4.0 \pm 1.2

Wann and Randel (1990) demonstrated that uterine manipulation resulted in increased PGFM (PGF metabolite) concentrations in multiparous cows. Velez et al. (1991) reported that uterine manipulation at weekly intervals decreased fertility in mature cows. An inference can be drawn from these two studies as to why the once-daily suckling treatment effects did not parallel historical observations. The minimal, but inevitable, uterine manipulation that occurs during transrectal ultrasonography may have triggered a $\text{PGF}_{2\alpha}$ response, altering the normal endocrine environment and, therefore, skewing responses to treatment.

The increase in PGFM concentration in response to uterine manipulation is not seen in primiparous cows (Wann and Randel, 1990) but, other factors may have influenced their lack of response to once-daily suckling treatment. The fact that all cows were maintained on extremely lush pastures may have caused the treatment effects to be minuscule or nonexistent, as seen with primiparous cows, due to high nutrient intake and excess energy reserves (Short et al., 1990). The fact also remains that total primiparous cow numbers used in the study were low and therefore, may not have been a truly representative sample of the population. Most of the primiparous cows were 3 yr of age as well. This may have caused them to deviate from the traditional 2 yr old primiparous cow response to treatment.

Ovulation

Ovulation before d 88 occurred in 42 of 44 (95%) cows overall. Murphy et al. (1990) noted that first ovulation is rarely associated with estrus. Similar events were seen here with 40 of 42 (95%) first ovulations not being accompanied by behavioral estrus. Furthermore, 39 of 42 (93%) first ovulations were followed by a subsequent short cycle (≤ 17 d). Mean cycle length was $9.4 \pm .9$ d for multiparous cows and 10.4 ± 1.4 d for primiparous cows (Table 10). This agrees with Perry et al. (1991a) and Short et al. (1990) who reported that the short luteal phase is a common occurrence after the first ovulation in postpartum cows. Copelin et al (1989) indicated that $\text{PGF}_{2\alpha}$ plays a role in the short-lived CL associated with short estrous cycles. These authors demonstrated that immunization against $\text{PGF}_{2\alpha}$ prevented the development of a short-lived corpus luteum.

The two cows that did not ovulate before d 88 were primiparous, and one was once-daily suckled and the other was a control. One multiparous and primiparous cow showed estrous behavior at first ovulation, but the subsequent estrous cycle was short. One multiparous cow experienced two silent ovulations and finally showed estrus before the third ovulation.

The ovulatory events seen herein are similar to past research in *Bos tarus* cows that showed 16 of 18 (89%) and 14 of 18 (78%) experiencing a silent first ovulation and short subsequent cycle (Murphy et al., 1990).

Conception

First service conception rates were not influenced ($P>0.10$) by parity.

Primiparous and multiparous cows achieved 41.7% (5/12) and 60% (18/30) first service conception rates, respectively. Once-daily suckling showed no statistically significant ($P>0.10$) effect on first service conception rate. Primiparous once-daily suckled cows had a 33% (2/6) first service conception rate, while control cows showed a rate of 50% (3/6). Multiparous cows did not ($P=1.0$) show any influence of suckling on first service conception rates, with once-daily suckled and control cows having a first service conception rate of 60% (9/15) each. Overall pregnancy rate at one month before weaning for multiparous cows was 97% (29/30). Once-daily suckled multiparous cows had a 100% pregnancy rate. Primiparous cows attained a 79% (11/14) pregnancy rate with the once-daily suckled cows showing the numerically higher pregnancy rate of 86% (6/7). These data reaffirm observations by Browning et al. (1994) in which cumulative pregnancy rates were greater for multiparous cows than for first-calf heifers. Furthermore, Odde et al. (1986) reported that once-daily suckled cows had higher breeding season pregnancy rates than did controls; emphasizing that cows approaching or maintaining average body condition from parturition to conception achieve higher pregnancy rates (Houghton et al., 1990).

SUMMARY AND CONCLUSIONS

Results from this study show that ovarian function is resumed shortly after parturition in *Bos indicus* cows. This is characterized by follicular development and formation of a dominant follicle. Ovulation of a dominant follicle is the event that ends postpartum anestrous. Parity influences postpartum interval and total number of follicular waves experienced from parturition through first normal estrus. Primiparous cows experience more follicular waves and therefore grow more dominant follicles prior to first estrus. It is failure of ovulation and not lack of development of a dominant follicle that causes primiparous cows to have a prolonged postpartum interval.

Once daily suckling hastened return to estrus and reduced the number of follicular waves in multiparous cows. Postpartum fertility was not affected by suckling treatments. The total number of primiparous cows used in the current study was small and may not have been enough of a representative sample of the population for effects of once-daily suckling to parallel those seen in previous studies.

The incidence of first ovulation being associated with behavioral estrus is low. Short estrous cycles usually follow first ovulation. Functional luteal tissue is formed after first ovulation. Luteal tissue resulting from first ovulation expresses reduced progesterone concentration when compared to that of the luteal tissue resulting from the second ovulation. It seems obligatory that postpartum Brahman cows experience a silent ovulation with formation of functional luteal tissue prior to resumption of normal estrous cyclicity.

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