OVARIAN ACTIVITY IN POSTPARTUM, EARLY PREGNANT AND NORGESTOMET SYNCHRONIZED DAIRY CATTLE

By

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ABSTRACT

Studies to monitor bovine ovarian function with regard to follicular growth and turnover, and corpus luteum (CL) growth and function, were carried out during three different reproductive states: the postpartum anestrus period, early pregnancy and during the artificial control of the estrous cycle with the synthetic progestin norgestomet. Ovarian function was monitored using a combination of ultrasound imaging and progesterone (P4) profiling.

Growth of large antral follicles (> 10mm) was found to commence very early in the postpartum period and ovulation occurred as early as the first week postpartum. Short first postpartum estrous cycles (< 18 days) were observed in a minority of the animals studied (4/10) and the occurrence of a short first cycle was not associated with an early ovulation following parturition. Growth of large antral follicles occurred in a wave-like pattern during the postpartum estrous cycles with most cycles being composed of two waves of growth, the second wave resulting in the growth of the ovulatory follicle.

A wave-like pattern of growth of large dominant follicles was also seen through the first 60 days of pregnancy. There was no difference between pregnant and non pregnant cows in the size of the dominant follicle found on day 20. In
addition no effect of the CL could be found on the side on which the dominant follicle was found, it was as likely to be on the ipsilateral ovary to the CL as on the contra lateral.

The gonadotrophin inhibitor norgestomet did not effect follicular dynamics in the presence of the CL, however in the absence of a CL the dominant follicle present was maintained for the duration of the norgestomet treatment and then went on to ovulate upon norgestomet removal. In addition there was no new growth of antral follicles in the absence of a CL. Norgestomet did not effect the temporal relationship between the onset of standing estrus, the LH surge and ovulation.

The results of the three studies suggest that a wave-like pattern of growth of large antral follicles is a characteristic of the bovine ovary regardless of the reproductive state.
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FORWARD

The hormonal factors involved in the growth of ovarian follicles and growth and regression of the corpus luteum in the bovine are quite well documented, however, the actual changes in follicular populations on different days of the estrous cycle have only recently been reported. The use of ultrasound imaging in recent years has allowed the continual monitoring of individual animals over a period of time, thus permitting actual changes in follicular populations and the growth and regression of the corpus luteum to be visualized. This thesis describes the follicular dynamics and corpus luteum growth and function during three different physiologic states: the postpartum anestrus period, early pregnancy and during the exogenous control of the estrous cycle with the progestin norgestomet. Each chapter is treated as a separate paper with an introduction, materials and methods, results and discussion. A general discussion of the relevance of each study to the other two and the possible implications and future research which the present studies suggest is presented in the final chapter.
LIT REVIEW

CHAPTER 1

LITERATURE REVIEW

One of the main characteristics of a functional ovary in sexually mature mammals is the growth and maturation of follicles, the rupture of a select few in the process of ovulation and atresia of the vast majority. This process begins early in embryonic and fetal life with the differentiation of the female ovary, the formation of a primordial follicle reserve and the subsequent growth and differentiation of follicles.

FORMATION OF THE GONAD

The ovary differentiates from the embryonic gonad which develops as a thickening along the ventral cranial area of the mesonephros, the primordial kidney (Witschi, 1951; Hamilton and Mossman 1972). There have been several different theories as to the origin of gonadal cells. In studies of amphibian gonads, Witschi (1948) concluded that the gonad was composed of two types of tissues: the cortex, derived from coelomic epithelium, secretes cortexin; and the medulla, derived from mesonephros, secretes medullarin. He proposed that these secretory products act antagonistically and that an
excess of one product causes the atrophy of the opposing tissue type. In the female excess cortexin causes the degeneration of the medulla and leads to the development of the ovary.

Burns (1961) proposed another model in which primary sex cords proliferate from germinal epithelium and form the medullary cords. In this model an ovary develops as the result of a second proliferation of germinal epithelium forming the cortical cords. The medullary cords which would form the testes in a male are pushed into the medulla where they degenerate.

A third model based on studies of fetal human (Pinkerton et al., 1961) and bovine (Gropp and Ohno 1966) ovaries suggest that gonadal blastema originates from mesonephros in much the same manner as that described by Witschi (1951). Gropp and Ohno (1966) found that in the bovine fetus the mesonephric tissue extends from the glomerular tuft of the developing kidney to the gonadal ridge where they mix with mesenchyme and germ cells.

In an extensive review on the development of the mammalian gonad, Byskov (1986) suggests that the primary tissue type involved in gonad formation is the mesonephros with some celomic epithelium, however there are no primary or secondary proliferations. In the cow formation of the genital ridge occurs at about 30 days of gestation (Byskov, 1986).
PRIMORDIAL GERM CELLS: ORIGIN AND MIGRATION

Primordial germ cells (PGCs) are the germ cells of both the male and female that originate extragonadally and migrate to the undifferentiated gonad. In what is now a classic experiment, Everett (1943) transplanted embryonic mouse genital ridges at different stages of development under the kidney capsule of adult mice. Examination two weeks later revealed that transplants derived from 9.5 to 10 day fetuses contained no PGCs whereas transplants from 11 to 14 day old fetuses did. Everett concluded that germ cells were extragonadal in origin and that they invaded the genital ridge after day 10 in the fetal mouse.

In the intervening years many studies have been done in an effort to trace the cell line from which the PGCs arise. Despite these efforts it is still not possible to determine when, and from where, these cells arise (Byskov, 1986). However, several characteristics of these cells have shed some light on their origin. The peripheral cytoplasm has been found to have high alkaline phosphatase activity (McKay et al., 1953). This characteristic has been used to establish the migratory pathway from the yolk sac to the genital ridge in the mouse embryo (Chiquoine, 1954).

The mechanisms involved in the movement of the PGCs from the yolk sac to the developing gonads are also not clear. Several hypotheses have been proposed and it is likely that
translocation involves a combination of mechanisms. Pseudopodia-like structures have been reported on the surface of PGCs in several species including the mouse (Clark and Eddy, 1975), rat (Eddy, 1974) and human (Kuwana and Fujimoto, 1983). It has also been reported that morphogenic movements of the tissues in which the PGCs are embedded help to transport the cells (Jeon and Kennedy, 1973; Snow, 1981). Finally, Witschi (1948) suggested that PGCs were directed to their destination by chemoattractants produced in the gonadal ridge.

Once the PGCs arrive at the gonadal ridge they are quickly enclosed in germ cell compartments where they proliferate and differentiate under the regulation of the surrounding somatic cells.

DIFFERENTIATION OF THE OVARY

Development up to the point where the PGCs have come to rest in the genital ridge is identical in both male and female embryos. Alfred Jost (1959) suggested that it is at this stage that differentiation to either testes or ovaries takes place. He proposed that in the absence of secretions from primordial testes the reproductive tract would develop along the female line. This has since become the central paradigm of sexual development (Wilson, 1985).
One of the first signs of differentiation of the ovary is the observation that germ cells are entering meiosis (Peters, 1970). However, in some species such as the sheep, cow, pig and rabbit meiosis is delayed while the germ cells become enclosed in germ cell cords (Mauléon, 1969) which then proceed to break up by approximately 1/2 term. The first cells to enter meiosis are found in the central portion of the gonad adjacent to the mesonephric connection. This is true whether meiosis is immediate or delayed (Byskov, 1986). The connection of meiotic cells with the mesonephros has led to the proposal that the mesonephros is responsible for initiating meiosis (Byskov, 1975) and that intercellular bridges help synchronize divisions. Species with a delay period prior to the initiation of meiosis exhibit a transitory secretion of sex steroids which are thought to inhibit meiosis. Once the secretions have ceased meiosis is initiated (Byskov, 1986). Meiosis is arrested in the last phase of the meiotic prophase, the diplotene stage, when the oocyte becomes surrounded by granulosa cells and a basal lamina to form a follicle (Peters, 1978).

As with meiosis, follicle formation always starts in the inner portion of the ovary immediately after the first oocytes reach the diplotene stage (Mossman and Duke, 1973). Granulosa cells arise from mesonephric derived cells (Byskov and Lintern-Moore, 1973) and are initially connected to the rete cords. Formation of the basal lamina breaks this connec-
tion and thus follicles become independent units (Hashimoto and Eguchi, 1955).

The stimulus for follicle formation is not known. Granulosa cell proliferation occurs in vitro without hormones (Baker and Neal, 1973; Challoner, 1975). In addition, treatment of adult mice with anti-gonadotrophins does not interrupt early follicular growth (Nakano et al., 1975), although gonadotrophins can accelerate the rate of growth of pre-antral follicles (Hansel and Convey, 1983). It has long been held that further growth of follicles to the antral stage requires stimulation by gonadotrophins: hypophysectomy results in the arrest of antral follicular growth and atrophy of the reproductive tract due to the decrease in circulating sex steroids normally produced by antral follicles (Gulyas et al., 1977). Treatment of rats with antibodies for follicle stimulating hormone (FSH) blocks the development of the pool of ovulatory follicles (Welschen and Dullaart, 1976). In addition, administration of exogenous gonadotrophins, namely (FSH), pregnant mares serum gonadotrophin (PMSG) or their analogues, stimulates antral follicular growth in prepubertal heifers and induces a superovulatory response in heifers and mature cows (Casida et al., 1943; Onuma et al., 1970; Seidel et al., 1971).
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THE WAVE PATTERN HYPOTHESIS OF FOLLICULAR GROWTH

The bovine ovary contains, on average, over 100,000 follicles at birth (Erickson, 1966). Throughout life, primordial follicles enter a pool of growing follicles, with a select few going on to ovulation, while most regress and become atretic. Smeaton and Robertson (1971), marking individual follicles with India ink showed that a sequence of follicles grow, regress and are then replaced by other follicles during a single estrous cycle in the sheep. Similar findings have been reported in cows (Matton et al., 1981). Rajakoski (1960) put forth the hypothesis that follicular growth in the cow takes place in a wave-like pattern with a single cycle consisting of two waves. The first wave of growth occurring between day 1 and day 12 of the cycle (day 1 = day of estrus) and the second wave of growth commencing on day 13 and culminating in the selection and ovulation of a single follicle at the subsequent estrus. A similar pattern of follicular growth has been proposed in sheep (Brand and DeJong, 1973). Although this hypothesis has come under some criticism (Spicer and Ekternkamp, 1986) the idea has persisted.

