Dedicated to my mother, Elsa, and my husband, Gregory
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In Experiment 1, a total of 155 cows with ovarian cysts was sequentially allocated to 3 groups on the day of diagnosis (Day 0). Cows in Group 1 (n = 55) were treated with GnRH (100 µg, im) on Days 0 and 7, PGF2α (25 mg, im) on Day 14, GnRH (100 µg, im) on Day 16, and timed inseminated (TAI) 16-20 h later. Cows in Group 2 (n = 49) were treated with GnRH on Day 0, PGF2α on Day 7, GnRH on Day 9, and TAI 16-20 h later. Cows in Group 3 (n = 51) were treated with GnRH on Day 7, PGF2α on Day 14, GnRH on Day 16, and TAI 16-20 h later. Pregnancy was determined by rectal palpation 45-50 d after TAI. On both Days 0 and 7, cows in all groups were subjected to ultrasonography (U/S) and blood samples were obtained for determination of progesterone concentration (P4). Cows in Groups 1 and 2 were more likely to have a CL and high P4 on Day 7 compared to cows in Group 3. There was no significant difference in pregnancy rate (PR) between cows in all groups, but cows with a CL on Day 7 were more likely to become pregnant. It was concluded that administering GnRH to cows with ovarian cysts 7 days
prior to the initiation of the Ovsynch protocol did not increase PR, but cows with a CL on Day 7 were more likely to become pregnant.

In Experiment 2 (Part A), a total of 228 postpartum (pp) dairy cows was sequentially allocated to 2 groups between Days 7 and 9 pp. Cows in Group 1 (n=114) were treated twice with PGF2α (25 mg, im) 8 h apart on Days 8 and 15 pp, and once on Days 22 and 36 pp. Cows in Group 2 (n=114) served as untreated controls. Vaginoscopy and rectal palpation were done on Days 22 and 58 pp. Cows in both groups were inseminated at estrus, or TAI approximately 130 to 134 days pp. Pregnancy was determined by rectal palpation between 45-50 d after insemination. It was concluded that sequential administration of PGF2α in the immediate postpartum period reduced the prevalence of a mucopurulent discharge, and both the size of the cervix and previously pregnant uterine horn only on Day 58 pp. There was no difference in PR between cows in both groups.

In Experiment 2 (Part B), a total of 418 postpartum dairy cows was sequentially allocated to 2 groups on Day 7 pp. Cows in Group 1 (n=209) were treated twice with PGF2α (25 mg, im) 8 h apart on Days 7 and 14 pp, and once on Days 21 and 35 pp. Cows in Group 2 (n=209) served as untreated controls. All cows were subjected to the Presynch and Ovsynch protocols on Days 49 and 75 pp, respectively. Pregnancy was determined by U/S between Days 29 and 32 after TAI. There was no significant difference in the conception rate to first service between the groups.
CHAPTER 1
INTRODUCTION

The profitability of a commercial dairy farm is based in part on the calving interval of the cows. In order to maximize the economic profitability of the farm, cows must return to ovarian cyclicity, express estrus and be bred within 85 days postpartum. The optimal calving interval is 365 days.

There are two physiologic factors which influence reproductive success in the postpartum dairy cow. The first is ovarian cyclicity, and the second is uterine health. Parturition is a very traumatic event, and the ability to control ovarian and uterine events in the postpartum cow could play an important role in achieving subsequent fertility. In this study, two experiments were performed. The first experiment investigated the use of gonadotropin releasing hormone (GnRH) to improve ovarian cyclicity in lactating dairy cows diagnosed with ovarian follicular cysts. The second experiment investigated the use of prostaglandin F2α in the early postpartum period to improve uterine health and improve fertility in lactating dairy cow.

Ovarian follicular cysts, also known as cystic ovarian degeneration, cystic ovaries or anovular follicles, is the most common abnormality of follicular function in cattle (Farin and Estill, 1993). It is most often seen in the postpartum dairy cow within the first 60 days post partum (Farin and Estill, 1993). Approximately 6–19% of dairy cows develop ovarian follicular cysts (McLeod and Williams, 1991; Garverick 1997; Silvia et al., 2002). Cystic ovarian degeneration increases days open by 22 to 64 days and increases the number of cows culled from the herd. Each occurrence of ovarian follicular
cysts has been estimated to cost $137 in reduced milk production and veterinary expenses (Silvia et al., 2002). The development of programs to prevent and/or treat cystic ovarian degeneration will benefit the dairy farmer.

The resumption of ovarian cyclicity is dependent on a number of factors including clearance of bacterial contamination from the uterus (Sheldon et al., 2002). Bacterial contamination of the uterus occurs within the first week post partum (Elliott et al., 1968) with spontaneous contamination, clearance and recontamination occurring up to seven weeks post partum (Griffin et al., 1974). Some cows have the ability to clear these infections but others do not and the reasons for this variability between cows are unknown. Bacterial contamination of the uterus has a direct effect on the ability of the cow to conceive and maintain a conceptus. Conception and the maintenance of pregnancy are, therefore, dependent on a healthy uterine environment.

The incidence of endometritis is greatest during the first 14 days post partum based on cultures of uterine fluids and uterine biopsies (Griffin et al., 1974). Failure to clear bacterial contamination by first ovulation post partum and corpus luteum formation could place the contaminated uterus under the influence of progesterone. Progesterone makes the uterus more prone to uterine infection (Hawk et al., 1964) with the incidence of severe endometritis increasing around Day 15 to Day 21 postpartum. This increase in the severity of endometritis coincides with the time of first post partum ovulation (i.e., 15 to 28 days post partum).

The hypothesis of Experiment 1 is based on research done in cows without ovarian cysts. This research demonstrated that cows in the early luteal phase of the estrous cycle at the time of initiation of the Ovsynch protocol had a higher pregnancy rate compared to
cows at other stages of the estrous cycle (Vasconcelos et al., 1999; Moreira et al., 2000a). The hypothesis of Experiment 1 was that GnRH administered to cows at the time of diagnosis of ovarian cysts would induce an early luteal phase and thus increase pregnancy rates to a protocol for synchronization of ovulation and timed insemination (Ovsynch protocol). The Ovsynch protocol consists of a single intramuscular injection of 100 µg of GnRH, followed seven days later by PGF2α (25 mg, intramuscularly). The second dose of GnRH (100 µg, im) is administered 48 hours after PGF2α with artificial insemination occurring 16 to 24 hours later. The objectives of Experiment 1 were two-fold. The first objective was to evaluate the ovarian response 7 days following diagnosis and treatment of cows with ovarian cysts with GnRH. The second objective was to determine the pregnancy rate of cows with ovarian cysts subjected to treatment with GnRH 7 days prior to the initiation of the Ovsynch protocol.

The hypothesis of Experiment 2 was that sequential administration of PGF2α during the early postpartum period would reduce the incidence of mucopurulent discharge, size of the cervix, size of the previously pregnant uterine horn and increase first service pregnancy rates. The objective of Experiment 2, Part A was to determine the effect of sequential administration of PGF2α in the immediate postpartum period on the incidence of mucopurulent discharge, size of the cervix and size of the previously pregnant uterine horn and first service pregnancy rates. The objective of Experiment 2, Part B was to evaluate the effect of sequential administration of PGF2α in the immediate postpartum period on first service conception rate in postpartum dairy cows subjected to a timed insemination protocol consisting of Presynch-Ovsynch protocol. The Presynch portion of the timed insemination protocol consisted of two intramuscular injection of 25
mg PGF2α given 14 days apart. The Ovsynch protocol began 12 days after the second dose of PGF2α.
Gonadotropin-releasing Hormone (GnRH)

Chemical Nature of the Hormone

Gonadotropin-releasing hormone (GnRH), also known as luteinizing hormone releasing hormone (LHRH), is a decapeptide produced in the arcuate, suprachiasmatic and preoptic nuclei of the hypothalamus in response to hormonal and integrated neuronal signals. The amino acid sequence of GnRH was first elucidated as early as 1971. The primary structure of GnRH has been established in the pig (Matsuo et al., 1971), sheep (Amoss et al., 1971; Burgus et al. 1972) and human placenta (Tan and Rousseau, 1982). GnRH is synthesized from a precursor molecule, which begins with a 23 amino acid signal sequence, followed by the GnRH decapeptide and ending with a 56 amino acid GnRH-agonist associated peptide.

There are between 800 and 2500 GnRH neurons located throughout the brain of vertebrates. These neurons originate in the olfactory placode outside the CNS and migrate inwards during fetal development (Silverman, 1984). Immunohistochemical techniques have been used to elucidate the location of the GnRH containing cell in the bovine hypothalamus and infundibulum. GnRH-positive cell bodies have been located singly or in small clusters in the infundibular nucleus (INFN), and in large discrete clusters in the ventromedial nucleus (VMN) with their axons projecting into the rostral hypothalamus (the grey matter of the lamina terminalis and medial preoptic area). The majority of axons were found within the middle region of the hypothalamus within the
dorsomedial nucleus, VMN, INFN, and lateral hypothalamic area, the periventricular nucleus (Dess and McArthur, 1981).

Embryonic GnRH neurons in primary cultures of olfactory placodes from ovine embryos begin pulsatile secretion of GnRH by 17 to 24 days, indicating that these neurons must mature prior to the onset of pulsatility (Duittoz and Batailler, 2000). GnRH is synthesized in neuroendocrine cell bodies and transported down axons that terminate in the median eminence where it is released in a pulsatile manner (Silverman, 1984; Rodriguez and Wise, 1989; Naor et al., 1998). In the median eminence, GnRH is released into the surrounding capillaries that drain into the hypothalamo-hypophyseal portal vessels and transported to the pituitary where it stimulates the secretion of luteinizing hormone (LH) and follicle stimulating hormone (FSH) from gonadotrophs (Garverick and Smith, 1993).

Luteinizing hormone and FSH are released into the systemic circulation, transported to the ovary where they stimulate follicular growth, ovulation, formation of the corpus luteum and steroidogenesis. The ovary synthesizes estrogen, progesterone, inhibin and activin. The gonadotropins and ovarian products regulate the release of GnRH by both negative and positive feedback mechanisms.

**Neurotransmitters and GnRH Secretion**

Neurotransmitters, growth-factors, neuromodulatory peptides and ovarian steroids positively and negatively affect GnRH and/or LH release (Kordon et al., 1994; Gore and Roberts, 1997; Gazal et al., 1998; Moenter et al., 2003). Most studies have been carried out in rats and immortalized GnRH neuron (GT1 cell lines). Since some of these substances do not have receptors on the GnRH neuron, it is thought that they influence GnRH gene expression through interneuronal pathways, or by directly binding to a
specific membrane receptor. Receptor binding leads to the activation of second-messenger pathways to cause GnRH and/or LH release (Gore and Roberts, 1997).

Glutamate and norepinephrine (NE) have been shown to stimulate GnRH/LH release and gene expression in vivo, and GnRH release in GT1 cells. A single injection of the neurotransmitter glutamate analog, N-Methyl-D-L-aspartic acid (NMDA) causes a 30% increase in GnRH levels within 15 to 60 minutes (Gore and Roberts, 1997). A similar increase in GnRH mRNA and plasma LH is seen in GnRH neurons (Petersen et al., 1991; Liaw and Barraclough, 1993; Gore and Roberts, 1997). The catecholamine, norepinephrine, stimulates GnRH release from intact rats and rabbits and plays a physiological role in the onset of puberty (Gore and Roberts, 1997). Dopamine stimulates and inhibits GnRH release in vivo while dopamine receptor-1 (D1) stimulates GnRH release in GT1 cells (Gore and Roberts, 1997).

There are direct connections between gamma-amino-butyric acid-ergic (GABAergic) and GnRH neurons. It is thought that through this direct connection, GABA plays an inhibitory role in GnRH/LH release and causes a blockage of the LH surge (Gore and Roberts, 1997). Opiates have also been shown to inhibit the GnRH/LH release and gene expression in rats and rabbits (Gore and Roberts, 1997).

Cannulation of the third ventricle of adult cattle has allowed for sampling, detection and quantification of GnRH secretion and has provided a means to monitor the central regulation of reproduction (Gazal et al., 1998). Gazal et al. (1998) demonstrated that GnRH is secreted into the cerebrospinal fluid in a pulsatile manner.

**Control of Secretion**

The GnRH neurosecretory system is diffuse and contains individual neurons located in different areas of the brain (Silverman, 1984). The activity of GnRH neurons is
regulated by integrated signals from the brain and these include photoperiod, steroid hormones, nutrition and stress (Moenter et al., 2003). However, in the absence of other cells types, in pure GnRH neuronal cell cultures, and GT1 cell, GnRH is secreted spontaneously in a rhythmic pattern (Martinez de la Escalera et al., 1992). Martinez de la Escalera et al. (1992) suggested that a model of cell-to-cell communication via intracellular contacts could explain synchronization of pulses in experiments where a single cell-coated coverslip per superfusion chamber was used. It was also suggested that there is a diffusible mediator, which would explain how two separated coverslips synchronized GnRH pulses, and that GnRH itself may be the diffusible mediator (Martinez de la Escalera et al., 1992).

The release of GnRH is episodic in both sexes. In females, however, there is an interruption in the episodic release of GnRH by a GnRH surge. This GnRH surge coincides with the pre-ovulatory LH surge, and lasts longer than the LH surge. Yoshioka et al. (2001) demonstrated that there is a preovulatory increase in GnRH secretion into the cerebrospinal fluid (CSF) in the third ventricle following the administration of PGF2α in heifers. The GnRH surge was shown to coincide with the LH surge and also with the onset of standing estrus (Yoshioka et al., 2001).

While it is known that GnRH is secreted in a pulsatile manner, little is known about the mechanisms generating this rhythmic secretion. It has been shown that GnRH neurons produce a high-frequency rhythm characterized by oscillations in intracellular calcium levels, burst firing of action potentials, and a low-frequency rhythm with a period corresponding to neurosecretion of GnRH (Moenter et al., 2003). Low frequency
rhythms result in secretion of GnRH and can be modified by the effect of steroids (Moenter et al., 2003).

The frequency of GnRH secretion varies during the estrous cycle and the reproductive state of the animal from once every 30 minutes to once every few hours (Moenter et al., 2003). Secretion of GnRH can be controlled at many levels. It may be regulated at the transcriptional, post-transcriptional and post-translational levels (Gore and Roberts, 1997).

The biosynthesis and release of GnRH are strictly controlled by afferent neurons to the GnRH neurons in the hypothalamus (Peter and Burbach, 2002). Cell specific expression and biosynthetic regulation rely on transcription from the gene promoter for which the 5’-flanking region of the peptidergic gene contains essential elements (Peter and Burbach, 2002). The amount of GnRH secreted from GnRH-containing neurons is controlled by GnRH gene expression. GnRH gene expression is limited by the rate of transcription of GnRH gene, polyadenylation and 5’ capping of the RNA, processing of its primary transcript to the mature mRNA, and transport of the mRNA from the nucleus into the cytoplasm. Once in the cytoplasm, mRNA levels are determined by the turnover of the molecule. Further regulation takes place at the level of mRNA translation into proGnRH peptide, maturation of GnRH and the rate of degradation and release of GnRH into the portal circulation, which affect the level of GnRH reaching the gonadotrophs (Gore and Roberts, 1997).

Estrogen controls LH and FSH synthesis through a negative feedback loop to the anterior pituitary and hypothalamus. Estrogen has been shown to downregulate mRNA levels and reporter gene activity in immortalized hypothalamic GnRH neurons (Peter and
Burbach, 2002). Progesterone also causes repression of GnRH promoter activity (Peter and Burbach, 2002). Neuropeptide Y (NPY) is a potent orexigenic substance, produced in the hypothalamus, anterior pituitary and adipose tissue. Neuropeptide Y forms a neuromodulatory link between nutritional status and the central reproductive axis (McShane et al., 1992). Infusion of high concentrations of NPY into the third ventricle inhibits GnRH pulses (Gazal et al., 1998).

**Function of GnRH**

The number of GnRH receptors in the anterior pituitary changes throughout the estrous cycle. Maximum numbers of the GnRH receptor occur just prior to the preovulatory surge in the cow, and their numbers decline thereafter (Nett et al., 1987; Turzillo and Nett, 1999). Luteolysis and decreasing progesterone concentrations may be the trigger for increased expression of the GnRH receptor gene (Turzillo and Nett, 1999). The GnRH pulse frequency increases when progesterone concentrations decline, and the negative feedback signal due to high concentration of P₄ is removed. This increase in GnRH pulse frequency increases the expression of GnRH receptors in the anterior pituitary.

The preovulatory follicle produces large amounts of estradiol, which enhance GnRH receptor gene expression. The increasing levels of estradiol and decreasing levels of progesterone increase the numbers of GnRH receptors on the gonadotrophs, and increases pituitary sensitivity to GnRH (Turzillo and Nett, 1999). In the rat, the number of GnRH receptors is less during pregnancy and lactation compared to the estrous cycle. It has been reported that when up to 50% of GnRH receptors are blocked with a GnRH antagonist, ewes still respond fully to GnRH with LH release (Wise et al., 1984).
Ovarian hormones influence the numbers of GnRH receptor. In ewes, estradiol-17β has been shown to increase the number of GnRH receptors (Clarke et al., 1988). *In vitro* studies using ovine pituitary cell cultures showed an increase in GnRH receptor expression in response to estrogen and decreased expression in response to progesterone (Laws et al, 1990a, 1990b). Inhibin caused an increase in the expression of GnRH receptors and, when combined with estradiol-17β, led to greater expression of GnRH receptors that either hormone singly (Laws et al, 1990b).

In the immediate postpartum period, the pituitary response to GnRH is reduced. Within the second week post partum, however, the pituitary responds to endogenous and exogenous administration of GnRH with release of LH in dairy cows (Cummins et al 1975). Garverick et al. (1980) showed that administration of GnRH within the second week post partum to dairy cows with large follicles was followed by ovulation and subsequent formation of a CL. Similarly, a single injection of 100 micrograms of GnRH 12 to 14 days post partum initiated cyclic ovarian activity as evidenced by a palpable corpus luteum and plasma progesterone ≥ 1.0 ng/ml by Day 9 post-treatment (Zaied et al 1980). Zaied et al. (1980) also suggest that GnRH treatment 12 to 14 days post partum may be useful in reducing abnormal ovarian activity. They found that 30% of non-treated postpartum cows developed ovarian cysts prior to conception compared to 12.5% GnRH-treated cows. Kittock et al. (1973) demonstrated that administration of GnRH to cows with ovarian follicular cysts led to an increase in LH secretion, return to normal cyclicity, and all cows expressed estrus 20 to 24 days after treatment.
Mechanism of Action

GnRH binds to receptors in the cell membrane of the gonadotrophs in the anterior pituitary. The GnRH receptor is a member of the seven transmembrane domain receptor coupled to a G-protein (G<sub>q</sub>). Receptor binding leads to sequential activation of different phospholipases to provide calcium (Ca<sup>2+</sup>) and lipid-derived messenger molecules. Initially, phospholipase C is activated followed by activation of phospholipase A<sub>2</sub> (PLA<sub>2</sub>) and phospholipase D (PLD). Following receptor binding, GnRH stimulates a GTP-protein (G<sub>q</sub>). Activation of the G-protein results in Ca<sup>2+</sup>-independent stimulation of phospholipase C (PLC). Activation of PLC leads to the generation of second messenger inositol 1,4,5-trisphosphate (IP<sub>3</sub>) and diacylglycerol (DAG) from phosphatidylinositol 4, 5-bisphosphate (PIP<sub>2</sub>). Phosphatidylinositol 4, 5-bisphosphate is a minor phospholipid constituting approximately 0.5% of the membrane phospholipids. Phosphatidylinositol 4, 5-bisphosphate and DAG are required for Ca<sup>2+</sup> mobilization and PKC activation. Calcium is mobilized from intracellular pools stored in the endoplasmic reticulum (ER) and by means of L-type voltage-sensitive Ca<sup>2+</sup> channels in the cell membrane (Naor et al., 1998; Ruf et al., 2003).

During exocytosis, Ca<sup>2+</sup> and phosphokinase C (PKC) act in parallel and exert an additive response on LH and FSH secretion. Calcium and PKC are also required during stimulated mitogen-activated protein kinase (MAPK) activity. Mitogen-activated protein kinase can activate transcription factors such as c-fos to mediate gene transcription with subsequent protein synthesis. In addition, MAPK can lead to stimulation of phosphokinase A<sub>2</sub> (PKA<sub>2</sub>) with subsequent LH and FSH synthesis and release (Naor et al., 1998; Ruf et al., 2003).
The signal cascade leading to the LH and FSH gene regulation is still not fully understood. Activation of the second messenger system leads to protein phosphorylation, gene transcription and biosynthesis of FSH and LH. Pituitary release of LH and FSH is down-regulated in the presence of continuous GnRH secretion (Belchetz et al, 1978). High frequency GnRH pulses favor LH release while low frequency pulses favor FSH release (Wildt et al., 1981). The changes in GnRH pulse frequency are essential for normal reproductive function (Moenter et al., 2003).

**Prostaglandins**

Prostaglandins were independently isolated from human seminal plasma in the 1930s by Goldblatt and von Euler. Prostaglandins are formed by most mammalian tissues and by tissues of lower vertebrates and certain invertebrates (Samuelsson et al., 1978). All mammalian cells types have the capacity for converting the membrane bound fatty acids into prostaglandins (Granström, 1981; Murray et al., 1996). Prostaglandins act as local hormones, having important physiological and pharmacologic activities (Murray et al., 1996). There are many stimuli (hormonal, nervous, other chemical, mechanical stimuli) known to activate phospholipase and initiate prostaglandin synthesis (Granström, 1981). The products formed and the amounts produced will vary within the same tissue under different conditions (Granström, 1981).

Prostaglandins are C$_{20}$ carboxylic acids. They have a central five-membrane ring with two side chains, 7 and 8 carbon in length, respectively, attached to adjacent positions on the ring. Depending on the number of double bonds (one, two or three double bonds) on the side chain they are designated to the ‘1’, ‘2’ or ‘3’ series (Granström, 1981). The type of prostaglandin (A, B, C, …I) depends on the arrangement of functional groups in the molecule (Champe and Harvey, 1994). The substituents in the
molecule determine its biological activity, most importantly those at position C-9 and C-11 in the ring and C-15 in the side chain (Granström, 1981). The prostaglandins that are biologically active compounds have a hydroxyl group (-OH group) at C-15 and a double bond at C-13 (Granström, 1981).

Prostaglandins belong to a group of unsaturated fatty acids called eicosanoids. Eicosanoids are not stored in cells, but are released upon synthesis and their biosynthesis is limited by the availability of free precursor fatty acid (Katzung, 1995). Eicosanoids include the prostanoids, leukotrienes and lipoxins (Murray et al., 1996).

Eicosanoids are produced through two different pathways. Leukotrienes and lipoxins are produced through the lipoxygenase pathway. Prostaglandins and thromboxanes are produced through the cyclooxygenase pathway, and each pathway competes with the other for arachidonic acid (Katzung, 1995). Not all cell types make all of these products (Katzung, 1995). The prostanoids consists of prostaglandins, prostacyclins and thromboxanes. They act as local hormones and function through a G-protein to elicit their biological effects (Katzung, 1995).

**Biosynthesis**

The precursor for prostaglandins is arachidonic acid (AA), which is an essential fatty acid that forms part of the glycerophospholipids found in the lipid bilayer of the cell membrane. Cleavage of arachidonic acid from membrane lipids and other lipid esters by phospholipase is activated by both specific and nonspecific stimuli (Katzung, 1995). Arachidonic acid is produced from the interaction of phospholipase $A_2$ (PLA2) with membrane phospholipids (Katzung, 1995). Phospholipase $A_2$ and other phospholipases cleave esterified arachidonic acid from the 2- position of glycerophospholipids (Dennis, 1987).
Free AA is converted to PGG₂, an unstable product, by oxygenation and cyclization of the pentane ring catalyzed by the enzyme prostaglandin endoperoxide synthase (prostaglandin G/H synthase or cyclooxygenase; COX). Cyclooxygenase is a membrane-bound hemoprotein (Katzung, 1995). The initial unstable product (PPG₂) is quickly reduced to PGH₂ by COX. Microsomes from cow uteri have been shown to possess strong endoperoxide F₂α reductase activity (Wlodawer et al., 1976).

The next substance produced from PGH₂ depends on the cell in which the reaction occurs (Sun et al., 1977). In tissues containing the cytosolic enzyme, prostaglandin D synthase, PGH₂ is converted to PGD₂. However, PGE₂ is produced if the tissue contains the membrane bound enzyme, prostaglandin E synthase. Reduced glutathione is required for both processes. Prostaglandin E 9-ketoreductase and 15-hydroxyprostaglandin dehydrogenase convert PGE₂ into PGF₂α (Sun et al., 1977; Katzung, 1995).

