HORMONAL, ESTRUAL, OVULATION AND MILK TRAITS IN POSTPARTUM DAIRY COWS FOLLOWING MULTIPLE DAILY INJECTIONS OF OXYTOCIN

by

ROBERT EDWIN STEWART

B.S., West Texas State University, 1983

A MASTER'S THESIS

submitted in partial fulfillment of the

requirements for the degree

MASTER OF SCIENCE

Department of Animal Sciences and Industry

KANSAS STATE UNIVERSITY

Manhattan, Kansas

1986

Approved by:

Jeffrey A. Stevenson

Major Professor

ACKNOWLEDGEMENTS

The author extends his sincere thanks and appreciation to his major advisor, Dr. Jeffrey S. Stevenson, for the time and expertise given throughout the experiment and the preparation of this thesis. Appreciation is also extended to Dr. G.H. Kiracofe and Dr. J.L. Morrill of the graduate committee for their assistance, encouragement, and constructive criticism during the degree program.

The author would also like to thank M.C. Lucy, B. Newton, K. Frantz, B. Carinder, L. Rosenkrans, and E. Carpenter for their time and assistance. Support from the faculty and staff in the Department of Animal Sciences and Industry is also gratefully acknowledged.

TABLE OF CONTENTS

LIST OF TABLES

Page

HORMONAL, ESTRUAL, OVULATORY AND MILK TRAITS IN POSTPARTUM DAIRY COWS FOLLOWING MULTIPLE DAILY INJECTIONS OF OXYTOCIN

LIST OF FIGURES

Page

HORMONAL, ESTRUAL, OVULATORY AND MILK TRAITS IN POSTPARTUM DAIRY COWS FOLLOWING MULTIPLE DAILY INJECTIONS OF OXYTOCIN

REVIEW OF LITERATURE

Effect of Lactation on Postpartum Estrus and Ovulation

Lactation and(or) suckling in cattle, sheep and swine can prolong intervals from parturition to first ovulation and estrus. Nonlactating cows and ewes ovulate and show estrus earlier than their lactating contemporaries (Mallampati et al., 1969; Radford et al., 1978; La Voie et al., 1981). In sows, lactation inhibits ovulation and ovarian follicular development (Peters et al., 1969). Also, suckling lengthens the period of anestrus compared with milking in cattle (Wiltbank and Cook, 1958; Wagner et al., 1969; Moller, 1970).

Marion and Gier (1968) reported that high milk-producing cows have longer postpartum intervals from calving to ovulation, and increasing frequency of milking tended to lengthen that interval (Carruthers and Hafs, 1980). Interval to first estrus was 9 d longer in genetically high milk-producing cows compared with genetically low producers (Saiduddin et al., 1968).

Frequency and intensity of suckling also affects the duration of anestrus and anovulation. Montgomery (1982) showed that restricting beef cows to once daily suckling by their calves decreased the interval to first ovulation, whereas cows suckling more than one calf had increased intervals (Wetteman et al., 1978). Postpartum interval to first estrus in ewes lambing during the breeding season also was lengthened as frequency of suckling increased (Fletcher, 1971).

Mastectomized beef cows had shorter postpartum intervals to estrus than nonsuckled intacts (Short et al., 1972). Ewes with denervated mammary glands ovulated sooner than intact ewes (Kann and Martinet, 1975), and mammillectomized sows had considerable ovarian follicular development, even when piglets were present (Peters et al., 1969). These findings suggest that the

suckling stimulus and not lactation per se may be the key inhibitory factor that delays the return of ovarian cyclicity.

Postpartum interval to first estrus is longer than the interval to first ovulation (Saiduddin et al., 1968), because few cows exhibit estrus before first ovulation. Morrow et al. (1966) reported that occurrence of ovulation without estrus in lactating dairy cows preceding first postpartum ovulation was 79% and that it decreased with each succeeding estrus, which agrees with recent work of Stevenson and Call (1983) and Benmrad and Stevenson (1986).

Lactation in cattle and ewes increases the frequency of silent ovulations (Graves et al., 1968; Schirar and Martinet, 1982). Although hormones released at suckling or milking are involved in the suppression of early postpartum estrous behavior, Kann and Martinet (1975) demonstrated that denervation of the mammary gland in the ewe failed to prevent silent estrus. Perhaps the threshold level of E-17β necessary for behavioral estrus is altered by lactational events, thereby reducing the classical estrual response before ovulation. Convey et al. (1983) suggested that suckling may prolong postpartum anestrus by a neural-mediated inhibition of gonadotropin secretion or by an inhibitory effect of hormones released by suckling on gonadotropin secretion and(or) their action at the ovary.

Postpartum Hormonal Secretion

Luteinizing Hormone

Postpartum Concentrations. Concentrations of luteinizing hormone (LH) are low early postpartum (Erb et al., 1971). Frequency of LH pulses and mean concentrations of LH in serum increase in the anestrous cow during the first 4 wk postpartum until a certain threshold is reached and ovulation is stimulated (Schallenberger and Hutterer, 1982). However, reduced LH is not associated with reduced content of LH in the anterior pituitary (Walters et al., 1982b), nor with

decreased content of hypothalamic gonadotropin-releasing hormone (GnRH; Carruthers et al., 1980; Moss et al., 1985). Frequency of LH pulses could be involved in the induction of preovulatory follicular determinants that regulate subsequent luteal function, as indicated by recent work of Mollett et al. (1983). They administered iv pulses of GnRH every 2 h for 72 h prior to a larger single bolus of GnRH to early postpartum (d 6 to 8) dairy cows. These cows had estrous cycles of normal duration, whereas cows receiving saline pulses before the GnRH challenge had short estrous cycles with reduced luteal phases. Schallenberger (1985) demonstrated that intervals between LH pulses are longer during short cycles, so a short luteal phase may result partly from infrequent LH stimulation.

Magnitude of LH release appears to be associated with estrous expression. Schallenberger and Prokopp (1985) observed two types of preovulatory-like LH surges in postpartum cows. No cow expressed estrus after low magnitude releases of LH without concomitant increase in follicle-stimulating hormone (FSH) before 30 d postpartum, but all cows showed estrus after parallel high magnitude releases of both LH and FSH after 20 d postpartum.

Effects of Suckling and Weaning. Suckling is associated with low concentrations of LH in serum of cows for up to 30 d postpartum (Radford et al., 1978) and with decreased frequency and amplitude of episodic pulses of LH (Carruthers et al., 1980). Lamming et al. (1982) demonstrated that in absence of intense suckling stimuli in the cow, repeated GnRH injections could induce cyclic ovarian function and estrous cycles.

Decreased concentrations of LH due to suckling may result from decreased ability of the anterior pituitary to respond to GnRH. Walters et al. (1982b) demonstrated that removal of the suckling stimulus increased pituitary responsiveness to GnRH and resulted in increased concentrations of LH in

serum. This agrees with Convey et al. (1982) who reported that pituitary explants from cows after weaning of their calves released more LH in vitro when challenged with GnRH than explants from suckled cows. In addition, pulses of GnRH to pituitary explants before the GnRH challenge increased the LH response. This GnRH priming of the pituitary could explain why nonsuckled cows have greater frequency and amplitude of LH pulses in response to frequent releases of GnRH than those of suckled cows (Carruthers et al., 1980).

Hinshelwood et al. (1985) concluded that suckling has a direct effect on the hypothalamic-pituitary axis because concentrations of LH in serum were lower in suckled than nonsuckled cows. Cows nursing two calves showed a significant delay in the initiation of episodic secretion of LH compared with cows nursing only one calf (Lamming et al., 1982).

Effect of Milking. Milked cows with delayed intervals to ovulation had lower pulse frequencies of LH than those of normal cows (Stevenson and Britt, 1979; Peters et al., 1981). However, milking has a less inhibitory effect than suckling on secretion of LH early postpartum. Fewer suckled than milked cows released LH after estradiol administration (Stevenson et al., 1983), suggesting that suckling is a more potent inhibitor of estradiol-induced release of LH than milking.

Feedback of Steroids. Animals with infrequent pulsatile release of LH are unable to express a stimulatory feedback response of LH to estradiol-17ß (E-17ß; Schallenberger and Hutterer, 1982). Ability of milked cows to display positive feedback to E-17ß is resumed within 10 d postpartum for LH, but E-17ß failed to increase LH (Schallenberger et al., 1982) or caused a delayed response (Stevenson et al., 1983) when cows were suckled rather than milked. In addition, the latter authors reported that postpartum interval to peak LH decreased and

magnitude of peak LH increased in cows that were repeatedly challenged with E-17ß weekly for 4 wk postpartum.

After an initial period of pituitary refractoriness to GnRH stimulation, a certain minimal frequency of LH pulses was necessary for E-17ß to evoke its stimulatory feedback (Schallenberger and Prokopp, 1985), and until LH secretion achieved that pulse frequency, E-17ß continued to reduce pulsatile LH release. Pope (1982) reported that E-17ß frequently reached preovulatory concentrations in postpartum cows, but displayed a pattern different from that during the estrous cycle. He also suggested that the first ovulation postpartum that is preceded by an LH surge and sometimes by a pre-estrual rise in progesterone (P) serves as an inhibitory control on E-17ß and changes the pattern of E-17ß secretion to that typical of the estrous cycle.

When P declines below a certain threshold in the presence of low E-17ß, the negative feedback of E-17ß on LH might be eliminated, thus allowing the frequency and amplitude of LH to increase sufficiently to induce ovulation, but not estrus (Schallenberger et al., 1984b). This pre-estrual rise in P is not caused by LH alone, because Hixon et al. (1983) showed that other factors in addition to LH stimulate P secretion. Luteinized follicles secreted pre-estrual P early postpartum in primiparous suckled beef cows (Castenson et al., 1976). Progesterone could be of adrenal origin because Erb et al. (1971) reported that levels of P are increased in the adrenal gland during 7 to 30 d postpartum. This might be a possible source for P when no luteal tissue is present.

Suckling does not apppear to influence E-17ß (Walters et al., 1982a), but there are conflicting studies on how it might affect P. Carruthers and Hafs (1980) and Dunlap et al. (1981) showed no effect, but La Voie et al. (1981) reported that suckling altered the occurrence and magnitude of the first postpartum rise of P in serum. In addition, suckling was shown to reduce adrenal

P compared with milking (Wagner et al., 1969). A possible reason for this discrepancy is that the increase in P might be very abrupt and short-lived in some cows and therefore easily missed without frequent sampling.

Follicle-stimulating Hormone

Postpartum Concentrations. Immediately postpartum, there is an absence of episodic release of follicle-stimulating hormone (FSH) but by 5 d postpartum FSH in serum has increased (Lamming et al., 1982; Schallenberger et al., 1982) and pituitary content of FSH is high compared with content of FSH observed in subsequent estrous cycles (Saiduddin et al., 1968). A certain threshold concentration of FSH appears to be necessary for induction of the first postpartum ovulation, as pre-treatment with a progestin enhanced the lifespan of an induced corpora lutea (CL) in postpartum beef cows only when FSH was above certain concentrations in serum (Garcia-Winder et al., 1986). However, increasing the frequency of FSH pulses with lowered amplitude does not appear to alter size or numbers of ovarian follicles (Spicer et al., 1986). Suppressed preovulatory FSH might result in abnormal follicular development and predispose the ovulatory follicle to inadequate luteinization after ovulation. Low concentrations of preovulatory FSH possibly may be due to high concentrations of E-17ß in serum, which might cause follicles to secrete P instead of E-17ß (Erb et al., 1971). This would allow FSH to increase and influence follicular development. The CL of first ovulations generally are smaller than normal and have shorter lifespans (Marion and Gier, 1968) and P in serum also is lower during the first than second estrous cycle (Edgerton and Hafs, 1973).

Effect of Suckling and Weaning. Suckling and weaning had no effect on pituitary concentrations of FSH in postpartum dairy and beef cows (Carruthers et al., 1980; Walters et al., 1982b). However, suckled cows had lower basal concentrations of FSH in serum than in cows whose calves were weaned (Convey

et al., 1982; Short et al., 1982). Removal of the suckling stimulus appears to increase the sensitivity of the anterior pituitary to stimulation by GnRH resulting in increased release of both LH and FSH.