One problem has been that until recently most studies on follicular dynamics in the bovine have been restricted to slaughterhouse studies or studies involving rectal palpation. Both of these methods have their limitations. The major limitation with sampling from a slaughterhouse is that it
precludes monitoring individual animals over a period of time perhaps obscuring the dynamic nature of follicular growth. In the past decade ultrasound imaging has been used in numerous studies of follicular growth and atresia. Pierson and Ginther (1987a) described a two wave estrous cycle in heifers, while Sirois and Fortune (1988) suggest that the majority of heifers studied have three waves of follicular growth during a single cycle. A third study found both two and three waves of growth in heifers (Savio et al., 1988). One common theme in all of the studies using ultrasound imaging is that there is a wave-like pattern of growth of large dominant follicles during the cycle and that the dominant follicle at the time of luteolysis is the follicle that ovulates.

SELECTION OF THE OVULATORY FOLLICLE

The process by which the ovulatory follicle is selected is as yet unknown. Removal of the corpus luteum (CL) in cows (Hammond and Bhattacharya, 1944) and sheep (Smeaton and Robertson, 1971) results in ovulation within 48 to 72 hours. However it is not the dominant follicle that is present at the time of CL removal (Smeaton and Robertson, 1971) or prostaglandin F$_{2\alpha}$ (PGF$_{2\alpha}$) induced luteolysis (Ireland and Roche, 1982) which ovulates, a new follicle or follicles grow while the old ones become atretic. These results suggest that
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selection of the ovulatory follicle has occurred prior to the induced luteolysis or CL removal, that is selection takes place early in the cycle or even during the previous cycle. However, conflicting evidence would suggest that selection of the ovulatory follicle takes place relatively late in the cycle. Removal of follicles by cautery (Tsonis et al., 1982) or x-irradiation (Draincourt and Mariana, 1982) does not result in a lengthening of the follicular phase. However it is possible that selection of follicles from the pool of preantral follicles has already taken place and that the ovulatory follicles undergo rapid growth once luteolysis commences.

OVULATION

Ovulation occurs as the result of a complex mechanism involving changes in endocrine patterns, biochemical changes within the ovulatory follicle and neuromuscular mechanisms.

A gonadotropin surge triggers the onset of ovulation and results from the decrease in circulating progesterone (P₄) after luteolysis and an increase in circulating estradiol-17β (E₂) from the ovulatory follicle. Pelletier and Thimonier (1975), in experiments with castrated female rats, demonstrated that E₂ treatment initially results in a decrease in luteinizing hormone (LH) due to negative feedback, but with
continued treatment there is a switch to positive feedback resulting in the LH surge preceding ovulation. Simultaneous administration of $P_4$ supresses the $E_2$ positive feedback.

The increased LH secretion enhances steroid synthesis and secretion by the ovulatory follicle and decreases the $E_2:P_4$ ratio in the follicular fluid as a result of a switch from $E_2$ production to $P_4$ production (Gerard et al., 1979). Lipner and Green (1971) found that inhibition of $P_4$ synthesis blocked ovulation. It appears that $P_4$ acts to stimulate collagenase activity in the follicular wall (Rondell, 1970).

Increases in prostaglandins ($\text{PGF}_{2\alpha}$ and $\text{PGE}_2$) are seen somewhat later than the increase in steroids. Ainsworth et al. (1975) showed that prostaglandins do not start to increase in follicular fluid until about 30 hours after the LH surge in the pig and don't reach their peak until about 40 hours. Inhibition of intra follicular prostaglandin synthesis has been shown to block ovulation in rabbits and sows (Armstrong, 1975). It is believed that $\text{PGF}_{2\alpha}$ is involved in follicular rupture via its rupturing effects on lysosomal membranes (Weiner and Kaley, 1972).

Finally neuromuscular systems appear to be involved in the rupturing of the ovulatory follicle. Inhibition of $\beta$-and-renoergic receptors delays ovulation and reduces the ovulation rate in rabbits (Virutamasen et al., 1976).
FORMATION AND FUNCTION OF THE CORPUS LUTEUM

After ovulation the ruptured follicle reorganizes to form a transitory gland, the CL. The main luteotrophin in most domestic farm species is LH (Hansel and Convey, 1983). The CL has been found to be composed of two different cell types in the bovine, ovine and in swine: small thecal derived cells and large cells primarily derived from granulosa cells (Hansel, 1988). The small thecal derived cells make up approximately 95% of the cells of the corpus luteum found during a normal estrous cycle and are very responsive to LH and cAMP modulators. Additionally, these small cells increase both basal and LH stimulated P₄ synthesis in response to PGF₂α challenges while the large cells decrease LH stimulated synthesis in response to the same challenge, leading to the belief that the luteotrophic effects of PGF₂α may be mediated via the large cells (Hansel 1988).

LUTEOLYSIS

In normal repetitive estrous cycles it is necessary for the CL to undergo luteolysis causing the fall in circulating P₄ and allowing the animal to come into estrus. A large body of evidence has shown that the luteolysin is PGF₂α of uterine
LIT REVIEW

origin (Thatcher et al., 1984). Removal of the uterus extends the lifespan of the CL (Ginther et al., 1967). Sectioning of the broad ligament ipsilateral to the CL has the same effect (Hixon and Hansel, 1974). Finally, administration of exogenous PGF$_2\alpha$ induces luteolysis. A complete mechanism for the induction of luteolysis in the nonpregnant cow has been outlined—(Thatcher et al., 1988). E$_2$ from a mid cycle dominant follicle induces the formation of endometrial oxytocin receptors, oxytocin synthesized and secreted by the CL then binds to these receptors and stimulates the endometrium to secrete PGF$_2\alpha$ which then causes luteolysis.

In the case of pregnancy it is essential that the CL be maintained beyond its normal estrous cycle lifespan. In the bovine and ovine, conceptus secretory products which can prevent the secretion of endometrial PGF$_2\alpha$ have been isolated (Thatcher et al., 1989). It is suggested that these trophoblastic proteins act by stimulating the synthesis of an endometrial prostaglandin synthetase inhibitor, thus suppressing the synthesis and secretion of PGF$_2\alpha$ and preventing luteolysis. There is also evidence to suggest that secretion of any PGF$_2\alpha$ that is synthesized is in an exocrine direction into the lumen of the uterus in pregnant animals as opposed to the secretion in an endocrine direction in cycling animals (Thatcher et al. 1984).
In the past most studies on follicular dynamics and corpus luteum growth have had to rely on slaughterhouse material or rectal palpation, however in the last decade the use of ultrasound imaging has become prominent in the study of ovarian function.

There are basically two types of ultrasound scanners used for anatomic visualization: sector scanners, which use a single pizoelectric crystal which rotates and produces a pie shaped image, and linear array scanners, which use a linear row of crystals and produce a square image. The main feature of each type is the probe which contains the crystal or crystals. These pizoelectric crystals expand and contract rapidly when excited by electric current. The expansion and contraction displaces molecules and in this way the waves are propagated through the tissues. At tissue interfaces the sound is reflected back and received by the probe, again exciting the pizoelectric crystals which then convert the energy back to electricity. The frequency of the electric impulse is then converted into varying shades of grey by the ultrasound imager producing an image on the screen.

The density of the tissue determines the frequency at which the wave impulse is reflected, fluid absorbs the waves and therefore sends back no impulse, producing a black area in the image. Very dense tissue such as cartilage or bone
sends back a high frequency impulse producing a very bright or hyperechogenic image, other tissues produce images of varying shades of grey and in this way the image can be analysed and structures visualized.

The frequency of the probe determines the depth to which the sound waves will penetrate and the level of resolution of the image. A high frequency probe will provide the best resolution but will not penetrate very deeply. A low frequency probe will penetrate deeper at the expense of resolution. The frequency of the probes range between 2 and 9 megahertz (mHz).

Most previous studies examining the reproductive tract of the bovine have used linear array scanners equipped with rectal probes. The entire probe can be inserted into the rectum and laid over the uterus or ovaries for examination. In addition most modern machines can freeze the image and structures can be measured using callipers built into the system. Callibration of the calipers can be checked using a phantom. Previous studies in the bovine have also validated the measurement of structures by measurement of the same structures after slaughter (Pierson and Ginther, 1987a).

The present thesis involves ultrasound imaging used in conjunction with progesterone profiles to study follicular dynamics and corpus luteum growth and function during three different reproductive states: during the postpartum period,
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a time where very little work has been done on follicular dynamics; during early pregnancy, when it has been hypothesized that the influence of the corpus luteum on follicular growth on the ipsilateral ovary changes from positive to negative; and finally under the influence of the norgestomet, a progestin which is used as an estrous synchronizing agent in beef cattle.
OVARIAN DYNAMICS AND THE RESUMPTION OF ESTROUS CYCLES
IN POSTPARTUM DAIRY COWS

ABSTRACT

Ten postpartum dairy cows were monitored for ovarian activity by using a combination of ultrasound imaging and progesterone profiles. Blood and milk samples were collected and ovaries scanned rectally starting 2 and 14 days postpartum, respectively, and continued until the end of the second estrous cycle. Ultrasound imaging revealed the presence of a corpus luteum in four of ten animals by 14 days postpartum and in eight of ten by 25 days. This was later confirmed by progesterone determination. There were no differences between primiparous and pluriparous animals with regard to time to first postpartum ovulation, duration of 1st estrous cycle, peak plasma progesterone levels or time to uterine involution. Time to first observed estrus was greater (P<0.1) in primiparous than pluriparous cows. The second postpartum estrous cycle was significantly longer (P<0.05) with greater plasma progesterone concentrations than the first cycle. Maximum corpus luteum diameter as measured by ultrasound imaging was not correlated to the length of the
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1st or 2nd postpartum estrous cycles or to the maximum plasma progesterone in the 1st estrous cycle but was positively correlated to the maximum plasma progesterone in the 2nd postpartum estrous cycle. Follicular growth occurred in two waves in most of the postpartum estrous cycles. Whole milk progesterone profiles were similar in pattern to the plasma profiles.

INTRODUCTION

Ovarian follicular dynamics and the return to normal cyclic activity in postpartum dairy cattle are not well understood. The postpartum period is characterized by a behavioral anestrus lasting from 20 to 70 days (Callahan et al., 1971; Schams et al., 1978). The first behavioral estrus is often preceded by a short estrous cycle characterized by an absence of overt signs of estrus and a short luteal phase with subnormal progesterone (P_4) blood concentrations (Robertson, 9172; Edgerton and Hafs, 1973; Manns et al., 1973). The cause of these "short" first cycles is not certain but several ideas have been put forth including FSH deficiency (Ramirez-Godinez et al., 1982) and premature luteolysis by prostaglandin F_2α (PGF_2α) from the involuting uterus (Troxel et al., 1980; Hanzen, 1986). Another factor reported to influence the length of the anestrous period is
parity; primiparous cows have longer calving to first estrus intervals than pluriparous cows (King and McLeod, 1983).