There are two cyclooxygenases: (I and II) that have a 60% homology and which are pharmacologically different (Katzung, 1995). Cyclooxygenase I is a constitutive form and synthesizes basal levels of prostaglandin. Cyclooxygenase II (COX II) is an inducible form of the enzyme. Cyclooxygenase II is induced by a variety of ligands (Herschman, 1994), and is regulated at transcriptional and posttranscriptional levels (Sirois and Richards, 1992). Its induction is inhibited by glucocorticoids (Herschman, 1994). Cyclooxygenase II is rapidly and transiently expressed, and leads to a bolus of prostaglandin production in response to stimulation (Herschman, 1994).

In response to pituitary glycoprotein hormones ovarian granulosa cells induced COX II (Herschman, 1994). Exposure of preovulatory follicles to FSH and LH rapidly and transiently induced the COX II message (Herschman, 1994). Prostaglandin synthesis
in the female reproductive cycle depends on the expression of COX II in response to pituitary glycoprotein hormones (Herschman, 1994).

**Metabolism**

Prostaglandins are rapidly inactivated in the body. Enzymes that catabolize prostaglandins are found in the lung, kidney, spleen, adipose tissue and intestines (Katzung, 1995). Oxidation of the secondary alcohol group at C-15 is catalyzed by the enzyme, 15-hydroxyprostanoate dehydrogenase (PGDH). The main sources of PGDH are the lungs, spleen and kidney (Kindahl, 1980). The lungs have the highest enzyme activity and with its vast vascular bed can render large amounts of prostaglandins biologically inactive (Katzung, 1995; Davis et al., 1980). After oxidation at C-15, the $\Delta^{13}$ double bond is reduced by $\Delta^{13}$ reductase resulting in 15-keto-13, 14-dihydro compounds, which are the main plasma metabolites (Kindahl, 1980). The main plasma metabolite of PGF2$\alpha$ in male calves is 15-keto-13, 14-dihydro PGF2$\alpha$. Urinary excretion was completed in approximately 6 hours, with recovery of 80% of the injected PGF2$\alpha$ (Kindahl, 1980). In heifers the main metabolite of PGF2$\alpha$ is 15-keto-13, 14-dihydro PGF2$\alpha$ (Kindahl et al., 1976).

**Mechanism of Action**

The eicosanoids are short-lived, highly potent local mediators that produce an astonishing array of biological effects by binding to specific cell surface receptors (Katzung, 1995). All binding appears to involve a G-protein linkage (Katzung, 1995). Receptor binding initiates a signal transduction pathway, which links the regulatory substance (PGF2$\alpha$) with its intracellular effect(s).
Prostaglandin F2α is released from the uterus, and transferred from the utero-ovarian vein to the ovarian artery by a countercurrent mechanism. On reaching the ovary, PGF2α binds to high and low affinity-binding sites (receptors) located in the plasma membrane of the corpus luteum (Samuelsson et al., 1978). These receptors are G protein-coupled receptors designated FP and subtyped into FPA and FPB. The high affinity-binding site requires calcium ions in order to be detected (Samuelsson et al., 1978).

Prostaglandin F2α receptors are coupled to phospholipase C (PLC). Receptor binding activates PLC and induces hydrolysis of phosphatidylinositol 4, 5-bisphosphate to generate two second messengers, inositol 1,4,5-triphosphate (IP3) and 1,2-diacylglycerol (DAG). Inositol 1,4,5-triphosphate diffuses into the cytoplasm and is involved in the liberation of intracellular calcium (Ca2+) from the endoplasmic reticulum.

Calcium is required for the activation of PKC and DAG increasing PKC’s affinity for Ca2+. Activation of PKC leads to the opening of calcium channels. Calcium and PKC promote protein phosphorylation and this eventually leads to the inhibition of progesterone secretion and regression of the corpus luteum (Samuelsson et al., 1978).

**Function in the Reproductive Tract of the Cow**

During the bovine estrous cycle, PGF2α is released for 2 to 3 days as rapid pulses with duration of 1 to 5 hours prior to and during luteolysis (Kindahl et al., 1976; Kindahl, 1980). A 9-keto-reductase enzyme that reduces PGE2 to PGF2α can also form PGF2α. However, this enzyme is not present in the bovine uterus (Kindahl, 1980).

The precise release of PGF2α throughout the bovine estrous cycle presupposes that there is an inhibiting factor in the uterus. This inhibiting factor is important for the
regulation of the physiologic PG biosynthesis and thus regulates its production to prevent premature lysis of the corpus luteum. Wlodawer et al. (1976) noted that an inhibiting factor was found in bovine uterine preparations that suppressed the fatty acid cyclooxygenase. Knickerbocker et al. (1986) noted that the bovine conceptus suppressed uterine production of PGF2α production during pregnancy recognition by what was then called bovine conceptus secretory proteins (CPS) and is now known as interferon tau (INF-τ). However, the suppression of PGF2α release from the endometrium is regulated by a number of hormones; estrogen, progesterone (Salamonsen et al., 1990; Xiao et al. 1998), oxytocin and endothelin-1 (ET-1).

The bovine endometrium contains large quantities of AA and has the ability to metabolize AA into a variety of products (Salamonsen and Findlay, 1990). Ovarian steroids influence the expression of the COX gene in bovine endometrial cells (Xiao et al. 1998). Progesterone directly influenced the basal secretion of PGF2α by the endometrium (Xiao et al. 1998). Progesterone has been shown to stimulate basal PGF2α secretion by bovine endometrial cells and tissues. However, it inhibits oxytocin-induced PGF2α secretion while in luteal cell culture while estrogen stimulated only PGF2α secretion.

Prostaglandins have also been shown to have a direct effect on the bovine myometrium. (Patil et al., 1980). In vitro studies carried out on the bovine myometrium indicate that PGF2α has the ability to increase uterine tone and motility (Patil et al., 1980). However, the extent to which motility is enhanced is dependent on the stage of the estrous cycle. During the follicular phase, the myometrium is more active and contractions are more frequent and stronger than in the luteal phase. Contractions occur
on average 9 times per 10 minutes, with mean amplitude of 12 mm. During the luteal phase, the frequency of contractions is less, on average 6 per 10 minutes, with mean amplitude of 5 mm. Under the influence of PGF2α, there is a general increase in mean contraction and strength in both stages of the estrous cycle (Patil et al., 1980). Zetler et al. (1969) demonstrated that PFG2α had a similar effect on the fallopian tubes, increasing motility.

**Prostaglandin F2α and its Uses in the Dairy Cow**

Prostaglandins are widely used in herd management due to their luteolytic properties. Prostaglandin F2α has been use in cattle as an abortifacient (Johnson, 1981). Vandeplassche et al. (1974) induced abortion in cases of pathological gestation in cattle by intrauterine infusion (into the horn ipsilateral to the ovary with the CL) of 10.5 to 45 mg of PGF2α. In cases where the cows carried a mummified fetus, 3 of 5 expelled the fetus. However, in cases of maceration, the fetus was not expelled. There was rapid regression of the CL within 4 to 5 day of intrauterine infusion, followed by estrus within 6 to 7 days.

Prostaglandin F2α has also been use in cattle to induce parturition usually from 240 days gestation or within 20 days of predicted term (Johnson, 1981; Schultz and Copeland, 1981). Bosc et al. (1975) compared the use of dexamethasone to an analogue of PGF2α (ICI 79939) for the induction of parturition in cattle. Parturition was successfully induced when prostaglandin was administered in 2 mg intramuscular doses five-hours apart using a dosage which ranged from 4 to 10 mg. Plasma progesterone decreased to a low level before parturition following two successive injections. This decrease in progesterone
concentration was attributed to lysis of the CL of pregnancy. However, cows in which parturition was induced had a higher incidence of retained fetal membranes.

Prostaglandin F2α has been used to treat unobserved estrus in lactating dairy cattle with a corpus luteum (Eddy, 1977; Seguin et al., 1978; Seguin, 1981) and for estrus synchronization. Intramuscular administration of the PGF2α analogue, clorprostenol (ICI 80, 996) in dairy cattle with unobserved estrus and a palpable mature CL resulted in cows exhibiting estrus and being inseminated sooner than those treated with saline. In one study (Eddy, 1977), intramuscular administration of 500µg clorprostenol resulted in 69% of unobserved estrus cows coming into heat within 8 days of treatment.

Prostaglandin F2α has been used in cattle for postpartum infections: pyometra, metritis and endometritis (Ott and Gustafsson, 1981a, 1981b). Pyometra is defined as a condition associated with accumulation of purulent material in the uterus, persistence of a CL and anestrus (Roberts, 1971). The mode of action of PGF2α in the treatment of cows with postpartum infection is based on its luteolytic activity. In cases of pyometra, treatment leads to the regression of the CL resulting in emptying of the uterus. Prostaglandin F2α has been shown to stimulate the myometrium and may aid in the physical evacuation of purulent material from the uterus (Ott and Gustafsson, 1981a, 1981b). Gustafsson et al. (1976) demonstrated that PGF2α administered both intramuscularly and intravenously at various dosages will effect resolution of postpartum and post insemination pyometra in dairy cows not previously treated with antibiotics (systemically or intrauterine) or any other drug. Gustafsson et al. (1976) treated 26 cows (23 with postpartum pyometra and 3 with post insemination pyometra). Eighty-five percent responded by emptying the uterus and exhibited estrus 3 to 4 day following
treatment. Sixty-five percent became pregnant following insemination beginning at the second estrus after treatment. Several other studies have shown the beneficial effect of using PGF2α in cows with pyometra (Jackson, 1977; Fazeli et al., 1980; Ott et al, 1981a; Paisley et al., 1986; Gilbert et al., 1992). Prostaglandin F2α has also been use in cattle with acute and chronic endometritis (Jackson, 1977; Ott et al., 1981a, 1981b; Paisley et al., 1986; Pepper et al., 1987; Gilbert et al., 1992; Sheldon et al., 1998; Heuwieser et al., 2000; Knutti et al., 2000).

**Estrous Cycle of the Cow**

There are four stages of the estrous cycle: estrus, metestrus, diestrus and proestrus. The estrous cycle can also be classified as luteal or follicular depending on the dominant structures on the ovaries. In the luteal phase, the CL is the dominant structure, and in the follicular phase the preovulatory follicle is the dominant structure. The average length of the estrous cycle in the heifer is 20 days with a range of 18 to 22 days, and in the cow is 21 days with a range of 18 to 24 days (Roberts, 1971).

**Follicular Phase**

The follicular phase consists of proestrus and estrus. It starts with corpus luteum regression and ends with ovulation of the dominant follicle. The follicular phase is approximately 4 to 5 days in the cow.

In the event that pregnancy does not occur, the uterus releases prostaglandin F2α (PF2α; Kindahl et al., 1976). Following release of PF2α from the endometrium, the corpus luteum of the luteal phase undergoes functional and structural regression. At this time there is a sharp decline in the blood progesterone (P₄) concentration (Kindahl et al., 1976). The sudden fall in P₄ removes the negative feedback on the developing follicles
leading to the selection and acceleration of preovulatory follicular growth (Savio et al., 1990).

The preovulatory follicle produces increasing concentrations of estradiol-17β and this influences the sex centers in the brain to induce estrus. Peak concentrations of estradiol-17β coincide with estrus. At the level of the hypothalamus, estradiol-17β induces a GnRH surge, which induces the preovulatory LH surge and FSH release from the anterior pituitary. There is considerably less estradiol-17β during the 18 to 20 hour period of estrus as compare to proestrus. At the level of the pituitary, estradiol increases the responsiveness of gonadotrophs to GnRH, which results in an increase in the LH pulse amplitude.

The duration of estrus is approximately 18 to 20 hours, and ovulation occurs 10 to 12 hours after estrus. During estrus, the cow or heifer will stand to be mounted by the bull or by herd-mates. During estrus, the vulva of the cow sometimes becomes swollen, the mucous membranes of the vagina are hyperemic, and a clear, viscous, mucous discharge may be seen hanging from the vulva.

Ovulation is spontaneous and occurs 10 to 12 hours after estrus in cows and 3 hours earlier in heifers (Roberts, 1971). The ovulatory process consists of the following: cytoplasmic and nuclear maturation of the oocyte; disruption of the cumulus cell cohesiveness among the cells of the granulose layer and thinning and rupture of the external follicular wall, with expulsion of the mature oocyte (Hafez et al., 2000).

**Luteal Phase**

The luteal phase is the period following ovulation and involves luteinization and development of the CL. It consists of metestrus (Days 1 to 4) and diestrus (Days 5 to 18). In this stage, the corpus luteum develops from the remnants of the pre-ovulatory follicle
and produces increasing quantities of progesterone. Progesterone is usually detectable in blood by day 5 after ovulation and CL formation. The CL also produces oxytocin and estrogen.

In the non-pregnant cow, the CL undergoes regression by day 17 after ovulation. Luteolysis occurs as a consequence of the interaction between the CL and prostaglandin F2α (PGF2α) released from the uterus. If pregnancy occurs, uterine production of PGF2α is blocked by the action of interferon tau (INF-τ) on the uterus. The up-regulation of oxytocin receptors in the endometrium is inhibited by the secretion of INF-τ from the trophodectoderm from days 12 to 25 in cattle (Farin et al. 1990). The CL is required for the maintenance of pregnancy in the cow.

During the luteal phase, the quantity of progesterone produced by the CL increases. The concentration of progesterone impacts LH secretion patterns. When progesterone is low, the LH pulse is characterized by high frequency and low amplitude. When progesterone is high, the LH pattern is characterized by a low frequency and high amplitude.

**Hormonal Control of the Estrous Cycle**

Hormones secreted by the hypothalamus, anterior pituitary, ovary and uterus regulate the estrous cycle. The hypothalamus integrates information from higher brain centers, involving internal and external signals, such as nutritional status, season, photoperiod, presence of a male and others (Garverick and Smith, 1993).

Gonadotropin-releasing hormone (GnRH) is produced by the neurosecretory cells within the hypothalamus and regulates the release of gonadotropins (luteinizing hormone, LH and follicle stimulating hormone, FSH) from the anterior pituitary (Smith and Jennes,
GnRH is produced in the cell bodies of neurosecretory cells located in the arcuate, suprachiasmatic and preoptic nuclei of the hypothalamus. GnRH is then transported along the axons to the median eminence, where it is released into the hypothalamo-hypophyseal portal circulation and travels to the anterior pituitary (Garverick and Smith, 1993). GnRH is released in a pulsatile manner in response to appropriate physiological cues. Luteinizing hormone and FSH are secreted in a pulsatile manner in response to GnRH secretion (Moenter et al., 1991).

Luteinizing hormone is a glycoprotein hormone produced by the gonadotrophs in the anterior pituitary. It consists of alpha- and beta-subunits. The alpha-subunit is common to several hormones, including FSH and thyroid stimulating hormone (TSH), and the beta-subunit is unique to the particular hormone. LH is a short-lived molecule, having a half-life of approximately 30 minutes.

Follicle-stimulating hormone is a glycoprotein produced in the anterior pituitary by gonadotrophs. It is composed of a common alpha-subunit and specific beta-subunit. FSH stimulates the growth and maturation of ovarian follicles and in the presence of LH stimulates secretion of estrogen from ovarian follicles.

Luteinizing hormone and FSH are secreted in a pulsatile manner from the anterior pituitary in response to the pulsatile release of GnRH. Tonic levels are controlled by the negative feedback mechanism from the ovaries.

The pulsatile secretion of LH varies throughout the estrous cycle (Rahe et al., 1980; Walters et al., 1984a, 1984b; Schallenberger et al., 1985). Rahe et al. (1980) suggests that the LH pattern throughout the estrous cycle is dependent on the stage of the cycle and ovarian steroids influence the LH pattern.
Studies have been done in ovariectomized animals to investigate the effect of steroids on the LH release. Ovariectomy in the cow results in increases in the pulse frequency and amplitude of LH and FSH (Schallenberger and Peterson, 1982). The pattern of secretion is related to circulating concentrations of progesterone. In the early luteal phase when progesterone concentrations are low, the LH pulse frequency is much higher than that seen in the mid-luteal phase; 8.0 pulses/12 hours and 3.6 pulses/12 hours respectively (Walters et al., 1984b). In the early luteal phase the pulse frequency of LH and FSH are similar. However, as the luteal phase progresses, the FSH pulse frequency becomes greater than the LH pulse frequency (Walters et al., 1984b).

Luteinizing hormone release is suppressed by exogenous progesterone (Beck et al., 1976). In the luteal phase, progesterone and estrogen are the main factors, which decrease the frequency of the LH pulses in cattle. Individually these hormones can suppress the LH pulse, but when administered together there is a more profound inhibiting effect on LH pulse frequency. Stumpf et al. (1993) showed that when estradiol-17β was administered to ovariectomized cows the LH pulse frequency was $0.97 \pm 0.07$ pulses/hour and when progesterone was administered the LH pulse frequency was $0.52 \pm 0.08$ pulses/hour. However, when both estradiol-17β and progesterone were given concomitantly the LH pulse frequency increased to $14 \pm 0.07$ pulses/hour.

**Effect of Season**

The effect of season or time of year on the bovine estrous cycle may be thought of as two fold. There is the effect of photoperiod on the estrous cycle and the effect of “heat stress” or high environmental temperatures on the estrous cycle.

High environmental temperatures reduce estrus behavior. Abilay et al. (1975) demonstrated that the duration of estrus was reduced on average by 5.5 hours in
Guernsey heifers maintained at 18.2 °C. It is thought that the reduction in estrus behavior is as a consequence of the decrease in circulating estradiol (Wolfsenson et al., 1997; Roth et al., 2001) noted during periods of heat stress. There is a reduction in the numbers of mounting episodes per estrus during heat stress (Nebel et al, 1997). Badinga et al. (1985) demonstrated that in Florida there is a decrease in conception rate in from 48% in March to 18% in July with recovery occurring in November. Imtiaz Hussain et al. (1992) noted that only 36.8% of cows expressed estrus, even though all cows were cycling based on progesterone concentrations.

High environmental temperatures have been shown in affect cortisol levels. There is an increase in cortisol levels in dairy cows that experience heat stress (Wise et al., 1988; Imtiaz Hussain et al., 1992) However, Abilay et al. (1975) demonstrated that there was a decrease in plasma cortisol levels in heat stressed heifers. Cortisol levels have been linked to GnRH release. Increased cortisol levels inhibit GnRH secretion, which in turn decreases LH secretions (Dobson and Smith, 2000). Cortisol has been shown to have a direct effect on the pituitary gland to depress both basal and GnRH-stimulated LH release in the cow (Li and Wagner, 1983; Padmanabhan et al., 1983).

High environmental temperatures have been shown to have an effect on the gonadotropins, luteinizing hormone (LH) and follicle-stimulating hormone (FSH). The effect of high environmental temperatures on LH is inconsistent. Vaught et al. (1977) found no difference in the frequency of preovulatory increase of luteinizing hormone or in the interval between the preovulatory increase and ovulation in either lactating or non-lactating cows exposed to summer heat stress. This is in contrast to findings by Wise et
al. (1988). There was a decrease in the number of LH pulses on Day 5 of the estrous cycle in heat stressed cows compared to controls (Wise et al., 1988).

Ovarian follicles are susceptible to high environmental temperatures. Roth et al. (2000) demonstrated that there are alterations in the pattern of growth and development of medium-sized follicles associated with an increase in plasma FSH concentration in the first follicular wave following acute heat stress. Wolfensson et al. (1997) demonstrated that estradiol concentration in follicular fluid and androstenedione production by thecal cells were lower in dominant follicles collected in autumn than those collected in winter. This finding by Wolfensson et al. (1997), indicate the carry over effect of heat stress. Roth et al. (2001) investigated the delayed effect of heat stress on medium-sized and preovulatory follicles at 20 and 26 days after acute heat exposure. It was noted that the number of medium-sized follicles that emerged during the first follicular wave after heat stress was the same in both control and heat-stressed cows. However, there were more healthy medium-sized follicles in the control than heat-stressed cows (56% vs 38%) but this was not significantly different. In healthy medium-sized follicles, estradiol production by granulosa cells and androstenedione production by thecal cells were lower and follicular fluid progesterone concentration was higher in heat-stressed than in control cows. In preovulatory follicles the viability of the granulose cells, concentration of androstenedione in follicular fluid and androstenedione production by thecal cells were lower in heat-stressed than in control cows (Roth et al., 2001).

Guzeloglu et al. (2001) investigated the effect of acute heat stress on long-term follicular dynamics and biochemical characteristics of dominant follicles in non-lactating dairy cows. They found that there was no difference in estradiol or progesterone
concentration in the follicular fluid of the dominant follicle between heat stressed and control cows. However, there was a significant difference in the size of the dominant follicle between the control and heat stressed cows. The dominant follicle size was greater in control cows than in heat stressed cows. Guzeloglu et al. (2001) noted that heat stress reduces follicular dominance during a follicular wave based on the increase in the number of class 3 follicles on Days 7 and 8. Roth et al. (2000) investigated the immediate effect of heat stress on plasma FSH and inhibin in Holstein dairy cows. It was demonstrated that there was a larger cohort of medium sized follicles (6-9 mm) during the second follicular wave of the estrous cycle of heat stressed cows compare to controls. This increase growth was associated with higher plasma concentrations of FSH, which lasted for 4 more days in heat stressed cows than in controls. The increase in plasma FSH was also associated with a decrease in plasma concentrations of inhibin.

There is an increase in the length of the estrous cycle in cows experiencing heat stress. Wilson et al. (1998a) noted that the second wave dominant follicle was less likely to ovulate in heat stressed lactating dairy cows. In heat stressed cow, 18% ovulated compared to controls 91%. The average day of luteolysis was delayed by 9 days in heat stressed cows. Similar finding were found in heifers. Heat stress inhibited the growth and function of the dominant follicle, such that heat stressed heifers had three follicular waves and there was a delay in corpus luteum regression (Wilson et al., 1998b).

High environmental temperatures have been shown to affect corpus luteum function. These observations were made based on progesterone concentrations. In some studies progesterone concentration was elevated (Abilay et al, 1975; Vaught et al., 1977) in cows experiencing heat stress. However, in other studies progesterone concentration
was unchanged (Wise et al., 1988) and yet in other studied progesterone concentrations were lowered during heat stress (Howell et al., 1994). Wilson et al. (1998a, 1998b) demonstrated that there was delayed regression of the corpus luteum in lactating dairy cows and heifer subjected to controlled heat stress.

Heat stress reduced feed intake (Fuquay, 1981; Rensis and Scaramuzzi, 2003). Reduced dry matter intake will prolong the period of negative energy balance in the postpartum dairy cow. Negative energy balance leads to decreased plasma insulin, glucose and insulin-like growth factor I (IGF-I; Rensis and Scaramuzzi, 2003). These factors are required for the normal growth and function of ovarian follicles and thus the estrous cycle.

**Folliculogenesis and Ovarian Dynamics in the Dairy Cow**

**Follicular Development**

An ovarian follicle is a spherical aggregation of cells that contain the developing gamete (Banks, 1986). Oogenesis begins during embryologic development and continues throughout fetal development until shortly after birth. Multiplication halts in meiotic prophase. At this stage the germ cell is termed a primary oocyte (Noden, 1985). Follicular development proceeds through further development of the primordial follicle into a primary follicle; primary to secondary follicle and finally a mature follicle. Follicular growth and maturation is under the influence of gonadotropins from the pituitary (Noden, 1985).

The primordial follicle contains the primary oocyte. At this stage the primary oocyte has paired homologous chromosomes and each has replicated to form two chromatids (Noden, 1985). At the time the oocyte is undergoing the first stages of meiosis follicular cells surround the primary oocyte to form a primordial follicle. Once
the primordial follicle is activated it becomes the primary follicle. Histologically, the primordial follicle appears as a single layer of flattened follicular cells surrounding the primary oocyte (Banks, 1986).

Activation of the primordial follicle results in the development of the primary follicle, involving alterations in the primary oocyte, follicular cells and stromal elements. Histologically, the primary follicle appears as single layer of cuboidal or columnar follicular cells surrounding the primary oocyte. At the stage, accumulation of yolk granules can been seen in the primary oocyte (Banks, 1985).

The secondary follicle consists of the primary oocyte separated from the follicular cells by the zona pellucida. The follicular cells are actively dividing and are now know as the membrana granulosa. As the secondary follicle continues to develop, the stromal cells differentiate into theca interna and theca externa, separated from the membrana granulosa by a basement membrane. The theca interna consists of large, epithelioid cells and an extensive vascular network. The theca externa consists of a fibroblastic layer of cells (Banks, 1986).

The tertiary follicle develops from the secondary follicle. Histologically, the tertiary follicle consists of cuboidal and columnar follicular cells surrounding the primary oocyte. Within the layers of follicular cells small, fluid filled periodic-acid-Schiff-positive spaces can been seen. Further secretion of follicular fluid by the granulosa cells leads to the formation of a follicular antrum seen in the mature follicle. The mature follicle is also known as the Graafian follicle or preovulatory follicle. The preovulatory follicle ranges form 15 to 17 mm in diameter in the bovine ovary.
Follicles protrude from the ovary and are recognized as a smooth fluctuant structures on palpation. The size of the follicle increases to approximately 20 to 25 mm in diameter by the middle of the estrous cycle. The increase in follicular fluid within the antrum is reflected by the tension in the palpable follicle, which increases up to six to twelve hours before ovulation (Zemjanis, 1970).