Prostaglandin $F_2\alpha$

Postpartum Concentrations. Concentrations of prostaglandin $F_2\alpha$ (PGF) are high at parturition and decrease for 2 to 3 wk in cows with normal parturitions (Lindell et al., 1982b; Troxel et al., 1984). This postpartum release of PGF is correlated with relatively shorter intervals to involution (Kindahl et al., 1982). However, cows with periparturient disorders (uterine disorders, retained placentas, dystocia) had prolonged release of PGF and longer intervals to involution (Kindahl et al., 1982; Lindell et al., 1982b; Fonseca et al., 1983). Prostaglandin $F_2\alpha$ is metabolized almost entirely to 13,14-dihydro-15-keto-prostaglandin $F_2\alpha$ (PGFM) during a single passage through the lungs (Piper et al., 1970; Davis et al., 1980).

Sources of PGF. The bovine uterus is the primary source of PGF during the early postpartum period because cows hysterectomized on day of parturition had undetectable levels of PGFM and intacts had elevated levels that declined to basal concentrations by 10 to 15 d postpartum (Guilbault et al., 1984). In addition, hysterectomy early postpartum resulted in retained luteal function of the first postpartum CL (Lindell et al., 1982a). Partial removal of the uterus in cycling ewes did not delay the return to estrus but complete removal prolonged the estrous cycle (Wiltbank and Casida, 1956).

Luteolytic Effect of PGF. Immunization against PGF prolonged the luteal phase of the estrous cycle (Fairclough et al., 1981). Exogenous administration of PGF caused complete luteolysis and precocious estrus (Milvae and Hansel, 1983) with concentrations of PGFM in serum increasing during luteal regression (Webb et al., 1981). Uterine PGF is secreted into the uterine vein and reaches the

ovary directly via a veno-arterial transfer in the utero-ovarian pedicle (McCracken et al., 1971). This transfer mechanism allows small amounts of PGF to reach the ovary without passing through the pulmonary circulation where it is metabolized to PGFM (Piper et al., 1970).

In cows with normal parturition, a potential suppressing effect of uterine PGF on resumption of ovarian function might be exerted for about 2 wk postpartum (Schallenberger et al., 1984a). The luteolytic effect of PGF appears to be exerted locally because Ginther et al. (1967) demonstrated that estrous cycles were extended and luteal function persisted when the intact uterine horn was contralateral to the ovary bearing the CL in unilaterally hysterectomized heifers. These data agree with conclusions by Morrow et al. (1968) in which they observed that the previously gravid horn appeared to decrease the proportion of first ovulations on the ipsilateral ovary during the first 20 d postpartum. In addition, Lindell et al. (1983b) reported that during periods when elevated concentrations of PGF in serum were present, levels of P remained low, indicating absence of luteal function.

Steroidal Influences. During the estrous cycle, E-17ß and P influence secretion of PGF from the uterus. Roberts et al. (1975) demonstrated that mechanical stimulation of the ovine uterus caused release of PGF very early and very late in the cycle, when E-17ß was elevated and P was low. A prior exposure of the uterus to P is necessary to get maximal uterine release of PGF after injection of E-17ß (McCracken et al., 1981). Only peaks of PGF around the time of luteal regression were associated with peaks of E-17ß, and the E-17ß-induced release of PGF was mediated directly by the uterus (Barcikowski et al., 1974). Ewes immunized against E-17ß had low levels of PGF similar to those of hysterectomized ewes (Caldwell et al., 1972). Since there is considerable ovarian follicular activity and elevated levels of E-17ß soon after

parturition (Pope, 1982), part of the increased release of PGF might be due to E-17ß secreted by ovarian follicles.

Hormones Released at Milking or Suckling

Cortisol

Concentrations of cortisol (C) in serum are higher in lactating cows than non-lactating cows, whereas suckling increased C greater than milking (Wagner and Oxenreider, 1972). Increases in C have been reported for lactating cows at milking (Smith et al., 1972) and at nursing (Dunlap et al., 1981). The latter group also observed decreased concentrations of LH in serum for 45 min after suckling, which is in agreement with the work of Forrest et al. (1980) who reported a similar decrease in LH for 1 h after suckling. However, increased C and decreased LH in serum cannot be directly attributed to suckling because Dunlap et al. (1981) and Wagner and Oxenreider (1972) observed that increased serum C before nursing was due to the dam's visual observation of the calf.

Other work also has shown that release of C might be part of an inhibitory process that decreases LH during suckling. Li and Wagner (1983b) demonstrated a direct action of C on the anterior pituitary in which C decreased both basal and GnRH-stimulated LH release. In addition, induced hyperadrenal activity decreased concentrations of P during the luteal phase of the estrous cycle (Li and Wagner, 1983a), but other nonglucocorticoid steroids also might be involved in that inhibition. Schallenberger et al. (1982) observed that C decreased amplitude, but not frequency, of LH release in postpartum cows.

The response of C to suckling decreased as the postpartum period increased (Ellicott et al., 1979; Dunlap et al., 1981), indicating that hyperadrenal activity may be involved in the mechanism by which suckling prolongs postpartum anestrus.

Prolactin

Suckling may occur without concomitant release of prolactin (PRL) and PRL release occurs independent of suckling (Convey et al., 1983). In lactating ewes with denervated mammary glands, Kann and Martinet (1975) demonstrated that PRL did not prolong postpartum anestrus. Other studies have confirmed the same results in cattle. Carruthers and Hafs (1980) reported that suckling had a similar effect on PRL as cows milked four times daily. Schallenberger et al. (1982) also reported that PRL did not prolong postpartum anestrus. Prolactin secretion does not affect actions or concentrations of steroids or gonadotropins because suppression of endogenous PRL release did not potentiate P or E-17β treatment effects in postpartum beef heifers (Williams and Ray, 1980).

Oxytocin

Sources. The neurohypophyseal hormone oxytocin is produced by the hypothalamus and released from the posterior pituitary along with neurophysin-I/II (Wathes and Swann, 1982). Oxytocin also is released from the CL since Flint and Sheldrick (1983) demonstrated that ovarian venous concentrations of oxytocin increase and decrease with formation and regression of the CL in sheep. Wathes et al. (1983) reported that neurophysin-I/II and oxytocin were found in extracts of bovine CL, suggesting local synthesis of oxytocin. Rodgers et al. (1983) later provided evidence that oxytocin is stored in large luteal cells that are capable of oxytocin synthesis. Ovariectomy also resulted in decreased levels of oxytocin (Schams et al., 1982b).

Causes of Release. Afferent neural receptors are present in the skin and teats of bovine mammary glands and, when stimulated by the calf or milker, they transmit nerve impulses to the hypothalamus and pituitary (Goodman and Grosvenor, 1983). These tactile impulses cause oxytocin to be released from the

neurohypophysis into the peripheral circulation where it causes contractions of myoepithelial cells in the mammary glands.

Other methods of causing oxytocin release include vaginal distension (Roberts, 1971) and mechanical stimulation of the uterus (Roberts et al., 1975). Insufflation of air into the vagina caused oxytocin release only in estrous cows (Schams et al., 1982a). In addition, extension of the birth canal by the fetus at parturition induces release of oxytocin (Schams and Prokopp, 1979).

Binding Sites and Effects on Tissues. Since oxytocin stimulates contraction of smooth muscle, several different parts of the reproductive tract of some species have been examined for potential binding sites for oxytocin. Eiler et al. (1984) demonstrated that oxytocin was more effective than PGF in stimulating uterine motility in postpartum cows. In sows and rats, Soloff and Swartz (1974) showed that oxytocin is bound with similar high affinity by the uterus and mammary glands. Binding of oxytocin in oviducts of rats was similar to binding in the uterus, but there was no binding of oxytocin in the ovary (Soloff, 1975). Virutamasen et al. (1973, 1976) reported that oxytocin increased ovarian contractions in monkeys and rabbits, but suggested that this may be due to a local oxytocin-induced release of PGF. The endometrium of the ovine uterus has a higher binding capacity for oxytocin than that of the myometrium, and oxytocin enhanced release of PGF only from endometrial tissue (Roberts et al., 1976).

Luteolytic and Luteotrophic Effects. Oxytocin given to the cow during the first 6 d of the estrous cycle will reduce duration of the cycle from 8 to 12 d (Armstrong and Hansel, 1959; Hansel and Wagner, 1960). Ewes immunized against oxytocin had prolonged luteal phases (Sheldrick et al., 1980). Uterine dilatation during the first 7 d of the estrous cycle has similar effects as exogenous oxytocin on reducing the duration of the cycle (Hansel and Wagner,

1960). Work done by Armstrong and Hansel (1959) in which hysterectomized heifers failed to respond to exogenous oxytocin also indicates that the luteolytic action of oxytocin is exerted only in the presence of an intact uterus. Oxytocin may regulate the release of PGF from the uterus because palpation of the reproductive genitalia caused levels of oxytocin and PGF in serum to increase (Roberts et al., 1975). Small doses of exogenous oxytocin, however, stimulated production of P by the CL (Donaldson and Takken, 1968), whereas Tan et al. (1982) showed that high doses of oxytocin inhibited production of P.

Effects of Other Hormones. Actions of E-17ß and P also may have roles in regulating the ability of oxytocin to stimulate release of PGF. When anestrous ewes were given only oxytocin, PGF release was not significantly altered, but when oxytocin was preceded by E-17ß treatment, levels of PGF in serum increased markedly (Sharma and Fitzpatrick, 1974). Garcia-Villar et al. (1984) also showed that the uterus of the ovariectomized ewe responded to oxytocin only after administration of E-17ß. Hanzen (1982) reported that uterine motility in postpartum cows was related to elevated levels of E-17ß, PGF, and oxytocin. Higher doses of exogenous oxytocin failed to increase levels of PGF, but rather the amount of PGF released depended on the priming dose of E-17ß than on the challenging dose of oxytocin (Newcomb et al., 1977).

Progesterone appears to be inhibitory to the actions of oxytocin on the uterus. The E-17ß-primed uterus of rabbits in vitro had increased contractile responses to oxytocin, which were abolished after treatment with P (Nissenson et al., 1978). McCracken et al. (1981) have suggested that P initially inhibits E-17ß-induced formation of oxytocin receptors in the uterus, but after a period of time P loses this ability and appears to enhance uterine secretion of PGF. They also proposed a hypothesis to explain how P enhances uterine release of PGF. They suggested that P increased formation of lipid droplets in ovine

endometrial cells, and thus an arachidonic cascade increased levels of PGF. Reduction in P might allow nuclear receptors of E-17ß to be replenished, and E-17ß to induce receptors of oxytocin, and then interaction with oxytocin might increase uterine secretion of PGF (McCracken et al., 1984).

Results of Sheldrick and Flint (1983) agree with the previous hypothesis. They found there was no release of oxytocin in response to a luteolytic dose of PGF in hysterectomized ewes. These data indicate that luteal oxytocin is unlikely to be involved in the intra-luteal events mediating PGF-induced luteolysis, but rather in a systematic action involving uterine secretion of PGF.

Flint and Sheldrick (1983) suggested that luteal secretion of oxytocin may amplify the luteolytic signal, ensure rapid completion of luteal regression, and also lead to the cessation of each individual episodic secretion of PGF. Hour-long surges of PGF release every 6 h are observed, equivalent to the regeneration time of oxytocin receptors in the uterus (McCracken et al., 1984).

Effect on Postpartum Intervals. Cameron et al. (1964) reported that oxytocin administered daily in doses of 75 or 100 USP units for 42 d had no effect on intervals from parturition to ovulation, estrus and completion of uterine involution in lactating dairy cows. This led the authors to suggest that other hormones released at suckling may act alone or interact with oxytocin to delay estrus. Injection of 40 IU oxytocin thrice daily for 35 d following calving failed to lengthen postpartum intervals in mastectomized or intact beef cows (Short et al., 1972). However, Fletcher (1973) demonstrated that postpartum ewes injected with 5 IU oxytocin ten times daily for 17 d after lambing had significantly shorter intervals to first ovulation and first estrus than suckled, restricted suckled (thrice daily), and nonlactating ewes.

Effect on Milk Yields. Hansel and Wagner (1960) demonstrated that milk production declined in lactating dairy cows given 100 to 400 USP units daily for

6 d, and that it returned to pretreatment yields 2 d after termination of the injections.