Previous studies have relied either on slaughterhouse material (Morrow et al., 1966) which precludes monitoring individual animals over a period of time or on rectal palpation (Saiduddin et al., 1968) in which a measure of accuracy and precision is lost (Landsverk and Kalberg, 1988). Ultrasound imaging has been used effectively to identify ovarian follicles and corpora lutea in cycling heifers (Pierson and Ginther, 1987) and cows (Rajamahendran and Walton, 1988) on a continuous basis, however, there appear to be no reports on the use of ultrasound imaging in the study of ovarian function in the postpartum cow.

The purpose of the present study is to utilize both ultrasound imaging and P₄ hormone profiles for the monitoring of ovarian activity in postpartum dairy cows, and to evaluate the reliability of whole milk progesterone concentrations as a means of assessing postpartum ovarian activity.

MATERIALS AND METHODS

Ten postpartum dairy cows (8 Holsteins, 2 Ayrshires) were selected at random from the University of British Columbia dairy herd between October 1987 and January 1988. The cows ranged from 2 to 6 years of age. Three animals were
first calvers. Calves were weaned within 24 hours of birth. Animals were kept in separate calving pens away from the herd for a minimum of two weeks following parturition with the first calvers generally remaining in the pens longer than the pluriparous cows. Cows were checked for signs of estrus at both a.m. and p.m. milkings. All cows received a mix of alfalfa-cubes and concentrate in equal proportions starting at 4 kg per day and increasing with increased milk production.

Animals were scanned three times per week using a real-time linear array ultrasound scanner (Tokyo Keiki LS300, Tokyo Keiki Co. Ltd., Tokyo, Japan) and a 5 MHz rectal transducer starting 14 days postpartum and continuing until the end of the second postpartum estrous cycle. Ovaries were scanned in several planes to identify all follicles and to determine the presence of a corpus luteum. Landmarks on the ovary such as a corpus luteum, other follicles and the poles of the ovary as well as the orientation of the ovary were used to identify and monitor individual follicles. The image was frozen and the largest follicle on each ovary and the corpus luteum, if present, were measured using a built-in caliper system. Prints of the image were made using a video processing unit (Mitsubishi Electronics Co. Ltd., Tokyo, Japan.). Uterine involution was determined by rectal palpation. The point at which the uterus stopped decreasing in size and had regained its tone was considered the point at
which uterine involution was complete.

Blood samples were collected from a tail vein three starting 2 days postpartum. Samples were centrifuged, plasma was separated and then stored at -20°C until analysis. Whole milk samples were collected at the afternoon milking on the same days as the plasma and preserved with potassium dichromate tablets at 5°C until analysis. Plasma and milk samples were analysed for P₄ using a coated tube radioimmunoassay kit (Coat-A-Count, Diagnostic Products Corp., Los Angeles, CA). Plasma, milk or reference standard (0.1 ml) was added to tubes coated with a specific antibody. Reference standards contained between 0 and 40 ng/ml P₄. Buffered I¹²⁵ P₄ (1 ml) was added to all tubes, vortexed and left to incubate for three hours. Tubes were decanted after the incubation period and cleaned with a cotton swab above the 1 ml mark to remove excess plasma, milk or tracer. Tubes were counted for 1 minute using a gamma counter (Packard Auto-Gamma 500). Coefficients of variation within and between plasma assays were 7.7 and 8.3% respectively. The limit of assay sensitivity was 0.05 ng/ml. The coefficients of variation for the milk assays were 9.2 and 12.4% and the limit of sensitivity was 0.05 ng/ml.

Primiparous and pluriparous animals were compared using a Student's t test for time to first postpartum ovulation, time to first observed estrus, duration of 1st and 2nd estrus cycles, peak plasma P₄ and time to uterine involution. A
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Student's t test was also used to compare characteristics of the 1st and 2nd postpartum estrous cycles among all animals. Correlations between maximum CL diameter, peak P4 concentrations and duration of the estrous cycles were determined by least squares linear regression and Pearson r correlation.

RESULTS

The time to first ovulation after parturition ranged from 10 to 55 days (Table 2.1). There was no difference between primiparous and pluriparous animals with regard to return to cyclic activity. The interval from calving to first observed estrus was highly variable ranging from 17 to 139 days postpartum. Primiparous animals tended (P<0.1) to have a longer behavioral anestrus period than pluriparous animals (Table 2.1). Uterine involution was generally complete by 4 weeks postpartum and there was no significant difference between primiparous and pluriparous animals.

Ultrasound imaging revealed the ovaries to be active with regard to follicular growth during the anovulatory period in all animals. Ovaries were characterized by several small (3-8mm) and medium (9-15mm) sized follicles during this time (Plate 2.1). One of two animals (84001) with a prolonged anovulatory period developed a large persistent follicle on the left ovary (Plate 2.2) attaining a maximum diameter of
Table 2.1. Mean interval in days (± S.D.) from calving to first ovulation, first estrus, uterine involution, length of the 1st and 2nd postpartum estrous cycles and maximum progesterone (P₄) concentration (ng/ml) in dairy cows (n = 10).

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<thead>
<tr>
<th></th>
<th>Overall</th>
<th>Primiparous</th>
<th>Pluriparous</th>
</tr>
</thead>
<tbody>
<tr>
<td>1st ovulation</td>
<td>21 ± 14</td>
<td>16 ± 7</td>
<td>24 ± 17</td>
</tr>
<tr>
<td>1st estrus</td>
<td>59 ± 38</td>
<td>89 ± 55</td>
<td>46 ± 23</td>
</tr>
<tr>
<td>Uterine involution</td>
<td>27 ± 5</td>
<td>25 ± 1</td>
<td>27 ± 6</td>
</tr>
<tr>
<td>1st cycle length</td>
<td>17 ± 7a</td>
<td>15 ± 7</td>
<td>18 ± 8</td>
</tr>
<tr>
<td>2nd cycle length</td>
<td>23 ± 5a</td>
<td>21 ± 1</td>
<td>24 ± 6</td>
</tr>
<tr>
<td>P₄ 1st cycle</td>
<td>3.1 ± 1.2a</td>
<td>3.1 ± 1.2</td>
<td>3.0 ± 1.7</td>
</tr>
<tr>
<td>P₄ 2nd cycle</td>
<td>4.4 ± 1.0a</td>
<td>4.7 ± 0.8</td>
<td>4.3 ± 1.0</td>
</tr>
</tbody>
</table>

a difference within groups p<0.05
OVARIAN ACTIVITY IN POSTPARTUM COWS

47mm by 36 days postpartum. The right ovary remained relatively inactive with only small follicles (<4mm) being observed while the persistent follicle developed. Shortly after the persistent follicle attained its maximum diameter larger follicles started to develop on the contralateral ovary with one finally ovulating 42 days postpartum. The persistent follicle had begun to regress at this point but was still approximately 40mm in diameter.

The other animal with an extended anovulatory period appeared to have normal follicular development and turnover. One follicle grew to a diameter of 20mm by 24 days postpartum but failed to ovulate. There was a continual turnover of follicles until one finally ovulated at 55 days postpartum.

There was no difference between primiparous and pluriparous animals with regard to duration of the 1st or 2nd estrous cycles or peak plasma P₄ levels (Table 2.1). Four of ten animals had a first postpartum cycle shorter than 14 days, five of ten had a cycle of 18-21 days and one animal had a 1st cycle greater than 21 days in duration. The side of pregnancy appeared to have no effect on the side of first postpartum ovulation: four of ten animals had their first ovulation on the same side as the previous pregnancy. The second postpartum cycle was significantly longer (P<0.05) and had higher peak progesterone levels (P<0.05) than the first postpartum cycle.
Plate 2.1.
ULTRASOUND IMAGES OF THE OVARIES IN POSTPARTUM DAIRY CATTLE

Picture taken 14 days PP. Note small follicles on left and right ovaries.

Image of ovaries 48 days PP. Note medium and small follicles on left and small follicles on right ovary.

Picture taken 7 days after the first PP ovulation. Note CL on left and 19mm follicle on right.
Plate 2.2.
ULTRASOUND IMAGES OF THE OVARIES IN POSTPARTUM DAIRY CATTLE

Ovaries 8 days after first PP ovulation. Note large CL (L) and 2 medium sized mid cycle follicles (R).

Ultrasound image of a large follicle (L) and a CL with a central cavity (R) after spontaneous ovulation.

Picture of above two days later. Note decrease in follicle size (L) and CL cavity (R).
There was no correlation between maximum corpus luteum
diameter and maximum plasma P₄ concentration in the first
postpartum estrous cycle however there a strong correlation
(r=0.88) in the second cycle (Fig. 2.1). Duration of the
first or second postpartum estrous cycles were not cor-
related to the maximum diameter of the corpus luteum.

The return to reproductive cycles followed one of two
distinct patterns (Fig 2.2): a very short anovulatory period
of two weeks or less, followed by a normal lengthed estrous
cycle with an adequate luteal phase and a normal P₄ profile
(early), or, a prolonged anovulatory period of three weeks or
greater, followed by a short estrous cycle with an inadequate
luteal phase with a subnormal P₄ profile (delayed).

There were generally two waves of follicular growth
during an estrous cycle, each wave resulting in a large
dominant follicle, as revealed by ultrasound imaging; only
three of twenty-one cycles had three waves, all others had
two waves, including short cycles (Fig. 2.3a, b & c). Typi-
 Typically the first wave of growth commenced on day 2.5 ± 0.9
of normal lengthed cycles (day 0 = estrus) and resulted in a
large mid cycle follicle of 16.5 ± 2.5 mm in diameter. This
follicle was maintained until day 14.1 ± 1.0 of the cycle
when it began to regress. The second wave of follicular
growth commenced on day 15.8 ± 1.8 and resulted in a large
ovulatory follicle (20.9 ± 3.9 mm). Early during each wave of
Fig 2.1 Correlation between corpus luteum diameter and circulating progesterone in postpartum dairy cattle
Fig 2.2 Patterns of return to reproductive cycles in postpartum dairy cattle
growth a single follicle appeared to become dominant and continued growing while other visible follicles on each ovary began to decrease in size.