In terms of the endocrinology, the development of the follicle can be divided into four stages. The initial stage of development is independent of the gonadotropins, follicle stimulating hormone (FSH) and luteinizing hormone (LH). In this stage follicles are usually < 3 mm in size. Next follows a stage in which the follicle is dependent on FSH, the follicles range from 3 to 10 mm in diameter. In the thirds stage the follicle is dependent on pulsatile LH and ranges from 10 mm to preovulatory size. The final stage is dependent on the preovulatory LH surge for ovulation (Thatcher et al., 2002).

**Follicular Waves**

Ovarian follicles develop in waves. Rajakoski (1960) first put this theory forward after he conducted extensive studies of ovaries taken at slaughter. Pierson and Ginther (1984) later confirmed the existence of follicular waves using ultrasonography.

Cattle either have two or three follicular waves during a single estrous cycle. In cows that have 2 follicular waves, recruitment occurs on Day 0 (day of ovulation) and day 10 of the estrous cycle. In cows with 3 follicular wave cycles, recruitment occurs on Days 0, 9 and 16 of the estrous cycle (Ginther et al., 1989a; Ginther et al., 1989b). Follicular waves occur in cyclic cows and heifers and in dairy and beef heifers prior to the onset of cyclicity, in prepubertal calves as early as 2 weeks of age (Evans et al. 1994) and during most of pregnancy (Ginther et al.1996). Follicular growth begins in the fetal ovaries, with the final maturation stages occurring days prior to ovulation.
Follicular dynamics in the cow consists of three distinct phases: recruitment, selection and dominance. Recruitment is the process by which a group of follicles begins to mature and grow under the influence of gonadotropins. In selection, a single follicle is chosen and avoids atresia with the potential to ovulate. In dominance, the selected follicle inhibits the recruitment of a new cohort of follicles (Lucy et al., 1992).

Hoak and Schwarts (1980) showed that the secondary increase in FSH levels following the preovulatory LH surge recruits follicles for the next estrous cycle in the rat. Adams et al., (1992) showed that the FSH surge in heifers began 2-4 days before ultrasonically detectable emergence of a follicular wave (follicles ≤5 mm). FSH peaked 1 or 2 days before emergence and began to decrease at the time of deviation, when the follicles of a wave begin to diverge into a dominant follicle and subordinate follicles (follicle 6-7 mm). One of these follicles becomes dominant and has the potential to ovulate.

In a two-wave estrous cycle, a group of small follicles (≤5 mm) is recruited and begins to grow under the influence of follicle-stimulating hormone (FSH; Adams, et al 1992). Later a single follicle becomes dominant and continues to grow while subordinate follicles will eventually undergo atresia. In the presence of the CL and high progesterone concentration, the dominant follicle of the first wave does not ovulate and regresses. A second follicular wave develops with recruitment of another group of follicles around midcycle. The dominant follicle of this second wave is functional at the time of CL regression and decreasing progesterone concentration and this follicle ovulates after luteolysis.
In a three-wave cycle the second dominant follicle fails to ovulate and a third follicular wave develops. The dominant follicle of the second wave is usually smaller than the first and third dominant follicle (Lucy et al., 1992). Cows with a three-wave cycle have a longer estrous cycle, as the third dominant follicle requires time to develop prior to ovulation (Lucy et al., 1992).

**Regulation of Follicular Growth by FSH and LH**

Follicular deviation or selection has been defined as the beginning of the greatest difference in the growth rates between the largest follicle (dominant follicle) and the second largest follicle (largest subordinate follicle) at or before the largest subordinate follicle reaches its maximum diameter (Ginther et al., 1996). The follicle that becomes dominant develops receptors for luteinizing hormone (LH) around the time of deviation, while the subordinate follicles do not. Under the influence of declining FSH concentrations and increasing LH concentrations, the subordinate follicles regress and the dominant follicle continues to develop. In the event that luteolysis does not occur with a resultant fall in progesterone concentrations, the dominant follicle will not ovulate and undergoes atresia.

The dominant follicle secretes both inhibin and estradiol. Inhibin is also secreted by follicle greater than 3 mm and also by atretic follicles. Estradiol and inhibin feed back on the pituitary and hypothalamus and decreases the secretion of FSH from the pituitary.

Estradiol biosynthesis is dependent on a two-cell system. Theca cells and granulosa cells individually are not equipped with the enzymes necessary for biosynthesis of estradiol from cholesterol. However, together these cells are capable of producing estradiol. Theca cells are the sites of androgen synthesis in follicles. Under the influence of LH androgen secretion is increased. The end product in steroidogenesis in theca cells
is progesterone. Androstenedione produced by the theca cells is transported into the granulosa cells where it is converted into testosterone. Under the influence of FSH, testosterone is converted into estradiol within the granulosa cells (Hansel and Convey, 1983).

**Production of Estrogen**

Prior to the LH surge and luteinization of the granulosa and theca cells, the major hormone produced by the ovary is estrogen, estradiol-17β. The granulosa and theca cells individually are unable to produce estradiol, since both lack the enzymatic pathways to do so. In order to produce estradiol, cholesterol must be converted to androgens and androgens to estradiol. Thecal cells are capable of the former, but not the latter, while granulosa cells are capable of the latter (Niswender et al., 2000).

**Corpus Luteum in the Cow**

The corpus luteum (CL) is a transient endocrine structure, which secretes progesterone, oxytocin (Fields et al., 1992), neurophysin, relaxin and other substances, which act in an autocrine and/or paracrine manner. There is some evidence that the bovine CL may produce estrogen. Kimbal and Hansel (1974) used a dextrand-charcoal adsorption method to show that estrogen-binding proteins (estrogen receptor, ER) were present in the bovine corpus luteum. Recent work by Okuda et al. (2001) demonstrated that estradiol 17β is produced in the bovine CL.

The major secretory hormone is progesterone, which is essential for the establishment and maintenance of pregnancy. In the non-pregnant animal, the life span of the CL determines the length of the estrous cycle.
Corpus Luteum Development

The corpus luteum is formed from the remnants of the ovarian follicle following ovulation. Simmons and Hansel (1964) postulated that any hormone that could stop oxytocin from shortening the bovine estrous cycle had luteotropic properties. The luteotropic property of luteinizing hormone (LH) was later confirmed by Donaldson et al., (1965b, 1965c).

Ovulation is initiated by a preovulatory LH surge. Rupture of the follicular wall is believed to involve enzymatic hydrolyzation of connective tissue by LH-induced collagenases, proteases and plasmins (Banks, 1986). The oocyte and its surrounding follicular fluid are simultaneously ejected from the ruptured follicle. The granulosa and theca cells collapse into the cavity. The hemorrhage associated with ovulation clots, forming the \textit{corpus hemorrhagicum}. This stage is a transitory stage.

The granulosa and thecal cells undergo hypertrophy and division to become the large and small luteal cells of the corpus luteum, respectively. The production of highly specific antibodies against granulosa and thecal surface antigens proved that as the CL ages the small luteal cells undergo structural and morphological changes to become large luteal cells (Alila et al., 1984). This substantiates earlier work by Donaldson and Hansel (1965a) using histology and histochemical staining techniques. They demonstrated that granulosa cells stop dividing by Day 4 of the estrous cycle and small luteal cells derived from thecal cells continue to respond to luteotropins – grow and develop into large luteal cells (Donaldson and Hansel, 1965a).

Ursely and Leymarie (1979) identified three types of cells in collagenase dispersed luteal preparations. These included small (10-20\(\mu\)m in diameter) steroidogenic
cells, large (>25 \(\mu\)m diameter) steroidogenic cells and numerous small (<10 \(\mu\)m diameter) non-steroidogenic cells consisting mainly of vascular cells (endothelial cells, erythrocytes leukocytes) and connective tissue cells (Hansel and Blair, 1996).

The non-steroidogenic cells of the bovine corpus luteum consist of macrophages and endothelial cells which account for approximately 14% of the volume and approximately 53% of the cells of the mature CL (Parry et al., 1980; O’Shea et al., 1989). Macrophages are important for their phagocytic activity and for participation in the immune response involved in the regression of the CL (Pate, 1994). Endothelial cells are those that line the microvasculature and are thought to secrete substances which are involved in both luteotropic and luteolytic processes. Fields and Fields (1996) reported the presence of five different phenotypes of endothelial cells from the bovine CL. Type 1 cells are believed to be true endothelial cells, while Type 5 cells displays characteristic of immature granulosa cell, and it has been suggested to be a putative stem cell for renewal of luteal cells (Fields and Fields 1996). Fibroblasts comprise approximately 10% of all luteal cells and approximately 6% of the total volume of the bovine CL (Fields and Fields 1996).

The steroidogenic luteal cells make up the majority of the bovine CL. The small luteal cells and the large luteal cells together account for approximately 70% of the volume of the bovine CL (Parry et al., 1980; O’Shea et al., 1989). The small luteal cells make up approximately 28% of the volume of the CL and are known for its low basal production of progesterone. When stimulated with LH, these cells increase their production of progesterone. The large luteal cells comprise 3% of the luteal cells. However, they comprise approximately 40% of the volume of the CL (O’Shea et al.,
1989). The large luteal cells do not respond to LH. However, they do secrete oxytocin and are the steroidogenic cells, which produce the majority of basal progesterone.

During the early development of the bovine CL, there is rapid growth and increase in size. The CL has a six-fold increase in size during early luteal phase of the estrous cycle (Zheng et al., 1994). Along with an increase in weight, there is also an increase in the amount of progesterone produced and secreted.

**Function of the Corpus Luteum**

The major hormone produced by the CL is progesterone. Progesterone functions in determining the length of the estrous cycle and is required for the maintenance of pregnancy in the cow.

The preovulatory LH surge results in luteinization of the granulosa and thecal cells to large and small luteal cells, respectively. The luteinization process results in a shifting of the steroidogenic pathway such that the major secretory product is progesterone. This process includes increased expression of enzymes required for conversion of cholesterol into progesterone. There is an increase in cytochrome P-450 side chain cleavage (P-450\textsubscript{scC}) enzyme and 3β-hydroxysteroid dehydrogenase/Δ\textsuperscript{5},Δ\textsuperscript{4} isomerase (3β-HSD) and decrease expression in the enzymes that convert cholesterol to estradiol–17α-hydroxylase cytochrome P-450 and aromatase cytochrome (Niswender et al., 2000).

In the female reproductive tract, prostaglandin E2 (PGE2) is considered luteotrophic (Pratt et al., 1977). Intrauterine administration of PGE2 protects the CL against induced and spontaneous luteolysis (Pratt et al., 1977; Henderson et al., 1977). Prostaglandin E2 stimulates the production of progesterone (P4) through the activation of cyclic-AMP – protein kinase A (PKA) pathway (Kotwica et al., 2003).
Prostaglandin E2 binds to its receptor, a G protein-coupled receptor, (EP) located in the cell membrane. There are four receptor subtypes, EP1, EP2, EP3 and EP4. Only EP2 and EP4 are coupled to adenylate cyclase. Activation of adenylate cyclase leads to an increase in cAMP that in turn activates PKA leasing to a cascade of intercellular signals with the eventual activation of genes for progesterone syntheses. Prostaglandin E2 EP2 receptor is highly expressed in the large luteal cells. The EP2 receptor is the major cAMP-generating PGE2 receptor in the bovine CL (Arosh et al. 2004) and the large luteal cells produce 80% of progesterone (Diaz et al., 2002)

**The Corpus Luteum and Pregnancy**

In the event of pregnancy, the secretion of PGF2α from the uterus is blocked, luteolysis does not occur and progesterone concentration is maintained. Between Day 15 and 17 of the estrous cycle, the bovine conceptus produces a signal, which prevents luteolysis induced by the pulsatile release of PGF2α (Kindahl et al., 1981; Asselin et al. 1996). The signal molecule, for the recognition of pregnancy in cows was first identified as bovine trophoblast protein-1 (bTP-1), which was later called interferon-τ (INF-τ). Bovine trophoblast protein-1 (bTP-1) is a 172-amino acid interferon. (Klemann et al., 1990).

In the cow, INF-τ prevents the luteolysis by down-regulation of oxytocin receptors (Meyer et al., 1996). Reducing the number of oxytocin-receptors available for oxytocin binding attenuates oxytocin-stimulated secretion of PGF2α (Meyer et al., 1996). This prevents oxytocin-stimulated PGF2α release and subsequent luteolysis.

Recombinant bovine interferon-τ (rbINF-τ) also causes a decrease in the expression of cyclooxygenase II (COX II) and prostaglandin F synthase (PGFS; Xiao et al., 1998).
Cyclooxygenase II is an inducible rate-limiting enzyme for the conversion of arachidonic acid to PGG\textsubscript{2} and PGH\textsubscript{2}, the precursors of PGF\textsubscript{2} and PGE\textsubscript{2}. Prostaglandin F synthase (PGFS) is responsible for the reduction of PGH\textsubscript{2} to PGF\textsubscript{2} and PGD\textsubscript{2} to 9α, 11β-PGF\textsubscript{2} (a stereoisomer of PGF\textsubscript{2α}). Recombinant bINF-τ has been shown to decrease COX II mRNA in epithelial cells (the primary source of PGF\textsubscript{2α}) and to increases COX II mRNA and prostaglandin synthesis in stromal cells (the primary source of PGE\textsubscript{2}; Xiao et al., 1998). Xiao et al. (1998) also demonstrated that there was a reduction in PGFS mRNA in both epithelial and stromal cells, and this was associated with an increase in PGE\textsubscript{2}: PGF\textsubscript{2α} ratio.

Bovine interferon-τ has been shown to shift the primary prostaglandin (PG) produced by the endometrium from PGF\textsubscript{2α} to PGE\textsubscript{2} (Asselin et al., 1997; Xiao et al., 1998). Prostaglandin E is thought to be a luteotrophic agent (Pratt et al., 1977). Asselin et al. (1997) showed that cultured bovine endometrial cells treated with bovine recombinant INF-τ (rINF-τ), in the presence and absence of oxytocin had a net PGE\textsubscript{2}: PGF\textsubscript{2α} ratio of 3.8 and 7.7 respectively. The conclusions made by Asselin et al. (1997) were rINF-τ regulates PGs by stimulating PGE\textsubscript{2} preferentially and rINF-τ transforms the response to OT from stimulation of PGF\textsubscript{2α} to stimulation of PGE\textsubscript{2}.

Progesterone is necessary for the maintenance of pregnancy in the bovine. The normal CL produces more progesterone than required to maintain the embryo until Day 15 of pregnancy. Normal embryo development proceeds to Day 15 when the levels of total progesterone production exceed a threshold value by approximately 100 µg of total luteal progesterone; 15-day CL normally contain nearly 300 µg of progesterone (Hansel and Blair, 1996).
Luteolysis

Luteal regression is caused by a pulsatile release of prostaglandin F2α primarily from the intercaruncular region of the surface epithelium of the uterus (Asselin et al. 1996) in the late luteal phase. It is thought that the pulsatile secretion of PGF2α is generated by a positive feedback loop between luteal and/or hypophyseal oxytocin and uterine PGF2α. Regression of the CL is essential for normal cyclicity, and allows the development of a new ovulatory follicle. However, prevention of luteolysis is necessary for establishment and maintenance of pregnancy (Okuda et al., 2002).

Prostaglandin F2α secretion from the bovine endometrium varies during the estrous cycle. During the follicular phase and at estrus, Prostaglandin F2α is at its highest and then declines at early to mid-luteal phase of the estrous cycle (Kindahl et al., 1981). PGF2α is released from the uterus in a series of short pulses 2 to 3 days during and after luteolysis (Kindahl et al., 1981). Schramm et al. (1983) demonstrated that the CL is sensitive to pulsatile administration of PGF2α resulting in luteolysis. Skarzynski and Okuda (1999) demonstrated that long-lasting stimulation with PGF2α desensitizes luteal PGF2α receptors in the cow and luteolysis fails to occur.

Oxytocin released from the corpus luteum acts upon the uterus to stimulate production of PGF2α, which in turn causes luteal regression. Prostaglandin F2α has the ability to cause further release of oxytocin from the ovine corpus luteum. Oxytocin has been proven to be essential for the initiation of luteolysis in the ewe (Silvia et al., 1991). It has been shown that oxytocin administered during the early stages of the estrous cycle (Days 2-6) causes PGF2α release, which is measurable in uterine venous blood (Milvae
et al., 1980). However, the level of oxytocin in the CL, plasma and oxytocin mRNA in the CL are all low during normal luteolysis (Hansel and Blair, 1996).

It has been shown that exogenous administration of PGF2α stimulates the utero-ovarian release of PGF2α in the ewe (Wade and Lewis, 1996). Similarly, Kotwica et al. (1997) and Okuda et al. (2002) reported that administration of exogenous PGF2α increased PGF2α release from the uterus on day 18 of the estrous cycle. It has been demonstrated by Skarzynski and Okuda (1999) that PGF2α activates protein kinase C (PKC) and increases intracellular calcium mobilization, which may in turn stimulate PGF2α production in the endometrium.

It has been reported by Schams and Berisha (2002) that exposure to progesterone or inhibition of progesterone action by a progesterone antagonist in early to mid-diestrus regulates the onset of uterine release of PGF2α from endometrium causing shortening or extension of the interestrous interval in sheep and cows. Sheep that were treated with onapristone (progesterone antagonists) on day 4, 6 and 8 had a longer estrous cycle. The controls had an estrous cycle length of 17.0 ± 0.5 days while those treated with onapristone had a cycle length of 22.0 ± 0.8 days.

Endothelin-1 (ET-1), a 21 amino acid peptide is a potent vasoconstrictor originally isolated from porcine aortic endothelial cells (Yanagisawa et al, 1988). Kisanuki et al. (2001) demonstrated using gene-knockout technology in mice that ET-1 is produced primarily by endothelial cells. It has been shown to be a modulator in female reproduction where it serves to inhibit premature luteinization of follicular cells in porcine ovaries (Flores, J.A., 2000) and in the propagation of the luteolytic process in the ewe (Hinckley and Milvae, 2001).
The ET gene is transcribed to produce the preprohormone, preproET-1 (ppET-1). PreproET-1 undergoes posttranslational processing to produce big ET-1, which is further processed to mature ET-1. Big ET has little biologically active while ET-1 is the biologically active form. The final processing stage from big ET-1 to the active form, ET-1 is carried out by endothelin-converting enzyme-1 (ECE-1; Xu et al, 1994). Endothelin-converting enzyme-1 is a membrane bound protein belonging to the zinc-binding metalloendopeptidases (Xu et al, 1994), and has several isoforms (Meidan and Levy, 2002). Endothelin-converting enzyme-1 mRNA is highly expressed in steroidogenic tissues, especially in the ovaries (Xu et al, 1994).

Endothelin-1 binds two receptors subtypes, ETA (for aorta) and ETB (type B receptors; for bronchus; Meidan and Levy, 2002). These receptors belong to the seven-transmembrane G protein-coupled receptor superfamily. The ETA receptor has a higher affinity for ET-1, while ETB binds all three endothelins (ET-1, -2 and –3) equally (Meidan and Levy, 2002).

Hinckley and Milvae (2001) demonstrated that ET-1 has an inhibitory effect of on progesterone synthesis by luteal cells in the ewe. They demonstrated that ET-1 inhibited basal and LH-stimulated progesterone production by dispersed ovine cells and that inhibition was removed by pre-incubation with BQ123 (a highly specific endothelin ETA receptor antagonist). They also showed that intramuscular administration of ET-1 at midcycle reduced plasma progesterone concentrations for the remainder of the estrous cycle. It has been shown that administration of a luteolytic dose of PGF2α stimulates gene expression of ET-1 in ovine CL collected at mid-cycle, and intra-luteal
administration of BQ123 on Days 8 and 9 of the estrous cycle removes the luteolytic effect of PGF2α (Hinckley and Milvae, 2001).

Hinckley and Milvae (2001), working with ewes, supported the hypothesis that ET-1 plays an integral part in PGF2α-mediated luteolysis. Following pretreatment with a subluteolytic dose of PGF2α, intramuscular administration of ET-1 caused a rapid decline in plasma progesterone concentrations and shortened the length of the estrous cycle in the ewe (Hinckley and Milvae, 2001).

The ETA receptor gene is expressed in the two luteal cell types enriched from bovine CL and the thecal- and granulosa-derived luteinized cells of the CL in vivo. However, the endothelial cells of the CL have higher levels of ETA receptor mRNA levels than in each of the steroidogenic luteal cell types (Mamluk et al, 1999). Little is known about the ETB receptor in the CL.

In theca-derived luteal cells, LH and forskolin downregulate ETA receptors, while in granulosa-derived luteal cells, insulin-like growth factor-I (IGF-I) inhibits the ETA receptor mRNA levels (Mamluk et al, 1999). This suggests that during the early stages of luteinization, when both IGF-I and LH are at their peak levels, the expression of ETA receptor is suppressed (Meidan and Levy, 2002).

Levy et al. (2003) showed that the ECE-1 gene is expressed by both bovine ovarian endothelial and steroidogenic cells using enriched follicular and luteal cell subpopulations and in situ hybridization techniques. Different ECE-1 isoforms exist based on the different N-terminal cytoplasmic tails, and are located in different intracellular areas. The intracellular ECE-1a isoform is present only in ET-1-expressing endothelial cells (Levy et al. 2003). The membrane-bound ECE-1b isoform is expressed
in both steroidogenic and endothelial cells of the preovulatory follicle and the CL. Insulin and IGF-I upregulate ECE-1 expression when cultured with granulosa cells with concomitant increase in progesterone production while ET-1 and LH down regulate ECE-1 levels in steroidogenic cells.

The levels of ECE-1 mRNA are lowest in the bovine CL during the early stages of CL development (Days 2-4). As the CL matures (Days 7-12), ECE-1 levels increase and remain elevated in the late CL (Days 13-16). ECE-1 levels decline in the regressed CL (Days 20+; Levy et al. 2003). Levy et al. (2003) suggested the following model for ET-1 biosynthesis in the ovaries. Endothelial cells expressing ppET-1 and the two endothelin-converting enzyme-1 (ECE-1) isoforms are capable of secreting both the precursor, big ET-1 and mature ET-1. Steroidogenic cells only express the cell surface form of ECE-1, ECE-1b, without ppET-1. In order to produce ET-1, steroidogenic cells are dependent on the extracellular supply of big ET-1. Endothelial cells provide the precursor (big ET-1) and the steroid-secreting cells convert it into mature ET-1, ensuring that ET-1 is generated near its site of action, the ETA receptor, to ensure that the short lived ET-1 is active.

Involution of the Bovine Uterus

Macrosopic Changes

The postpartum interval is the period from parturition to the first postpartum estrus accompanied by ovulation. During this period, several processes take place. The uterus involutes to its previously non-gravid state, the endometrium regenerates, bacterial contaminants are cleared from the reproductive tract and ovarian cyclicity resumes.

During the process of uterine involution, there is a reduction in the size of the uterus accompanied by loss of tissue and regeneration of the endometrium. The reduction
in the size of the uterus is contributed to by strong muscular contractions that occurs every three to four minutes during the first 24 hours post partum (Moller 1970b). After calving, the uterus decreases in a logarithmic fashion in weight, length and diameter of the previously pregnant horn. The greatest changes in these parameters occur rapidly in the first few days after parturition. At calving, the uterus of a cow weighs approximately 9 kg. At Day 4 to Day 7 post partum, the uterine horns can be palpated cranial and ventral to the pelvis (Morrow et al., 1969c). Over a period of 10 days the uterus rapidly involutes to approximately 3 kg (Gier and Marion, 1968). The rapid reduction in size is accompanied by an increase in uterine tone from Day 10 to Day 14 post partum and the previously pregnant horn decreases from approximately 12 cm to 7 cm in diameter (Morrow et al., 1969c). The period of rapid involution coincided with the first estrus in normal cows and is associated with discharge of uterine lochia (Morrow et al., 1969c).

Following the rapid reduction in the size of the uterus, the rate of involution decreases. The rate of involution decreases such that the uterus weighs about 1 kg by 20 to 30 days post partum and 750 g by 50 days post partum (Gier and Marion, 1968). The length of the previously gravid horn decreases to half its length at parturition by Day 15 post partum and by Day 30 it is a third its size at parturition (Gier and Marion, 1968). The postpartum uterus decreases to half its gravid diameter by Day 5 postpartum.