Uterine Involution

Effects of Lactation

Uterine involution is similar between milked and suckled cows (Wagner and Hansel, 1969; Moller, 1970), but there is more rapid acceleration of uterine regression in lactating cows than in nonlactating cows (Schirar and Martinet, 1982). The uteri of suckled cows were nearly involuted by 30 d postpartum, whereas more time was required for nonsuckled cows (Lauderdale et al., 1968; Riesen et al., 1968).

Effect of Previously Gravid Uterine Horn

The previously gravid uterine horn influences where the first postpartum CL forms before 20 d postpartum (Saiduddin et al., 1967; Morrow et al., 1968). This could be due to the enlargement of the uterine horn and the increased time required for involution compared with the previously nongravid horn (Marion and Gier, 1968). Even though the CL of pregnancy has degenerated by d 7 postpartum (Wagner and Hansel, 1969), the ovary bearing that CL had less follicular estrogen and fewer healthy follicles than the contralateral ovary (Bellin et al., 1984). The latter authors also demonstrated that suckling decreased the amount of follicular estrogen and percentage of healthy follicles on both ovaries.

As previously discussed, release of PGF from the uterus is correlated with uterine involution in cows with and without periparturient disorders. Cows with normal parturitions have relatively shorter intervals to uterine involution, whereas older cows and those with abnormal parturitions required longer intervals to involution (Morrow et al., 1966; Fonseca et al., 1983).

Influence of the uterus diminishes as involution progresses (Foote and Petersen, 1968) and the frequency of ovulations adjacent to the previously gravid horn increases as diameter of the horn decreases (Marion and Gier, 1968). Whether reinitiation of normal estrous cycles depends on the return of the uterus to its nongravid size and function has not yet been determined (Schirar and Martinet, 1982), since both milked and nonlactating cows begin their estrous cycles well before uterine involution is completed (Oxenreider and Wagner, 1971; Stevenson and Britt, 1979).

Postpartum Ovarian Activity

Ovarian Follicular Development

Ovaries increase in size from 8 to 20 d postpartum (Callahan et al., 1972), and follicular development, which is low on the day after parturition, increases considerably by d 10 (Saiduddin et al., 1968). Increased follicular activity may be caused by an increased release of GnRH, or by enlargement of some follicles after the onset of follicular atresia (Schirar and Martinet, 1982). Kesler and Garverick (1982) suggested that postpartum follicular cysts develop when hypothalamic and pituitary control of LH release appears to be less responsive to ovarian or exogenous E-17ß. They also reported that spontaneous reestablishment of ovarian cycles occurred in 60% of cows that developed cysts before the first postpartum ovulation. Follicular growth by d 5 postpartum and considerable follicular activity before the first initial rise in P were observed in beef and dairy cattle (Morrow et al., 1966; Carter et al., 1980; Rawlings et al., 1980).

Effect of Lactation on Follicular Development

Lactation apparently influences follicular development since suckled cows have smaller and fewer follicles and lower concentrations of follicular estrogen than nonsuckled cows (Bellin et al., 1984). In addition, Carter et al. (1980)

demonstrated that nonsuckled cows had six times more follicular volume than suckled cows on d 5 postpartum. Lactating sows also have limited follicular growth (Crighton and Lamming, 1969). Frequency of suckling or milking also affects follicular development. Twice daily or multiple suckling reduces follicular growth, but there is great similarity in ovarian follicular activity between milked cows and cows nursing only one calf (Moller, 1970). High milk-producing dairy cows have been shown to have an increased incidence of follicular cysts (Marion and Gier, 1968).

Short Estrous Cycles

The first estrous cycle in postpartum cattle is usually shorter in duration than subsequent cycles. Ramirez-Godinez et al. (1982) reported the first cycle in suckled beef cows whose calves were weaned at 28 to 34 d postpartum lasted only 8 to 10 d, whereas Fonseca et al. (1983) observed in dairy cows that the first cycle was 4 d shorter than the second. Carter et al. (1980) noted that earlier first ovulation was associated with a shorter interval between the first and second ovulations. The CLs that develop from first ovulations are smaller than normal and have shorter lifespans (Lauderdale et al., 1968; Marion and Gier et al., 1968) and P in serum also is lower during the first cycle (Edgerton and Hafs, 1973).

Incidence of short luteal phases during the first estrous cycle increases if the first ovulation occurs relatively early postpartum (Kindahl et al., 1982), possibly as a result of continuously high concentrations of luteolytic PGF. Other possible causes could include an insufficient LH surge and/or infrequent LH stimulation of follicles (Schallenberger, 1985).

REFERENCES CITED

Armstrong, D. T. and W. Hansel. 1959. Alteration of the bovine estrous cycle with oxytocin. J. Dairy Sci. 42:533.

Barcikowski, B., J. C. Carlson, L. Wilson and J. A. McCracken. 1974. The effect of endogenous and exogenous estradiol-17β on the release of prostaglandin $F_{2\alpha}$ from the ovine uterus. Endocrinology 95:1340.

Bellin, M. E., M. M. Hinshelwood, E. R. Hauser and R. L. Ax. 1984. The influence of suckling and side of CL of pregnancy on folliculogenesis in postpartum cows. Biol. Reprod. 31:849.

Benmrad, M. and J. S. Stevenson. 1986. Gonadotropin-releasing hormone and prostaglandin $F_{2\alpha}$ for postpartum dairy cows: Estrus, ovulation, and fertility traits. J. Dairy Sci. 69:800.

Caldwell, B. V., S. A. Tillson, W. A. Brock and L. Speroff. 1972. The effect of exogenous progesterone and estradiol on prostaglandin F levels in ovariectomized ewes. Prostaglandins 1:217.

Callahan, C. J., R. E. Erb, A. H. Surve and R. D. Randel. 1972. Variables influencing ovarian cycles in postpartum dairy cows. J. Anim. Sci. 33:1053.

Cameron, N. W. and O. T. Fosgate. 1964. Effects of oxytocin upon the reproductive tract of the lactating bovine. J. Dairy Sci. 47:79.

Carruthers, T. D. and H. D. Hafs. 1980. Suckling and four-times daily milking: Influence on ovulation, estrus, and serum luteinizing hormone, glucocorticoids and prolactin in postpartum Holsteins. J. Anim. Sci. 50:919.

Carruthers, T. D., E. M. Convey, J. S. Kesner, H. D. Hafs and K. W. Cheng. 1980. The hypothalamo-pituitary gonadotrophic axis of suckled and

non-suckled dairy cows post partum. J. Anim. Sci. 51:949.

Carter, M. L., D. J. Dierschke, J. J. Rutledge and E. R. Hauser. 1980. Effect of gonadotropin-releasing hormone and calf removal on pituitary-ovarian function and reproductive performance in postpartum beef cows. J. Anim. Sci. 51:903.

Castenson, P. E., A. M. Sorenson, Jr., C. R. Cobos and J. L. Fleeger. 1976. Source of postpartum P and 20ß-OHP preceding estrus in heifers. J. Anim. Sci. 43:277 (Abstr.).

Convey, E. M., D. L. Walters, R. E. Short, V. Padmanabhan and K. Leung. 1982. Pituitary function in suckled and weaned cows postpartum. In: H. Karg and E. Schallenberger (Ed.) Factors Influencing Fertility in the Postpartum Cow. pp 229-233. Martinus Nijhoff, The Hague.

Convey, E. M., H. A. Tucker and R. E. Short. 1983. Acute effect of suckling on gonadotropin, prolactin and glucocorticoid concentrations in serum of intact and ovariectomized beef cows. Theriogenology 20:661.

Cornette, J. C., K. L. Harrison and K. T. Kirton. 1974. Measurements of prostaglandin $F_{2\alpha}$ metabolites by radioimmunoassay. Prostaglandins 5:155.

Crighton, D. B. and G. E. Lamming. 1969. The lactational anoestrus of the sow: The status of the anterior pituitary-ovarian system during lactation and after weaning. J. Endocrinol. 43:507.

Davis, A. J., L. R. Fleet, F. A. Harrison and F. M. Maule Walker. 1980. Pulmonary metabolism of prostaglandin $F_{2\alpha}$ in the conscious non-pregnant ewe and sow. J. Physiol. 301:86P (Abstr.).

Donaldson, L. E. and A. Takken. 1968. The effect of exogenous oxytocin on corpus luteum function in the cow. J. Reprod. Fertil. 17:373.

Dunlap, S. E., T. E. Kiser, G. B. Rampacek, R. R. Kraeling and F. N. Thompson.

1981. Effect of suckling on cortisol, progesterone and luteinizing hormone in postpartum beef cows. Theriogenology 16:185.

Edgerton, L. H. and H. D. Hafs.. 1973. Serum luteinizing hormone, prolactin, glucocorticoid and progestin in dairy cows from calving to gestation. J. Dairy Sci. 56:451.

Eiler, H., F. M. Hopkins, C. S. Armstrong-Backus and W. A. Lyke. 1984. Uterotonic effect of prostaglandin F and oxytocin on the postpartum cow. Amer. J. Vet. Res. 45:1011.

Ellicott, A. R., D. Gimenez, D. M. Henricks and T. E. Kiser. 1979. Influence of suckling on cortisol levels in beef heifers. J. Anim. Sci. 49 (Suppl. 1):295.

Erb, R. E., A. H. Surve, C. J. Callahan, R. D. Randel and H. A. Garverick. 1971. Reproductive steroids in the bovine. VII. Changes post partum. J. Anim. Sci. 33:1060.

Fairclough, R. J., J. F. Smith and L. T. McGowan. 1981. Prolongation of the oestrous cycle in cows and ewes after passive immunization with PGF antibodies. J. Reprod. Fertil. 62:213.

Fletcher, I. C. 1971. Relationships between frequency of suckling, lamb growth, and postpartum oestrous behavior in ewes. Anim. Behav. 19:108.

Fletcher, I. C. 1973. Effects of lactation, suckling and oxytocin on postpartum ovulation and estrus in ewes. J. Reprod. Fertil. 33:293.

Flint, A. P. F. and E. L. Sheldrick. 1983. Evidence for a systemic role for ovarian oxytocin in luteal regression in sheep. J. Reprod. Fertil. 67:215.

Fonseca, F. A., J. H. Britt, B. T. McDaniel, J. C. Wilk and A. H. Rakes. 1983. Reproductive traits of Holsteins and Jerseys. Effects of age, milk yield, and clinical abnormalities on involution of cervix and uterus, ovulation, estrous cycles, detection of estrus, conception rate, and days open. J.

Dairy Sci. 66:1128.

Foote, W. D. and D. W. Peterson. 1968. Relationship between side of pregnancy and side of subsequent ovarian activities in beef and dairy cattle. J. Reprod. Fertil. 16:415.

Forrest, P. K., R. C. Rhodes and R. D. Randel. 1980. Effect of bleeding stress and variable suckling intensity upon serum luteinizing hormone in Brangus heifers. Theriogenology 13:321.

Garcia-Villar, R., P. L. Toutain and Y. Ruckebusch. 1984. Differential responsiveness to oxytocin of the uterus and cervix in the ovariectomized ewe. Anim. Reprod. Sci. 7:421.

Garcia-Winder, M., P. E. Lewis, D. R. Deaver, V. G. Smith, G. S. Lewis and E. K. Inskeep. 1986. Endocrine profiles associated with life span of induced corpora lutea in postpartum beef cows. J. Anim. Sci. 62:1353.

Gill, J. L. and H. D. Hafs. 1971. Analysis of repeated measurements of animals. J. Anim. Sci. 33:331.

Ginther, O. J., C. O. Woody, S. Mahajan, K. Janakiraman and L. E. Casida. 1967. Effect of oxytocin administration of the oestrous cycle of unilaterally hysterectomized heifers. J. Reprod. Fertil. 14:225.

Goodman, G. T. and C. E. Grosvenor. 1983. Symposium: Milk ejection. Neuroendocrine control of the milk ejection reflex. J. Dairy Sci. 66:2226.

Graves, W. E., J. W. Lauderdale, E. R. Hauser and L. E. Casida. 1968. Relation of postpartum interval to pituitary gonadotropins, ovarian follicular development and fertility in beef cows. Res. Bull. 270:23. Univ. Wisconsin, Madison.

Guilbault, L. A., W. W. Thatcher, M. Drost and S. M. Hopkins. 1984. Source of F series prostaglandins during the early postpartum period in cattle. Biol.

Reprod. 31:879.