The P₄ profiles from the whole milk samples followed a similar pattern to those from the plasma samples (Fig. 2.4). Milk progesterone concentrations tended to start increasing and generally peaked on the same days as did the plasma concentrations, however, peak milk P₄ concentrations tended to be much more variable and much higher than the plasma concentrations.
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Fig. 2.3. Growth of dominant follicles in 3 representative postpartum dairy cows.
Fig. 2.4 Comparison between plasma and milk progesterone in postpartum dairy cattle
OVARIAN ACTIVITY IN POSTPARTUM COWS

DISCUSSION

The average time to the first postpartum ovulation of 21 days and the finding that 8 of 10 animals ovulated by 25 days postpartum agree well with earlier findings based on slaughterhouse and hormonal studies showing luteal activity in 88% of animals by 35 days after calving (Ball and Lamming, 1983) and 95% by 50 days (Morrow et al., 1966). Ultrasound imaging revealed by the presence of a corpus luteum that four animals had ovulated by 14 days postpartum. This was later confirmed by the P₄ assays which showed an increase in circulating P₄ in these animals. Scanning prior to 14 days postpartum was not possible due to the size of the uterus. Ovarian follicles have been detected early in the second week postpartum by rectal palpation (Saiduddin et al., 1968) and slaughterhouse studies have found ovarian changes due to follicle growth 5 to 7 days postpartum (Morrow et al., 1966). The interval from calving to the first observed estrus was generally longer and much more variable. Only one animal of the ten was observed in estrus at the first postpartum ovulation. During the present study primiparous animals tended (P<0.1) to take longer to show their first postpartum estrus than pluriparous animals. This agrees with studies in beef cattle (King and McLoed, 1984). However, time to first observed estrus must be looked at with caution, it depends greatly on the efficiency of estrus detection. Primiparous
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animals were housed in calving pens isolated from the main herd for a longer period than pluriparous animals. Additionally, animals housed in calving pens were kept in a separate holding pen during milking and therefore their exposure to other animals in estrus was minimal. These practices may contribute to the apparently longer period of anestrus seen in the first calvers in the University of British Columbia herd. Another contributing factor may be the behavior of the animals. First calving heifers usually are smaller and less aggressive which may be expressed as less mounting behavior than normally seen in pluriparous cows, however few studies have looked at this.

Only two animals (83002 & 84001) had prolonged anovulatory periods greater than 25 days, one of which (84001) developed a large persistent follicle on the left ovary. The spontaneous ovulation of a 26mm follicle on the contralateral ovary corresponded with the regression of the persistent follicle. The fact that the large follicle had begun to regress as indicated by ultrasonographic imaging suggests that it had stopped secreting large amounts of estrogens. It also suggests that it had not become luteinized and was not secreting P4. This was confirmed by the P4 assays which showed the animal to have basal P4 levels until after ovulation.

The other animal (83002) with an extended anovulatory period appeared to have normal follicular development and
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turnover but failed to ovulate early in the postpartum period. The reason for this failure is not known but may include insufficient estradiol secretion to produce an LH surge, a prolonged postpartum refractory period of the hypothalamus-pituitary axis, inadequate LH available to produce a surge or insufficient LH receptors on the pre-ovulatory follicle. The first postpartum estrous cycle in this cow was characterized by a small corpus luteum of 23mm with a 5 day life span and very low P₄ secretion supporting the possibility of an LH deficiency. Hypophysectomy in the first three days following ovulation results in early regression of the CL (Niswender et al., 1986). The second postpartum cycle was of normal duration with normal plasma P₄ concentrations.

Overall the first postpartum estrous cycle was found to be significantly shorter with lower peak P₄ levels than the second cycle, thus confirming previous reports (Robertson, 1972; Edgerton and Hafs, 1973; Manns et al., 1983). However, looking at individual animals it was found that the decrease in first cycle length was due to a greatly reduced luteal phase in four of the ten cows studied, the remaining six cows all had cycles which fell in the normal range of 18-22 days and normal P₄ profiles. The cause of the short luteal phase is not known. Maximum diameter of the corpus luteum in the first postpartum luteal phase could not be correlated to its duration. Additionally, one animal with a cycle less than 14
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days had a corpus luteum of greater than 30mm suggesting that
diameter of the corpus luteum is not the limiting factor in
the first postpartum cycle. Plasma P₄ was lower in the first
cycle and was not correlated to the diameter of the corpus
luteum. It is likely that corpora lutea of cycles less than
14 days in length begin to regress before they reach their
peak P₄ production.

There has been the suggestion that PGF₂α secreted during
uterine involution may be responsible for premature luteo-
lysis early in the postpartum period (Hanzen, 1986) but
several observations in the present study appear to contra-
dict this possibility. Two of the four animals which had
first postpartum cycles less than 14 days in duration were
the two animals with prolonged anovulatory periods. Previous
studies suggest that PGF₂α levels have returned to basal
concentrations by three weeks post calving (Lindell et al.,
1983) and therefore prostaglandin should not have been a
factor in shortening the cycle length in these two animals.
The remaining two animals with short first postpartum estrous
cycles (82031 & 85018) ovulated on day 22 and day 23 post-
partum. Uterine regression in both animals was complete by 26
days postpartum suggesting that PGF₂α levels would have been
elevated early in the cycles, a time when PGF₂α has no effect
on cycle length (Fredriksson et al., 1988). These obervations
suggest that factors other than PGF₂α may be involved in
determining the duration of the first postpartum estrous
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cycle. The present study would not appear to support the hypothesis of inadequate FSH (Ramirez-Godinez et al., 1982) as all animals had growth of antral follicles starting early in the postpartum period. It is possible that inadequate LH support during the luteal phase or poor response to LH due to insufficient receptors may be the cause of the inadequate luteal phase, however it is impossible to tell from the present study.

Several reports suggest that the corpus luteum of the just terminated pregnancy may have a negative effect on follicular growth in that ovary (Dufour and Roy, 1985). The first postpartum ovulation in the present study was unaffected by the side of pregnancy, four of ten animals had their first ovulation on the same side as the previous pregnancy. However, two of these animals were animals with an anovulatory period of greater than 28 days. Follicular growth may have been influenced by the side of pregnancy, at 14 days postpartum the largest follicle was on the same side as the previous pregnancy in only two animals lending some support to the possible negative influence of the corpus luteum of pregnancy on follicular growth early in the postpartum period.

The second postpartum cycle was generally much less variable in length than the first cycle, although one animal did have a prolonged cycle of 36 days. As with the first cycle the duration of the second estrus cycle was not cor-
related to the maximum diameter of the corpus luteum, however, there was a strong correlation ($r=0.88$) between maximum corpus luteum diameter and peak plasma $P_4$ concentrations. It seems reasonable to suspect that the diameter of the ovulatory follicle is positively correlated to the maximum corpus luteum diameter, however the present study was unable to confirm this. Animals were scanned only three times per week so not all animals were scanned on the day of estrus or ovulation and therefore the maximum diameter of the ovulatory follicle was not known in all animals.

Ultrasound imaging revealed that follicular growth occurred in two waves in the vast majority of cycles. This pattern of growth supports the findings of Peirson and Ginther (1987) in normally cycling heifers. Short cycles also appeared to have two waves of follicle growth however the resulting mid cycle follicle was not maintained but rather began to regress immediately. Others have reported that the majority of animals studied had three waves of growth (Sirois and Fortune, 1988; Savio et al., 1988). In the present study only 3 of 21 cycles followed had three waves and of those three, two were cycles of greater than 22 days.

The $P_4$ profiles from whole milk samples followed the plasma profiles closely with regard to the pattern of secretion and confirmed conclusions drawn from the ultrasound images. As has been previously reported (Mather et al., 1988; Kassa et al., 1986) milk $P_4$ levels appear to provide a
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reliable means of assessing luteal function in the postpartum dairy cow and can now be taken advantage of by the commercial dairy farmer using a cowside progesterone kit to identify truly anestrous / anovulatory animals.

Ultrasound imaging was useful for monitoring ovarian activity in postpartum animals. Its non invasive nature allows animals to be monitored repetitively so growth of ovarian structures can be followed in a single animal.
The effects of pregnancy on follicular dynamics, and corpus luteum growth and function are somewhat contentious. In an effort to learn more about the effects of pregnancy, the reproductive tracts of 16 bred dairy cows were monitored using a linear array ultrasound device equipped with a rectal probe through the first 60 days of pregnancy or a return to estrus. In addition, blood samples were collected for progesterone determination. Of the sixteen cows, eleven were diagnosed pregnant on the basis of visualization of an embryo with a heartbeat by 25 days post artificial insemination. There were two embryonic mortalities between 28 and 32 days. A two wave pattern of follicular growth and atresia, with each wave resulting in a large dominant follicle, was seen in both pregnant and non pregnant cows. Pregnant cows had significantly more follicles (P<0.05) than non pregnant cows; however there was no difference in the size of the largest follicle present on the ovaries. A wave-like pattern of growth of large follicles continued throughout the study.
FOLLICULAR AND CL GROWTH IN PREGNANT COWS

period in the pregnant cows. Progesterone profiles for the first 18 days, corpus luteum growth rate and maximum size of the corpus luteum were similar in both pregnant and non pregnant cows. It is concluded that pregnancy has little effect on the growth of dominant follicles during the first 60 days of pregnancy and hypothesized that large follicles serve as a safeguard to insure a quick return to estrus in the event of embryonic mortality. It is also concluded that pregnancy has little effect on corpus luteum growth and function for the first 60 days other than maintenance beyond its normal lifespan.

INTRODUCTION

Cycling cows generally have two or three waves of follicular growth during a normal estrous cycle (Rajamahendran and Taylor, 1988; Sirois and Fortune, 1988; Peirson and Ginther, 1987) each wave consisting of a pool of small follicles at the start of the wave, followed by a period of growth and ending with the growth of a large dominant follicle while the others become atretic. It is uncertain whether this wave-like pattern of follicular growth and atresia occurs in pregnant animals. Earlier studies (Peirson and Ginther, 1987; Rexroad and Casida, 1978) showed that after the first twenty days of pregnancy follicular growth
FOLLICULAR AND CL GROWTH IN PREGNANT COWS

slows, the size of the largest follicle decreases and the
corpus luteum (CL) has a negative effect on follicular growth
on the ipsilateral ovary. One study (Guilbault et al., 1986)
found that by 17 days post estrus pregnant cows had fewer
atretic follicles in the smaller size classes while larger
size classes had a higher proportion of atretic follicles
than non pregnant cows. It was concluded that the presence of
a conceptus promotes a more rapid turnover of small follicles
to medium sized ones and then limits further growth by
increasing atresia. In addition, it has been suggested that
one of the major differences between pregnant and non
pregnant cows with regard to ovarian function is the absence
of a large ovulatory follicle in pregnant animals thus
removing the luteolytic effects of estradiol-17β (E₂)
(Thatcher et al., 1988).