Gier and Marion (1968) noted that the cervix in the postpartum cow decreases in diameter from 15 cm on Day 2 postpartum, 9-11 cm by 10 days post partum; 7-8 cm on Day 30 post partum to 5-6 cm 60 days post partum. Morrow et al. (1969c) noted that at Day 4 to Day 7 post partum the cervix was palpated cranial to the pelvis. There is an initial decrease in diameter between days 5 and 10 followed by relaxation of the cervix at
day 10 post partum when final sloughing of the caruncular masses occur (Gier and Marion, 1968). The relaxation of the cervix is interpreted on palpation and a slight enlargement and is associated with discharge of lochia from the uterus and first postpartum estrus (Morrow et al., 1969c). Involution of the cervix continues gradually until Day 30 post partum in normal cows and until Day 35 post partum in abnormal cows (Morrow et al., 1969c). Any other changes taking place after Day 30 to Day 35 post partum could not be detected by transrectal palpation.

There is a difference in the rate of reduction between the myometrium and the endometrium. During the first few days post partum, the myometrium reduces in size, while the endometrium becomes edematous during the first day post partum. Endometrial edema then resolves slowly over the next 5 days and by Days 6 to 8 post partum it has disappeared and the endometrium regresses rapidly (Gier and Marion, 1968).

Further tissue losses after Day 19 consist of reduction of blood vessels, regression of uterine glands, and contraction of tissue with reduction in cell numbers and cell volume. The caruncles further reduce in size. On Day 19 the caruncles are 15 to 20 mm in diameter. By Day 39 the caruncles appear as smooth knobs 10 to 15 mm in diameter and by Days 50 to 60 the caruncles are reduced to circular cratered cones 8 to 10 mm in diameter across the base and 4 to 6 mm across the crown (Gier and Marion, 1968). At the end of the regression changes the caruncles appear as rows of white disks in a pink endometrium (Gier and Marion, 1968). Involution of the uterus is considered to be complete by 40 to 60 days post partum, when caruncles have regressed to a smooth, oblong, epithelium-covered, avascular knob (Gier and Marion, 1968). Garcia and Larsson
(1982) reported that complete uterine involution occurred between Days 41 to 50 post partum.

Uterine lochia is a mixture of normal and degenerate cells from the mucosa, maternal placenta and blood from the shedding of the fetal placenta (Moller, 1970b). A considerable amount of blood is found in the lumen of the uterus following parturition. By Day 4 post partum the blood becomes mixed with sloughed caruncular material. By Day 12 post partum, however, the luminal content changes to a lymph-like fluid that decreases in quantity as the postpartum period progresses (Gier and Marion, 1968). This fluid (lochia) is palpable in the uterus from Days 8 to 13 post partum. Uterine lochial fluid diminishes from 1,400 to 1,600 ml at Day 2 to naught by Day 21 to 25 post partum. The amount that is seen as a vaginal discharge varies from animal to animal and is usually seen between Days 5 to 10 post partum (Moller, 1970b).

**Microscopic Changes**

In the cow, fetal cotyledons fuse with maternal caruncles of the uterine mucosa to form placentomes (Hafez, 1993). The fusing of the two tissues forms the primary anchoring system of the placenta, keeping the maternal and fetal tissues in apposition (Eiler, 1997). The secondary anchoring system consists of root-like penetration of caruncle crypts by cotyledon villi and adhesive fluid between fetal and maternal epithelium. The secondary anchoring system functions to hold fetal and maternal epithelia together for physiologic exchange (Eiler, 1997).

After parturition, the septa and crypts of the caruncles contain remnants of chorionic villi (Gier and Marion, 1968; Archbald, et al 1972). The remnants of chorioallantoic cells in the maternal crypts undergo necrosis and mineralization and are
phagocytized or expelled in the lochia and are not observed in the caruncles after Day 11 post partum (Archbald et al., 1972).

The medium-sized and small arteries of the caruncle undergo progressive vascular degeneration from Days 1 to 19 post partum (Archbald et al., 1972). Gier and Marion (1968) found that within the first 2 days post partum, caruncular blood vessels constrict and the septum and crypts undergo necrosis. The smooth muscle cells of the tunica media undergo hydropic degeneration and pyknosis and the tunica media undergoes fibrinoid necrosis (Archbald et al., 1972).

Archbald et al. (1972) noted that sloughing of the superficial areas of the caruncles began on Day 1 post partum and necrotic changes in the stratum compactum were noted on Day 5 post partum with sloughing of the superficial layer occurring by Days 6 and 7 post partum. The stratum compactum is reduced almost to the level of the inter-caruncular area by Day 15 post partum (Archbald et al., 1972). By Day 5 post partum, there is a necrotic layer 1 to 2 mm thick over the stratum compactum and cellular organization is lost leaving only blood vessels and clumps of leukocytes (Gier and Marion, 1968).

By Day 10 post partum, most of the necrotic material is removed, and, all of the caruncular mass involved in the placentomes sloughs by Day 15 post partum, leaving stubs of blood vessels extending beyond the stratum compactum. At 19 days postpartum, the arterioles within the stratum compactum and beyond disappear, leaving the caruncle relatively smooth (Gier and Marion, 1968).

The leukocytes found in clinically normal cows consist of lymphocytes and plasmacytes. Histiocytes and occasional polycytes are also found in the clinically normal
cow. In cows that have a uterine infection, organized lymphocytic nodules are found in the stratum compactum.

Tissue regeneration occurs in all areas of the endometrium. However, the earliest to show full regeneration is the inter-caruncular epithelium, which occurs by 8 days post partum (Gier and Marion, 1968). Archbald et al. (1972) reported that at no time during uterine involution was the inter-caruncular area devoid of an epithelial layer.

Tissue regeneration of the uterine epithelium begins just after parturition (Gier and Marion, 1968) with focal areas of epithelial cells found on the caruncular surface on Day 1 post partum (Archbald et al., 1972). These epithelial cells seen on Day 3 and 5 are later sloughed along with the superficial layer of the caruncle due to necrosis of the stratum compactum of the caruncle between Days 5 and 6 (Archbald et al., 1972). The epithelial cells are pleomorphic, and contain large hyperchromatic nuclei and granular cytoplasm.

Archbald et al. (1972) observed degenerated and regenerated epithelial cells in the inter-caruncular epithelium on Day 1 post partum. The degenerative cells were confined to the basal area of the epithelium and characterized by pyknosis and vacuolation of the cytoplasm. Regenerated cells were interspersed among degenerated cells and characterized by large, hyperchromatic nuclei and granular cytoplasm. Degeneration and regeneration of the epithelial cells occurred simultaneously until Day 15 when degenerative cells were no longer observed (Archbald et al., 1972).

In the event of bacterial contamination, the uterine epithelium may be totally or partially destroyed (Gier and Marion, 1968). Re-epithelialization of the caruncle is complete by Day 25 post partum, 10 days after obvious sloughing is complete (Gier and
Marion, 1968). Archbald et al. (1972) found that a layer of epithelial cells covered the entire caruncular surface by Day 19 post partum.

The epithelial cells of the uterine endometrial glands exhibit a pattern of simultaneous degeneration and regeneration similar to that observed in the intercaruncular area (Archbald et al., 1972). Microscopic changes in the myometrium begin on Day 3 post partum and progress to Day 27 post partum. These changes include granular degeneration of the sarcoplasm, vacuolation of the muscle cell and atrophy of the nucleus. By Day 31, the fibers appear normal and the myometrium is greatly reduced in size in comparison to that in the early postpartum period. Necrosis of the muscle fibers was not observed during postpartum myometrial regression (Archbald et al., 1972). Prior to Day 3 post partum the muscle fibers of the uterus contract from 750-800 µm to 400 µm and by Day 3 are approximately 200 µm (Moller, 1970b). These changes in the size of the muscle fibers have been attributed to glycogen formation leading to degeneration and absorption (Moller, 1970b).

**Factors Affecting Uterine Involution**

Parity has been shown to influence the interval from parturition to complete uterine involution. Marion et al. (1968) found that the average interval from parturition to uterine involution was significantly shorter in primiparous cows (34.0 days) compared to pluriparous cows (40.6 days). Based on rectal palpation, Morrow et al. (1969c) noted that multiparous cows of six lactations or more had larger uteri than primiparous cows, and took a longer time to involute. However, Moller (1970a) and Miettinen (1990) found no difference in the rate of uterine involution between primiparous and multiparous cows.

Cervical involution is influenced by parity. Miettinen (1990) found that there was a difference in the size of the cervix with the cervix being larger in multiparous cows.
However, there was no difference in the time the cervix took to involute. In contrast, Otlenacu et al. (1983) found that involution of the cervix occurred earlier in primiparous than in multiparous cows. Based on rectal palpation Morrow et al. (1969c) noted that multiparous cows of six lactations or more had a larger cervix than primiparous cows, and took a longer time to involute. On Day 10 and Day 20 post partum there was a significant difference of 1.2 and 1.0 cm in the diameter of the cervix. However, by Day 30 post partum the cervix was of similar sizes between 3.2 and 4.1 cm in diameter and by Day 50 post partum the cervix was between 2.9 and 3.3 cm in diameter.

Otlenacu et al. (1983) noted that the time for complete involution of the cervix in cows with a normal discharge was less than that for cows with an abnormal uterine discharge. The greatest difference in cervix diameter between cows with normal and abnormal discharge was 10 mm at three weeks postpartum (Otlenacu et al. 1983).

Time of year, or season, has been shown to influence the rate of uterine involution. Cows calving in the spring and summer have shorter intervals from parturition to uterine involution than cows which calve in the winter, regardless of parity (Marion et al., 1968). Cows calving in the summer have variable intervals from parturition to uterine involution. Marion et al. (1968) found that in cows calving in the summer with an ambient temperature of $\geq 38^\circ$C, the heat stress increased the interval from parturition to uterine involution.

Retained fetal membranes have been shown to increase the interval from parturition to the completion of uterine involution. Marion et al. (1968) found that it required 50.1 ± 4.9 days for the uterus to involute in pluriparous cows with retained fetal membranes, and 45.1 ± 3.2 days for primiparous cows. It was also noted that the rate of
uterine involution was affected more in primiparous cows than in pluriparous cows with retained fetal membranes (Marion et al, 1968).

Morrow et al. (1969c) defined an abnormal cow as a cow that experienced any of the following: abortion, dystocia, twins, retained fetal membranes, metritis, milk fever, acute mastitis, ketosis or other debilitating disease. Abnormal cows had larger uteri in the early postpartum period, especially cows with retained fetal membranes and metritis. At Day 10 and Day 20 post partum, there was a significant difference of 1.3 cm and 0.8 cm respectively between normal and abnormal cows. Abnormal cows had a combined diameter of both uterine horns on rectal palpation of 14.9 cm on Day 10 and 8.4 cm on Day 20 post partum, respectively. The gross palpable involution of the uterus was notable up to Day 25 post partum in normal cows, and Day 30 post partum in abnormal cows. Any changes that occurred after Day 25 and Day 30 post partum could not be felt on transrectal palpation (Morrow et al., 1969c).

Metabolic disturbances can have a negative effect on uterine involution. Cows or heifers that experience hypocalcaemia have a reduction in uterine contractions (Roberts, 1989; Al-Eknah and Noakes, 1989) and rate of uterine and cervical involution (Fonseca et al., 1983). Kamgarpour et al. (1999) investigated subclinical hypocalcaemia in Friesian cows. Subclinical hypocalcaemia was defined as occurring if the plasma total calcium (PTCa) fell below 2.0 mmol/L. Cows calving in the winter and experiencing subclinical hypocalcaemia took longer to complete uterine and cervical involution in comparison to normocalcaemic cows, with hypocalcaemic cows taking 6 more days to reach a mean size. Similar findings were noted in cows that calved in the summer and experienced subclinical hypocalcaemia.
The bovine uterus is invaded and colonized by bacteria within the first two weeks postpartum (Elliot et al., 1968; Griffin et al., 1974). In most cases the uterus clears this infection by the third or fourth week post partum. Not all cows clear the bacterial infection and subsequently develop endometritis. Endometritis has been shown to delay uterine and cervical involution. Endometritis delays the resumption of ovarian cyclicity, increases the interval from calving to ovulation and delays the return of prostaglandin F$_{2\alpha}$ metabolite (PGFM) to basal levels (Lindell et al., 1985; Del Vecchio et al., 1992). Endometritis accounts for approximately 20% of reproductive disorders in postpartum dairy cows (Coleman et al., 1985). Moller (1970a) examined the rate of uterine involution between milked cows and cows suckling three or four calves and found that there was no difference in the rate of involution between milked and suckled cows.

Steroid hormones have variable effects on the interval from parturition to uterine involution. In cattle, the first postpartum dominant follicle develops on the ovary contralateral to the previously gravid uterine horn. However, the presence of an estradiol-secreting dominant follicle in the ipsilateral ovary is a marker of subsequent fertility (Sheldon et al., 2000a). Based on their findings, Sheldon et al. (2000b) attempted to promote a dominant follicle on the ipsilateral ovary using equine chorionic gonadotrophin (eCG). Cows treated intramuscularly with 250 iu eCG or 750 iu eCG on Day 14 post partum showed no difference in the rate of uterine involution compared with those treated with a placebo. Treatment with eCG, or the presence of a follicle > 8 mm in diameter in the ovary ipsilateral to the previously gravid uterine horn, did not affect the rate of uterine involution (Sheldon et al. 2000b). The intramuscular administration of estradiol benzoate approximately 48 hours after calving had no effect on the intervals
from calving to the completion of involution or between the intervals from calving to the first ovulation (Tian and Noakes, 1991).

Sheldon et al. (2003) infused estradiol benzoate into the previously gravid uterine horn on Days 7 and 10 postpartum, and monitored uterine involution by ultrasonography. There was no effect of estradiol treatment on the diameter of the previously gravid or nongravid uterine horns. They concluded that utero-ovarian signaling in the direction from the uterus to the ovary may be more important during the postpartum period.

Administration of oestradiol into the uterine lumen increased uterine pathogenic anaerobic bacterial contamination (Sheldon et al., 2004). Estradiol benzoate was infused into the previously gravid uterine horn on Days 7 and 10 postpartum. Animals treated with estradiol benzoate had higher bacterial load on Day 14, than on Days 7 or 21, attributable to pathogens associated with endometritis, *Prevotella melaninogenicus* and *Fusobacterium necrophorum*.

These finding by Sheldon et al. (2000a, 2000b, 2003, 2004) and others indicate that estradiol administration in the early postpartum period had no effect on uterine involution (Tian and Noakes, 1991) and may be detrimental by promoting endometritis (Sheldon et al 2004). More first postpartum dominant follicles are selected in the ipsilateral ovary when there is a lower uterine bacterial load (Sheldon et al., 2002).

Exogenous progestogens have been administered to cows in an attempt to improve uterine involution. Melengestrol acetate (MGA) was feed to Holstein cows at 1 mg per cow per day for 14 days beginning 14 to 18 days post partum in one group of cows. In a second group MGA was fed in a similar dosage and 500 micrograms of estradiol benzoate was administered intramuscularly 48 hours after the last feeding of MGA. The
third group consisted of control cows that received no treatment. Based on rectal palpation, there was no significant difference in the rate of involution (Britt et al., 1974).

Subcutaneous administration of 0.05 mg and 1.0 mg estradiol 17β given every other day from Day 3 post partum was shown to have no effect on the rate of involution. The oral administration of 300 mg medroxyprogesterone acetate every other day from Day 3 postpartum had no effect on the rate of uterine involution. However, daily administration of 30 mg of progesterone has been shown to increase the time for the uterus to involute completely in both intact and ovariectomized cows (Marion et al., 1968). Removal of ovarian hormone by ovariectomy has been shown to significantly reduce the time for uterine involution in pluriparous cows (Marion et al., 1968).

The administration of prostaglandin F2α has been shown to decrease the time for complete involution of the uterus as detected by rectal palpation (Lindell and Kindahl, 1983). Lindell (1982) demonstrated that there is a massive release of PGF2α postpartum, which continues for 2 to 3 weeks. It was also deduced from this study that cows that had a shorter interval from parturition to uterine involution had a longer period of postpartum PGF2α release.

In a study by Tian and Noakes (1991) five groups of five cows were treated intramuscularly with a single dose of either 100mg progesterone in oil, 25 mg dinoprost tromethamine (PGF2α tromethamine), 5 mg oestradiol benzoate, 1.2 mg long-acting oxytocin analogue, carbonectin or 5 ml sterile water 48 hours after parturition. There was no statistical difference in the rate of uterine involution.
Methods to Assess Uterine Involution

Uterine involution may be studied by observing the reduction in the size of the vulva, vagina, cervix and the uterus (body and horns). These observations may be made using the following: 1) palpation per rectum of the cow’s reproductive tract from calving to completion of uterine involution (Marion et al., 1968; Moller, 1970a; Garcia and Larsson 1982); 2) removal of the reproductive tracts at slaughter at predetermined intervals from calving (Gier and Marion, 1968; Moller 1970a); 3) transrectal ultrasonography of the reproductive tract (Mateus et al., 2002; Wehrend et al., 2003; 4) cervical forceps (Wehrend et al., 2003)

Garcia and Larsson (1982) performed rectal palpation of the cervix, uterus and ovaries. They assessed uterine size, tone, symmetry and location of the uterus in pelvic cavity as indicators of uterine involution. Uterine involution was considered complete when the uterus was in the normal position in the pelvic cavity, the uterine horns were equal or almost equal in size and there was no enlargement in the thickness of the uterine wall. Marion et al., (1968) used rectal palpation twice weekly to monitor uterine involution, using criteria set by Gier and Marion (1968) as a marker for complete uterine involution.

Moller (1970b) refers to Rasbech’s (1950) four-stage sequence in involution, which can be followed by rectal palpation. During the first stage (1-8 days post partum), the vagina can be palpated as a band approximately 8 cm in width within 24 hours post partum. The cervix is not distinguishable until Day 3 and by Days 4 and 5 has enough tone to be distinguished from the uterus and is usually located at the anterior edge of the pelvic floor. The surface of the uterus feels hard and corrugated and the uterine caruncles can only be felt through the uterine wall when it is relaxed. In the second stage (8-10 days
post partum) the entire uterus can be palpated. The surface is smooth and soft with fluctuation in the post-gravid horn. The caruncles are palpable as hazel-nut shaped structures and the cervix is firm and lies within the pelvic cavity in younger animals. During the third stage (10-18 days post partum) the uterus feels like a soft plastic body; caruncles and fluctuations are less pronounced; the cervix is firm and continues to decrease in size until it is similar to that of the post-gravid uterine horn. During the fourth stage (18-25 days post partum) there is an increase in uterine tone and the previously gravid horn reduces to a similar size to the non-gravid uterine horn.

Moller (1970b) reports that authors on the subject of uterine involution agree that the previously pregnant horn rarely returns to its pre-gravid size. Although the uterus continues to undergo involutional changes after 25-30 days (Gier and Marion, 1968), these changes are small and difficult to detect by rectal palpation (Moller, 1970b). However, Marion et al. (1968) concluded that uterine involution monitored by rectal palpation take 40 days.

Gier and Marion (1968) used reproductive tracts collected from slaughtered cows to assess involutional changes. They removed the excess fat and vagina from the cows to access the changes that occur during the process of involution. The remainder of the tract was then measured. The weight, length and diameter of the previously pregnant uterine horn were used to access the rate of regression. The diameter of the cervix was measured to assess uterine involution.

Wehrend et al (2003) used a combination of ultrasonography and cervical forceps to measure the rate of uterine and cervical involution. Cervical forceps were positioned in the cervical canal and the position verified by ultrasonography. The distance of the legs
of the forceps was proportional to the opening of the tip of the forceps. A gauge table was used to determine the degree of the opening of the intracervical tip of the forceps by measuring the distance of the extracorporeal legs of the forceps.

**Endocrinology of the Immediate Postpartum Period in the Cow**

The corpus luteum of pregnancy secretes progesterone throughout pregnancy and regresses about 2 days prior to parturition (Garverick and Smith, 1993). The placenta also contributes progesterone in the latter part of pregnancy. At the end of pregnancy there is a rapid increase in the secretion of estrogen (Hoffmann and Schuler, 2002). Prior to calving, progesterone and estrogen concentrations are high (Hoffmann and Schuler 2002).

The progesterone concentration begins to decrease about two days prior to parturition and is low following calving. Following parturition, there is a rapid decline in circulating concentrations of estrogen. The fall in progesterone and estrogen removes the inhibitory block on the hypothalamic-pituitary axis (Garverick and Smith, 1993; Noakes, 1996). The inhibitory effects of estradiol are thought to operate through inhibition of the expression of mRNA coding for the common \( \alpha \)-subunit and specific \( \beta \)-subunit of FSH and LH (Garverick and Smith, 1993). Messenger RNA expression of the subunits is low following parturition and increases thereafter.

During gestation, LH is secreted in a pulsatile manner. However, the pulse frequency and amplitude decrease as gestation progresses. Pituitary LH content and plasma LH concentrations are low shortly after calving and generally increase during the postpartum period (Schallenberger et al., 1982; Garverick and Smith, 1993). Short-term episodic surges of LH increase as time post partum increases and LH responsiveness to
GnRH is low at parturition and increases as time post partum increases (Garverick and Smith, 1993). The frequency of the pulsatile LH secretory pattern increases just prior to the first ovulatory surge of LH (Garverick and Smith, 1993).

Ovariectomy in the early postpartum cow has a profound effect on the tonic secretion of gonadotropin from the pituitary. Schallenberger et al. (1982) showed that ovariectomy performed in the early postpartum period caused a two-fold increase in mean LH values as well as the amplitude and frequency of pulsatile release. A similar response was seen for FSH. However, the increase in amplitude was less pronounced than that seen for LH.

Following parturition, FSH concentration is within normal range (Garverick and Smith, 1993). Schallenberger et al. (1982) noted pulsatile FSH release during the first day post partum. The frequency of pulsatile LH release coincided with FSH pulses. However, there were additional pulses between the FSH and LH pulses that coincided during the early postpartum period (Schallenberger et al., 1982). Increasing progesterone concentrations caused an immediate suppression of frequency and basal LH, but not FSH secretion. This is similar to that seen during normal cyclicity (Schallenberger et al., 1982).

The administration of estrogen has no effect on LH secretion on Days 0 and 5 post partum. However, by Day 10 post partum and beyond, administration of estradiol elicited an LH surge with increasing magnitudes within 12 to 24 hours. Cows possessing a functional CL did not respond to estrogen with an LH surge. However, this was not the case for FSH. FSH surges also occurred in cows at 5 days post partum (Schallenberger et al., 1982).
Schallenberger et al. (1982) showed that first calf heifers could be induced to cycle early in the post partum period when GnRH is administered on an hourly basis at 500 micrograms followed by challenge with 1mg estradiol-benzoate.

It has been hypothesized that the following sequence of endocrine events occur in the normal cow after calving based on studies carried out by themselves and others (Peters and Lamming, 1986). Gonadotropin releasing hormone (GnRH) is secreted from the hypothalamus immediately after calving. However, the quantity or frequency of secretion is inadequate to cause sufficient release of LH and FSH from the pituitary to restore normal ovarian cyclicity. The concentration of FSH rises quickly after parturition and stimulates follicular development. The plasma concentration of LH and the frequency of LH pulses increase gradually with time post partum. Secretion of LH and FSH stimulates follicular growth and estradiol production. There is a gradual recovery in the positive feedback mechanism such that by two weeks postpartum normal ovarian cyclicity returns.

**Resumption of Ovarian Cyclicity Post Partum**

The interval from parturition to first observed estrus ranges from 30 to 76 days in the dairy cow (Roberts, 1971). However, observation of estrus may not be a reliable method to estimate the resumption of ovarian activity since some cows experience “silent” estrus. As time progresses post partum, the percentage of cows in which silent estrus occurs decreases (Roberts, 1971). Other methods of determining the onset of cyclicity have evolved which are more accurate. The milk progesterone assay determines the resumption of ovarian cyclicity by the presence of elevated progesterone concentrations (Noakes, 1996). Using milk progesterone concentrations, Bulman and Wood (1980) determined that approximately 50 percent of cows resumed normal
cyclicity by 20 days postpartum and greater than 90 percent by 40 days post partum
(Noakes, 1996).

Ultrasonography has been used to follow the development of ovarian follicles in
the post partum cow. Sheldon et al. (2002) detected follicular development by 7 to 10
days post partum with emergence of a dominant follicle within 14 days of parturition and
ovulation of this dominant follicle by 17 to 18 days post partum. This interval was
increased in cows with high milk production, in cows nursing calves or being milked four
times a day, in cows on a poor or on low plane of nutrition and in older cows with greater
than four parturitions (Roberts, 1971).

In cows with a normal postpartum period, a mature follicle develops and ovulates
with subsequent development of a CL by Days 13 to 15 after calving (Roberts, 1971).
The first estrous cycle is usually shorter than the normal 20 to 21 days (Roberts 1971).
Terqui et al. (1982) found that the earlier ovarian activity occurs the more likely cows
will experience a short luteal phase. The CL associated with the short estrous cycle has a
short life span as a result of lack of luteotropic support, failure of the luteal tissue to
recognize a luteotropin, or enhanced secretion of a luteolytic agent (Hafez, 1993).