Hansel, W. and W. C. Wagner. 1960. Luteal inhibition in the bovine as a result of oxytocin injections, uterine dilatation and intrauterine infusions of seminal and preputial fluids. J. Dairy Sci. 43:796.

Hanzen, Ch. 1982. Uterine motility in cattle prior to and post parturition (preliminary results). In: H. Karg and E. Schallenberger (Ed.) Factors Influencing Fertility in the Postpartum Cow. pp 61-66. Martinus Nijhoff, The Hague.

Hinshelwood, M. M., D. J. Dierschke and E. R. Hauser. 1985. The effect of suckling on the hypothalamic-pituitary axis in postpartum beef cows, independent of ovarian secretions. Biol. Reprod. 32:290.

Hixon, J. E., G. J. Pijanowski, P. G. Weston, R. D. Shanks and W. C. Wagner. 1983. Evidence for an oscillator other than luteinizing hormone controlling the secretion of progesterone in cattle. Biol. Reprod. 29:1155.

Kann, G. and J. Martinet. 1975. Prolactin levels and duration of post-partum anoestrus in lactating ewes. Nature 257:63.

Kesler, D. J. and H. A. Garverick. 1982. Ovarian cysts in dairy cattle: A review. J. Anim. Sci. 55:1147.

Kindahl, H., L.-E. Edqvist, K. Larsson and A. Malmqvist. 1982. Influence of prostaglandins on ovarian function postpartum.. In: H. Karg and E. Schallenberger (Ed.) Factors Influencing Fertility in the Postpartum Cow. pp 173-196. Martinus Nijhoff, The Hague.

Lamming, G. E., A. R. Peters, G. M. Riley and M. W. Fisher. 1982. Endocrine regulation of post-partum function. In: H. Karg and E. Schallenberger (Ed.) Factors Influencing Fertility in the Postpartum Cow. pp 148-172. Martinus Nijhoff, The Hague.

Lauderdale, J. W., W. E. Graves, E. R. Hauser and L. E. Casida. 1968. Relation of postpartum interval to corpus luteum development, pituitary prolactin activity, and uterine involution in beef cows. Res. Bull. 270:42. Univ. Wisconsin, Madison.

La Voie, V., D. K. Han, D. B. Foster and E. L. Moody. 1981. Suckling effect on estrus and blood progesterone in post-partum beef cows. J. Anim. Sci. 52:802.

Li, P. S. and W. C. Wagner. 1983a. Effects of hyperadrenal states on luteinizing hormone in cattle. Biol. Reprod. 29:11.

Li, P. S. and W. C. Wagner. 1983b. In vivo and in vitro studies on the effect of adrenocorticotropic hormone or cortisol on the pituitary response to gonadotropin-releasing hormone. Biol. Reprod. 29:25.

Lindell, J.-O., H. Kindahl, L.-E. Edqvist and G. Tufresson. 1982a. Effect of hysterectomy on the postpartum prostaglandin levels in the cow. Acta Vet. Scand. 23:144.

Lindell, J.-O., H. Kindahl, L. Jansson and L.-E. Edqvist. 1982b. Post-partum release of prostaglandin $F_2\alpha$ and uterine involution in the cow. Theriogenology 17:237.

Mallampati, R. S., A. L. Pope and L. E. Casida. 1969. Effect of suckling on post-partum anestrus, the pituitary and the ovary in ewes lambing in different seasons. J. Anim. Sci. 29 (Abstr.): 194.

Marion, G. B. and H. T. Gier. 1968. Factors affecting bovine ovarian activity after parturition. J. Anim. Sci. 27:1621.

McCracken, J. A., W. Schramm, B. Barcikowski and L. Wilson. 1981. The identification of prostaglandin $F_2\alpha$ as a uterine luteolytic hormone in the sheep and the endocrine control of its synthesis. Acta Vet. Scand. Suppl.

77:71.

McCracken, J. A., W. Schramm and W. C. Okulicz. 1984. Hormone receptor control of pulsatile secretion of $PGF_{2\alpha}$ from the ovine uterus during luteolysis and its abrogation in early pregnancy. Anim. Reprod. Sci. 7:31.

McLeod, B. J., W. Haresign and G. E. Lamming. 1982. The induction of ovulation and luteal function in seasonally anoestrous ewes treated with small-dose multiple injections of GnRH. J. Reprod. Fertil. 65:215.

Milvae, R. A. and W. Hansel. 1983. Luteolytic effect of 13,14-dihydro-$PGF_{2\alpha}$ in heifers. J. Reprod. Fertil. 67:203.

Moller, K. 1970. Uterine involution and ovarian activity after calving. New Zealand Vet. J. 18:140.

Mollett, T. A., M. F. Smith and H. A. Garverick. 1983. Endocrine responses of postpartum dairy cows to repetitive GnRH administration. J. Dairy Sci. 66 (Suppl. 1): 228 (Abstr.).

Montgomery, G. W. 1982. Influence of suckling frequency and bromocryptine treatment on the resumption of ovarian cycles in post-partum beef cattle. Theriogenology 17:551.

Morrow, D. A., S. J. Roberts, K. McEntee and H. G. Gray. 1966. Postpartum ovarian activity and uterine involution in dairy cattle. J. Amer. Vet. Med. Assoc. 149:1596.

Morrow, D. A., S. J. Roberts and K. McEntee. 1968. Latent effects of pregnancy on postpartum ovarian activity in dairy cattle. J. Anim. Sci. 27:1408.

Moss, G. E., J. R. Parfet, C. A. Marvin, R. D. Allrich and M. A. Diekman. 1985. Pituitary concentrations of gonadotropins and receptors for GnRH in suckled beef cows at various intervals after calving. J. Anim. Sci. 60:285.

Newcomb, R., W. D. Booth and L. E. A. Rowson. 1977. The effect of oxytocin treatment on the levels of prostaglandin F in the blood of heifers. J. Reprod. Fertil. 49:17.

Nissenson, R., G. Flouret and O. Hechter. 1978. Opposing effects of estradiol and progesterone on oxytocin receptors in rabbit uterus. Proc. Natl. Acad. Sci. USA 75:2044.

Odde, K. G., G. H. Kiracofe and R. R. Schalles. 1985. Suckling behavior in range beef calves. J. Anim. Sci. 61:307.

Oxenreider, S. L. and W. C. Wagner. 1971. Effect of lactation and energy intake on postpartum ovarian activity in the cow. J. Anim. Sci. 33:1026.

Peters, A. R., G. E. Lamming and M. W. Fisher. 1981. A comparison of plasma LH concentrations in milked and suckled post-partum cows. J. Reprod. Fertil. 62:567.

Peters, J. B., N. L. First and L. E. Casida. 1969. Effects of pig removal and oxytocin injections on ovarian and pituitary changes in mammillectomized sows. J. Anim. Sci. 28:537.

Piper, P. J., J. R. Vane and J. H. Wylie. 1970. Inactivation of prostaglandins by the lungs. Nature 225:600.

Pope, G. S. 1982. Oestrogens and progesterone in plasma and milk of post-partum dairy cattle. In: H. Karg and E. Schallenberger (Ed.) Factors Influencing Fertility in the Postpartum Cow. pp 248-276. Martinus Nijhoff, The Hague.

Radford, H. M., C. D. Nancarrow and P. E. Mattner. 1978. Ovarian function in suckling and non-suckling beef cows post-partum. J. Reprod. Fertil. 54:49.

Ramirez-Godinez, J. A., G. H. Kiracofe, R. R. Schalles and G. D. Niswender. 1982. Endocrine patterns in the postpartum beef cow associated with

weaning: A comparison of the short and subsequent normal cycles. J. Anim. Sci. 55:153.

Rawlings, N. C., L. Weir, B. Todd, J. Mann and J. H. Hyland. 1980. Some endocrine changes associated with the post-partum period of the suckling beef cow. J. Reprod. Fertil. 60:301.

Riesen, J. W., S. Saiduddin, W. T. Tyler and L. E. Casida. 1968. Relation of postpartum interval to corpus luteum development, pituitary prolactin activity, and uterine involution in dairy cows. Res. Bull. 270:27. Univ. Wisconsin, Madison.

Riley, G. M., A. R. Peters and G. E. Lamming. 1981. Induction of pulsatile LH release, FSH release and ovulation in post-partum acyclic beef cows by repeated small doses of Gn-RH. J. Reprod. Fertil. 63:559.

Roberts, J. S. 1971. Progesterone-inhibition of oxytocin release during vaginal distension: Evidence for a central site of action. Endocrinology 89:1137.

Roberts, J. S., B. Barcikowski, L. Wilson, R. C. Skarnes and J. A. McCracken. 1975. Hormonal and related factors affecting the release of prostaglandin $F_2\alpha$ from the uterus. J. Steroid Biochem. 6:1091.

Roberts, J. S., J. A. McCracken, J. E. Gavagan and M. S. Soloff. 1976. Oxytocin-stimulated release of prostaglandin $F_2\alpha$ from ovine endometrium in vitro: Correlation with estrous cycle and oxytocin-receptor binding. Endocrinology 99:1107.

Rodgers, R. J., J. D. O'Shea, J. K. Findlay, A. P. F. Flint and E. L. Sheldrick. 1983. Large luteal cells the source of luteal oxytocin in the sheep. Endocrinology 113:2302.

Sagi, R., R. C. Gorewit and D. B. Wilson. 1980. Role of exogenous oxytocin in eliciting milk ejection in dairy cows. J. Dairy Sci. 63:2006.

Saiduddin, S., J. W. Riesen, W. J. Tyler and L. E. Casida. 1967. Some carryover effects of pregnancy on post-partum ovarian function in the cow. J. Dairy Sci. 50:1846.

Saiduddin, S., J. W. Riesen, W. J. Tyler and L. E. Casida. 1968. Relation of postpartum interval to pituitary gonadotrophins, ovarian follicular development and fertility in dairy cows. Res. Bull. 270:15. Univ. Wisconsin, Madison.

SAS. 1982. SAS User's Guide: Statistics. Statistical Analysis System Institute, Inc., Cary, NC.

Schallenberger, E. and G. Hutterer. 1982. Frequent pulsatile LH secretion is a prerequisite for a stimulatory feedback action of oestradiol and the re-establishment of oestrous cycles post partum in dairy cows. Acta Endocrinol. (Copenh.) 99, Suppl. 246:16.

Schallenberger, E., U. Oerterer and G. Hutterer. 1982. Neuroendocrine regulation of postpartum function. In: H. Karg and E. Schallenberger (Ed.) Factors Influencing Fertility in the Postpartum Cow. pp 123-147. Martinus Nijhoff, The Hague.

Schallenberger, E., H.-J. Breitinger, S. J. Oschmann, D. Schams and D. L. Walters. 1984a. Hysterectomy postpartum: Changes in the secretory pattern of gonadotrophins, ovarian steroids and ovarian oxytocin. Acta Endocrinol. Suppl. 264:71 (Abstr.).

Schallenberger, E., D. Schams, B. Bullerman and D. L. Walters. 1984b. Pulsatile secretion of gonadotropins, ovarian steroids, and ovarian oxytocin during prostaglandin-induced regression of the corpus luteum in the cow. J. Reprod. Fertil. 71:493.

Schallenberger, E. 1985. Gonadotropins and ovarian steroids in cattle 3.

Pulsatile changes of gonadotropin concentrations in the jugular vein postpartum. Acta Endocrinol. 109:37.

Schallenberger, E. and S. Prokopp. 1985. Gonadotrophins and ovarian steroids in cattle. IV. Re-establishment of the stimulatory feedback action of oestradiol-17ß on LH and FSH. Acta Endocrinol. 109:44.

Schams, D. and H. Karg. 1969. Zeitlicher verlauf und analytische erfassbarkeit des endogenen bzw. Exogen erhohten blutspiegels an luteinishier ungshormon beim rind. Zuchthygiene 4:61.

Schams, D. and S. Prokopp. 1979. Oxytocin determination by RIA in cows around parturition. Anim. Reprod. Sci. 2:267.

Schams, D., G. Baumann and W. Leidl. 1982a. Oxytocin determination by radioimmunoassay in cattle. II. Effect of mating and stimulation of the genital tract in bulls, cows and heifers. Acta Endocrinol. 99:218.

Schams, D., A. Lahlow-Kassi and P. Glatzel. 1982b. Oxytocin concentrations in peripheral blood during the oestrous cycle and after ovariectomy in two breeds of sheep with low and high fecundity. J. Endocrinol. 92:9.