Conceptus removal studies and the transfer of embryos to
non bred cows (Betteridge et al., 1980; Northey and French,
1980) have shown that the conceptus transmits a signal to the
maternal compartment approximately 15 days after fertiliz-
ation to prevent luteolysis by prostaglandin F₂α (PGF₂α). Whether this signal includes a luteotrophic component has not
been shown, although some reports suggest that pregnant cows
have higher plasma (Lukaszewska and Hansel, 1980) and milk
(Lamming et al., 1989; Bulmann and Lamming, 1978) progester-
terone (P₄) concentrations than non pregnant animals as early
as 10 days post estrus.
FOLLICULAR AND CL GROWTH IN PREGNANT COWS

The aim of the present study was to further elucidate the effects of the early conceptus on follicular dynamics, and CL growth and function. Ultrasound imaging was used to monitor ovarian dynamics with regard to follicular growth and atresia, CL growth and identification of embryonic vesicles and the embryo proper. The incidence of embryonic mortality and plasma P₄ were also measured.

Materials and Methods

Sixteen postpartum dairy cows ranging from 2½ to 8 years of age were selected at random from the University of British Columbia dairy herd. All cows were housed in a closed freestall barn and received a mix of alfalfa cubes and concentrate in equal proportions. Total ration was adjusted according to the cows milk production. All cows were bred approximately 12 hours after the onset of standing estrus between 60 and 90 days post calving.

Ultrasound imaging commenced one day post AI. Cows were scanned daily until 20 days and then thrice weekly until 60 days of pregnancy or a return to estrus. Ultrasound examinations were performed using a linear array real-time ultrasound device (Tokyo Keiki LS300, Tokyo Keiki Co. Ltd. Tokyo, Japan) equipped with a 5 MHz rectal probe. After the evacuation of feces, the probe was placed intrarectally and
FOLLICULAR AND CL GROWTH IN PREGNANT COWS

the reproductive tract examined. The ovaries were scanned in several planes to identify all follicles ≥ 2mm in diameter and to provide an image of the CL with its greatest cross sectional area. Desired images were frozen on the screen, measurements taken using a built-in caliper system and a hard copy made using a video processing unit (Mitsubishi Electronics Co. Ltd., Tokyo, Japan). The uterine horns and body were scanned over their entire length and in several planes in order to examine uterine contents, and to identify embryonic vesicles and the embryo proper. As with the ovaries, desired images were frozen and hard copies made.

Blood samples were collected from a tail vein into heparinized tubes prior to each ultrasound examination. Plasma was separated and stored at -20°C for P₄ analysis. P₄ was measured by a commercially available solid phase RIA system (Diagnostics Corp., Los Angeles, California). Plasma or reference standard (0.1 ml) was added to tubes coated with a specific antibody. Reference standards contained between 0 and 40 ng/ml P₄. Buffered I¹²⁵ P₄ (1.0 ml) was added to all tubes, vortexed and left to incubate for three hours. Tubes were decanted after the incubation period and cleaned with a cotton swab above the 1 ml mark to remove excess plasma or tracer. Tubes were counted for 1 minute in a gamma counter (Packard Auto-Gamma 500). Intra and inter assay coefficients of variation were 6.2% and 9.8%, respectively and the limit of detection was 0.05 ng/ml.
FOLLICULAR AND CL GROWTH IN PREGNANT COWS

Animals returning to estrus prior to the identification of an embryo proper were deemed to be non pregnant. Total number of follicles, diameter of the largest follicle present, diameter of the CL and $P_4$ concentrations on specific days post breeding were compared between pregnant and non pregnant cows using an analysis of variance for repeated measures (SAS, 1985). Total number of follicles on the CL bearing ovary and on the contralateral ovary were also compared using an ANOVA for repeated measures.

RESULTS

Changes in the appearance of the uterus, development of the fetus and images of the CL bearing ovary in a representative pregnant cow are shown in Plates 3.1 & 3.2. Presumptive embryonic vesicles were seen in 14 of the 16 cows examined (Table 3.1); however only 11 animals were diagnosed pregnant based on visualization of an embryo proper with a heartbeat. There were two embryonic mortalities, as diagnosed by the cessation of the embryonic heartbeat, one between 28 and 30 days post breeding and the other between day 30 and 32. These two cows returned to estrus 40 and 63 days post breeding, respectively, shortly after luteolysis had taken place.

In the first 24 days following breeding, all animals had two waves of follicular growth. The first wave resulted in a
Plate 3.1. Ultrasound images of the CL bearing ovary and the gravid uterine horn in a pregnant dairy cow.

Note large mid-cycle follicle (short arrow) and CL (long arrow).

Note embryonic vesicle (long arrow) now visible in the uterus. Mid-cycle follicle is now decreasing in diameter.

Embryo proper is clearly visible (long arrow). Mid-cycle follicle is no longer visible. No change in diameter of CL.
Plate 3.2. Ultrasound images of the CL bearing ovary and the gravid uterine horn in a pregnant dairy cow.

- **Jul 22/88 11:50**
  - ID: 82019
  - Probe: 5 MHz
  - Distance: 18 mm
  - Area: Pa*e 1.35 DPVS
  - POST fl 16MM EMBR*HEARTBEAT OBSER
  - RIGHT OVARY 30X25MM CL 7MM FOLLICLE

  Fetal limb buds are now visible (short arrow). Also note presence of the amnion (long arrow).

- **Aug 02/88 13:10**
  - ID: 82019
  - Probe: 5 MHz
  - Distance: 45 D AVS
  - Area: RCOUR RV
  - Page 2
  - 45 DAYS POST AI
  - 25MM EMBR HEARTBEAT OBSER
  - R OVARY 32X23MM CL 16MM FOLLICLE

  Note fetal heart surrounded by pericardium (short arrow). Also note new dominant follicle on the CL bearing ovary (long arrow).

- **Aug 17/88 12:43**
  - ID: 82019
  - Probe: 5 MHz
  - Distance: 60 D AVS
  - Area: Page 2
  - 60 DAYS POST AI
  - R OVARY 33X26MM CL 12MM FOLLICLE

  Fetal limbs are clearly visible (short arrows). Also note ossification of the skull (long arrow).
Table 3.1. Intrauterine structures visualized on different days post breeding using ultrasound imaging in dairy cattle.

<table>
<thead>
<tr>
<th>Structures visualized</th>
<th>% Animals</th>
<th>Days post breeding</th>
</tr>
</thead>
<tbody>
<tr>
<td>Presumptive Embryonic Vesicles</td>
<td>88% (14/16)</td>
<td>15.4 ± 2.7</td>
</tr>
<tr>
<td>Embryo Proper</td>
<td>69% (11/16)</td>
<td>23.9 ± 1.5</td>
</tr>
<tr>
<td>Embryonic Heartbeat</td>
<td>69% (11/16)</td>
<td>25.0 ± 1.1</td>
</tr>
<tr>
<td>Embryonic Heartbeat</td>
<td>56% (9/16)</td>
<td>60</td>
</tr>
</tbody>
</table>
mid cycle follicle which attained its maximum size 8.8 ± 1.5 days post breeding and persisted until day 16.1 ± 2.0 when it was replaced by a second dominant follicle from the second wave of follicular growth. There was no difference between pregnant and non pregnant cows in the maximum diameter attained by either of the dominant follicles (Table 3.2), however, the second dominant follicle resulted in ovulation in non pregnant cows whereas in pregnant animals this follicle decreased gradually in size. Pregnant cows had significantly (P<0.05) more visible follicles on days 8 to 12, 15 to 18 and 19 to 24 than non pregnant cows (Table 3.3). The difference tended (P<0.1) to be greater on days 15 to 18 and 19 to 24 than on days 8 to 12.

In pregnant cows, the wave-like pattern of follicular growth continued through the first sixty days of pregnancy (Fig. 3.1), each wave resulting in a large dominant follicle corresponding to the mid cycle and ovulatory follicles of a normal estrous cycle. There was no difference in diameter of these later follicles when compared to the corresponding type in a normal cycle, although there was a tendency (P<0.1) for the dominant follicle found between day 56 and 60 to be smaller than its corresponding mid cycle follicle found between day 8 and 12 (14.7 ± 3.0mm vs 17.1 ± 0.8mm). No interaction between the side of the CL and side of the dominant follicle or total number of follicles on the ipsilateral or contralateral ovary could be established, (Fig. 3.2).
Fig. 3.1 Diameter of the largest follicle on the right and left ovaries of pregnant dairy cow (82019)
Fig. 3.2 Total number of follicles on the CL bearing and contralateral ovaries of early pregnant dairy cows.

Day 0 = day of breeding
Table 3.2. Comparison of the diameter (± SEM) of the largest follicle present on the ovaries during the first 24 days of early pregnant and non pregnant dairy cows.

<table>
<thead>
<tr>
<th>Pregnancy status at day 25 based on ultrasonography</th>
<th>Diameter of largest follicle (mm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Days post breeding</td>
<td></td>
</tr>
<tr>
<td>8 - 12</td>
<td>15 - 18</td>
</tr>
</tbody>
</table>

| Pregnant (n=11) | 16.6 ± 0.8a | 15.6 ± 0.5a | 17.1 ± 0.8a |
| Non pregnant (n=5) | 16.8 ± 0.8a | 16.6 ± 0.6a | 17.6 ± 0.8a |

Values within columns with different superscripts are significantly different (P < 0.05).
Table 3.3. Comparison of the total number (± SEM) of visible follicles on both ovaries during the first 24 days between early pregnant and non pregnant dairy cows.

<table>
<thead>
<tr>
<th>Pregnancy status at day 25 based on ultrasonography</th>
<th>Number of follicles</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Days post breeding</td>
</tr>
<tr>
<td></td>
<td>8 - 12</td>
</tr>
<tr>
<td>Pregnant (n=11)</td>
<td></td>
</tr>
<tr>
<td>15.5 ± 0.4&lt;sup&gt;a&lt;/sup&gt;</td>
<td>16.7 ± 0.5&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Non pregnant (n=5)</td>
<td></td>
</tr>
<tr>
<td>12.0 ± 0.6&lt;sup&gt;b&lt;/sup&gt;</td>
<td>11.0 ± 0.7&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

Values within columns with different superscripts are significantly different (P < 0.05).
The CL achieved its maximum cross sectional diameter by 9.2 ± 1.7 days post breeding in both groups of animals. There was no difference between pregnant and non pregnant cows in the maximum area of the CL (Table 3.4). No increase in the size of the CL was observed at any stage of the study period after day 10 in pregnant animals (Fig. 3.3). CL regression commenced 3.2 ± 1.8 days prior to the onset of estrus in non pregnant cows.