The first ovulation post partum usually occurs on the contralateral ovary.
Transrectal palpation of the ovaries has shown that approximately 90 percent of corpora
lutea formed within 15 days post partum occur on the ovary opposite the previously
pregnant horn and 60 percent in cows ovulating between 15 and 20 days after parturition
(Roberts, 1971). This has been substantiated by sequential transrectal ultrasonography.

The first postpartum dominant follicle is usually found on the ovary contralateral to
the previously pregnant uterine horn, that is, opposite the ovary containing the CL of
pregnancy (Foote et al., 1968; Kamimura et al., 1993; Nation et al., 1999). This led many to speculate that the CL of pregnancy may have a local inhibitory effect on folliculogenesis. Dufour et al. (1985) concluded that the CL of pregnancy and/or the conceptus have a carry-over effect on the rate of growth of the antral follicles even after parturition. Bellin et al. (1984) found that the ovary with the CL of pregnancy had smaller follicles and lower follicular estrogen concentrations than those of the ovary without a CL. It was also noted that only 18% of the follicles on the ovary with a CL were healthy compared to 48% of follicles in the ovary without a CL (Bellin et al., 1984). However, recent work done by Sheldon et al. (2002) suggested that the CL of pregnancy does not have a local inhibitory effect on postpartum folliculogenesis. It was further suggested that the previously pregnant uterine horn shortly after parturition may play more of a role.

It has been shown that the presence of a large follicle on the ovary ipsilateral to the previously pregnant uterine horn within 4 weeks of parturition was associated with improved subsequent fertility (Sheldon et al., 2000). Bridges et al. (2000a) reported similar findings in beef cows, where less cows ovulated from the ipsilateral ovary. However, ovulation from the ipsilateral ovary tended to increase fertility (Sheldon et al., 2000a).

There are several factors that have a negative impact on the resumption of ovarian cyclicity in dairy cows. The interval from calving to first ovulation increased in cows with high milk production, cows nursing calves or being milked four times per day, cows on a poor or on low plane of nutrition, and in older cows with greater than four...
parturitions (Roberts, 1971). Bellin et al. (1984) found that suckled cows had smaller and fewer follicles and lower follicular concentrations than nonsuckled cows.

There is a close association between the time to first ovulation and negative energy balance in dairy cows (Zurek et al., 1995). The exact mechanism by which energy balance influences reproduction is not completely understood. However, one mechanism may be through the suppression of GnRH and the LH pulse frequency required for follicles to grow to the preovulatory stage (Schillo, 1992).

**Cystic Ovarian Disease in the Dairy Cow**

Cystic ovarian degeneration, ovarian follicular cysts, cystic ovarian disease, ovarian cysts and cystic ovaries are terms used to describe the same condition. Ovarian cysts are follicles that fail to ovulate at the time of estrus (Garverick, 1999), and commonly occur in lactating dairy cows. They can also occur in beef cows and dairy heifers. Ovarian cysts have been defined as follicular structures 2.5 cm or greater in diameter that persists for an extended period of time in the absence of a CL (Garverick, 1999). Ovarian cysts can be classified as follicular cysts or luteal cysts. Follicular cysts are thin walled, may be single or multiple and affect one or both ovaries (Elmore, 1986). Higher concentrations of estradiol are usually found in follicular cyst fluid than in fluid from luteal cysts and normal follicles (Odore et al, 1999). Luteal cysts are thick-walled, usually occurring singly, and affect one ovary and have higher concentrations of progesterone in their cystic fluid than follicular cysts and normal follicles (Odore et al, 1999).

Bane (1964), in a review of fertility and reproductive disorders in Swedish cattle, quoted Henricson reporting that the risk of ovarian cysts increased from 0.3% for heifers to 8-10% for cows four to five years old and that Henricson found the incidence of ovarian cysts was higher in the winter months than in the summer months. Bane analyzed
the frequency of ovarian cysts in relation to the month of calving and found that cows calving in the winter had a higher risk of developing ovarian cysts in the following reproductive period (Bane, 1964). Lopez-Gatius et al. (2002) indicated that ovarian cysts occur most commonly in cows calving in the summer. Garverick (1999) estimated the incidence of ovarian follicular cysts between 10 –13% in the United States.

The calving date influences the frequency of ovarian cysts. Cows that calved in April-May were less likely (2.2%) to develop ovarian cysts while cows calving in October had a higher frequency (9%) of ovarian cysts (Bane, 1964).

In Sweden between 1954 and 1961, the frequency of animals with ovarian cysts in five-year-old cows fell from 10.8 % to 5.1%. This was brought about by the culling of bulls with a high frequency of cystic ovarian degeneration among their daughter (Bane, 1964) suggesting that there may be a hereditary aspect.

Spontaneous recovery occurs in some cows that develop ovarian cysts. López-Gatius et al. (2002) found that in cows diagnosed with cysts 43-49 days postpartum, only 38.8% recovered spontaneously while in cows diagnosed with cysts 57-63 days postpartum, 71.4% recovered spontaneously. Erb and White (1981) reported that the highest incidence of ovarian cysts occurs in the first 60 days of lactation, while Morrow et al. (1969b) reported that there was a peak in incidence between 14 and 40 days post partum.

**Predisposing Factors**

López-Gatius et al. (2002) reported that ovarian cysts occur most commonly in cows calving in the summer, in high milk producers, in older cows, and in cows that body condition score increased during the prepartum period. The major risk factor for the presence of cysts at the time of insemination was the development of cysts early (43-49
days postpartum) in the postpartum period (López-Gatius et al., 2002). The risk of having a cyst at the time of insemination was 36.6 times higher in cows with early cysts (López-Gatius et al., 2002).

**Pathogenesis**

Postpartum uterine infections may increase secretion of PGF2α and cortisol associated with the formation of cystic ovaries in dairy cows (Bosu and Peter, 1987; Peter et al., 1989). In a study carried out on cows that calved normally but subsequently developed ovarian cysts, a correlation between postpartum uterine infections and the development of ovarian cysts was made. In 88.9% and 11.1% of cows that subsequently developed ovarian cysts endometrial swabs yielded bacterial growth densities of +3 and +2 respectively, while 35.1% of cows that did not develop ovarian cysts yielded bacterial growth densities of +1 and +2 (5.4%) (Bosu and Peter, 1987). Further, consistently high cortisol levels were detected prior to cyst detection and in association with high bacterial growth densities in the uterus prior to cyst detection (Bosu and Peter, 1987).

Cysts have been experimentally induced using anti-LH serum, estradiol valerate and adrenocorticotropic hormone (ACTH) administration. Cysts have also been induced by daily exogenous injection of 30 mg of estradiol-17β and 150 mg of progesterone dissolved in alcohol for 7 days (Cook et al., 1990; Garverick, 1999).

Ovarian cysts have been induced by ACTH administration in the preovulatory period. Adrenocorticotropic hormone has been shown to block the preovulatory LH surge, possibly through cortisol-mediated inhibition of LH release (Refsal et al, 1987). The mode of action of opioids is by inhibiting the release of hypothalamic GnRH. Through their action on GnRH, they are thought to suppress LH secretion from the
pituitary in the prepubertal period and modulate LH during the estrous cycle. It has been established that gonadal steroids suppress LH secretion by negative feedback on the hypothalamic-pituitary axis, and this action may be brought in part by intermediate opioidergic neurons (Brooks et al., 1986).

It has been demonstrated that follicular cysts can be induced using estradiol benzoate by stimulating a GnRH/LH surge in the absence of a preovulatory follicle. Subsequent exposure of the cystic cow to progesterone can resolve the cystic condition by reinitiation of GnRH/LH surges in response to estradiol (Gümen et al, 2002).

Gümen and Wiltbank (2002) demonstrated in cows that an initial GnRH/LH surge and subsequent ovulation could be induced with high levels of estradiol, but estradiol induction of a subsequent GnRH/LH surge required exposure to progesterone. Follicular cysts were induced using an intravaginal progesterone insert (IPI)/PGF2α/estradiol benzoate (EB) protocol, and indicated that the effect is mediated at the level of the hypothalamus (Gümen and Wiltbank, 2002).

Odore et al., (1999) suggested that ovarian cysts might be due to alterations in the feedback regulatory mechanism. This hypothesis was based on findings that indicated a difference in the concentration of luteinizing hormone receptors and follicle stimulating receptors in the ovary and pituitary between normal and cystic cows.

Silvia et al. (2002) proposed the following model for how intermediate levels of progesterone could lead to the development of ovarian follicular cysts in the dairy cow. During the follicular stage in cows without ovarian cysts, the tonic center of the hypothalamus secretes GnRH at a high frequency stimulating a frequency mode of LH secretion by the anterior pituitary. The high frequency LH secretion promotes maturation
of the dominant follicle. This follicle in turn secretes increasing concentrations of
estradiol that eventually reach a threshold adequate to trigger the surge center of the
hypothalamus to release GnRH in quantities that will trigger a preovulatory LH surge and
induce ovulation of the dominant follicle. However, in cows with ovarian cysts, the
intermediate progesterone levels make the surge center of the hypothalamus insensitive to
estradiol. The preovulatory GnRH/LH surge is blocked and ovulation fails to occur.
However, the tonic center of the hypothalamus is unaffected by intermediate
progesterone concentrations and thus the high frequency, tonic pattern of LH is
maintained. This tonic pattern of LH provides the cysts with the gonadotrophic support it
requires to function (Silvia et al., 2002).

**Cyst Dynamics**

Ovarian cysts are dynamic structures. They regress and are replaced with other
cystic structures (turnover), or spontaneous recovery occurs characterized by ovulation of
a new follicle at a site different from that of the original cysts (Cook et al. 1990). Cook et
al. (1990), induced ovarian cysts with twice daily subcutaneous injections of 15 mg
estradiol-17β and 37.5 mg of progesterone for 7 days starting on Day 15 post partum. Of
the 23 cows that became cystic, 7 ovulated (spontaneous recovery), 13 showed cystic
turnover and 3 persisted (Cook et al. 1990). In no case did the marked cyst ovulate. In
another study carried out on cows that calved normally over a one-year period, 12 out of
47 cows were found to be cystic. Follicular cysts were detected on the ovary as early as 8
days post partum. In 10 cows, serial rectal and ultrasound examination of the cysts
indicated an increase in size confirming the dynamic nature of ovarian cysts (Bosu and
Peter, 1987). In 4/12 cows (33.3 %) the cyst regressed before Day 28 post partum and in
the remaining 8/12 cows (66.6%) the cyst regressed between Days 29 and 57 post partum (Bosu and Peter, 1987). In a study of 29 heifers undergoing daily transrectal ultrasound in order to follow follicular dynamics, one heifer developed follicular cysts. This heifer regressed the corpus luteum in a normal manner, developed a normal preovulatory size follicle on the ovary, exhibited standing heat, but the follicle failed to ovulate. This follicle lost follicular dominance and a new follicular wave emerged. Two dominant follicles emerged from this wave. When one reached preovulatory size, the heifer once again exhibited estrus but did not ovulate. This process continued through a third follicular wave and finally the heifer was successfully treated using the Ovsynch protocol after emergence of the fourth follicular wave (Wiltbank et al., 2002).

**Diagnosis**

Due to the dynamic nature of ovarian cysts, weekly rectal palpation and/or ultrasonic observations will detect those cows with cysts more readily than monthly or bimonthly examinations. The diagnosis of ovarian cysts is usually based on rectal palpation of a fluid filled structure greater than 2.5 cm in diameter on one or both ovaries in the absence of a CL. The diagnosis may be confirmed using transrectal ultrasonography and the type of cysts (luteal or follicular cysts) determined. Luteal cysts may also be confirmed by progesterone assay of milk or serum. The most common sign of cows that have been diagnosed as cystic is a lack of estrus (Farin and Estill, 1993). Nymphomania has also been noted in cows that have follicular cysts (Farin and Estill, 1993).

In order to differentiate between cystic follicles and a large preovulatory follicle, the characteristic of the uterus must be evaluated. On rectal palpation a diagnosis of cystic ovaries will be made when the uterus has no tone and is unresponsive to
manipulation. The uterus of a cow in estrus has tone and responds to manipulation by increase coiling of the horns.

**Treatment**

Although cows with ovarian cysts have been shown to recover spontaneously, only a portion of cows affected with cystic ovarian disease recovers spontaneously and within a time frame that is economical to the dairy farmer. Morrow et al (1966) reported that only 48% of cows affected with follicular cysts recovered spontaneously. Kesler and Garverick (1982) estimated that even less cows (approximately 20%) with cystic ovaries will spontaneously recover and return to normal cyclicity.

One of the earliest methods of resolving ovarian cysts was manual rupture via rectal palpation. Repeated manual rupture of cysts at 6 to 10 day intervals, especially follicular cysts in the early postpartum period, has produced 37% (Roberts, 1971) to 45% recovery rates (Kesler and Garverick, 1982; Ijaz et al., 1987). However, manual rupture can cause injury to the tissue of the ovary and its surrounding structures, causing hemorrhage, promoting adhesions and infertility (Roberts, 1989; Elmore, 1986).

Gonadotropin-releasing hormone (GnRH) is commonly used to treat ovarian cysts regardless of the type of cysts. Exogenous administration of GnRH triggers an LH surge that results in ovulation of a LH-responsive follicle at the time of treatment or luteinization of the cyst (Kittok, et al. 1973; Cantley et al. 1975). Repeated use of GnRH intravenously 120 minutes apart has been shown to cause a LH surge similar to the preovulatory LH surge in normal cycling cows; the second dose corresponding to peak LH serum concentrations in cows with cystic follicles (Kittok et al. 1973). The LH surge is induced with 30 minutes of GnRH administration and remains elevated for 4 hours (Cantley et al. 1975). However, the response of the cow with ovarian cysts differs from
that of cows without ovarian cysts that have an elevated LH concentration for up to 10 hours which may due to luteinization of the cystic structure as a result of ovarian response to GnRH induced LH release (Cantley et al. 1975). Cow treated with GnRH have been shown to return to estrus within 20 to 24 days (Kittok et al. 1973) after treatment. Cows with ovarian cysts treated with a single intramuscular injection of either 50, 100 or 250 µg GnRH exhibited estrus by 22 days and most cows exhibited estrus 18 to 23 days post-treatment (Bierschwal et al. 1975). The 100 µg dosage had the best response of the three dosages with 82% returning to estrus and 87% of those that returned to estrus conceiving (Bierschwal et al. 1975).

In order to shorten the interval between GnRH treatment and estrus, PGF2α has been used 9-14 days after GnRH treatment (Neil, 1991). However, the need for estrus detection has been eliminated with the introduction of the Ovsynch protocol (Pursley, 1995). This protocol uses a GnRH/PGF2α/GnRH treatment scheme which has proven successful in treating cystic ovarian degeneration (Bartolome et al., 2000; Gumen et al., 2003).

Human chorionic gonadotropin (hCG) is used for its high luteinizing hormone activity (Kesler and Garverick, 1982). It has the same effect as GnRH. However, hCG has the ability to induce antibody production where as GnRH because of its small molecular size is not likely to induce an immune response (Bierschwal, et al. 1975). A dose of hCH between 2,500 and 10,000 IU, given intramuscularly or intravenously will have the same effect as GnRH (treatment response and post treatment fertility). However it can cause undesirable effects (Neil, 1991)
Progesterone has been shown to resolve ovarian cysts. Methods of administering progesterone have included injections, intravaginal devices or ear implants. Zulu et al. (2003) investigated the use of a progesterone releasing intravaginal device (PRID) in the treatment of cows with ovarian cysts. Their study confirmed that follicular and luteal cysts could be successfully treated with PRID; resulting in ovulation 2-4 days after removal of the device with subsequent formation of a CL.

Prostaglandin F2α (PGF2α) has been used alone in the treatment of ovarian cysts (Chavatte et al., 1993). It is the treatment of choice for luteal cysts or cysts containing luteinized tissue. Luteal cysts respond by regression with subsequent follicular development and estrus in 2-5 days (Kesler and Garverick, 1982; Neil, 1991). It has been shown that there is a higher PGF2α receptor concentration in luteal cysts than in follicular cysts (Odore et al., 1999) and this may explain the success of prostaglandin treatment. Prostaglandin F2α is generally believed to be ineffective in follicular cysts, but some cows with progesterone as low as 0.5 ng/ml will respond positively (like luteal cysts; Neil, 1991).

**Timed Artificial Insemination Programs in the Dairy Cow**

One of the factors affecting pregnancy rate in dairy herds is the detection of estrus. In some instances estrous behavior is reduced. Reduced estrus behavior has been noted in cows treated with bovine somatotropin (bST) (Kirby et al., 1997). It has been shown that the physiological state of lactation is associated with lower concentrations of estradiol during proestrus than levels found in non-lactating dairy cows and thus behavioral estrus is reduced in these cows. During lactation progesterone levels are also
lower than in non-lactating dairy cows. In addition it has been shown that cows in lactation have a lower reproductive rate than do heifers.

Heat stress is another factor that decreases the expression of estrus behavior (Abilay et al., 1975; Nebel et al., 1997). However, lack of heat detection can play a major role in reducing pregnancy rate. The development of programs for estrus synchronization and timed insemination have eliminated the need for estrus detection, and have been shown to increase conception rates to artificial insemination programs. It should be noted however, that pregnancy rate is affected by a number of factors, such as anestrus, low conception rates and increased embryo mortality. In many regions of the world another factor which influences the dairy cow, especially high producing dairy cows is heat stress.

In an attempt to return the high producing lactating dairy cow to normal reproductive function, various pharmacological programs have been devised. These programs manipulate ovarian function. The primary goal of estrous synchronization programs is to synchronize estrus and ovulation effectively, such that cows may be inseminated at a predetermined time without estrus detection and reduction in fertility (Rathbone et al., 2001).

Several synchronization protocols have developed over the years for synchronization of estrus. The basis of these protocols rely on one of the following: 1) regression of the CL with PGF2α or its analogue with the cows returning to estrus and ovulating within 2 to 4 days of the (final) injection of PGF2α; 2) the use of exogenous progesterone or synthetic progestins to prevent estrus and ovulation for a long enough period to allow for regression of the CL; upon removal of the exogenous progesterone or
synthetic progestins the cows should return to estrus and ovulate (Rathbone et al., 2001). Although these pharmacological substances can be used alone, the conception rates were found to be low and thus they have been used in combination with other reproductive drugs to increase conception rates to timed insemination.

**Prostaglandin F2α**

Prostaglandin F2α given randomly during the estrous cycle between Days 5 and 16 will cause luteolysis of the CL and cows should exhibit estrus between 2 and 4 days post treatment. The variability in return to estrus and ovulation depends on the stage of the dominant follicle at the time of luteolysis. If luteolysis occurs when the dominant follicle is viable, then estrus and ovulation will occur in a relatively short period from treatment. However, if luteolysis occurs when the dominant follicle is nonresponsive, then the dominant follicle of the following wave will grow and become the ovulatory follicle and the interval between treatment and ovulation will be longer (Kastelic et al., 1990). For these reasons the use of one luteolytic dose of PGF2α with insemination at a fixed time yielded low conception rates.

**Combination of Gonadotropin-releasing Hormone (GnRH) and PGF2α**

Pursley et al. (1995) introduced a new method for synchronizing the time of ovulation in cattle using GnRH and PGF2α (Ovsynch program). In this protocol, GnRH is given at a random stage of the estrous cycle and PGF2α is given seven days later. A second GnRH injection is administered 2 days after PGF2α and the cow or heifer was bred 24 hours later. The first GnRH injection either caused ovulation of the dominant follicle or had no effect. Next, PGF2α caused regression of the CL, which resulted from the initial injection of GnRH. The seven-day waiting period between the initial GnRH
and PGF2α injection gives the CL enough time to mature and become responsive to PGF2α. The final GnRH injection caused ovulation of the dominant follicle. The period of 48 hours between PGF2α and GnRH allowed for a new follicle to emerge, grow to preovulatory size and become sensitive to the LH surge induced by GnRH to cause ovulation.

This study demonstrated that the Ovsynch protocol was more suited to lactating dairy cows than heifers. Ninety percent of lactating dairy cows ovulated to the first GnRH injection in comparison to fifty percent of heifers.

There are stages within the estrous cycle when the initiation of an Ovsynch protocol will cause a reduction in pregnancy rates. Initiation of the Ovsynch protocol during late luteal phase of the estrous cycle, between Days 13 to 17, may lead to premature lysis and regression of the CL. These cows are asynchronous and exhibit estrus prior to the second GnRH injection. In these cases insemination within 16 to 20 hours after the last GnRH injection will be ineffective and conception is not likely (Moreira et al., 2000a). In the early stages of the estrous cycle, Days 2 to 4, the dominant follicle is not yet sensitive to LH and will not respond to GnRH. These follicles will ovulate to the second GnRH injection. Follicles that fall into this category will range in age from 11 to 13 days, and have been shown to be less fertile.

The early luteal phase, between Days 5 and 11 of the estrous cycle, is the optimal time to initiate the Ovsynch protocol to achieve acceptable pregnancy rates. As a result it becomes important to presynchronize lactating dairy cows to an optimal stage in the estrous cycle at which time initiation of the Ovsynch protocol is most effective.
Presynch-Ovsynch

In this presynchronization program PGF2α is given twice, 14 days apart, and the Ovsynch protocol is initiated 12 days after the last PGF2α injection. Presynchronization programs have been shown to increase the first-service pregnancy rates in comparison with initiation of the Ovsynch protocol at random stages of the estrous cycle in lactating dairy cows (Moreira et al, 2001). The increase in the pregnancy rate has been attributed to the initiation of the Ovsynch protocol in the most favorable stage of the estrous cycle.

Presynchronization beginning on Day 22 post partum with a second injection 14 days later has been shown to lower the incidence of metritis-pyometra, ovarian cysts, improve luteal activity rate on Day 50 post partum, improve estrus detection rate, increase ovulation rate and increase pregnancy rate (López-Gatius et al., 2003).

Postpartum Endometritis

Endometritis is defined as inflammation of the endometrium. The term is descriptive and refers to the extent and anatomical distribution of the inflammatory process. There are a plethora of papers published on the topic of postpartum uterine infection. However, the definition of uterine infection varies and the terms endometritis and metritis have been used interchangeably in the literature (Bretzlaff, 1987; Lewis, 1997). The following terms have been used to define uterine infection: metritis, endometritis, and pyometra.

Postpartum endometritis is a pathologic condition usually diagnosed during the intermediate postpartum period (Ball et al., 1984; Olson et al 1984) during routine postpartum examination of the cow or heifer. It is commonly characterized by the absence of estrus, a vaginal discharge of creamy-white or yellow pus and a large doughy
uterus that fails to involute. Postpartum endometritis is the most common cause of infertility in cows. It delays uterine involution (Tennant and Peddicord, 1968; Griffin et al., 1974), prolongs the time to first estrus, increases the number of services per conception and prolongs the interval to calving (Griffin et al., 1974). Lewis (1997) stated that as many as 40% of postpartum cows within a herd may be diagnosed and treated for uterine infections. Coleman et al. (1985) stated that endometritis was a common reproductive disorder accounting for 20% of the reproductive disorders in dairy cows. However, Ruder et al. (1981) found that the percentage of cows with endometritis could be as high as 67%. It has been reported that each lactating dairy cow with a uterine infection can cost a farmer up to $106. This costs consists of treatment, loss in milk production (associated with the infection and as a consequence of milk discarded due to antimicrobial therapy) and the loss of cows due to culling for reproductive disorders associated with and as a direct cause of uterine infection (Lewis, 1997).

Pathology

Ball et al. (1984) defined the postpartum period as the period from parturition to complete involution. This period was further divided into the ‘early postpartum period’, ‘intermediate period’ and ‘postovulatory period’.

The ‘early postpartum period’ is the period following parturition until the pituitary becomes sensitive to GnRH, and usually lasts 8 to 14 days. The ‘intermediate period’ begins when the pituitary becomes responsive to GnRH and ends with the first postpartum ovulation. The ‘postovulatory period’ starts with the first postpartum ovulation and ends with the completion of involution.

During the early postpartum period bacteria invade the uterus (Elliot et al., 1968; Griffin et al., 1974; Peter and Bosu, 1987). Elliot et al., (1968) recovered bacteria from
93% of uteri examined between 3 to 15 days post partum. The major groups of bacteria isolated were *Streptococcus*, *Staphylococcus*, *Micrococcus*, *Archanobacteria* formally known as *Corynebacteria*, *Pseudomonas* and *Escherichia* (Elliot et al., 1968). Griffin et al. (1974) found 92% of uteri sampled infected with bacteria between Days 1 and 7 postpartum, and 96% infected between Days 8 and 14 post partum. The fertility of cows were not affected by the variable uterine flora or endometritis found within the first two weeks post partum. However, cows that had a uterine infection with *Archanobacterium pyogenes* following Day 21 postpartum developed severe endometritis and were infertile to the first service (Griffin et al., 1974).