Schirar, A. and J. Martinet. 1982. Postpartum ovarian activity and its interaction with the uterus in resuming cyclic activity postpartum. In: H. Karg and E. Schallenberger (Ed.) Factors Influencing Fertility in the Postpartum Cow. pp 67-94. Martinus Nijhoff, The Hague.

Sharma, S. C. and R. J. Fitzpatrick. 1974. Effect of oestradiol-17ß and oxytocin treatment on prostaglandin F alpha release in the anoestrous ewe. Prostaglandins 6:97.

Sheldrick, E. L., M. D. Mitchell and A. P. F. Flint. 1980. Delayed luteal regression in ewes immunized against oxytocin. J. Reprod. Fertil. 59:37.

Sheldrick, E. L. and A. P. F. Flint. 1983. Regression of the corpora lutea in

sheep in response to cloprostenol is not affected by loss of luteal oxytocin after hysterectomy. J. Reprod. Fertil. 68:155.

Short, R. E., R. A. Bellows, E. L. Moody and B. E. Howland. 1972. Effects of suckling and mastectomy on bovine postpartum reproduction. J. Anim. Sci. 34:70.

Short, R. E., D. L. Walters, E. M. Convey, R. B. Staigmiller and C. C. Kaltenbach. 1982. Role of pulsatile LH release in resumption of ovarian cycles in postpartum beef cows. In: H. Karg and E. Schallenberger (Ed.) Factors Influencing Fertility in the Postpartum Cow. pp 220-224. Martinus Nijhoff, The Hague.

Skaggs, C. L., B. V. Able and J. S. Stevenson. 1986. Pulsatile or continuous infusion of luteinizing hormone-releasing hormone and hormonal concentrations in prepubertal beef heifers. J. Anim. Sci. 62:1034.

Smith, V. G., E. M. Convey and L. A. Edgerton. 1972. Bovine serum corticoid response to milking and exteroceptive stimuli. J. Dairy Sci. 55:1170.

Soloff, M. S. and T. L. Swartz. 1974. Characterization of a proposed oxytocin receptor in the uterus of the rat and sow. J. Biol. Chem. 249:1376.

Soloff, M. S. 1975. Oxytocin receptors in rat oviduct. Biochem. Biophys. Res. Comm. 66:671.

Spicer, L. J., E. M. Convey, H. A. Tucker and S. E. Echternkamp. 1986. Effects of intermittent injections of LHRH on secretory patterns of LH and FSH and ovarian follicular growth during postpartum anovulation in suckled beef cows. J. Anim. Sci. 62:1317.

Stevenson, J. S. and J. H. Britt. 1979. Relationship among luteinizing hormone, estradiol, progesterone, glucocorticoids, milk yield, body weight and postpartum ovarian activity in Holstein cows. J. Anim. Sci. 48:570.

Stevenson, J. S. and E. P. Call. 1983. Influence of early estrus, ovulation, and insemination on fertility in postpartum Holstein cows. Theriogenology 19:367.

Stevenson, J. S., M. F. Spire and J. H. Britt. 1983. Influence of the ovary on estradiol-induced luteinizing hormone release in postpartum milked and suckled Holstein cows. J. Anim. Sci. 57:692.

Tan, G. S., R. Tweedale and J. S. G. Briggs. 1982. Effects of oxytocin on the bovine corpus luteum of early pregnancy. J. Reprod. Fertil. 66:75.

Troxel, T. R., M.J. Opsomer and D. J. Kesler. 1984. The effect of days postpartum, indomethacin and oxytocin on prostaglandin metabolite concentrations in postpartum suckled beef cows. Theriogenology 22:187.

Virutamasen, P., K. H. Wright and E. E. Wallach. 1973. Monkey ovarian contractility - the relationship to ovulation. Fert. Steril. 24:763.

Virutamasen, P. Y. Smithasiri and A-R Fuchs. 1976. Intra-ovarian pressure changes during ovulation in rabbits. Fert. Steril. 27:188.

Wagner, W. C. and W. Hansel. 1969. Reproductive physiology of the postpartum cow. I. Clinical and histological findings. J. Reprod. Fertil. 18:493.

Wagner, W. C., R. Saatman and W. Hansel. 1969. Reproductive physiology of the postpartum cow. II. Pituitary, adrenal and thyroid function. J. Reprod. Fertil. 18:501.

Wagner, W. C. and S. L. Oxenreider. 1972. Adrenal function in the cow. Diurnal changes and the effects of lactation and neurohypophyseal hormones. J. Anim. Sci. 34:630.

Walters, D. L., C. C. Kaltenbach, T. G. Dunn and R. E. Short. 1982a. Pituitary and ovarian function in postpartum beef cows. I. Effect of suckling on serum and follicular fluid hormones and follicular gonadotropin receptors.

Biol. Reprod. 26:640.

Walters, D. L., R. E. Short, E. M. Convey, R. B. Staigmiller, T. G. Dunn and C. C. Kaltenbach. 1982b. Pituitary and ovarian function in postpartum beef cows. II. Endocrine changes prior to ovulation in suckled and nonsuckled postpartum cows compared to cycling cows. Biol. Reprod. 26:647.

Wathes, D. C. and R. W. Swann. 1982. Is oxytocin an ovarian hormone? Nature 297:225.

Wathes, D. C., R. W. Swann, S. D. Birkett, D. G. Porter and B. T. Pickering. 1983. Characterization of oxytocin, vasopressin and neurophysin from the bovine corpus luteum. Endocrinology 113:693.

Webb, R., M. D. Mitchell, J. Falconer and J. S. Robinson. 1981. Temporal relationships between peripheral plasma concentrations of oxytocin, progesterone, and 13,14-dihydro-15-keto prostaglandin $F_{2\alpha}$ during the estrous cycle and early pregnancy in the ewe. Prostaglandins 22:443.

Wetteman, R. P., E. J. Turman, R. D. Wyatt and R. Totusek. 1978. Influence of suckling intensity on reproductive performance of range cows. J. Anim. Sci. 47:342.

Williams, G. L. and D. E. Ray. 1980. Hormonal and reproductive profiles of early postpartum beef heifers after prolactin suppression or steroid-induced luteal function. J. Anim. Sci. 50:906.

Wiltbank, J. N. and L. E. Casida. 1956. Alteration of ovarian activity by hysterectomy. J. Anim. Sci. 15:134.

Wiltbank, J. N. and A. C. Cook. 1958. The comparative reproductive performance of nursed cows and milked cows. J. Anim. Sci. 17:640.

HORMONAL, ESTRUAL, OVULATORY AND MILK TRAITS IN POSTPARTUM DAIRY COWS FOLLOWING MULTIPLE DAILY INJECTIONS OF OXYTOCIN

Summary

Release of oxytocin at time of suckling or milking may delay onset of estrous cycles in postpartum cows. Twenty lactating Holsteins of mixed parity were given 100 mU oxytocin (n=10) or 2 ml saline (control; n=10) via jugular catheters at 0530, 0930, 1730 and 2130 h daily from calving (d 0) until d 28 postpartum. Cows were milked twice daily at 0130 and 1330 h. Blood was collected thrice weekly (M, W, F at 0530 h) for 12 wk and analyzed by radioimmunoassay (RIA) for progesterone and 13,14-dihydro-15-keto prostaglandin $F_{2\alpha}$ (PGFM) in serum. On d 12, blood was collected via jugular catheters every 15 min for 6 h and concentrations of luteinizing hormone (LH), cortisol, and PGFM were determined by RIA. Rate of involution of the reproductive tract was estimated twice weekly by palpation per rectum. Overall mean, baseline concentrations, number of pulses/6h and pulse duration of LH on d 12 were similar among treatment groups. However, oxytocin appeared to reduce ($P<.10$) pulse amplitude of LH in multiparous cows ($.4 \pm .2$ vs. $8 \pm .1$ ng/ml), but not in primiparous cows compared with controls. Concentrations of cortisol and PGFM in serum on d 12 were unaffected by treatment. The average interval from calving to first ovulation based on changes of progesterone in serum and the interval to first estrus were both similar between treatment groups. Rates of involution of the cervix and uterus were also similar between treatments. Changes in body weight were not influenced by treatment, but multiparous cows lost ($P<.01$) more weight early postpartum, whereas

primiparous cows maintained their body weight. Milk yield, percentage protein in milk, and somatic cell counts were not different between treatments. However, percentage fat in milk tended to be higher ($P<.10$) in oxytocin-treated cows than in controls ($3.99 \pm .22$ vs. $3.68 \pm .21$%). These data suggest that multiple daily injections of oxytocin did not affect: 1) length of anestrus and anovulation in postpartum dairy cows, 2) LH release, and 3) rates of cervical and uterine involution.

Introduction

Lactation in cattle prolongs intervals from parturition to first ovulation (Radford et al., 1978; La Voie et al., 1981). Suckling delayed establishment of cyclic ovarian activity after calving longer than twice-daily milking in cattle (Wiltbank and Cook, 1958; Moller, 1970). Montgomery (1982) showed that restricting beef cows to once daily suckling decreased postpartum intervals to ovulation, whereas cows that nursed more than one calf had longer intervals (Wetteman et al., 1978).

The suckling stimulus and not lactation *per se* may be the key inhibitory factor that delays the return of ovarian cyclicity, because ewes with denervated mammary glands ovulated sooner than unoperated controls (Kann and Martinet, 1975), and mammillectomized sows had considerable ovarian follicular development even when piglets were present (Peters et al., 1969). Hormones released at milking or suckling, such as cortisol and oxytocin, could be involved in this inhibitory process. Whereas several studies have addressed the potential role of cortisol (Smith et al., 1972; Wagner and Oxenreider, 1972; Dunlap et al., 1981), there is a paucity of information concerning the role of oxytocin as a potential inhibitor of ovarian function in cattle (Cameron et al., 1964; Short et al., 1972). The present study was conducted to determine the effects of multiple

daily injections of oxytocin on postpartum intervals to ovulation and estrus, and postpartum hormonal and milk secretion.

Materials and Methods

Experimental Design. Twenty lactating Holstein cows of mixed parity were assigned to two treatment groups at parturition during January and February, 1985. Cows were distributed across treatment based on their previous lactation yield (305-d mature equivalent yield) and heifers were distributed across treatments based on their potential estimated breeding value (PEBV) from the Dairy Herd Improvement records. All cows were fitted with jugular catheters within 12 h after calving and were assigned to receive (iv) either 100 mU oxytocin (n=7 primiparous and n=3 multiparous cows) or 2 ml saline (n=6 primiparous and n=4 multiparous cows) four times daily at 0530, 0930, 1730, and 2130 h, equally spaced around twice daily milkings at 0130 and 1330 h. Dosage of oxytocin (100 mU/2 ml saline) was based on evidence presented by Sagi et al. (1980) in which a similar iv dose was sufficient to induce milk let-down and increase concentrations of oxytocin in serum comparable to a normal milking procedure. Treatments were designed to expose treated cows to six physiological doses of oxytocin per day (four given exogenously and two from normal milkings), or one every 4 h. Administration of oxytocin continued through d 28 postpartum.

Animal Handling. Cows were housed on concrete in a free-stall confinement facility exposed to the environment. The diet consisted of a concentrate mix (16% protein) containing equal parts corn and sorghum grain, soybean meal, 1.5% sodium bicarbonate, and minerals in a self feeder and alfalfa hay fed ad libitum. Observation for signs of estrus were made throughout the day by the herdsman and milkers, in addition to two periods of estrous detection in the morning (0500 to 0900 h) and evening (1700 to 1800 h). Cows

were weighed once weekly (0600 to 0700 h) for the duration of the experiment. Uterine and cervical regression were estimated by twice weekly palpation per rectum until gross involution was completed. Each cow was palpated by two individuals and the measurements from each were averaged. Daily milk production was recorded for each cow and milk samples were analyzed once weekly for percentages of butterfat and protein in milk (Mulispec M, Multispec Ltd., England) and for concentrations of somatic cells in milk (Fossomatic 215, A/S N. Foss Electric, Denmark) at the Kansas DHI Laboratory, Manhattan, KS.