Circulating P₄ rose from basal concentrations 3.7 ± 1.1 days post breeding and peaked on day 13.2 ± 2.0 (Fig. 3.4). There was no difference between pregnant and nonpregnant cows in the peak levels of circulating P₄ achieved (4.8 ± 1.1 ng/ml for pregnant vs 5.3 ± 0.9 ng/ml for non pregnant cows). However in non pregnant cows P₄ began to decline 4.6 ± 1.1 days prior to estrus in contrast to pregnant cows in which P₄ levels remained relatively constant throughout the remainder of the study period.
Table 3.4. Comparison of the diameter (mm ± SEM) of the corpus luteum during the first 24 days post breeding of early pregnant and non pregnant dairy cows.

<table>
<thead>
<tr>
<th>Pregnancy status at day 25 based on ultrasonography</th>
<th>Diameter of the corpus luteum (mm)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Pregnant (n=11)</strong></td>
<td>Days post breeding</td>
</tr>
<tr>
<td></td>
<td>8 - 12</td>
</tr>
<tr>
<td></td>
<td>15 - 18</td>
</tr>
<tr>
<td></td>
<td>19 - 24</td>
</tr>
<tr>
<td>Pregnant (n=11)</td>
<td>31.1 ± 0.7a</td>
</tr>
<tr>
<td></td>
<td>31.3 ± 1.0a</td>
</tr>
<tr>
<td></td>
<td>30.2 ± 1.6a</td>
</tr>
<tr>
<td>Non pregnant (n=5)</td>
<td>29.2 ± 1.0a</td>
</tr>
<tr>
<td></td>
<td>30.2 ± 1.5a</td>
</tr>
<tr>
<td></td>
<td>23.8 ± 2.4b</td>
</tr>
</tbody>
</table>

Values within columns with different superscripts are significantly different (P < 0.05).
Fig. 3.3 Diameter of the corpus luteum in early pregnant and non pregnant dairy cows as determined by ultrasound imaging.
Fig. 3.4 Circulating plasma progesterone in early pregnant and non pregnant dairy cows

- Day 0 = day of breeding
DISCUSSION

The presence of presumptive embryonic vesicles in 88% of observed cows, an embryo proper in 69% and a final pregnancy rate of 56% agrees well with reports indicating fertilization rates in dairy cattle as high as 90% but final pregnancy rates in the 50 to 60% range after embryonic mortality (Hunter, 1984). The majority of embryonic mortality takes place between 14 and 17 days of gestation, approximately the time when the embryo transmits a signal for the maternal recognition of pregnancy (Betteridge et al., 1980; Northey and French, 1980). Another critical period appears to be around 30 days of gestation or when placentome formation is taking place. Data from the present study agrees well with the timing of embryonic loss and final pregnancy rates. One concern is that structures visualized prior to the identification of the embryo proper may not be conceptus structures. Normal uterine secretions could be confused for embryonic vesicles. However, in the present study vesicles were judged to be of embryonic origin based on three criteria: size, 2 to 3mm in diameter; location, initially only in the uterine horn ipsilateral to the CL, then elongating and spreading to both horns by 19 days post breeding; and definition, well defined structures with distinct borders. Uterine secretions tend to be much less
FOLLICULAR AND CL GROWTH IN PREGNANT COWS

well defined and very diffuse throughout the uterus as visualized by ultrasonography.

Contrary to previous reports, (Peirson and Ginther, 1987; Rexroad and Casida, 1975) cows in the present study continued to have a regular pattern of follicular growth through the first 60 days of pregnancy. Large dominant follicles were present at periods corresponding to the mid cycle and ovulatory periods. The CL did not influence the side on which the largest follicles were located, they were just as likely to be on the ipsilateral ovary as on the contralateral ovary to the CL. There was no change in the total number of follicles through the study period in pregnant cows. The proportion of large (> 10mm) and medium sized (7 to 10mm) follicles was similar to corresponding periods of a normal estrous cycle. This is in contrast to reports suggesting an increased rate of atresia in the larger size classes of follicles during pregnancy (Guilbault et al., 1986). Although atresia cannot be measured using ultrasound imaging, the absence of any decrease in the proportion of large and medium sized follicles would argue against any increase in atresia. One would expect a decrease in the number of follicles in the larger size classes and an increase in the number of follicles in the smaller size classes if the rate of atresia were less in small follicles and greater in medium sized follicles.

The physiological significance of the large follicles is
not immediately clear. A bovine chorionic gonadotrophin analogous to pregnant mares serum gonadotrophin has been hypothesized (Thatcher et al., 1988) but whether large follicles become luteinized as in the horse has not been shown. One study showed that large follicles from pregnant cows had higher \( P_4 : E_2 \) ratios than follicles of the same size in cycling cows (Choudary et al., 1968); however this is usually taken as a measure of atresia not luteinization. It is possible that large follicles secrete \( E_2 \) even during pregnancy. It is hypothesized that \( E_2 \) from the mid cycle dominant follicle stimulates the synthesis of endometrial oxytocin receptors and that oxytocin produced by the CL then stimulates the synthesis and secretion of \( \text{PGF}_2\alpha \) by the endometrium and results in luteolysis (Hansel, 1988). However, it appears that the \( E_2 \) - oxytocin - \( \text{PGF}_2\alpha \) pathway for luteolysis is uncoupled at the level of the uterus (Thatcher et al., 1988) and therefore physiological levels of \( E_2 \) would not be luteolytic. Increases in \( E_2 \) around day 20 in pregnant cows have not been reported and increases afterwards are usually attributed to be of conceptus origin (Robertson and King 1979).

The embryonic mortalities suggest an intriguing possibility, large follicles may have no significant physiological role for pregnancy, but are present as a safeguard in the event of early embryonic mortality. The presence of large follicles ensures a relatively fast return to estrus in two
FOLLICULAR AND CL GROWTH IN PREGNANT COWS

ways: First, a large follicle will initiate luteolysis once the influence of the conceptus is removed, secondly, a subsequent follicle will develop and be available for ovulation once progesterone has returned to basal levels, thus preventing a prolonged anestrus period following embryonic mortality.

The finding that pregnant cows had more follicles than cows subsequently diagnosed non pregnant is difficult to reconcile. Total numbers of visible follicles were similar until approximately day 7 to 8 post breeding. The pregnancy x period trend would suggest this was a real phenomenon and not an experimental artifact, however it is impossible to attribute a causal effect of pregnancy, it is just as likely that cows with more follicles tend to become pregnant or maintain their pregnancy when compared to cows with fewer follicles. Further studies for confirmation are required.

Results of the present study do not lend support to the concept of an early pregnancy luteotrophic factor. P₄ levels, CL growth rate and maximum diameter were similar in both pregnant and non pregnant cows until about 18 days post breeding when the decline of P₄ and decrease in CL size commenced due to luteolysis in the non pregnant group. After approximately day 10, the size of the CL remained constant for the duration of the study period in pregnant cows. This agrees with reports of no increase in CL weight or cell number after day 12 in pregnant cows (Hansel, 1988). There
does appear to be a slight increase in circulating $P_4$, although not significant, after approximately 30 days of pregnancy but the origin of this cannot be concluded from the present study. A study involving a large number of bred dairy cows found that pregnant cows tended to have higher milk $P_4$ levels as early as 10 days post estrus compared to cows that subsequently returned to estrus (Lamming et al., 1989), however the authors of that report suggest that the smooth progressive increase of $P_4$ in the pregnant cows does not lend support to an embryo derived luteotrophic factor.

In conclusion, it appears that follicular dynamics undergo very little change over the first 60 days of pregnancy. It is hypothesized that the growth of large follicles serves mainly as a safeguard in the event of early embryonic mortality and may have little physiologic significance for pregnancy itself. There was no evidence for an early luteotrophic factor as no increase in $P_4$ or CL size could be found over nonpregnant cows.
The effects of norgestomet implant treatment on follicular dynamics, corpus luteum growth and function as well as the temporal relationships between body temperature, standing estrus, LH surge and ovulation following implant removal were studied in 16 cycling Holstein heifers. Estrous cycles of the heifers were synchronized with two injections of prostaglandin F2α (PGF2α) and then heifers were implanted with a 9 day norgestomet ear implant either at the middle of the synchronized cycle (diestrus) or at the end of the synchronized cycle (proestrus). Follicular dynamics, corpus luteum growth and regression, and circulating progesterone were not affected by norgestomet treatment. The dominant follicle present at the time of norgestomet implantation in the proestrus group was maintained during the 9 day implant period in 6 of 8 heifers and ovulated after implant removal. Time to standing estrus and time to LH peak following implant removal
were very highly correlated with the time of ovulation (0.92 and 0.96, respectively). Onset of standing estrus and the LH peak and the onset of standing estrus and peak vaginal and rectal temperatures were also highly correlated (0.96, 0.82 and 0.81, respectively). It is concluded that any decrease in pregnancy rates with norgestomet is not due to asynchrony between estrus, the LH surge and ovulation but may be due to aging of the ovulatory follicle.

INTRODUCTION

The use of artificial insemination and embryo transfer in both dairy and beef operations has led to the widespread use of estrous synchronization as a reproductive management tool. There are two predominant methods of synchronization. The first method is based on the luteolytic activity of prostaglandin F₂α (PGF₂α) and its analogues, however Smith et al. (1985) reported that the PGF₂α analogue, Alfaprostol, did not consistently induce luteolysis until after 5 days post estrus in non lactating Hereford cows. A system involving two injections of PGF₂α or its analogues administered 10-12 days apart has also been used. In this system all treated cows should have a responsive corpus luteum (CL) at the time of the second injection and come into estrus within 72 - 96 hours. This method has resulted in pregnancy rates equal to
controls in some studies but not others (Hansel and Convey, 1983).

The second method of estrous synchronization is based on the use of progestins to block the luteinizing hormone (LH) surge following natural or induced luteolysis, and therefore suppress estrus and ovulation. Withdrawal of the progestin removes the gonadotrophin inhibition and results in estrus within 72 - 96 hours. 17α-acetoxy-11β-methyl-19-nor-preg-4-ene-3, 10-dione (Norgestomet) has been found to be a very potent progestin capable of suppressing both estrus and ovulation at very low concentrations (Wishart, 1972). The Syncro-mate B system, which involves norgestomet that has been incorporated in a hydrophylic polymer ear implant and used in conjunction with an injection of estradiol valerate administered on the day of implant to induce luteolysis, is used for estrous synchronization in the beef industry, however, lowered pregnancy rates have been reported in numerous studies (Walters et al., 1984; Rentfrow et al., 1987; Williams and Kovacik, 1987; Brown et al., 1988; Mikeska and Williams, 1988). It has been suggested that the lowered fertility rates may be due to either asynchrony between estrus, the LH surge and ovulation (Rentfrow et al., 1987) or a delay in the selection of the ovulatory follicle (Mikeska and Williams, 1988). Rajamahendran et al. (1989) reported a strong correlation between the onset of standing estrus, the LH surge and ovulation in dairy cows synchronized with 2
injections of PGF$_{2\alpha}$ given 12 days apart. Similar studies in cattle synchronized with norgestomet have not been reported.