During the intermediate period and into the postovulatory period, spontaneous clearance of the bacterial contaminant and recontamination occurs. It was noted that the composition of uterine flora changed throughout the first 7 weeks post partum as a result of spontaneous contamination, clearance and recontamination (Griffin et al., 1974). It is likely that as the cow or heifer begins to cycle and the uterus comes under the influence of high concentrations of estrogen, that infections are cleared and there is an increased rate of repair of the endometrium. Rowson et al. (1953) demonstrated that the bovine uterus is more susceptible to bacterial infection during the luteal phase of the estrous cycle when progesterone is the dominant hormone. It was also noted that under the influence of estrogen during the follicular stage of the cycle, the uterus was less susceptible to bacterial infection. Hawk et al. (1960; 1964) demonstrated that the uterus of heifers differed in their leukocytic response during the estrous cycle. The leukocytic response to an inoculum of *Escherichia coli* was more pronounced and occurred much faster during estrus than in the luteal phase.
During intermediate period and postovulatory period, intrauterine bacterial infection has been associated with short estrous cycles. Peter and Bosu (1987) demonstrated the temporal relationship between infection patterns and serum concentrations of LH, P₄ and PGF₂α metabolite (PGFM). They noted that cows with a short first estrous cycle length were those with uterine infections. High infection rates were associated with higher concentrations of PGFM before the first postpartum LH surge and ovulation. Following the LH surge, increasing concentration of PGFM was associated with increasing intensity of uterine infection. There was a further increase in PGFM prior to the decrease in P₄ and lysis of the CL. However, in cows with a normal estrous cycle length, low PGFM was noted prior to and following the LH surge and only prior to onset of luteolysis (Peter and Bosu, 1987).

**Pathogenesis**

Endometritis is a localized inflammation of the endometrium of the uterus associated with chronic postpartum infection with pathogenic bacteria. The primary bacterium associated with endometritis is *Archanobacterium pyogenes* (Elliot et al., 1968; Griffin et al., 1974; Peter and Bosu, 1987; Bonnett et al., 1991; Dohmen et al., 1995; 2000). Griffin et al. (1974) noted that 80% of the time *Archanobacterium pyogenes* was isolated form uteri with moderate or severe endometritis. Classification of the severity of endometritis was based on the number of neutrophils, histiocytes, plasma cells and lymphocytes in the biopsy specimen. Moderate endometritis was defined as medium infiltration of the stratum compactum and upper part of the stratum spongiosum and/or three to four foci of cellular reaction per section in a number of sections. Severe endometritis was defined as dense infiltration in the stratum compactum and stratum
spongiosum and/or five or more foci of cellular reaction per section in a number of sections (Griffin et al., 1974).

Dohmen et al. (2000) investigated the relationship between intra-uterine bacterial contamination, endotoxin levels and the development of endometritis in postpartum cows with dystocia or retained fetal membranes. They found that the presence of an abnormal cervical discharge at Day 14 post partum was higher in cows with retained fetal membranes compared to cows that experience dystocia. However, *Archanobacterium pyogenes* was isolated more often in cows with retained fetal membranes on Day 14 post partum than dystocia cows and was positively associated with an abnormal discharge on Days 14 and 28 post partum. Dohmen et al. (2000) suggested that the presence of *Escherichia coli* and lipopolysaccharides (endotoxins) in lochia early in the postpartum period predisposed the development of uterine infections by *A. pyogenes* and Gram-negative anaerobes.

Reduced neutrophil function plays a role in the development of endometritis. Neutrophils function in phagocytosis and elimination of bacterial contaminants in the uterus. In cows with retained fetal membranes, neutrophil functions such as chemotaxis and bacterial ingestion were reduced in comparison to health cows (Cia et al., 1994). The reduction in neutrophil function may increase the susceptibility of the postpartum dairy cow to infection.

**Diagnosis**

Diagnosis of endometritis may be made based using a combination of visual inspection of the vulva and tail, vaginoscopy, rectal palpation, uterine culture or biopsy and/or ultrasonography. Visual inspection may reveal crusts formed on the vulva and/or tail. There may be discharge attached to the ventral commissure of the vulva (Zemjanis,
Visual inspection of the vulva and tail alone is not adequate to diagnose endometritis.

Rectal palpation is the most common method used for diagnosing endometritis. However, this has been noted as an insensitive and non-specific method (Bretzlaff, 1987; Lewis, 1997). The results obtained through this method depend on the skill and experience of the individual. The size and consistency of the uterus and cervix along with the fluid content of the uterus are used to ascertain the presence of uterine infection compared to the “normal” finding for a given time postpartum.

Vaginoscopy is seldom used routinely as a diagnostic technique in dairy cows (Zemjanis 1970; Bretzlaff, 1987). Vaginoscopy, visual examination of the vagina, luminal content and external cervical os have been shown to be a superior means of diagnosing endometritis. LeBlanc et al. (2002) found that the prevalence of clinical endometritis was 16.9% and vaginoscopy was required to identify 44% of these cases. Visual and transrectal palpation of the uterus was not sufficient to identify cases of clinical endometritis.

Le Blanc (2002) suggested that the diagnosis of clinical endometritis should be made by the presence of purulent uterine discharge or cervical diameter > 7.5 cm after 20 DIM, or mucopurulent discharge after 26 days in milk. This definition of endometritis is based on the negative effect these criteria have on subsequent fertility. In the absence of vaginoscopy a combination of history, inspection and palpation may be used to diagnose clinical endometritis. In the absence of vaginoscopy the following criteria can be used to classify clinical endometritis (LeBlanc et al., 2002): presence of mucopurulent or
purulent discharge on the perineum, cervical diameter ≥7.5 cm, and presence of a uterine horn ≥8 cm in diameter.

Griffin et al. (1974) identified cases of endometritis using uterine biopsy and uterine culture. The severity of endometritis was determined on histological findings and the predominant bacterial flora evaluated by uterine culture. Recently, endometrial cytology has been used to identify cows with subclinical endometritis in the postpartum dairy cow (Kasimanickam et al., 2004). Endometrial cytology was performed on clinically normal cows, cows without evidence of an abnormal discharge on visual inspection of the vulva, tail and surrounding areas and vaginoscopy between Days 20 and Day 33 post partum. Clinical endometritis was defined as > 18% polymorphonuclear cells seen in endometrial cytology samples or fluid in the uterus at the first visit (Days 20 and Day 33 post partum) and as > 10% polymorphonuclear cells seen in endometrial cytology samples or fluid in the uterus at the first visit (Days 34 and Day 47 post partum).

In a similar fashion to LeBlanc et al (2002) and Kasimanickam et al (2004) defined clinical endometritis based on its impact on subsequent pregnancy rates. Although there was no evidence of abnormal discharge on the day of enrollment, 9.1% had a discharge within 24 hours. This indicated that evaluation of endometritis should be done on more than one occasion. It is interesting to note that 35.1 % of cows were diagnosed with subclinical endometritis by endometrial biopsy on the first visit and 34% on the second visit 2 weeks later. However, 48% were diagnosed with subclinical endometritis by the presence of fluid in the uterus on the first visit and 20.5% on the second visit 2 weeks later. Cows with subclinical endometritis were less like to become pregnant than those without subclinical endometritis (Kasimanickam et al., 2004).
Ultrasonography may be used to aid in the diagnosis of endometritis. One of the sonographic indications of endometritis is the appearance of fluid in the uterine lumen. This fluid accumulation must be distinguished from that which is seen with estrous secretions or the embryonic fluid of the early conceptus. Fluids that accumulate during estrus and early pregnancy are less echogenic than that of endometritis (Fissore et al., 1986).

In mild endometritis, there may be no fluid present or few fluid filled pockets found when scanning the uterus (Kahn et al., 1989). In cases of severe endometritis, the uterus may be distended with fluid and involve both horns (Kahn et al., 1989). The echogenicity of the fluid that accumulated during endometritis varies with the products of inflammation. Kahn et al. (1989) described the echogenic pattern displayed by the products of inflammation. Echogenic patterns range from small bright spots in mild cases to a “snow-storm effect” or, in severe cases, to almost bright white images on the screen. Real-time-scanning may reveal turbulences within the larger collections of fluid.

Since plasma levels of 13,14-dihydro,15-keto-PGF2α (PGFM), the stable metabolite of PGF2α, are elevated in spontaneous uterine infection, it has been suggested that these levels may aid in the diagnosis of endometritis in the postpartum cow (Del Vecchio et al., 1994). However, Archbald et al. (1998) were unable to correlate plasma levels of PGFM with abnormal uteri identified by per rectum palpation during Days 24 to 29 post partum in lactating dairy cows. In addition, Archbald et al. (1998) demonstrated that the postpartum rate of decline of plasma levels of PGFM was influenced by the presence of a corpus luteum (CL). In cows which had a CL, the rate of decline of PGFM was slower compared to cows without a CL. It has been reported that progesterone plays
an extremely important role in regulating pulsatile secretion of PGF2α from the bovine uterus (Mann et al., 1995) since it can completely restore the number of pulses and partially restore pulse magnitude when administered to ovariectomized ewes. However, a decrease in progesterone appeared to be essential for pulses of PGF2α to reach a maximum magnitude (Silvia et al., 1991).
CHAPTER 3
EXPERIMENT 1: AN EVALUATION OF PRETREATMENT WITH GnRH ON OVARIAN RESPONSE AND PREGNANCY RATE OF LACTATING DAIRY COWS WITH OVARIAN CYSTS SUBJECTED TO THE OVSYNCH PROTOCOL

Introduction

Bovine ovarian cysts are follicles that fail to ovulate at the time of estrus (Garverick, 1997; 1999). They are an economic problem in the dairy cow because these cows are infertile as long as the condition persists (Kesler and Garverick, 1982). The exact cause of ovarian cysts is not presently known, but some predisposing factors include age, stress, high milk production and genetics. It appears that an important component in the pathogenesis of this condition is the inappropriate, or lack of, release of hypothalamic gonadotropin-releasing hormone (GnRH) at the time of estrus (Refsal et al., 1987; Gümen et al., 2002; Silvia et al., 2002). One therapeutic approach involves the use of exogenous GnRH, which releases LH from the anterior pituitary and causes ovulation of an ovarian follicle, and/or luteinization of the ovarian cysts (Garverick, 1997; 1999).

The occurrence of ovarian follicular waves has been adequately demonstrated in the dairy cow without ovarian cysts (Rajakoski, 1960; Pierson and Ginther, 1984). It has been suggested that ovarian follicular waves also occur in cows with ovarian cysts (Garverick, 1999). The difference is that ovulation of follicles occur in cows without ovarian cysts, but does not occur in cows with ovarian cysts. It has further been suggested that the administration of GnRH to cows with ovarian cysts causes ovulation of a functionally mature follicle of an ovarian follicular wave (Garverick, 1999). An injection
of GnRH at random during the estrous cycle of cows without ovarian cysts will either cause ovulation or luteinization of large follicles present in the ovary and synchronize the recruitment of a new follicular wave (Thatcher et al., 1989; Macmillan and Thatcher, 1991). Gonadotropin releasing hormone can advance the follicular wave by increasing the rate of atresia in cows without ovarian cysts (Thatcher et al., 1989; Macmillan and Thatcher, 1991).

In cows without ovarian cysts, the initiation of a protocol for synchronization of ovulation and timed insemination (Ovsynch) at a specific stage of the estrous cycle can influence the reproductive responses to this protocol. The stage of the estrous cycle that appears to be the most appropriate for increased pregnancy rates using this protocol is the early luteal phase of the estrous cycle (Vasconcelos et al., 1999; Moreira et al., 2000a).

The hypothesis of this study was that GnRH administered to cows with ovarian cysts at the time of diagnosis will induce an early luteal phase of the estrous cycle which will be conducive to an increased pregnancy rate to a protocol for synchronization of ovulation and timed insemination. The objectives of this study were: i) to compare the ovarian response of cows with ovarian cysts to no treatment and ovarian response 7 days after treatment with GnRH; and, ii) to determine the pregnancy rate of cows with ovarian cysts subjected to Ovsynch and pretreatment with GnRH 7 days prior to the initiation of the Ovsynch protocol.

**Materials and Methods**

The study was conducted during the period of January 2002 to May 2003 in a dairy herd (approximately 700 milking cows) in north-east Florida. Cows were milked three times per day and were kept in covered, open-sided barns between milking. They were
fed a total mixed ration. The herd was visited weekly, and all reproductive health and management records were computerized.

At the time of diagnosis, cows with ovarian cysts were sequentially allocated to one of three groups (Day 0). The diagnosis of ovarian cysts was based on palpation of the ovaries and uterus per rectum, and by ultrasonographic examination of the ovaries. The criteria used on rectal palpation were the presence of multiple follicles on the ovary with at least one follicle being ≥ 17 mm diameter (Hatler et al., 2003), the absence of a corpus luteum (CL) on either ovary, and the lack of tonicity of the uterus (Archbald et al., 1991; Bartolome et al., 2000; Bartolome et al., 2002). On ultrasonographic examination of the ovaries, ovarian cysts were recognized by the hypoechogenicity of the structure, and the absence of a CL on either ovary (Pierson and Ginther, 1984). The size of ovarian cysts was determined using ultrasonography and the recorded size was based on the largest diameter observed on ultrasonography.

Cows in Group 1 were treated with GnRH (100 µg, im; Cystorelin, Rhode Merieux Inc., Athens, GA) on Day 0, and Day 7, PGF2α (25 mg, im; Lutalyse, Pharmacia Upjohn, Kalamazoo, MI) on Day 14, GnRH (100 µg, im) on Day 16, and timed inseminated 16-20 h later. Cows in Group 2 were treated with GnRH (100 µg, im) on Day 0, PGF2α (25 mg, im) on Day 7, GnRH (100 µg, im) on Day 9, and timed inseminated 16-20 h later. Cows in Group 3 were not treated with GnRH on Day 0, but were treated with GnRH (100 µg, im) on Day 7, PGF2α (25 mg, im) on Day 14, GnRH (100 µg, im) on Day 16, and timed inseminated 16-20 h later (Figure 3-1). Pregnancy was determined by rectal palpation of the uterus between 45-50 d after timed insemination using previously described techniques (Zemjanis, 1970).
Figure 3-1. Experiment 1 – Experimental Design

On both Days 0 and 7, cows in all groups were subjected to ultrasonographic examination of the ovaries. On both Day 0 and Day 7, blood samples for progesterone (P₄) concentration were obtained from all cows and placed on ice immediately after collection. Samples were centrifuged at 5000 x g for 15 minutes, and serum was stored at -20°C until assayed for progesterone using a solid-phase, no-extraction RIA previously described (Srikandakumar et al., 1986). Serum progesterone concentration on Day 0 was classified as high (> 0.5 ng/ml) or low (≤ 0.5 ng/ml). A serum progesterone concentration >1 ng/ml on Day 7 was used to confirm the presence of a functional corpus luteum.

**Statistical Analysis**

Baseline data for parity, DIM, time of year and P₄ on Day 0 were compared using Chi-square (P<0.05). The outcomes of interest for this experiment were ovarian response 7 days after treatment with GnRH, and pregnancy rate. Data for ovarian response on Day
7 were analyzed using logistic regression (Proc Logistic, SAS; SAS 9.0, 2002) adjusting for days in milk (DIM), time of year (October to February, and March to September), parity (1, 2, 3+) and P₄ on Day 0. Data for pregnancy rate were analyzed using logistic regression (SAS 9.0, 2002), adjusting for DIM, time of year, parity, P₄ on Day 0 and Day 7, and ovarian response on Day 7. The explanatory variables were evaluated using the backward elimination procedure and variables that significantly affected the outcomes remained in the model (Agresti, 1996). The level of significance was set at P ≤ 0.05.

Results

A total of 176 cows were diagnosed with ovarian cysts and enrolled on Day 0. Fifteen cows failed to complete assigned treatment or were culled prior to completion of the study, and six cows were inseminated to the wrong date and were removed from the study. A total of 155 cows completed the study; Group 1 (n=55), Group 2 (n=49), Group 3 (n=51). The following statistical analysis is based on the 155 cows that completed the study.

The baseline comparison for parity, time of year, day in milk, and P₄ on Day 0 is shown in Table 3-1. On Day 0, data for P₄ were present for 150/155 (96.8%) cows enrolled in the study. There was no significant difference (P > 0.12) in any of the variables among the groups.

On Day 7, data for ultrasonographic examination of the ovaries were available for 153/155 (98.7%) cows enrolled in this study. The percentage of cows in Group 3 (4%) with a CL on Day 7 was significantly less (P < 0.0001) than that of cows in Group 2 (72.9%) and Group 1 (50.9%). On Day 7, P₄ was present for 145/155 (93.5%) cows enrolled in this study. The percentage of cows in Group 3 (40.8%) with a P₄ > 1.0 ng/ml
on Day 7 was significantly less (P < 0.03) than that of cows in Group 2 (63.6%) and Group 1 (63.5%).

The adjusted odds ratios (AOR) and 95% confidence interval (CI) for the risk of the presence of a CL on Day 7 in lactating dairy cows with ovarian cysts treated with GnRH on Day 0 are shown in Table 3-2. Cows in Group 1 and Group 2 (treated with GnRH on Day 0) were more likely to have a CL on the ovary 7 days later (AOR: 21.30; 95% CI: 4.67-97.04; P = 0.04; AOR: 61.92; 95% CI: 13.11-292.41; P < 0.0001, respectively) compared to cows in Group 3 (non-treated cows). There was no effect of DIM, time of year, parity and P₄ on Day 0 for this outcome.

Table 3-1. Baseline data for heifers and cows that were diagnosed with ovarian cysts and successfully completed Experiment 1 for parity (1, 2, 3+), time of year (March-September/October-February), days in milk (DIM) and progesterone concentration (< 0.5 ng/ml / ≥ 0.5 ng/ml) on Day 0.

<table>
<thead>
<tr>
<th>Variable</th>
<th>Group 1</th>
<th></th>
<th>Group 2</th>
<th></th>
<th>Group 3</th>
<th></th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>n</td>
<td>%</td>
<td>n</td>
<td>%</td>
<td>n</td>
<td>%</td>
<td></td>
</tr>
<tr>
<td>Parity</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>17/55</td>
<td>30.9</td>
<td>13/49</td>
<td>26.5</td>
<td>13/51</td>
<td>25.5</td>
<td>0.85</td>
</tr>
<tr>
<td>2</td>
<td>22/55</td>
<td>40.0</td>
<td>23/49</td>
<td>47.0</td>
<td>26/51</td>
<td>51.0</td>
<td></td>
</tr>
<tr>
<td>3+</td>
<td>16/55</td>
<td>29.1</td>
<td>13/49</td>
<td>26.5</td>
<td>12/51</td>
<td>23.5</td>
<td></td>
</tr>
<tr>
<td>Time of year</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>0.17</td>
</tr>
<tr>
<td>March-September</td>
<td>34/55</td>
<td>61.8</td>
<td>35/49</td>
<td>71.4</td>
<td>40/51</td>
<td>78.4</td>
<td></td>
</tr>
<tr>
<td>October-February</td>
<td>21/55</td>
<td>38.2</td>
<td>14/49</td>
<td>28.6</td>
<td>11/51</td>
<td>21.6</td>
<td></td>
</tr>
<tr>
<td>Days in Milk</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>0.42</td>
</tr>
<tr>
<td>≤ 88</td>
<td>16/55</td>
<td>29.1</td>
<td>13/49</td>
<td>26.5</td>
<td>9/51</td>
<td>17.7</td>
<td></td>
</tr>
<tr>
<td>89-134</td>
<td>12/55</td>
<td>21.8</td>
<td>10/49</td>
<td>20.4</td>
<td>17/51</td>
<td>33.3</td>
<td></td>
</tr>
<tr>
<td>135-223</td>
<td>16/55</td>
<td>29.1</td>
<td>10/49</td>
<td>20.4</td>
<td>12/51</td>
<td>23.5</td>
<td></td>
</tr>
<tr>
<td>&gt;224</td>
<td>11/55</td>
<td>20.0</td>
<td>16/49</td>
<td>32.7</td>
<td>13/51</td>
<td>25.5</td>
<td></td>
</tr>
<tr>
<td>P₄ on Day 0</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>0.12</td>
</tr>
<tr>
<td>&lt; 0.5 ng/ml</td>
<td>34/52</td>
<td>65.4</td>
<td>33/49</td>
<td>67.4</td>
<td>24/49</td>
<td>49.0</td>
<td></td>
</tr>
<tr>
<td>≥ 0.5 ng/ml</td>
<td>18/52</td>
<td>34.6</td>
<td>16/49</td>
<td>32.6</td>
<td>25/49</td>
<td>51.0</td>
<td></td>
</tr>
</tbody>
</table>
Table 3-2. Percentage, adjusted odds ratios (AOR) and 95% confidence interval (CI) for the risk of finding a CL on Day 7 in cows with ovarian cysts treated with GnRH on Day 0.

<table>
<thead>
<tr>
<th>Variable</th>
<th>CL</th>
<th>AOR</th>
<th>95% CI</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group</td>
<td>n</td>
<td>%</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>28/55</td>
<td>50.9</td>
<td>21.30</td>
<td>4.67-97.04</td>
</tr>
<tr>
<td>2</td>
<td>35/48</td>
<td>72.9</td>
<td>61.92</td>
<td>13.11-292.41</td>
</tr>
<tr>
<td>3</td>
<td>2/50</td>
<td>4.0</td>
<td>1.00</td>
<td>Referent</td>
</tr>
</tbody>
</table>

The adjusted odds ratios (AOR) and 95% confidence interval (CI) for the risk of having a progesterone concentration greater than 1.0 ng/ml on Day 7 in lactating dairy cows with ovarian cysts treated with GnRH on Day 0 are shown in Table 3-3. Cows in Group 1 and Group 2 (treated with GnRH on Day 0) were more likely to have P₄ > 1.0 ng/ml 7 days later compared to cows in Group 3 (non-treated cows). Cows with P₄ < 0.5 ng/ml on Day 0 were more likely to have P₄ ≤ 1.0 ng/ml on Day 7 regardless of treatment. There was no association between DIM, time of year and parity with P₄ concentration on Day 7.

Table 3-3. Percentage, adjusted odds ratios (AOR) and 95% confidence interval (CI) for the risk of finding progesterone concentration > 1.0 ng/ml on Day 7 in cows with ovarian cysts treated with GnRH on Day 0.

<table>
<thead>
<tr>
<th>Variable</th>
<th>High (&gt;1.0 ng/ml)</th>
<th>AOR</th>
<th>95% CI</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group</td>
<td>n</td>
<td>%</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>33/52</td>
<td>63.5</td>
<td>3.52</td>
<td>1.43 – 8.71</td>
</tr>
<tr>
<td>2</td>
<td>28/44</td>
<td>63.6</td>
<td>3.74</td>
<td>1.46 – 9.59</td>
</tr>
<tr>
<td>3</td>
<td>20/49</td>
<td>40.8</td>
<td>1.00</td>
<td>Referent</td>
</tr>
<tr>
<td>P₄ on Day 0</td>
<td>n</td>
<td>%</td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt; 0.5 ng/ml</td>
<td>38/86</td>
<td>44.2</td>
<td>0.22</td>
<td>0.10 – 0.48</td>
</tr>
<tr>
<td>≥ 0.5 ng/ml</td>
<td>41/56</td>
<td>73.2</td>
<td>1.00</td>
<td>Referent</td>
</tr>
</tbody>
</table>
Data for risk of pregnancy were available for 127/155 (81.9%) cows that successfully completed the study. The risk of pregnancy at 45 to 50 days after timed insemination for cows in each group is shown in Table 3-4. There was no difference in the risk of pregnancy at 45 to 50 days between the groups. The adjusted odds ratios (AOR) and 95% confidence interval (CI) for the risk of pregnancy in lactating dairy cows with ovarian cysts treated with GnRH on Day 0 are shown in Table 3-4. Cows with ovarian cysts were 0.20 times less likely to become pregnant if inseminated between the periods of March to September compared to cows inseminated during the period of October to February, regardless of treatment. Cows with a CL on Day 7 were more likely to become pregnant compared to cows without a CL on Day 7. There was no effect of group, progesterone concentrations on Day 0 and Day 7 on risk of pregnancy.

Table 3-4. Percentage, adjusted odds ratios (AOR) and 95% confidence interval (CI) for the risk of pregnancy in lactating dairy cows with ovarian cysts treated with GnRH on Day 0.