Blood Collection. Blood was collected thrice weekly (Monday, Wednesday, and Friday at 0530 h) via coccygeal venipuncture during the first 12 wk postpartum. In addition, blood was collected via jugular catheters on either d 11, 12, or 13 postpartum (hereafter denoted as d 12 postpartum) every 15 min for 6 h, preceding the first daily injection of either saline or oxytocin (0530 h). Blood was chilled upon collection and held at 4 C for 24 h until serum was obtained by centrifugation. Serum was frozen at -20 C until assayed. All samples collected thrice weekly were radioimmunoassayed for progesterone (P) and 13,14-dihydro-15-keto-prostaglandin $F_2\alpha$ (PGFM). Serum samples collected on d 12 were analyzed for luteinizing hormone (LH), cortisol, and PGFM.

Radioimmunoassays. Concentrations of progesterone and cortisol in serum were quantified according to procedures previously validated in our laboratory (Skaggs et al., 1986). Intra- and inter-assay coefficients of variation of six assays for progesterone averaged 7.7 and 13.4% , respectively. Cortisol was quantified in one assay with an intra-assay coefficient of variation of 5.9%. Concentrations of LH in serum were radioimmunoassayed as described by Skaggs et al. (1986). Assay sensitivity was 43 pg/tube and the intra-assay coefficient of variation was 1.8%. Purified bovine LH (LER 1716-2) was iodinated and NIH-LH-B4 was used as the standard reference preparation.

Concentrations of PGFM in serum were quantified by radioimmunoassay. Duplicate 200 µl aliquots of serum were extracted after adding 50 µl .5 N acetic acid and 2 ml ethyl acetate. Samples were vortexed on a mechanical shaker for 5 min and frozen at -20 C for 30 min before decanting the solvent fraction into disposable culture tubes. Extracts were then dried under air in a water bath (40 C). Once dry, tubes were rinsed with an additional 1 ml ethyl acetate and redried. Recovery of tritiated PGFM extracted with 0.5 N acetic acid and ethyl acetate was 90% in four assays. Standard curves were prepared for each assay in triplicate at concentrations of 0, 5, 10, 25, 50, 100, 250 and 500 pg/tube of stock PGFM (Sigma D4143, St. Louis, MO) dissolved in 95% ethanol.

Goat anti-13,14-dihydro-15-keto-$PGF_2\alpha$ (gift from K. Kirton of The Upjohn Co., Kalamazoo, MI) was diluted at 1:70,000 in 0.1M phosphate-buffer saline containing 0.1% gelatin (PBSG, pH 7.0). At this dilution, approximately 50% of 14,000 cpm ([5,6,8,9,11,12,14(N)^{3}H] PGFM, 80 Ci/mM, Amersham TRK.517, Arlington Heights, IL) was bound after precipitation with 1 ml solution of dextran (.04%)-coated charcoal (.4%) in PBSG. Cross-reaction of PGFM antisera was <0.1% for $PGF_2\alpha$, 0.5% for 13,14-dihydro-$PGF_2\alpha$, <1% for C-16 urinary metabolites of $PGF_2\alpha$, 20% for 15-keto-$PGF_2\alpha$, and 100% for 13,14-dihydro-15-keto $PGF_2\alpha$ (Cornette et al., 1974). When 25, 50, 75, 100, 150 and 200 pg (dissolved in 100 µl of 95% ethanol) of exogenous PGFM were added to 100 µl serum from a postpartum cow, PGFM was recovered quantitatively: 26, 49, 80, 95, 140 and 185 (n = 4 each; average percent recovery = 98.25 $\pm$ 5.86; r = .99). Parallelism was determined by quantifying PGFM in different volumes of pooled postpartum serum. When 100, 150, 200 and 250 µl of pooled serum were assayed, concentrations of PGFM were 250, 220, 200 and 200 pg/ml, respectively. Various volumes of serum produced a binding curve that was parallel to the standard curves of PGFM. Assay sensitivity was 7 pg/tube and

intra- and inter-assay coefficients of variation were 7.3 and 16.3%, respectively.

Definitions. Concentrations of progesterone in serum during treatment and for 8 wk after treatment were used to determine occurrence of first and second ovulations and duration of estrous cycles. Day of ovulation was estimated by the following criteria (Stevenson and Call, 1983): 1) ovulation occurred on the day after observed estrus if serum progesterone was less than 1 ng/ml at estrus, 2) if estrus was unobserved, ovulation occurred 2 d before any increase in progesterone that exceeded .5 ng/ml but was less than 2 ng/ml, or 3) if estrus was unobserved, ovulation occurred 4 d prior to an increase in progesterone above 2 ng/ml.

An increase in serum LH was designated as an LH pulse (Riley et al., 1981; McLeod et al., 1982) when: 1) the highest LH concentration attained was 50% above the preceding nadir, 2) at least two consecutive LH values were between the peak value and the following nadir value, and 3) the rate of decline from LH peak values was not greater than the half-life of LH, which is approximately 35 min in bovine serum (Schams and Karg, 1969). Pulse magnitude was defined as the maximal level of LH associated with an LH pulse, and pulse amplitude was the concentration of LH resulting from the difference between basal concentrations and pulse magnitude. Overall average concentrations of LH in serum included all values, whereas the average baseline concentrations excluded all values associated with an LH pulse.

Statistical Analyses. Data were subjected to least-squares analysis of variance using the general linear models procedure of the Statistical Analysis System (SAS, 1982). A split-plot analysis of variance for repeated measurements was utilized to test LH data, including treatment (n=2), lactation (n=2; first and second or greater lactation), and their interaction. Treatment and lactation

were tested by the cow within treatment x lactation variance (Gill and Hafs, 1971). Other characteristics of LH were analyzed with a similar model without repeated measurements. Means were separated by orthogonal contrasts or by LSD (SAS, 1982).

Daily milk yields and milk constituents were divided into two 4-wk periods; a 4-wk period of treatment and a 4-wk post-treatment period. The model for these analyses included treatment, lactation, period, and all interactions.

Uterine and cervical involution, concentrations of PGFM from calving to 28 d postpartum, and changes in body weight were subjected to regression analysis. The polynomial regression equations used for involution and PGFM data were similar, both using the inverse of days postpartum and the square of the inverse of days postpartum as independent variables. The regression for body weight included days postpartum, the square of days postpartum, and the cube of days postpartum as independent variables. Parameter estimates for each cow from the above analysis were subjected to least-squares analysis of variance, and the model included treatment, lactation, and their interaction. Average parameter estimates derived for different treatment and lactation groups were used in the prediction equations.

Results

Hormonal Concentrations on Day 12 Postpartum. Characteristics of concentrations of LH in serum on d 12 postpartum are summarized in table 1. Overall mean and baseline concentrations during the frequent sampling period were similar between treatment groups. Administration of oxytocin had no effect on pulse frequency (pulses/6 h) or duration of pulses compared with those of control cows. Although there was no effect of parity for any characteristic of LH in serum, a treatment x parity interaction was present ($P<.10$) for pulse

magnitude and amplitude. The interaction for pulse amplitude of LH concentrations is illustrated in figure 1. Whereas control and treated primiparous cows had similar amplitudes (.48 ± .13 vs. .56 ± .11 ng/ml), oxytocin appeared to reduce LH pulse amplitude ($P<.10$) in treated multiparous cows compared with that of multiparous controls (.38 ± .16 vs .81 ± .15 ng/ml).

Concentrations of cortisol in pooled serum from the 6-h sampling period were similar for controls (6.3 ± 1.3 ng/ml) and treated cows (7.1 ± 1.3 ng/ml). Concentrations of cortisol in primiparous cows (7.4 ± 1.5 ng/ml) also were similar to those of multiparous cows (5.5 ± 1.5 ng/ml).

Concentrations of PGFM in serum on d 12 were not affected by treatment or parity (564 ± 12 vs 666 ± 12 pg/ml for control and oxytocin-treated cows, respectively; 657 ± 11 vs 535 ± 15 pg/ml for primiparous and multiparous cows, respectively).

Estrous and Ovulation Traits. Intervals to first and second ovulations and first estrus are shown in table 2. Treatment with oxytocin had no effect on any of the postpartum intervals examined. Interval to first ovulation averaged 26.5 d overall and ranged from 13 to 64 d across treatments. Two cows, one from each treatment group, remained anovulatory during the experiment and were excluded from this analysis. A normal luteal phase was characteristic of most cows after first ovulations. Only two cows (one from each treatment) had short estrous cycles (<17 d) and six (four treated and two controls) had long cycles (>24 d). Two of the four treated cows had prolonged periods (14 and 31 d) of low concentrations (<1 ng/ml) of progesterone between CL regression and the subsequent luteal phase. Interval to second ovulation averaged 51.5 d for both groups, and ranged from 30 to 71 d. One cow receiving oxytocin had an extended period (47 d) of high concentrations of progesterone (retained corpus

luteum) after first ovulation that continued until the end of the experiment (78 d postpartum).

Interval to first estrus was similar between controls (53 ± 9 d) and treated cows (48 ± 9 d). Interval to first estrus was longer than the interval to first ovulation because all cows failed to express estrus before first ovulation. Estrous activity increased by the second ovulation, with 13 of 17 (76%) cows showing estrus prior to second ovulation.

Involution of Reproductive Tract. Prior to 10 d postpartum, the reproductive tract was difficult to palpate, due to its depth in the body cavity and large amounts of tissue debris remaining from pregnancy. Rate of involution for the previously gravid uterine horn is shown in figure 2. Neither treatment nor parity influenced involution of the previously gravid or nongravid horn. Rate of cervical involution is shown in figure 3. Treatment with oxytocin did not affect rate of cervical involution. However, multiparous cows had larger ($P<.10$) cervixes than primiparous cows and cervical involution occurred earlier in younger than older cows. The differences tended to become less as gross involution approached completion.

Concentrations of PGFM during the first 4 wk postpartum are shown in figure 4. Concentrations of PGFM in serum were high (1 to 2 ng/ml) early postpartum, and rate of decline was similar for parity and treatment groups. However, rate of decline appeared to be faster earlier postpartum, whereas the rate of decline decreased after the first week. Concentrations of PGFM returned to baseline levels (~200 pg/ml) by 16 to 20 d postpartum.

Body Weight. Changes in body weight are shown in figure 5. Regression analysis indicated that parity ($P<.01$), but not treatment, influenced postpartum changes in body weight. Multiparous cows were heavier ($P<.01$) than primiparous cows, and multiparous cows lost more weight ($P<.01$) early postpartum, whereas

primiparous cows maintained their body weight during the same period. Around d 36 postpartum, multiparous cows previously treated with oxytocin started gaining weight, whereas control multiparous cows continued to lose weight. These differences were not significantly different. Primiparous cows started gaining weight by 28 d postpartum and continued to gain weight during the experiment.

Milk Yield and Milk Constituents. Daily milk yield, percentage fat and protein in milk, and number of somatic cells in milk are summarized in table 3. Daily milk yield was not affected by oxytocin treatment, even though oxytocin-treated cows appeared to produce less milk during both treatment and post-treatment periods. Milk production increased ($P<.001$) from the treatment period to the post-treatment period (22.9 vs 26.0 kg/d) as cows approached their peak milk yields. Although not shown in table 3, primiparous cows produced less ($P<.01$) milk than multiparous cows (20.3 vs 32.1 kg/d).

Percentage fat in milk tended to be higher ($P<.10$) in the oxytocin-treated cows during both periods. However, when averaged across the treatments in each period, percentage fat in milk declined ($P<.001$) from 4.15% to 3.53% . Primiparous cows also had lower ($P<.05$) milk fat than multiparous cows ($3.65 \pm .13$ vs. $4.18 \pm .19$%).

Oxytocin treatment did not alter percentage protein in milk. Protein in milk declined ($P<.001$) from $3.18 \pm .08$% during treatment in both groups of cows to $2.84 \pm .09$% during the post-treatment period. Percentage protein was higher ($P<.01$) in primiparous ($3.12 \pm .05$%) than in multiparous cows ($2.80 \pm .08$%).

Control and treated cows had similar concentrations of somatic cells in milk. Somatic cells declined ($P<.05$) from 510,000 during treatment to 153,000 during the post-treatment period. Parity had no influence on concentration of somatic cells.