The present study was conducted to monitor the follicular dynamics and CL growth and function during norgestomet treatment and to determine temporal relationships between changes in body temperature, the onset of standing estrus, the LH surge and ovulation following norgestomet implant removal.

MATERIALS AND METHODS

Sixteen cycling holstein heifers were selected from the University of British Columbia dairy herd. The heifers were initially scanned with a linear array ultrasound device (Tokyo Kekei LS300, Tokyo Japan) equipped with a rectal probe to determine the reproductive status of the animals.

Animals were then synchronized with 2 injections of PGF$_{2\alpha}$ (Lutalyse, Upjohn Co., Kalamazoo, MI) administered 11 days apart and randomly assigned to one of two treatments. Heifers in treatment 1 received a 6 mg norgestomet ear implant (Sanofi Canada Inc., Victoriaville, Quebec) between day 9 and day 11 [day 0 = day of estrus following PGF$_{2\alpha}$ synchronization (PGF estrus)] and heifers in treatment 2 received the ear implant once an ovulatory follicle was apparent and CL regression was underway according to the ultrasound imaging.
TIMING OF ESTRUS EVENTS DURING ESTRUS SYNCHRONIZATION

The implant was left in place for 9 days in all animals and all heifers received 5 mg of PGF$_{2\alpha}$ one day before implant removal.

Jugular blood samples were collected daily from day 1 post PGF estrus until implant removal and every 4 h thereafter until the subsequent ovulation. Plasma was separated and stored at -20°C until analysis for LH and P$_4$ using radioimmunoassay procedures described earlier (Vostermans and Walton, 1985; Rajmahendran and Taylor, 1990). The assay standard for the LH determination was NAIMMD-bLH-4 (2.2 x NIH-LH-B1). All results were then corrected to NIH-LH-B1. The inter and intra assay coefficients of variation were 19% and 6%, respectively, for LH and 13% and 7% for P$_4$. The sensitivities of the LH and P$_4$ assays were 0.1 and 0.05 ng/ml, respectively.

Vaginal and rectal temperatures were taken every 4 h from implant removal until ovulation. Animals were observed for estrus during and between the sampling periods after implant removal. Onset of standing estrus was defined as the time from implant removal until the first observation of the heifer standing to be mounted.

The ovaries were scanned by linear array ultrasonography daily from day 1 post PGF estrus until the onset of standing estrus after implant removal and then every 2 h until ovulation which was determined by the acute disappearance of the dominant follicle (Rajamahendran et al., 1989). The
ovaries were scanned in several planes to identify all visible follicles and the CL. Landmarks such as a CL, other follicles, the poles of the ovary and the orientation of the ovary were used to identify individual follicles. Appropriate images were frozen and structures measured using a built-in caliper system and then hard copies were made for a permanent record using a video processing unit (Mitsubishi Electronics Co. Ltd., Tokyo, Japan).

Heifers were bred approximately 12 h after the onset of standing estrus. Heifers not returning to estrus before 28 days post insemination were scanned using the ultrasound device for confirmation of pregnancy. Pregnancy was diagnosed on the basis of visualization of the embryo proper with a heartbeat.

Correlations between the onset of standing estrus, peak vaginal and rectal temperatures, time to peak LH and ovulation were made using Pearson r correlations. Comparisons between the two treatment groups with regard to the time to standing estrus and the time to ovulation were made using a Student's t test. In addition, timing of the onset of atresia of dominant follicles was compared between the two treatments using a t test. Heifers receiving the norgestomet implant during proestrus served as controls for the heifers receiving the implant during diestrus with regard to growth and atresia of dominant follicles and the onset of luteolysis.
RESULTS

All heifers had two or three waves of follicular growth following the PGF$_{2\alpha}$ synchronized estrus. All heifers receiving a norgestomet implant during diestrus had a functional CL (P$_4$ > 1ng/ml) at the time of implant. The timing of atresia of mid cycle follicles, the timing of the onset of luteolysis (Fig. 4.1) and circulating concentrations of P$_4$ (Fig. 4.2) were not affected by norgestomet treatment during diestrus. Ultrasound imaging showed that CL regression had commenced at the time of the PGF$_{2\alpha}$ injection administered the day before implant removal in heifers receiving the implant during diestrus, and this was later confirmed by P$_4$ analysis. The dominant follicle at the time of CL regression went on to ovulate in all heifers regardless of treatment. In heifers receiving an implant during proestrus, CL regression was well underway at the time of implant insertion according to both the ultrasound images and P$_4$ analysis (Fig. 4.2). The dominant follicle at the time of implant insertion ovulated in 2 of the 8 heifers within 48 h of the insertion. In the remaining 6 heifers the dominant follicle was maintained for the entire 9 day treatment period and went on to ovulate following implant removal (Plate 4.1).

All heifers receiving the norgestomet implant during diestrus were observed in standing estrus within 54 h of the
Fig. 4.1 Day of onset of atresia of mid-cycle dominant follicles and luteolysis in norgestomet synchronized heifers

Day of cycle

Time of Implant

- S.D.  - Atresia 1st follicle
- Atresia 2nd follicle - Luteolysis

*Day 0 = day of estrus
Fig 4.2 Plasma progesterone profiles in heifers implanted with norgestomet

Day of Cycle (day 0 = day of estrus)

- SEM
- Diestrus implant
- Proestrus implant
Plate 4.1. Ultrasound images of the left and right ovary of a heifer receiving a norgestomet ear implant during proestrus.

Image of left and right ovary on day of norgestomet implantation. Note presence of large dominant follicle on left ovary (long arrow) and many small follicles on both ovaries (short arrows).

Image taken 6 days after norgestomet implantation. The dominant follicle persists, however note the absence of small follicles.

22 hours after norgestomet implant removal the dominant follicle has yet to ovulate. Note the appearance of a new pool of small antral follicles (short arrow).

53 hours following norgestomet implant removal the dominant follicle has ovulated.
TIMING OF ESTRUS EVENTS DURING ESTRUS SYNCHRONIZATION

norgestomect implant removal. Two heifers receiving the implant during proestrus ovulated shortly after implant insertion and one other showed no signs of estrus following implant removal and therefore are not included in the temperature, LH and ovulation data. The remaining 5 heifers that received the norgestomect implant during proestrus were all observed in standing estrus within 54 h of the implant removal. Times to standing estrus, LH peak, temperature peaks and ovulation are summarized in Table 4.1. Standing estrus and the LH peak and standing estrus and peak vaginal and rectal temperatures were highly correlated (0.96, 0.82 and 0.81, respectively).

Cows that received the norgestomect implant during proestrus generally came into estrus faster and ovulated earlier than cows receiving the implant during diestrus (Fig 4.3) although there was no significant difference (P < 0.05).

Pregnancy rates as diagnosed by ultrasound imaging at 28 days post breeding are summarized in table 4.2. The overall pregnancy rate was 61.5% and there was no difference between treatments in the pregnancy rate.
Table 4.1. Mean interval in hours (± SD) from norgestomet implant removal to standing estrus, temperature peaks, LH peak and ovulation, and correlation coefficients (r) with the time to ovulation (n = 13).

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Mean</th>
<th>SD</th>
<th>r</th>
</tr>
</thead>
<tbody>
<tr>
<td>Standing Estrus</td>
<td>30.7</td>
<td>9.8</td>
<td>0.92*</td>
</tr>
<tr>
<td>Vaginal Temperature</td>
<td>36.4</td>
<td>9.9</td>
<td>0.51</td>
</tr>
<tr>
<td>Rectal Temperature</td>
<td>36.8</td>
<td>8.6</td>
<td>0.44</td>
</tr>
<tr>
<td>LH Peak</td>
<td>34.2</td>
<td>8.8</td>
<td>0.96*</td>
</tr>
<tr>
<td>Ovulation</td>
<td>60.1</td>
<td>11.4</td>
<td>1.00</td>
</tr>
</tbody>
</table>

* Significantly correlated (P < 0.01)
Fig 4.3 Time to estrus, LH peak and ovulation after norgestomet implant removal in dairy heifers
Table 4.2. Number of pregnancies in dairy cows synchronized with norgestomet ear implants.

<table>
<thead>
<tr>
<th>Treatment</th>
<th># Bred</th>
<th># Pregnant at 28 days</th>
</tr>
</thead>
<tbody>
<tr>
<td>Diestrus implant</td>
<td>8</td>
<td>5</td>
</tr>
<tr>
<td>Proestrus implant</td>
<td>5</td>
<td>3</td>
</tr>
<tr>
<td>Total</td>
<td>13</td>
<td>8</td>
</tr>
</tbody>
</table>
DISCUSSION

Data in the present study do not support the contention that there is asynchrony between estrus, the LH surge and ovulation in cows synchronized with norgestomet (Rentfrow et al., 1987). The correlations between the onset of standing estrus and the LH surge, standing estrus and ovulation, and the LH surge and ovulation were very high. Although both rectal and vaginal temperatures were correlated with standing estrus, the correlation with ovulation was not significant. The single best parameter for basing the time of insemination still appears to be the onset of standing estrus.

The temporal relationships between standing estrus, the LH surge and ovulation are very much in agreement with those reported in cows synchronized with PGF$_{2\alpha}$ (Rajamahendran et al. 1989). The present study further confirms that young animals ovulate later in relation to estrus and the LH surge than mature cows (Rajamahendran et al., 1989; Schams et al., 1977; Christenson et al., 1975).

Heifers receiving the implant during diestrus generally came into estrus later and had greater variability than animals receiving the implant during proestrus. This may be due to circulating concentrations of P$_4$ greater than basal at the time of implant removal in the heifers treated during diestrus whereas P$_4$ was basal at the time of removal in
TIMING OF ESTRUS EVENTS DURING ESTRUS SYNCHRONIZATION

Heifers receiving the implant during proestrus. The elevated P4 would cause negative feedback on the hypothalamic-pituitary axis, thus prolonging the time to estrus, the LH peak and ovulation and increasing the variation in heifers receiving the implant during diestrus. Administration of the PGF2α two days prior to implant removal rather than the day preceding removal should tighten the synchronization. The normal Syncro-mate B plan recommends an injection of estradiol valerate to induce luteolysis on the day of implant rather than PGF2α prior to removal. Guilbault et al. (1989) reported that estradiol valerate may not always be effective in initiating luteolysis and that this failure rate increases when given to cows with a developing CL.