<table>
<thead>
<tr>
<th>Variable</th>
<th>Pregnancy</th>
<th>AOR</th>
<th>95% CI</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>n</td>
<td>%</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Group</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>8/43</td>
<td>18.6</td>
<td>1.41</td>
<td>0.25 – 8.33</td>
</tr>
<tr>
<td>2</td>
<td>8/41</td>
<td>19.5</td>
<td>1.43</td>
<td>0.21 – 10.00</td>
</tr>
<tr>
<td>3</td>
<td>3/43</td>
<td>7.0</td>
<td>1.00</td>
<td>Referent</td>
</tr>
<tr>
<td>Season</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>March-September</td>
<td>8/89</td>
<td>9.0</td>
<td>0.20</td>
<td>0.06 - 0.61</td>
</tr>
<tr>
<td>October-February</td>
<td>11/38</td>
<td>28.9</td>
<td>1.00</td>
<td>Referent</td>
</tr>
<tr>
<td>Ovarian Response</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Follicle</td>
<td>5/72</td>
<td>7.3</td>
<td>0.19</td>
<td>0.04 - 0.86</td>
</tr>
<tr>
<td>CL</td>
<td>14/53</td>
<td>26.4</td>
<td>1.00</td>
<td>Referent</td>
</tr>
</tbody>
</table>
Discussion

The hypothesis of this study was that GnRH administered to cows with ovarian cysts at the time of diagnosis will induce an early diestral stage of the estrous cycle which will be conducive to an increased pregnancy rate to a protocol for synchronization of ovulation and timed insemination. This hypothesis was based on previous research in cows without ovarian cysts, which showed that cows in early diestrus at the time of initiation of the Ovsynch protocol had a higher pregnancy rate compared to cows subjected to this protocol at other stages of the estrous cycle (Vasconcelos et al., 1999; Moreira et al., 2000).

However, the results of this study did not fully substantiate this hypothesis. It appeared that while pretreatment with GnRH induced an early diestral stage of the estrous cycle (cows in Group 1), initiation of the Ovsynch protocol at this time (early diestrus) did not increase pregnancy rate compared to cows which were subjected to this protocol at the time of diagnosis (cows in Group 2). Our interpretation of these results is that the progesterone concentration in early diestrus when the Ovsynch protocol was initiated may not be an important factor in determining the response to GnRH 7 days later. This interpretation is supported by another significant finding in this study, which indicated that the level of progesterone at the time of initiation of the Ovsynch protocol (Day 0; cows in Group 2) was not associated with the presence of CL on the ovaries on Day 7.

It is speculated that the presence of an ovarian follicle that can respond to the effect of the GnRH-induced LH surge may be more important than the concentration of progesterone at the time of initiation of the Ovsynch protocol. The presence of ovarian follicular waves has been shown to occur in cows with ovarian cysts (Garverick, 1999).
However, it is impossible to determine the stage of these ovarian follicular waves when the diagnosis of ovarian cysts is made, and treatment with GnRH is initiated in these cows. Therefore, it may simply be a matter of chance that treatment with GnRH at the time of diagnosis coincides with the presence of an ovarian follicle, which can respond to the effects of the GnRH-induced LH surge. A similar situation may exist when GnRH is administered to initiate the Ovsynch protocol in cows without ovarian cysts. It is interesting to note that not all cows responded, as determined by the presence of a CL 7 days later, to the initial treatment with GnRH. In fact, this response was in the range of 50.9 to 70.9%. These values fall within the range of heifers and cows that ovulated to the first GnRH of the Ovsynch protocol (heifers: 54.2%; cows: 90%; Pursley et al., 1995).

In the present study, pre-treatment with GnRH 7 days prior to initiation of the Ovsynch protocol did not increase the risk of pregnancy. The risk of pregnancy achieved in Group 1 (cows pre-treated with GnRH) was 18.6% compared to Group 2 (Ovsynch) 19.5% and Group 3 (no treatment and Ovsynch started 7 d following diagnosis) 7.0%. Combining Groups 2 and 3, the risk of pregnancy is 13.25%. It is tempting to simply accept these percentages. However, due to the low power of this study, it is more likely to make a Type II error; a higher chance of saying that there is no difference between the treatments when there is. The risk of pregnancy for Group 2 and 3 in this study is lower than those obtained by Bartolome et al. (2000; 23.6% in cystic cows subjected to Ovsynch). However, the risk of pregnancy in Group 1 (18.6%), cystic cows pretreated with GnRH is similar to a conception rate of 15.1% obtained with a larger sample size (Bartolome et al., 2003) and higher than the 9% obtained by Gümen et al. (2003).
In the present study, cows with ovarian cysts treated with GnRH on Day 0 were more likely to have a CL 7 days later compared to untreated cows. This response was independent of the progesterone concentration on the day of diagnosis, days in milk, parity and time of year. The presence of a CL on Day 7 appeared to have a beneficial effect on fertility of these cows following the subsequent use of the Ovsynch protocol. In fact, cows with a CL on Day 7 were more likely to become pregnant compared to those without a CL on Day 7. Nevertheless, treatment with GnRH on Day 0 did not have any effect on the risk of pregnancy, even though it had a positive effect on the presence of a CL on Day 7.

Therefore, it is tempting to suggest that ovulation occurring without the use of exogenous GnRH (spontaneous ovulation) may also be an important factor affecting fertility in cows with ovarian cysts, subsequently subjected to the Ovsynch protocol. In support of this statement, it should be noted that spontaneous ovulation occurred in untreated cows with ovarian cysts (Group 3) since 2/50 (4%) possessed a CL on Day 7, and 3/43 (7%) of these cows in Group 3 conceived following subsequent treatment with the Ovsynch protocol. This is in agreement with previous research (Bierschwal et al 1975), which showed that spontaneous ovulation occurred in 6/28 (21.4%) untreated cows with naturally-occurring ovarian cysts 14 days after diagnosis, and 4/6 (66.7%) of these cows conceived to artificial insemination at estrus. Also, it has been reported that 1/8 (12.5%) untreated cows with experimentally-induced ovarian cysts possessed a CL 10 days after charcoal marking of these cysts (Cook et al., 1990). However, pregnancy was not an outcome in that study.
In the present study, since the rate of spontaneous ovulation was relatively low compared to that obtained through the use of exogenous GnRH, it would seem clinically prudent to administer GnRH at the time of diagnosis of this condition. In this way, it is more likely that most cows with ovarian cysts will respond with a CL on the ovaries 7 days later. In fact, the authors suggest that cows with ovarian cysts be treated with exogenous GnRH on the day of diagnosis, and that ultrasonographic examination of the ovaries be performed 7 days later. If there is a CL on the ovaries at this time, these cows should be treated with a luteolytic dosage of PGF2α, GnRH 2 d later, and timed inseminated 16-20 h after treatment with GnRH. However, if there is no CL on the ovaries on Day 7, these cows should be judiciously re-treated with GnRH until the presence of a CL is evident.

The results of this study showed that cows with ovarian cysts treated with GnRH at the time of diagnosis (Day 0) were more likely to have a P₄ concentration greater than 1 ng/ml on Day 7. However, cows with P₄ ≥ 0.5 ng/ml on Day 0 were more likely to have a P₄ concentration > 1 ng/ml on Day 7. This was observed in cows in all groups without regard to treatment. This phenomenon could probably be explained by the occurrence of spontaneous ovulation, and the presence of luteinized, anovulatory follicles in cows in Group 3 (non-treated), and the response to exogenously administered GnRH to cows in Group 1 and Group 2. It was also observed that cows with a P₄ concentration < 0.5 ng/ml on Day 0 were more likely to have a P₄ concentration on Day 7 ≤ 1.0 ng/ml. This could probably be explained by lack of response to GnRH by these cows.

Although P₄ concentrations in cows in Group 1 and Group 2 were significantly higher (P₄ > 1 ng/ml) than that observed in cows in Group 3, pregnancy rate was not
affected by P₄ concentrations on Day 7 in cows in all groups. This was in contrast to the observation that the presence of a CL on Day 7 (detected by ultrasonography) positively influenced subsequent pregnancy following timed insemination of cows in all groups. It is assumed that the presence of a mature CL on the ovary 7 days after the initiation of the Ovsynch protocol is an important component in the success of this protocol since this would determine the effectiveness of PGF2α administered at this time.

Therefore, a possible explanation for the apparent discrepancy between the presence of a CL and P₄ concentrations on Day 7 with respect to pregnancy may be a reflection of the presence of luteinized anovulatory follicles, developing corpora hemorrhagica, and mature CL on the ovaries at this time. It is, therefore, speculated that these luteinized anovulatory follicles and developing corpora hemorrhagica were not as responsive to the luteolytic dosage of PGF2α administered on Day 7 as would the mature CL present at this time. This speculation is supported by previous research (Nanda et al., 1988) which demonstrated that luteinized follicular cysts showed a poor response to PGF2α administered 7 days after treatment with GnRH, and that developing corpora lutea (corpora hemorrhagica) are unresponsive to the luteolytic effects of PGF2α (Lauderdale, 1975).

A valid criticism of this study revolves around the ability and accuracy of the investigators to make a diagnosis of ovarian cysts using per rectum palpation of the ovaries on one occasion. The criteria used to determine the presence of ovarian cysts were the presence of multiple follicles on one or both ovaries, the absence of a CL on the ovaries, and the lack of uterine tonicity. The palpable characteristics used in this study to identify a CL have been previously described (Zemjanis, 1970). In the opinion of the
authors, the criteria used to identify ovarian cysts using per rectum palpation of the ovaries and uterus can be justified, since a CL is not present in cows with ovarian cysts (because of a lack of ovulation), and the uterine tone that accompanies a functional follicle at the time of estrus is also not present in this condition. In addition, the absence of a CL was substantiated using ovarian ultrasonography and concurrent determination of plasma progesterone concentrations.

From the results of this study, it was concluded that administering GnRH to cows with ovarian cysts 7 days prior to the initiation of the Ovsynch protocol increased the proportion of cows with a CL on Day 7 but did not increase pregnancy rate. However, independent of treatment, the presence of a CL on Day 7 had a beneficial effect on pregnancy rate.
CHAPTER 4

EXPERIMENT 2: EFFECT OF SEQUENTIAL ADMINISTRATION OF PGF2α ON THE PREVALENCE OF MUCOPURULENT DISCHARGE, SIZE OF THE CERVIX, SIZE OF THE PREVIOUSLY PREGNANT UTERINE HORN AND FIRST SERVICE PREGNANCY RATE IN LACTATING DAIRY COWS

Introduction

It has been reported that the uterus of all postpartum dairy cows is invaded by opportunistic bacteria within the first 21 days post partum (Elliot et al., 1968). While most cows spontaneously eliminate these bacteria from the uterus, some do not, and the presence of these bacteria in the uterus could predispose these cows to periods of subsequent infertility. It is not known why the uterus of some postpartum dairy cows resist infection by these opportunistic bacteria. It has been shown that the ovarian sex hormones (estrogen and progesterone) could modulate the ability of the uterus to respond to bacterial invasion. In fact, estrogen has been shown to have a protective effect while progesterone has been shown to make the uterus more susceptible to infection (Rowson et al., 1953). There are relatively high blood concentrations of estrogen at parturition and during the immediate postpartum period. However, little is known about the estrogen concentrations at the level of the uterus at this time.

The first dominant ovarian follicle postpartum is usually formed on the ovary opposite to the previously pregnant uterine horn (contralateral ovary; Foote et al, 1968). With ovulation of this dominant follicle, usually between 10-12 days post partum, the uterus becomes subjected to physiologic concentrations of progesterone. In the presence of any intrauterine fluid or subclinical endometritis at this time, there is the possibility of
exacerbating the subclinical endometritis and this could lead to development of subsequent pyometra. In fact, it has been shown that treatment with GnRH in the immediate postpartum period could exacerbate a subclinical endometritis (Etherington et al., 1984). Therefore, while progesterone may be needed for proper uterine function, it would appear that there is the potential for normal or prolonged exposure of the uterus to progesterone to have a detrimental effect on the uterus at this time.

It has been reported that the presence of a large follicle in the ovary on the same side as the previously pregnant uterine horn (ipsilateral ovary) by 9 days post partum is a marker of subsequent improved fertility (Sheldon et al., 2000a). In addition, it has been suggested that elimination of bacterial contamination of the postpartum uterus may cause the selection of a dominant follicle in the ipsilateral ovary (Sheldon et al., 2003) in the early postpartum period.

There are conflicting reports on the effectiveness of exogenously administered prostaglandin F2 alpha (PGF2α) to increase the rate of uterine involution, cause evacuation of bacterial contamination from the uterus, and subsequently improve conception rate (Young et al., 1984; Etherington et al., 1988; Archbald et al., 1990; Risco et al., 1994). These reports indicate the use of PGF2α on either one or 2 occasions on random days postpartum without regard to the presence or absence of a functional corpus luteum (CL). Nevertheless, it has been suggested that exogenously administered PGF2α in the early postpartum period could have a direct beneficial effect on the uterus of cows that calved normally (Lindell et al., 1982) or abnormally (Risco et al., 1994), and this effect could occur in the absence of a CL.
However, we speculate that exogenous PGF2α would be more consistently effective in causing evacuation of bacterial contamination of the postpartum uterus if administered when there is a CL on the ovary. In most postpartum dairy cows, this would be approximately 20-24 days post partum since ovulation usually occurs within 10-12 days post partum. It is further speculated that consecutively lysing the CL with exogenous PGF2α at specific times post partum (sequential luteolysis) will result in exposing the uterine environment to normal concentration of progesterone for a reduced period of time. This reduced exposure of the uterine environment to normal concentration of progesterone in the early postpartum period could be beneficial to uterine health since progesterone has been shown to increase the susceptibility of the uterus to infection (Rowson et al., 1953). However, even though some cows may not have a CL on the ovary at the time of administration of PGF2α, it is speculated that a beneficial effect of exogenous PGF2α would occur through its direct effect on the uterus (Lindell et al., 1982; Risco et al., 1994).

Recent research (Lewis, 2003) in the ewe has suggested that a method for increasing uterine production of PGF2α could enhance the immune function of the uterus and its ability to resist uterine infections. However, the mechanism(s) by which this could be accomplished in the postpartum dairy cow are presently unknown. It has been shown that luteal oxytocin can stimulate the synthesis and release of endogenous uterine PGF2α at the time of luteolysis (Silvia et al., 1989). Nevertheless, it appeared that while administration of exogenous oxytocin was unable to compliment the effect of uterine PGF2α during diestrus, exogenous administration of PGF2α appeared to enhance the effect of endogenous PGF2α at this time (Archbald et al., 1994).
LeBlanc et al. (2002) defined clinical endometritis based on its impact on pregnancy rate. When vaginoscopy was performed clinical endometritis was defined as the presence of a purulent, foul-smelling or fetid discharge, or cervical diameter >7.5 cm between 20 and 33 DIM, or as a mucopurulent discharge after 26 DIM. In the event that vaginoscopic examination was not performed, the presence of mucopurulent or purulent discharge on the perineum, cervical diameter >7.5 cm and the presence of a uterine horn ≥ 8 cm in diameter were used to define clinical endometritis.

The Ovsynch protocol introduced by Pursley et al. (1995) uses a combination of GnRH and PGF2α to synchronize ovulation such that insemination may occur at a fixed time. Momcilovic et al. (1998) showed that the reproductive performance of lactating dairy cows was better when time-inseminated without the need for estrus detection was used compared with estrus detection and insemination at estrus. In the Ovsynch protocol GnRH is administered at a random stage of the estrous cycle followed seven days later by PGF2α. A second GnRH injection is administered 2 days after PGF2α and insemination occurs 16 to 20 hours following the second GnRH. However, it was noted that different pregnancy rates were achieved when the first GnRH was administered at different stages of the cycle. Reduced pregnancy rates occurred when GnRH was administered between Days 1 to 4 and 13 to 17 of the estrus cycle (Vasconcelos et al., 1999; Moreira et al., 2000a).

Pre-synchronization (Presynch) with two injection of PGF2α 14 days apart has been shown to increase the pregnancy rate to the Ovsynch protocol. The Presynch-Ovsynch protocol, in which PGF2α is given 14 days apart and the Ovsynch protocol initiated 12 days after the second injection of PGF2α has been shown to increase
pregnancy rate by 18 percentage units (25% to 43%; Moreira et al., 2000a). This increase in the pregnancy rate was attributed to starting the Ovsynch protocol during the early luteal phase, Days 5-11 of the estrous cycle.

The hypothesis of Experiment 2 was that sequential administration of PGF2α in the immediate postpartum period would decrease the prevalence of mucopurulent discharge, size of the cervix and previously pregnant uterine horn (PPH) and increase first-service conception rate in postpartum dairy cows. The objective of Experiment 2, Part A was to determine the effect of sequential administration of PGF2α in the immediate postpartum period on the prevalence of mucopurulent discharge, size of the cervix and previously pregnant uterine horn (PPH) and first-service pregnancy rate in postpartum dairy cows. The objective of Experiment 2, Part B was to evaluate the effect of sequential administration of PGF2α in the immediate postpartum period on first-service conception rate in postpartum dairy cows subjected to a timed insemination protocol consisting of Presynch-Ovsynch protocol.

**Experiment 2 – Part A**

**Materials and Methods**

The study was conducted during the period June to September 2003 in a commercial dairy herd of approximately 3,000 milking cows in north central Florida. These cows were milked 3 times per day and were kept in shaded areas between milking. They were fed a total mixed ration to meet or exceed the recommendations of National Research Council (National Research Council, 2001). The herd was visited weekly and all reproductive health and management records were computerized.
The prevalence of mucopurulent discharge by 50 days post partum in this herd was 20%. Mucopurulent discharge was detected by visual inspection of the perineum, vulva and tail. This was later confirmed by transrectal palpation of the reproductive tract with expulsion of purulent material from the vaginal canal and/or presence of fluid in the uterine horn(s). It was anticipated that the proposed treatment would reduce this prevalence to approximately 8%. A total of 102 cows per group provided a 95% confidence and 80% power to declare a difference in the prevalence of mucopurulent discharge between 20% and 8% as statistically significant.

A total of 228 cows was enrolled in this study. Cows were between 7 to 9 days post partum (pp). On the day of initiation of the study, cows were allotted to two groups using sequential randomization. All cows entered the study between the months of June and August 2003. For convenience, the day of initiation of the study was designated as Day 8 post partum.

Group 1 (n=114) formed the treated group and Group 2 (n=114) formed the control group. In Group 1, 45/114 (39.5%) were enrolled on Day 7 post partum, 31/114 (27.2%) were enrolled on Day 8 post partum and 38/114 (33.3%) were enrolled on Day 9 post partum. Similarly in Group 2, 38/114 (33.3%) were enrolled on Day 7 post partum, 44/114 (38.6%) were enrolled on Day 8 post partum and 32/114 (28.1%) were enrolled on Day 9 post partum (Table 4-1).

In addition, information concerning parity, dystocia (yes/no) and retained fetal membranes (yes/no) were recorded. Cows enrolled in the study were grouped into primiparous (first lactation) and multiparous (second lactation and higher; Table 4-1).
Dystocia was defined as heifers or cows requiring medium to heavy assistance to deliver their calf (two or more persons assisting for more than 15 minutes). Eighty-nine percent (203/228) of animals enrolled in the study required no assistance and were classified as calving normally with 106/114 (93.0%) and 97/114 (85.1%) in Group 1 and Group 2 respectively. A total of 25 required assistance and was classified as experiencing dystocia. Cows that underwent a Cesarean section or fetotomy were excluded from the study.

Retained fetal membranes (RFM) were defined as the failure to expel the placenta within 24 hours of parturition. In Group 1 and Group 2, 32/114 (28.1%) and 25/114 (21.9%) had RFM respectively (Table 4-1).

**Treatment**

Cows in Group 1 (n=114) were treated with two luteolytic dosage of PGF2α (25 mg intramuscularly: im) 8 hours apart on Days 8 and 15 post partum, and one luteolytic dosage of PGF2α (25 mg, im) once on Days 22 and 36 post partum. Cows in Group 2 (n=114) were not treated with PGF2α (25 mg, im) on Days 8, 15, 22 and 36 post partum and served as untreated controls (Figure 4-1).

At the termination of the treatment protocol cows followed the normal herd reproductive management practices. At the time reproductive management consisted of a voluntary waiting period of 100 days and detection of estrus using visual observation and a computerized system that utilized increased walking activity and an accompanied decrease in milk production (Afimilk®, S.A.E. Afikim, Israel). A specific individual was assigned the duty of observing these cows for estrus during each 8-hour shift. Cows that failed to show heat by 100 days post partum were subjected to the Ovsynch protocol.
Pregnancy was determined using rectal palpation 42-49 days after artificial insemination by on staff veterinarians.

**Group 1 (PGF2α; n=114)**

![Diagram of Group 1 experimental design]

**Group 2 (Control; n=114)**

![Diagram of Group 2 experimental design]

Figure 4-1. Experimental 2 – Part A: Experimental Design.

**Exclusion Criteria**

Animals were excluded or withdrawn from the study if they had any of the following: failed to complete the treatment scheme, were not examined at Day 22 post partum, were in the hospital barn and received systemic antibiotic therapy during the study period, underwent surgery (such as correction of displaced abomasum, Cesarean section, fetotomy), controls treated with PGF2α during the study period, and culled from the herd or died during the study period.

**Blood Sampling and Hormone Assay**

Blood samples were collected on Days 8, 15, 22, 36 and 50 post partum, to measure progesterone (P₄) concentrations. Blood samples were collected from the coccygeal vessels by venipuncture into 10 ml vacutainers without any anticoagulant and
placed on ice immediately after collection. Samples were centrifuged at 5000 x g for 15 minutes, and serum was stored at -20°C until assayed for progesterone.

Table 4-1. Distribution of cows enrolled in the study between June and August 2003 within Groups 1 and 2 based on days in milk (DIM), parity (primiparous/multiparous), dystocia (yes/no) and retained fetal membranes (yes/no).

<table>
<thead>
<tr>
<th>VARIABLE</th>
<th>GROUP 1 (Treated)</th>
<th>GROUP 2 (Control)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>n</td>
<td>%</td>
</tr>
<tr>
<td>DIM</td>
<td></td>
<td></td>
</tr>
<tr>
<td>7</td>
<td>45/114</td>
<td>39.5</td>
</tr>
<tr>
<td>8</td>
<td>31/114</td>
<td>27.2</td>
</tr>
<tr>
<td>9</td>
<td>38/114</td>
<td>33.3</td>
</tr>
<tr>
<td>Parity</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Primiparous</td>
<td>36/114</td>
<td>31.6</td>
</tr>
<tr>
<td>Multiparous</td>
<td>78/114</td>
<td>68.2</td>
</tr>
<tr>
<td>Dystocia</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td>8/114</td>
<td>7.0</td>
</tr>
<tr>
<td>No</td>
<td>106/114</td>
<td>93.0</td>
</tr>
<tr>
<td>Retained Fetal Membranes</td>
<td></td>
<td></td>
</tr>
<tr>
<td>No</td>
<td>82/114</td>
<td>71.9</td>
</tr>
<tr>
<td>Yes</td>
<td>32/114</td>
<td>28.1</td>
</tr>
</tbody>
</table>

Serum progesterone (P₄) concentrations were determined using a Coat-A-Count Kit (DPC® Diagnostic Products Incorporation, CA, USA) using solid-phase, no-extraction radioimmunoassay previously described (Srikandakumar et al., 1986). A standard curve was prepared using plain uncoated polypropylene tubes for total counts and non-specific binding and coated tubes. A volume of 100 μl of calibrators was added to each tube in increasing concentrations of 0.1, 0.25, 0.5, 1, 2, 5, 10 and 20 ng/ml.

Reference samples, ovariectomized, low and high (6.0 -7.0 ng/ml representative of luteal phase P₄ concentrations) were also used. The calibrators and reference samples were
performed in duplicate. A 100 µl serum sample was added to coated tubes and 1 ml of 125I-labelled P₄. Every sixth sample was done in duplicate. The tubes were incubated at room temperature for 3 hours, decanted, dried for 15 minutes and counted for 1 minute in a gamma counter. The gamma count for each tube was converted using the calibration curve to give the P₄ concentration of the serum samples.

Vaginoscopy

On Days 22 and 58 post partum, vaginoscopy was performed to determine the presence or absence of a mucopurulent vaginal discharge. The vulva and surrounding area were washed with water to remove fecal matter. Next the vulva was washed with iodine and water and dried with a paper towel. Lubricant was applied to a sterile disposable foil-lined cardboard vaginal speculum. The speculum was inserted into the vagina up to the level of the external cervical os and a penlight was used to visualize the vaginal walls, cervix and content of the vagina.

The cervix was evaluated to be either open or closed. Vaginal discharge was classified as normal, clear, mucoid (clear mucus), mucopurulent (presence of flecks of pus and mucus to purulent – thick creamy to cheesy exudates) or serous mucopurulent (pus, red-brown and/or foul smelling). The quantity of the discharge was also classified as slight, moderate or copious. The color of the vaginal wall and the external os of the cervix was evaluated and classified as pink, red and red and inflamed. These findings were recorded (Appendix A).