Discussion

Oxytocin was administered four times daily equally spaced around the twice daily milkings, to allow treated cows to receive six daily physiological doses of oxytocin in attempt to simulate the release of oxytocin caused by suckling or milking. Odde et al. (1985) observed that beef calves on range generally nurse around five times daily. The dosage of oxytocin administered (100 mU) was sufficient to induce milk let down, as milk leaking from teat ends of treated cows was observed after injections of oxytocin, and this dosage was sufficient to cause milk let-down in a normal milking procedure when given iv and quantitated in the serum of milked cows (Sagi et al., 1980).

Frequent daily administration of oxytocin for 28 d postpartum failed to affect intervals from parturition to ovulation and estrus. Our results agree with those of Cameron et al. (1964) and Short et al. (1972) who used once daily pharmacological doses of oxytocin (150 and 200 USP units/d and 40 IU thrice daily). However, Fletcher (1973) demonstrated that frequent injections (5 IU) given ten times daily during the first 17 d postpartum shortened intervals to first estrus and first ovulation in sheep.

Wiltbank and Cook (1958) demonstrated earlier that suckling lengthened anestrus more than milking. If the six daily exposures of oxytocin in treated cows simulated that which normally occurs in suckled or frequently milked cows, then treated cows should have had longer intervals to estrus and ovulation. However, occurrence of ovulation without estrus was similar in both treatment groups. None of the cows exhibited estrus behavior preceding the first ovulation, and only four (one control and three treated cows) failed to show estrus prior to the second ovulation. Other work has demonstrated that lack of estrous behavior is associated with early postpartum ovulations and that incidence of estrous activity increases with each subsequent ovulation in

postpartum cows (Morrow et al., 1966; King et al., 1976; Stevenson and Call, 1983).

Convey et al. (1983) suggested two methods by which suckling may prolong anestrus. Actual release of hormones induced by suckling may depress gonadotropin secretion via a neural-mediated inhibition, or hormones released at suckling may depress gonadotropin secretion and(or) inhibit ovarian activity. The latter thesis does not appear to be the case for oxytocin, because in our study oxytocin was introduced into the peripheral circulation and should have reached the ovary as it reached the mammary glands to induce milk let-down. Thus it appears that oxytocin alone does not prolong anestrus by inhibiting ovarian activity. In addition, oxytocin failed to inhibit secretion of LH as overall mean and baseline concentrations, and pulse frequencies of LH in serum did not differ between treatments. Suckling decreased concentrations of LH in serum (Radford et al., 1978), whereas its removal allowed LH to increase (Walters et al., 1982). Since administering oxytocin did not decrease further concentrations of LH in serum compared with controls, inhibition of LH by suckling or milking does not appear to be associated directly with oxytocin release. However, oxytocin tended to reduce the pulse amplitude of LH in multiparous cows. Perhaps endogenous release of oxytocin was sufficient in older, higher milk-producing cows to partially inhibit secretion of LH. Even though we noted no differences in intervals to ovulation and estrus due to parity, our data might explain why high milk-producing cows have longer postpartum intervals to first estrus (Marion and Gier, 1968; Saiduddin et al., 1968).

Actual release of oxytocin by the posterior pituitary due to nerve impulses caused by suckling or milking stimuli might affect gonadotropin secretion. Nerve impulses are transmitted to the hypothalamus and pituitary

(Goodman and Grosvenor, 1983) where they may have a direct effect on the hypothalamic-pituitary axis (Hinshelwood et al., 1985) and alter gonadotropin secretion. Concentrations of oxytocin resulting from exogenous administration of oxytocin may have been below a physiological dose necessary to alter function of the anterior pituitary (Armstrong and Hansel, 1959), yet elevated sufficiently above those physiological levels necessary to induce milk let-down.

Uterine involution was not affected by oxytocin treatment. Previous work demonstrated that rate of uterine involution was similar in milked and suckled cows (Wagner and Hansel, 1969; Moller, 1970). During lactation, the uterus is exposed to oxytocin and has binding receptors for oxytocin (Roberts et al., 1976). Frequent injections or exposure to oxytocin is probably associated with hastened uterine involution in lactating cows, because suckling increased rate of uterine involution (Riesen et al., 1968; Schirar and Martinet, 1982) and induced contractions of smooth muscles of uterus (Eiler et al., 1984). However, treatment with oxytocin in our study did not significantly hasten involution of the uterus. Oxytocin receptors in the uterus have a 6-h regeneration period once they have been occupied (McCracken et al., 1984), possibly limiting the effectiveness of oxytocin in inducing uterine contractions. In addition, our study indicated that rate of uterine involution is not affected significantly by parity, which is at variance with the work of Morrow et al. (1966), but does agree with data from Moller (1970).

Oxytocin stimulates release of prostaglandin $F_{2\alpha}$ (PGF) by the uterus (Roberts et al., 1976) and when administered on d 5 to 8 of the estrous cycle, oxytocin caused luteal regression and precocious estrus (Armstrong and Hansel, 1959). The latter authors also demonstrated that the uterus is necessary for oxytocin to stimulate release of PGF. Our dose (100 mU) of oxytocin may have been insufficient to cause uterine secretion of PGF or alter uterine involution,

but adequate to cause milk let-down. Perhaps the dosage of oxytocin is critical enough that cows can have estrous cycles of normal duration when exposed to suckling or milking stimuli, in association with its normal physiological levels of oxytocin, without experiencing aberrations in cyclic ovarian activity.

Whereas Hansel and Wagner (1960) showed that treatment with 100 to 400 IU of oxytocin (1,000 to 4000-fold greater dosages than what we utilized) for 6 d caused milk yield to decline, our study showed no such effect of oxytocin. It is possible that the numerically lower milk yield detected during treatment was due to milk leakage from the teats of cows treated with oxytocin. Increased milk yield during the post-treatment period (4 wk) of both groups of cows was most likely a result of cows reaching their peak production of milk, and not a result of treatment. Oxytocin had no effect on percentage protein or concentrations of somatic cells in milk, both of which declined after the treatment period. Somatic cells were higher in the treatment period because milk samples from the first week postpartum are elevated consistently (Schultz, 1977). Oxytocin tended to increase percentage fat in milk, but if true, its mechanism is unknown. Percentage fat also declined in both groups after the treatment period, probably as a result of higher milk production.

Results of this study indicated that oxytocin alone does not appear to inhibit reestablishment of postpartum ovarian cyclicity. However, we cannot preclude the possibility that oxytocin is part of an inhibitory complex of several neuroendocrine components that may originate in the hypothalamus and(or) pituitary to delay the initiation of ovarian cyclicity.

Literature Cited

Armstrong, D. T. and W. Hansel. 1959. Alteration of the bovine estrous cycle with oxytocin. J. Dairy Sci. 42:533.

Cameron, N. W. and O. T. Fosgate. 1964. Effects of oxytocin upon the reproductive tract of the lactating bovine. J. Dairy Sci. 47:79.

Convey, E. M., H. A. Tucker and R. E. Short. 1983. Acute effect of suckling on gonadotropin, prolactin and glucocorticoid concentrations in serum of intact and ovariectomized beef cows. Theriogenology 20:661.

Cornette, J. C., K. L. Harrison and K. T. Kirton. 1974. Measurements of prostaglandin $F_{2\alpha}$ metabolites by radioimmunoassay. Prostaglandins 5:155.

Dunlap, S. E., T. E. Kiser, G. B. Rampacek, R. R. Kraeling and F. N. Thompson. 1981. Effect of suckling on cortisol, progesterone and luteinizing hormone in postpartum beef cows. Theriogenology 16:185.

Eiler, H., F. M. Hopkins, C. S. Armstrong-Backus and W. A. Lyke. 1984. Uterotonic effect of prostaglandin F and oxytocin on the postpartum cow. Amer. J. Vet. Res. 45:1011.

Fletcher, I. C. 1973. Effects of lactation, suckling and oxytocin on postpartum ovulation and estrus in ewes. J. Reprod. Fertil. 33:293.

Goodman, G. T. and C. E. Grosvenor. 1983. Symposium: Milk ejection. Neuroendocrine control of the milk ejection reflex. J. Dairy Sci. 66:2226.

Hansel, W. and W. C. Wagner. 1960. Luteal inhibition in the bovine as a result of oxytocin injections, uterine dilatation and intrauterine infusions of seminal and preputial fluids. J. Dairy Sci. 43:796.

Hinshelwood, M. M., D. J. Dierschke and E. R. Hauser. 1985. The effect of suckling on the hypothalamic-pituitary axis in postpartum beef cows, independent of ovarian secretions. Biol. Reprod. 32:290.

Kann, G. and J. Martinet. 1975. Prolactin levels and duration of post-partum

anoestrus in lactating ewes. Nature 257:63.

King, G. J., J. F. Hurnik and H. A. Robertson. 1976. Ovarian function and estrus in dairy cows during early lactation. J. Anim. Sci. 42:688.

La Voie, V., D. K. Han, D. B. Foster and E. L. Moody. 1981. Suckling effect on estrus and blood progesterone in post-partum beef cows. J. Anim. Sci. 52:802.

McCracken, J. A., W. Schramm and W. C. Okulicz. 1984. Hormone receptor control of pulsatile secretion of $PGF_2\sigma$ from the ovine uterus during luteolysis and its abrogation in early pregnancy. Anim. Reprod. Sci. 7:31.

McLeod, B. J., W. Haresign and G. E. Lamming. 1982. The induction of ovulation and luteal function in seasonally anoestrous ewes treated with small-dose multiple injections of GnRH. J. Reprod. Fertil. 65:215.

Moller, K. 1970. Uterine involution and ovarian activity after calving. New Zealand Vet. J. 18:140.

Montgomery, G. W. 1982. Influence of suckling frequency and bromocryptine treatment on the resumption of ovarian cycles in post-partum beef cattle. Theriogenology 17:551.

Morrow, D. A., S. J. Roberts, K. McEntee and H. G. Gray. 1966. Postpartum ovarian activity and uterine involution in dairy cattle. J. Amer. Vet. Med. Assoc. 149:1596.

Odde, K.G., G. H. Kiracofe and R. R. Schalles. 1985. Suckling behavior in range beef calves. J. Anim. Sci. 61:307.

Peters, J. B., N. L. First and L. E. Casida. 1969. Effects of pig removal and oxytocin injections on ovarian and pituitary changes in mammillectomized sows. J. Anim. Sci. 28:537.

Radford, H. M., C. D. Nancarrow and P. E. Mattner. 1978. Ovarian function in suckling and non-suckling beef cows post-partum. J. Reprod. Fertil. 54:49.

Riesen, J. W., S. Saiduddin, W. T. Tyler and L. E. Casida. 1968. Relation of postpartum interval to corpus luteum development, pituitary prolactin activity, and uterine involution in dairy cows. Res. Bull. 270:27. Univ. Wisconsin, Madison.

Riley, G. M., A. R. Peters and G. E. Lamming. 1981. Induction of pulsatile LH release, FSH release and ovulation in post-partum acyclic beef cows by repeated small doses of Gn-RH. J. Reprod. Fertil. 63:559.

Roberts, J. S., J. A. McCracken, J. E. Gavagan and M. S. Soloff. 1976. Oxytocin-stimulated release of prostaglandin $F_{2\alpha}$ from ovine endometrium in vitro: Correlation with estrous cycle and oxytocin-receptor binding. Endocrinology 99:1107.

Sagi, R., R. C. Gorewit and D. B. Wilson. 1980. Role of exogenous oxytocin in eliciting milk ejection in dairy cows. J. Dairy Sci. 63:2006.

Saiduddin, S., J. W. Riesen, W. J. Tyler and L. E. Casida. 1968. Relation of postpartum interval to pituitary gonadotrophins, ovarian follicular development and fertility in dairy cows. Res. Bull. 270:15. Univ. Wisconsin, Madison.

SAS. 1982. SAS User's Guide: Statistics. Statistical Analysis System Institute, Inc., Cary, N.C.

Schams, D. and H. Karg. 1969. Zeitlicher verlauf und analytische erfassbarkeit des endogenen bzw. Exogen erhohten blutspiegels an luteinishier ungshormon beim rind. Zuchthygiene 4:61.

Schirar, A. and J. Martinet. 1982. Postpartum ovarian activity and its interaction with the uterus in resuming cyclic activity postpartum. In: H. Karg and E. Schallenberger (Ed.) Factors Infuencing Fertility in the Postpartum Cow. pp 67-94. Martinus Nijhoff, The Hague.

Schultz, L. H. 1977. Somatic cells in milk-physiological aspects and relationship

to amount and composition of milk. J. Food Prot. 40:125.