Possible reasons for a decreased fertility rate in cows synchronized with norgestomet may lie in the follicular dynamics of animals synchronized with the Synchromate B method. Mikeska and Williams (1988) suggested that retarded selection or maturation of the ovulatory follicle after implant removal may decrease conception rates in timed-inseminated females. The present study would suggest that selection of the ovulatory follicle is not affected by norgestomet treatment, however in the absence of the corpus luteum the selected follicle is maintained for a prolonged period of time, possibly leading to over maturation. With luteolysis occurring early after norgestomet implantation, due to the estradiol valerate injection in the normal Syncro-mate
TIMING OF ESTRUS EVENTS DURING ESTRUS SYNCHRONIZATION

B protocol, the ovulatory follicle would be selected and maintained for the remainder of the norgestomet treatment period. Maintenance of the selected follicle for a nine day period may effect its fertility. Pregnancy rates in the present study do not reflect any decrease in fertility in heifers where the ovulatory follicle had been maintained for the 9 day norgestomet treatment, however the number of animals is very small and a much larger number of animals would be required to reflect a difference.

In conclusion, it would appear that norgestomet treatment is an effective method of estrus synchronization with no effect on the relationship between the onset of standing estrus, the LH surge and ovulation following implant removal. It would appear that any decrease in pregnancy rate is not due to asynchrony between the above parameters, but may be due to a combination of the early luteolysis induced by the estradiol valerate injection at the beginning of the treatment period, as used in the normal Syncro-mate B plan, and the prolonged maintenance of the ovulatory follicle in the absence of the corpus luteum. The use of PGF$_{2\alpha}$ to induce luteolysis towards the end of the treatment period rather than estradiol valerate at the onset of treatment would seem to be a more effective protocol as it minimizes the chance of the ovulatory follicle growing and being maintained for a prolonged period of time before ovulation.
DISCUSSION

CHAPTER 5

GENERAL DISCUSSION

The previous three chapters have described three independent studies looking into follicular dynamics and corpus luteum function in dairy cattle during different reproductive states. Although these were independent studies several different concepts, themes and trends emerge. Many, if not all of these are based on circumstantial or secondary observations and for this reason the concepts are put forth merely as ideas for discussion, not hard bound conclusions. The present chapter will probably leave more questions asked than answered, however studies designed to shed some light on some of the questions raised will be proposed.

GROWTH OF DOMINANT FOLLICLES

From the three studies described it would appear that the wave-like pattern of growth of large dominant follicles is a characteristic of the bovine ovary during several different reproductive states. Waves of growth of large dominant follicles are seen in the anestrous period following parturition, during the first 60 days of pregnancy and even during treatment with a gonadotropin inhibitor, such as norgestomet,
as long as there is a corpus luteum present. Hansel and Convey (1983) point out that the pattern of secretion of gonadotrophins do not adequately explain the pattern of growth and regression of follicles. The observation that the pattern of growth of large dominant follicles is unaltered during the first 60 days of pregnancy, a period of low gonadotrophin activity, and under gonadotrophin inhibition in the presence of a CL serve to underline this fact.

Hansel and Convey (1983) suggest an intraovarian level of regulation of follicular growth, perhaps via the CL. The study using norgestomet for estrus synchronization may provide some support for a role of the CL in the regulation of follicular growth, however it would appear to be an endocrine effect as opposed to a strictly paracrine effect. In the presence of the corpus luteum norgestomet had no effect on the growth of large antral follicles, there continued to be the normal wave-like pattern of growth and this growth was independent of the side of the CL. However, once luteolysis had commenced there was a marked absence of turnover of large antral follicles. Moreover there was essentially no new growth of medium (7 to 10mm) or small (2 to 6mm) sized follicles.

Maintenance of the dominant follicle in the absence of the CL and continued turnover in the presence of a CL suggest a role for the CL in atresia of large dominant follicles. It is not possible from the present work to speculate on the
mechanism by which the CL acts but work elsewhere point to P₄ as a possible effector. Fortune (personal communication) administered P₄ to dairy cows once luteolysis had commenced in order to test if there would be a continued pattern of growth and atresia of follicles. When P₄ was administered at doses below a normal mid luteal concentration the dominant follicle at the time of luteolysis was maintained. When mid luteal doses were administered the wave-like pattern of growth and atresia was restored. These results combined with the norgestomet synchronization results (chapter 4) would suggest that mid luteal concentrations of progesterone may initiate atresia of large dominant follicles.

It is interesting that the first dominant follicle of an estrous cycle in cows reaches its maximum diameter between day 8 and 9 of the cycle and is maintained until day 13 to 15 before it begins to decrease in size whereas in cows with 3 waves of growth the 2nd mid cycle follicle is not maintained for any length of time. Timing of the onset of the decrease in size of the first dominant follicle corresponds closely with the time at which P₄ reaches its maximum circulating concentrations. In addition, heifers with three waves of follicular growth in a single estrous cycle have significantly longer luteal phases than heifers with 2 waves (Ginther et al., 1989). It is possible that in cows with 2 waves of growth luteolysis has already commenced, and therefore P₄ is falling, before the dominant follicle of the second wave has
reached its maximum diameter. In cows with three waves the CL is still secreting large amounts of P₄ when the second dominant follicle reaches its maximum size and it therefore becomes atretic and permits a third wave of growth.

In mature lactating cows the vast majority of cycles studied had two waves of follicular growth. In contrast, heifers generally had three waves of growth, and yet when the duration of the luteal phases are compared (fig. 3.4 and fig. 4.3, respectively) there is no difference. If one accepts the hypothesis that P₄ may play a role in atresia then the difference between heifers and mature cows could be explained on the basis of the size of the dominant follicles. The dominant follicles in heifers are generally smaller than those seen in mature cows while growth rates of the follicles would appear to be similar. The second dominant follicle achieves its maximum size earlier in heifers than in mature cows and therefore is subject to high levels of progesterone leading to atresia and a third wave.

SHORT LUTEAL PHASE

The postpartum study described in chapter 2 found that on average the first postpartum estrous cycle was shorter than subsequent cycles and this was mainly due to a shortened luteal phase. However, less than half of the cows studied actu-
DISCUSSION

ally had short first cycles while the remaining cows all had normal lengthed cycles. The interesting finding was that the short cycles were not associated with the earliest ovulations but were in fact associated with a delay in the return to estrus. It was concluded that the timing was not right for short cycles to be attributed to the secretion of PGF$_{2\alpha}$ from the involuting uterus and that the short cycles were more probably due to an LH deficiency or a deficiency in LH receptors. A result in the norgestomet synchronization study would appear to support a role for an LH deficiency. Two of the heifers implanted during proestrus went on to ovulate despite the norgestomet implant. Both of these heifers experienced short cycles and the P$_4$ profiles closely resembled those seen in the postpartum cows with short cycles. It is hypothesised that the norgestomet implant was put in place too late to block the LH surge but soon enough to block any post surge LH support that the CL may require for a normal estrous cycle lifespan. A simple experiment using norgestomet implanted so as to allow the LH surge but block any post surge release and LH or hCG replacement would help to show whether post surge LH support is indeed required for a normal lived CL.

A more puzzling question is why are short postpartum estrous cycles associated with a delay in the return to cycling activity? Growth of large dominant follicles appears to begin very shortly following parturition, if not even before.
DISCUSSION

Follicles reach ovulatory size early in the postpartum period even in cows with a prolonged anestrus. This would indicate that the availability of large sized follicles is not the limiting factor in the duration of the postpartum anestrous. Ovulatory sized follicles are available but for some reason fail to ovulate. This would again suggest either an LH deficiency or an LH receptor deficiency. An LH deficiency would help explain the association between the delay in return to estrus and short cycles.

An abnormally high LH pulse frequency during the postpartum period could be an explanation. LH "leakage" due to an increased LH pulse frequency could result in inadequate LH stores thus preventing an LH surge, estrus and ovulation. As LH stores slowly build, a point is reached at which there is enough to produce an LH surge but an insufficient amount to provide any post surge support thus resulting in an ovulation followed by a short lived CL. Although there is no direct evidence for such a mechanism there are some secondary observations which may support such a hypothesis. Treatment of anestrous cows and cystic cows with hCG will induce ovulation suggesting that the problem may not be in the receptor content. Additionally, norgestomet has been used in beef cattle to decrease the incidence of postpartum short cycles. Since norgestomet acts by blocking LH it is possible that it allows stores to be rebuilt so there is an adequate amount present for post surge support. Finally, Soules et al.
DISCUSSION

(1987) were able to induce short lived corpora lutea in women by increasing LH pulse frequency in the follicular phase. This resulted in a decrease in post surge LH pulse frequency, short lived CLs and inadequate P₄.

Experiments designed to show that there is an abnormally high LH pulse frequency in cows with a prolonged anestrous period are easy to conceive but difficult to execute. Frequent sampling of LH during the postpartum period should provide the answer, however samples must be taken on the order of every 10 minutes for several hours in order to determine pulse frequency. This and the fact that it is not possible to predict which animals will ovulate early and which will not make this a cumbersome study.

LOCAL EFFECT OF THE CORPUS LUTEUM ON FOLLICULAR GROWTH

Several reports suggest that the corpus luteum may have a local effect on follicular growth. Peirson and Ginther (1987b) suggest that the CL has a positive effect on follicular growth on the ipsilateral ovary during repetitive estrous cycles but after approximately 20 days of pregnancy the CL may have a negative influence. Guilbault et al. (1986) also suggest that during pregnancy the CL may increase follicular turnover in the ipsilateral ovary. No positive or negative influence of the CL could be established in any of
DISCUSSION

the three studies presented in the present thesis. Total number of follicles were similar on both the CL bearing ovary and the contralateral ovary. In addition the largest follicle was just as likely to be on the CL bearing ovary as the contralateral ovary in all three studies.

It does appear that the CL may have an endocrine effect on follicular growth, perhaps initiating turnover of follicles as discussed above. Further studies are required to better elucidate the relationship between the CL and follicular growth.

Overall ultrasound imaging proved to be a useful tool in the study of follicular dynamics and corpus luteum growth and regression. Its relatively non-invasive nature and its capacity to allow for the monitoring of individual animals over a period of time have helped in our understanding of the dynamic nature of follicular growth in the bovine ovary. A better understanding of ovary function is essential for better success in the treatment of some reproductive disorders, such as ovarian cystic condition, and for more consistent results in super ovulation and embryo transfer.
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