Short, R. E., R. A. Bellows, E. L. Moody and B. E. Howland. 1972. Effects of suckling and mastectomy on bovine postpartum reproduction. J. Anim. Sci. 34:70.

Skaggs, C. L., B. V. Able and J. S. Stevenson. 1986. Pulsatile or continous infusion of luteinizing hormone-releasing hormone and hormonal concentrations in prepubertal beef heifers. J. Anim. Sci. 62:1034.

Smith, V. G., E. M. Convey and L. A. Edgerton. 1972. Bovine serum corticoid response to milking and exteroceptive stimuli. J. Dairy Sci. 55:1170.

Stevenson, J. S. and E. P. Call. 1983. Influence of early estrus, ovulation, and insemination on fertility in postpartum Holstein cows. Theriogenology 19:367.

Wagner, W. C. and W. Hansel. 1969. Reproductive physiology of the postpartum cow. I. Clinical and histological findings. J. Reprod. Fertil. 18:493.

Wagner, W. C. and S. L. Oxenreider. 1972. Adrenal function in the cow. Diurnal changes and the effects of lactation and neurohypophyseal hormones. J. Anim. Sci. 34:630.

Walters, D. L., R. E. Short, E. M. Convey, R. B. Staigmiller, T. G. Dunn and C. C. Kaltenbach. 1982. Pituitary and ovarian function in postpartum beef cows. II. Endocrine changes prior to ovulation in suckled and nonsuckled postpartum cows compared to cycling cows. Biol. Reprod. 26:647.

Wetteman, R. P., E. J. Turman, R. D. Wyatt and R. Totusek. 1978. Influence of suckling intensity on reproductive performance of range cows. J. Anim. Sci. 47:342.

Wiltbank, J. N. and A. C. Cook. 1958. The comparative reproductive performance of nursed cows and milked cows. J. Anim. Sci. 17:640.

TABLE 1. CHARACTERISTICS OF LUTEINIZING HORMONE ON DAY 12 POSTPARTUM[a]

Characteristics	Control	Oxytocin
Overall mean, ng/ml	.27 ± .05	.28 ± .05
Baseline, ng/ml	.16 ± .03	.18 ± .03
No. of pulses/6 h	2.2 ± .3	2.7 ± .3
Magnitude of pulses, ng/ml	.80 ± .10	.65 ± .09
Amplitude of pulses, ng/ml	.62 ± .10	.47 ± .10
Duration of pulses, min	69 ± 6	67 ± 5

[a]Mean ± SE.

TABLE 2. POSTPARTUM INTERVALS (DAYS) TO OVULATION AND ESTRUS[a]

Treatment group	n[b]	First Ovulation	n	Second Ovulation	n	First estrus
Control	9	26 ± 6	9	52 ± 8	9	53 ± 9
Oxytocin	9	27 ± 6	8[c]	51 ± 8	5	48 ± 9

[a]Mean ± SE.

[b]One cow from each treatment remained anovulatory until end of the experiment and they were excluded from this analysis.

[c]One cow had persistent luteal function after the first ovulation.

TABLE 3. DAILY MILK YIELD AND MILK CONSTITUENTS IN CONTROL AND OXYTOCIN-TREATED COWS[a]

Milk traits	Control	Oxytocin
Daily milk yield, kg/d[b]		
Treatment period	24.1 ± .8	21.8 ± .8
Post-treatment period	27.4 ± .8	24.6 ± .9
Milk fat, %		
Treatment period	4.00 ± .21	4.30 ± .20[†]
Post-treatment period	3.36 ± .21	3.69 ± .23
Milk protein, %		
Treatment period	3.20 ± .08	3.16 ± .08
Post-treatment period	2.84 ± .08	2.83 ± .09
Somatic cell count, 10^3		
Treatment period	456 ± 221	565 ± 233
Post-treatment period	132 ± 217	176 ± 233

[a]Mean ± SE.

[b]Treatment period consisted of first 4 wk postpartum and post-treatment period was the following 4 wk. All means differ between periods when averaged across treatments ($P<.05$).

[†]Differs from controls ($P<.10$).

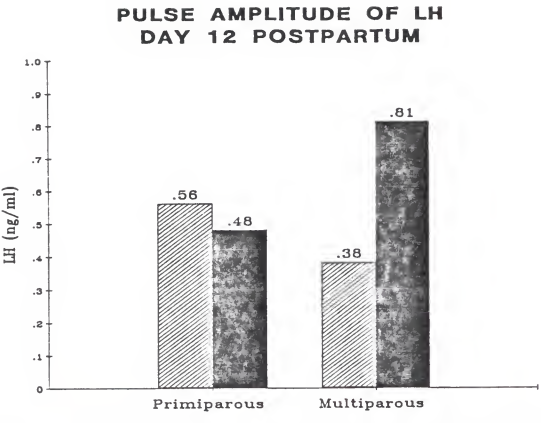

Figure 1. Pulse amplitude of serum luteinizing hormone pulses on day 12 postpartum for primiparous and multiparous cows. Stippled bars represent oxytocin-treated cows and solid bars represent control cows.

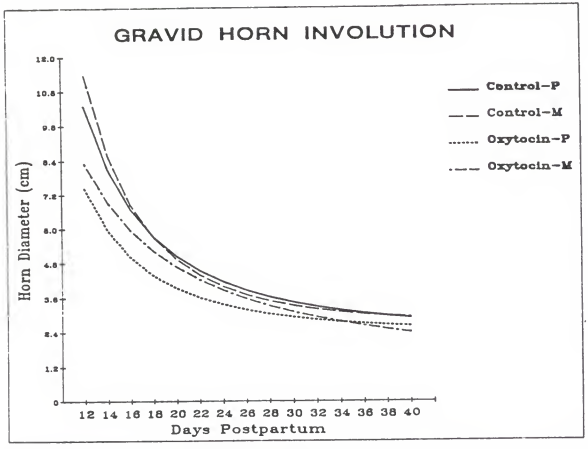

Figure 2. Rate of involution of previously gravid uterine horn for control and oxytocin-treated primiparous (P) and multiparous (M) cows.

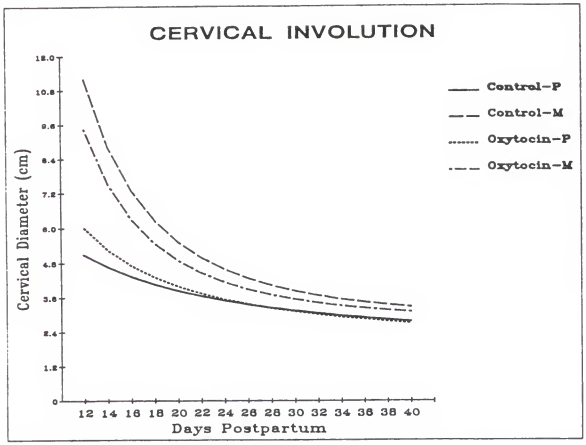

Figure 3. Rate of involution of the cervix for control and oxytocin-treated primiparous (P) and multiparous (M) cows.

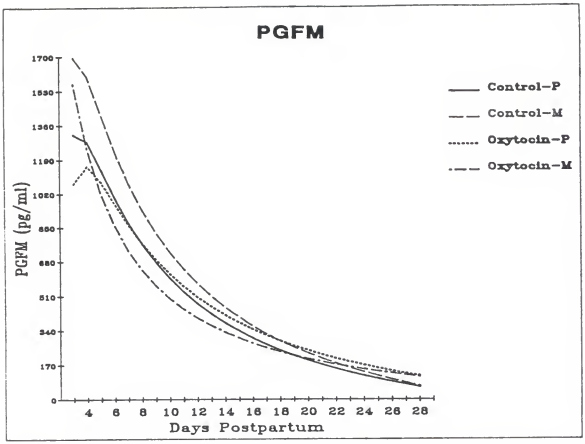

Figure 4. Rate of secretion of PGFM in serum for control and oxytocin-treated primiparous (P) and multiparous (M) cows.

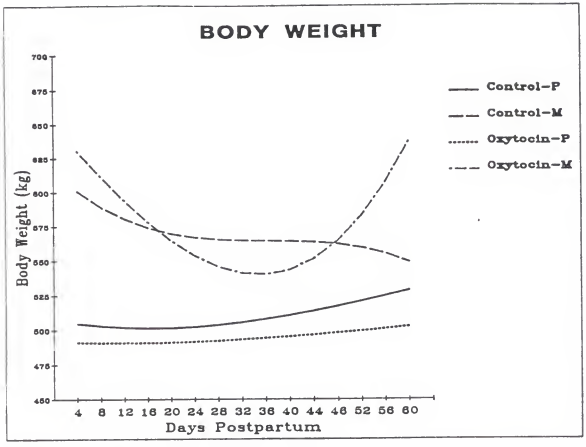

Figure 5. Rate of change of body weight for control and oxytocin-treated primiparous (P) and multiparous (M) cows.

HORMONAL, ESTRUAL, OVULATION AND MILK TRAITS IN POSTPARTUM DAIRY COWS FOLLOWING MULTIPLE DAILY INJECTIONS OF OXYTOCIN

by

ROBERT EDWIN STEWART

B.S., West Texas State University, 1983

AN ABSTRACT OF A MASTER'S THESIS

submitted in partial fulfillment of the

requirements for the degree

MASTER OF SCIENCE

Department of Animal Sciences and Industry

KANSAS STATE UNIVERSITY

Manhattan, Kansas

1986

ABSTRACT

Release of oxytocin at time of suckling or milking may delay initiation of ovarian cyclicity in postpartum cows. Thirteen primiparous and seven multiparous lactating Holsteins were given 100 mU oxytocin (n=10) or 2 ml saline (control; n=10) via jugular catheters at 0530, 0930, 1730 and 2130 h daily from calving (d 0) until d 28 postpartum. Cows were milked twice daily at 0130 and 1330 h. Blood was collected thrice weekly (M, W, F at 0530 h) for 12 wk. Concentrations of progesterone and 13,14-dihydro-15-keto $PGF_{2\alpha}$(PGFM) in serum were analyzed by radioimmunoassay (RIA). On d 12, blood was collected via jugular catheters every 15 min for 6 h and concentrations of luteinizing hormone (LH), cortisol, and PGFM were determined by RIA. Cervical and uterine involution were estimated twice weekly by palpation per rectum. Overall mean, baseline concentrations, number of pulses/6h and pulse duration of LH in serum on d 12 were similar for oxytocin-treated and control cows. However, oxytocin appeared to reduce ($P<.10$) pulse amplitude of LH in multiparous cows ($.4 \pm .2$ vs $.8 \pm .1$ ng/ml), but not in primiparous cows compared with controls. Concentrations of cortisol and PGFM in serum on d 12 were unaffected by treatment. Based on changes of progesterone in serum, oxytocin treatment did not affect interval to first ovulation (27 ± 6 vs 26 ± 6 d) or interval to first estrus (48 ± 9 vs 53 ± 9 d). Rates of involution for the previously gravid uterine horn and cervix were also similar between treatments. However, rate of cervical involution was more rapid in primiparous

than multiparous cows ($P<.10$). Regression of concentrations of PGFM in serum was similar for oxytocin-treated and control cows. Changes in body weight were not influenced by treatment, but multiparous cows lost ($P<.01$) more weight early postpartum, whereas primiparous cows maintained their body weight. Milk yield (23.2 $\pm$ .8 vs 25.7 $\pm$ .8 kg/d), percentage protein in milk (3.00 $\pm$ .09 vs 3.02 $\pm$ .08%), and somatic cell counts (368,900 $\pm$ 233,300 vs 294,000 $\pm$ 218,700) for oxytocin-treated and control cows, respectively, were similar. In contrast, percentage fat in milk tended to be higher ($P<.10$) in oxytocin-treated cows than in control cows (3.99 $\pm$ .22 vs 3.68 $\pm$ .21%). These results demonstrate that multiple daily injections of oxytocin failed to prolong anestrus and anovulation in postpartum dairy cows. There was no evidence that oxytocin inhibited secretion of LH or hastened the rate of cervical and uterine involution. It is concluded that exogenous oxytocin had no inhibitory role in preventing onset of cyclic ovarian function. However, these data do not preclude the possibility that oxytocin is part of an inhibitory complex of hormones and other factors that delays or inhibits the establishment of postpartum cyclic ovarian activity.