Transrectal Palpation of the Reproductive Tract

Prior to vaginoscopy, transrectal palpation of the reproductive tract was performed and the findings recorded (Appendix A). The data were noted as follows:
• Size of cervix (diameter of cervix: <20, 25, 30, 35, 40, 45, 50, >50mm)
• Previously pregnant horn (right/left)
• Size of the left horn (diameter of uterine horn: <20, 25, 30, 35, 40, 45, 50, >50mm)
• Size of the right horn (diameter of uterine horn: <20, 25, 30, 35, 40, 45, 50, >50mm)
• Fluid in right horn (absent/present)
• Fluid in left horn (absent/present)
• Structures on right and left ovaries (no significant structures (NSS), follicle and follicle size, CL – present/absent)

**Statistical Analysis**

All analysis was performed with SAS, version 9.0 (2001). Baseline comparisons for parity (primiparous and multiparous), DIM, dystocia (yes/no), retained fetal membranes (yes/no) and P₄ (≤1.0 ng/ml or > 1.0 ng/ml) on Day 8 post partum were compared using Chi-square (P≤0.05).

LeBlanc et al. (2002) defined clinical endometritis based on its impact on pregnancy rate. When vaginoscopy was performed clinical endometritis was defined as the presence of a purulent, or foul-smelling or fetid discharge, or cervical diameter >7.5 cm between 20 and 33 DIM, or as a mucopurulent discharge after 26 DIM. In the event that vaginoscopic examination was not performed, the presence of mucopurulent or purulent discharge on the perineum, cervical diameter >7.5 cm and the presence of a uterine horn ≥ 8 cm in diameter were used to define clinical endometritis.

Based on these definitions, three models were used to assess the effect of treatment on the criteria used to define clinical endometritis. Model 1 consisted of assessing the effect of treatment on the presence of purulent or foul-smelling or fetid discharge at Days 22 and 58 post partum. A purulent or foul-smelling discharge is defined here as a mucopurulent (presence of flecks of pus and mucus to purulent – a thick creamy to cheesy exudates) or serous mucopurulent (pus, red-brown and/or foul-
smelling) discharge. Model 2 consisted of assessing the effect of treatment on the presence of purulent or foul-smelling discharge and cervix > 50 mm at Day 22 and the presence of purulent or foul discharge and cervix > 30 mm at Day 58 post partum. Model 3 consisted of assessing the effect of treatment on the presence of purulent or foul-smelling discharge, cervix > 50 mm and the presence of a uterine horn ≥ 30 mm in diameter at Day 22 and the presence of purulent or foul-smelling discharge, cervix > 30 mm and the presence of a uterine horn ≥ 30 mm in diameter at Day 58 post partum. The diameter of the cervix and uterine horn were chosen based on the median value on Day 22 and Day 58 post partum.

The outcomes of interest for this experiment were the effect on treatment on Models 1, 2, and 3 on Days 22 and 58 post partum. Data for Models 1, 2, and 3 on both Day 22 and Day 58 post partum were analyzed using logistic regression (Proc Logistic, SAS 9.0) adjusting for parity (primiparous/multiparous), dystocia (yes/no) and RFM (yes/no) and a value of P ≤ 0.05 was considered statistically significant.

The association between treatment and first service conception rate was evaluated using logistic regression (Full Model; Proc Logistic, SAS 9.0) adjusting for group, parity (primiparous/multiparous), dystocia (yes/no), RFM (yes/no), and days to first service (≤130 days/>130 days). A value of P ≤ 0.05 was considered statistically significant.

**Experiment 2 – Part B**

This study was performed between October 2003 and March 2004 in a the same dairy herd as Experiment 1. The conception rate to timed insemination in this dairy herd was approximately 30%. It was anticipated that the proposed treatment would result in a 15% increase in conception rate to approximately 45%. A total of 127 cows per group
will provide 95% confidence and 80% power to declare a difference in conception rates between 30% and 45% statistically significant.

A total of 418 cows was used in this study, and cows were enrolled at 7 days post partum. At this time, 2 experimental groups were formed using sequential randomization. In addition, information concerning retained fetal membranes (yes/no), dystocia (yes/no) and parity (2, 3+) were recorded. No primiparous or first-calf heifers were enrolled in the study based on management discussions.

**Treatment**

Cows in Group 1 (n=209) were treated with two luteolytic dosage of PGF2α (25 mg intramuscularly: im) 8 hours apart on Days 7 and 14 post partum, and one luteolytic dosage of PGF2α (25 mg, im) once on Days 21 and 35 post partum. On Days 49 and 63 post partum, cows were treated with one luteolytic dosage of PGF2α (25 mg, im; Presynch). Cows were subjected to the Ovsynch protocol on Day 75 post partum and time inseminated on either Days 85 or 86 post partum (Figure 4-2).

Cows in Group 2 (n=209) were not be treated with PGF2α on Days 7, 14, 21 and 35 post partum and served as untreated controls. However, cows in Group 2 were treated with one luteolytic dosage of PGF2α (25 mg, im) once on both Days 49 and 63 post partum (Presynch). Cows in Group 2 were also subjected to the Ovsynch protocol starting on Day 75 post partum, 12 days following the last PGF2α injection of Presynch, and time inseminated on either Days 85 or 86 post partum (Figure 4-2).
**Exclusion Criteria**

Animals were excluded or withdrawn from the study for any of the following reasons; failure to complete the treatment scheme; animals with RFM or dystocia which later experienced metritis and were aggressively treated with penicillin, cephalosporin and/or PGF2α; animals that underwent surgery (correction of displaced abomasum); animals placed in the hospital barn that received systemic antibiotic therapy during the study period; animals assigned as controls treated with PGF2α during the study period; animals culled from the herd or which died during the study period.

**Pregnancy Diagnosis**

Pregnancy was determined using ultrasonography (ALOKA 500 with a 5 MHz probe) 29-32 days after timed insemination of cows in both groups. The presence of intrauterine fluid and a viable embryo were the criterion used to evaluate pregnancy status. The presence or absence of a heartbeat was the criterion used to determine viability of the embryo.
**Statistical Analysis**

Baseline comparison for parity (2, 3+), retained fetal membranes (yes/no), dystocia (yes/no), and abnormal calving (dystocia and/or RFM) was carried out to establish comparability of the groups using Chi-Square test. A value of $P \leq 0.05$ was considered statistically significant.

The outcome of interest was conception to first service. The effect of treatment (group) on conception to first service was evaluated using both Chi-Square and logistic regression (Proc Logistic, SAS 9.0). Included in the model were parity (2, 3+), retained fetal membranes (yes/no) and dystocia (yes/no). A value of $P \leq 0.05$ was considered statistically significant.

**Results**

**Experiment 2 – Part A**

**Day 8 post partum**

A total of 203 cows successfully completed the study with distribution as follows: Group 1, 101/114 (88.6%) and Group 2 102/114 (89.5%). The baseline comparison for parity (primiparous/multiparous), days in milk (DIM), dystocia (no/yes), retained fetal membranes (no/yes) and P₄ concentration ($\leq 1.0$ ng/ml / $>1.0$ ng/ml) at 8 days post partum are shown in Table 4-2.

**Day 16 post partum**

Blood was obtained and analyzed for $P₄$ for 97/101 (96.0%) of cows in Group 1, and 98/102 cows (96.1%) in Group 2. Group 1 had 5/97 cows (5.2%) with $P₄$ levels $>1.0$ ng/ml on Day 16 post partum, and Group 2 had 9/98 cows (9.2%) with $P₄$ levels $>1.0$ ng/ml on Day 16 post partum. Progesterone levels ranged from 0.1 ng/ml to 6.26 ng/ml.
for cows in Group 1, and from 0.1 ng/ml to 3.11 ng/ml for cows in Group 2. The mean and standard error of the mean were:

Group 1: 0.29 ± 0.07 ng/ml; Group 2: 0.29 ± 0.06 ng/ml, (P>0.05).

Table 4-2. Baseline data for heifers and cows that successfully completed the study for parity (primiparous/multiparous), days in milk, dystocia (no/yes), retained fetal membranes (no/yes) and progesterone concentration (≤ 1.0 ng/ml / >1.0 ng/ml) at 8 days post partum.

<table>
<thead>
<tr>
<th>VARIABLE</th>
<th>GROUP 1 (Treated)</th>
<th>Group 2 (Control)</th>
<th>P-Value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>n</td>
<td>%</td>
<td>n</td>
</tr>
<tr>
<td>Parity</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Primiparous</td>
<td>32/101</td>
<td>31.7</td>
<td>29/102</td>
</tr>
<tr>
<td>Multiparous</td>
<td>69/101</td>
<td>68.3</td>
<td>73/102</td>
</tr>
<tr>
<td>Day in Milk</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>7</td>
<td>40/101</td>
<td>39.6</td>
<td>32/102</td>
</tr>
<tr>
<td>8</td>
<td>27/101</td>
<td>26.7</td>
<td>42/102</td>
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<td>9</td>
<td>34/101</td>
<td>33.7</td>
<td>28/102</td>
</tr>
<tr>
<td>Dystocia</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>No</td>
<td>94/101</td>
<td>93.1</td>
<td>88/102</td>
</tr>
<tr>
<td>Yes</td>
<td>7/101</td>
<td>6.9</td>
<td>14/102</td>
</tr>
<tr>
<td>Retained Fetal Membranes</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>No</td>
<td>73/101</td>
<td>72.3</td>
<td>82/102</td>
</tr>
<tr>
<td>Yes</td>
<td>28/101</td>
<td>27.7</td>
<td>20/102</td>
</tr>
<tr>
<td>P₄ on Day 8 post partum</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>&gt;1.0 ng/ml</td>
<td>1/101</td>
<td>1.0</td>
<td>2/101</td>
</tr>
<tr>
<td>≤ 1.0 ng/ml</td>
<td>100/101</td>
<td>99.0</td>
<td>99/101</td>
</tr>
</tbody>
</table>

**Day 22 post partum**

On Day 22 post partum, blood was taken for P₄ evaluation, vaginoscopy and transrectal palpation of the reproductive tract were performed.

**P₄ evaluation:** Blood was obtained and analyzed for P₄ for 96/101 (95.0%) of cows in Group 1, and 99/102 (97.1%) in Group 2. Cows in Group 1 had 20/96 (20.8%) with P₄
levels $> 1.0$ ng/ml on Day 22 post partum, and cows in Group 2 had 27/99 (27.3%) with
P$_4$ levels $> 1.0$ ng/ml on Day 22 post partum. Progesterone levels ranged from 0.1 ng/ml
to 5.47 ng/ml in cows in Group 1 and from 0.1 ng/ml to 7.94 ng/ml in cows in Group 2.

The mean and standard error of the mean were:

Group 1: 0.78 ± 0.14 ng/ml; Group 2: 0.83 ± 0.15 ng/ml, (P>0.05).

Vaginoscopy: Of the 228 cows enrolled at 7-9 days post partum, one cow died
(1/228; 0.4%), two cows were culled (2/228; 0.9%) from the herd, and 8 cows were
placed in the hospital barn (8/228; 3.5%) for various ailments prior to vaginoscopy and
transrectal palpation of the reproductive tract. At Day 22 post partum, 14 cows were not
examined by vaginoscopy (14/228; 6.1%). Data for vaginoscopic examination were
available for 203/228 (89.0%) of the cows enrolled in the study. However in Group 1,
99/101 cows (98.0%) and Group 2, 100/102 cows (98.0%) were completely examined
and all the variables were recorded.

Group 1 (Treated): The cervix was visualized in 100/101 (99.0%) cows. In one
cow, the cervix could not be seen due to the large volume of fluid in the lumen of the
vagina. The cervix was open in 53/100 cows (53.0%). The vaginal wall and external os of
the cervix were pink in color in 69/101 cows (68.3%), red in 24/101(23.8%), and red and
inflamed in 8/101 (7.9%). In one cow, discharge type and discharge quantity were not
noted. An abnormal discharge (a mucopurulent discharge with presence of flecks of pus
and mucus, purulent, thick creamy to cheesy exudate or serous mucopurulent with pus,
red-brown and/or foul-smelling) was seen in 57/100 cows (57.0%) with 54/100 cows
(54.0%) classified as having a mucopurulent and 3/100 cows (3.0%) with a serous
mucopurulent discharge.
**Group 2 (Control):** The cervix was visualized in 100/102 (98.0%) cows. In one cow, vaginoscopy was not performed, and in a second cow the cervix was not classified as open or closed. The cervix was open in 58/100 cows (58.0%). The vaginal wall and external os of the cervix were pink in color in 72/101 cows (71.3%), red in 25/101 (24.7%), and red and inflamed in 4/101(4.0%). An abnormal discharge was seen in 53/101 cows (52.5%) with 51/101 (50.5%) classified as having a mucopurulent and 2/101 (2.0%) with a serous mucopurulent discharge.

**Transrectal palpation:** Data for transrectal palpation of the reproductive tract were available for 203/228 (89%) of the cows enrolled in the study. However in Group 1, 93/101 (92.1%) cows, and Group 2, 94/102 (92.2%) cows were completely examined and all the variables were recorded.

**Group 1 (Treated):** The size of the cervix was classified as “greater than” or “less than or equal to” the median value on Day 22 post partum. The median value was 50 mm in diameter with a range of <20 mm to >50 mm. There was a total of 38/101 cows (37.6%) with the cervix > 50 mm in diameter on rectal palpation. The previously pregnant horn (PPH) was identified as the larger of the two uterine horns. The majority of pregnancies appeared to have occurred in the right horn (67/101; 66.3%), and in 7/101 (7.0%) the horns were estimated to be the same size and were designated as non-distinguishable. The size of the PPH was classified as “greater than” or “less than or equal to” the median value. The median value on Day 22 post partum was 30 mm in diameter with a range of <20 to >50 mm in diameter. Both categories were almost evenly distributed with 47/100 (47.0%) > 30 mm in diameter. Fluid was present in the uterine horns of 25/92 cows (27.2%) and a palpable CL was identified in 37/97 cows (38.1%).
**Group 2 (Control):** The size of the cervix was classified as “greater than” or “less than or equal to” the median value on Day 22 post partum. The median value was 50 mm in diameter with a range of <20 mm to >50 mm. There was a total of 40/102 cows (39.2%) with the cervix > 50 mm in diameter on rectal palpation. The previously pregnant horn (PPH) was identified as the larger of the two uterine horns. The majority of pregnancies appeared to have occurred in the right horn (69/102; 67.7%), and in 9/102 cows (8.8%) the horns were estimated to be the same size and were designated as non-distinguishable. In one animal (1/102; 0.9%) there were adhesions of the uterine horns and the size could not be determined. The size of the PPH was classified as “greater than” or “less than or equal to” the median value. The median value on Day 22 post partum was 30 mm in diameter with a range of <20 to >50 mm in diameter. Both categories were almost evenly distributed with 49/101 (48.5%) > 30 mm in diameter. Fluid was present in the uterine horns of 18/90 (20.0%) cows and a palpable CL was identified in 58/97 cows (59.8%).

**Day 36 post partum**

Blood was obtained and analyzed for P₄ for 94/101 (93.1%) of cows in Group 1 and 95/102 (93.1%) in Group 2. Group 1 had 36/94 cows (38.3%) with P₄ levels > 1.0 ng/ml, and Group 2 had 42/95 cows (44.2%) with P₄ levels > 1.0 ng/ml. Progesterone levels ranged from 0.1 ng/ml to 12.90 ng/ml in Group 1 and from 0.1 ng/ml to 9.22 ng/ml in Group 2. The mean and standard error of the mean were:

Group 1: 1.80 ± 0.23 ng/ml; Group 2: 1.80 ± 0.22 ng/ml, (P>0.05).
Day 58 post partum

On Day 58 post partum, blood was taken for P4 evaluation, and vaginoscopy and transrectal palpation of the reproductive tract were performed.

**P4 evaluation:** Blood was obtained and analyzed for P4 for 92/101 (91.1%) of cows in Group 1 and 96/102 cows (94.1%) in Group 2. Group 1 had 45/92 cows (48.9%) with P4 levels > 1.0 ng/ml, and Group 2 had 47/96 cows (49.0%) with P4 levels > 1.0 ng/ml. Progesterone levels ranged from 0.1 ng/ml to 8.97 ng/ml in cows in Group 1, and from 0.1 ng/ml to 7.56 ng/ml in cows in Group 2. The mean and standard error of the mean were:

- Group 1: 2.45 ± 0.24 ng/ml; Group 2: 1.95 ± 0.21 ng/ml, (P>0.05).

**Vaginoscopy:** Of the 228 cows enrolled on Day 58 post partum, two cows died (2/228; 0.9%); three cows were culled (3/228; 1.3%) from the herd, four cows were placed in the hospital barn (4/228; 1.8%) for various ailments prior to transrectal palpation of the reproductive tract and vaginoscopic examination, and 7 were missed on Day 22 post partum which were removed from the study due to failure to complete the treatment regime. On Day 58 post partum a total of 9 cows was not examined by vaginoscopy (9/228; 3.9%).

**Group 1 (Treated):** A total of 93 animals was available for vaginoscopy. The cervix was visualized in 90/93 (96.8%) cows. The cervix was open in 46/90 cows (51.1%). The vaginal wall and external os of the cervix were pink in color in 60/92 (65.2%), red in 27/92 (29.4%), and red and inflamed in 5/92 (5.4%). An abnormal discharge was seen in 26/92 cows (28.3%), and in 24/92 cows (26.1%) this was classified as mucopurulent and 2/92 (2.2%) as serous mucopurulent.
Group 2 (Control): A total of 98 animals was available for vaginoscopy. The cervix was visualized in 97/98 (99.0%) cows. The cervix was open in 51/98 cows (52.0%). The vaginal wall and external os of the cervix were pink in color in 45/97 (46.4%), red in 47/97 (48.5%), and red and inflamed in 5/97 (5.1%). Abnormal discharge was seen in 34/98 cows (34.7%) with all abnormal discharge classified as mucopurulent.

Transrectal palpation: Data for transrectal palpation of the reproductive tract was available for 191/228 (83.7%) of the cows enrolled in the study.

Group 1 (Treated): A total of 92 animals was available for transrectal palpation of the reproductive tract. The size of the cervix was classified as “greater than” or “less than or equal to” the median value on Day 58 post partum. The median value was 30 mm in diameter with a range of <20 mm to >50 mm. There was a total of 38/92 (41.3%) with the cervix > 30 mm in diameter on rectal palpation. The previously pregnant horn (PPH) was identified as the larger of the two uterine horns. The majority of pregnancies occurred in the right horn (67/101; 66.3%), and in 7/101 cows (7.0%) the horns were estimated to be the same size and were designated as non-distinguishable. The size of the PPH was classified as “greater than” or “less than or equal to” the median value. The median value on Day 58 post partum was 30 mm in diameter with a range of <20 to >50 mm in diameter. Both categories were almost evenly distributed with 13/92 (14.1%) > 30 mm in diameter. Fluid was present in the uterine horns of 5/89 (5.6%) animals and a palpable CL was identified on an ovary in 64/92 cows (69.6%).

Group 2 (Control): A total of 99 animals was available for transrectal palpation of the reproductive tract. The size of the cervix was classified as “greater than” or “less than or equal to” the median value on Day 58 post partum. The median value was 30 mm
in diameter with a range of <20 mm to >50 mm. There was a total of 50/99 cows (50.5%) with the cervix > 30 mm in diameter on rectal palpation. The previously pregnant horn (PPH) was identified as the larger of the two uterine horns. The majority of pregnancies occurred in the right horn (69/102; 67.7%), and in 9/102 cows (8.8%) the horns were estimated to be the same size and were designated as non-distinguishable. In one animal (1/102; 0.9%), there were adhesions of the uterine horns and the size could not be determined. The size of the PPH was classified as “greater than” or “less than or equal to” the median value. The median value on Day 58 post partum was 30 mm in diameter with a range of <20 to >50 mm in diameter. Both categories were almost evenly distributed with 30/98 (30.6%) > 30 mm in diameter. Fluid was present in the uterine horns of 10/95 (10.5%) animals and a palpable CL was identified in 66/97 cows (68.0%).

**Model 1**

In Model 1, the effect of treatment on the presence of purulent or foul-smelling discharge at Days 22 and 58 post partum was evaluated. Progesterone concentrations (<1.0 ng/ml/≥1.0 ng/ml) on Days 8 and 15 post partum were not associated with the presence of purulent or foul-smelling discharge on Day 22 post partum (Chi-square: P = 0.66 and P = 0.41 respectively). However, P₄ concentrations on Day 22 (Chi-square: P = 0.02) were associated with the presence of purulent or foul-smelling discharge on Day 58 post partum, while P₄ concentrations on Day 36 post partum tended to be associated with the presence of purulent or foul-smelling discharge on Day 58 post partum (Chi-square: P = 0.08).

There was no effect of treatment on the presence of purulent or foul-smelling discharge at Days 22 and 58 post partum (P = 0.904 and P = 0.134, respectively).
adjusting for parity (primiparous/multiparous), RFM (yes/no) and dystocia (yes/no; Table 4-3 and Table 4-4). Primiparous cows (AOR: 2.87; 95%CI: 1.43 – 5.71; P = 0.003) and cows with retained fetal membranes (AOR: 8.40; 95%CI: 3.45 – 20.41; P < 0.0001) were more likely to have a purulent or foul-smelling discharge at Day 22 post partum regardless of treatment. There was a tendency for cows that experienced dystocia (P = 0.064) to have a purulent or foul-smelling discharge at Day 22 post partum regardless of treatment (Table 4-3). Similarly, cows with retained fetal membranes (AOR: 6.13; 95%CI: 2.92 – 12.99; P < 0.0001) and cows that experienced dystocia (AOR: 4.07; 95%CI: 1.47 – 11.24; P = 0.007) were more likely to have a purulent or foul-smelling discharge at Day 58 post partum regardless of treatment (Table 4-4). Parity was not associated with the presence of a mucopurulent vaginal discharge on Day 58 post partum (P = 0.397).

**Model 2**

In Model 2, the effect of treatment on the presence of purulent or foul-smelling discharge and cervix > 50mm at Day 22, and the presence of purulent or foul discharge and cervix > 30mm at Day 58 post partum were evaluated.

There was no association between progesterone concentrations (<1.0 ng/ml/≥1.0 ng/ml) on Days 8 and 15 post partum with the presence of purulent or foul-smelling discharge on Day 22 post partum (Chi-square: P = 0.31 and P = 0.76, respectively). There was a tendency for P₄ concentrations (<1.0 ng/ml/≥1.0 ng/ml) on Day 22 (Chi-square: P = 0.08) to have an association with the presence of purulent or foul-smelling discharge and cervix > 30mm at Day 58 post partum while P₄ concentrations on Day 36 post
partum had no association with the presence of purulent or foul-smelling discharge on Day 58 post partum (Chi-square: P = 0.14).

Table 4-3. Model 1—Percentage, crude odds ratio (COR), adjusted odds ratio (AOR), 95% confidence interval (CI) and P-value for the risk of finding a mucopurulent vaginal discharge by vaginoscopy on Day 22 post partum.

<table>
<thead>
<tr>
<th>VARIABLE</th>
<th>MUCOPURULENT DISCHARGE</th>
<th>COR</th>
<th>AOR</th>
<th>95% CI</th>
<th>P-VALUE</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>n</td>
<td>%</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Group</td>
<td></td>
<td></td>
<td></td>
<td></td>
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</tr>
<tr>
<td>Treated</td>
<td>56</td>
<td>56.0</td>
<td>1.15</td>
<td>1.04</td>
<td>0.56 – 1.94</td>
</tr>
<tr>
<td>Control</td>
<td>53</td>
<td>52.5</td>
<td>1.00</td>
<td>1.00</td>
<td>Referent</td>
</tr>
<tr>
<td>Parity</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Primiparous</td>
<td>42</td>
<td>68.9</td>
<td>2.41</td>
<td>2.87</td>
<td>1.43 – 5.71</td>
</tr>
<tr>
<td>Multiparous</td>
<td>67</td>
<td>47.9</td>
<td>1.00</td>
<td>1.00</td>
<td>Referent</td>
</tr>
<tr>
<td>RFM</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td>41</td>
<td>85.4</td>
<td>7.32</td>
<td>8.40</td>
<td>3.45 – 20.41</td>
</tr>
<tr>
<td>No</td>
<td>68</td>
<td>44.4</td>
<td>1.00</td>
<td>1.00</td>
<td>Referent</td>
</tr>
<tr>
<td>Dystocia</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td>17</td>
<td>61.9</td>
<td>4.07</td>
<td>3.13</td>
<td>0.94 – 10.42</td>
</tr>
<tr>
<td>No</td>
<td>92</td>
<td>25.8</td>
<td>1.00</td>
<td>1.00</td>
<td>Referent</td>
</tr>
</tbody>
</table>

Table 4-4. Model 1—Percentage, crude odds ratio (COR), adjusted odds ratio (AOR), 95% confidence interval (CI) and P-value for the risk of finding a mucopurulent vaginal discharge by vaginoscopy on Day 58 post partum.

<table>
<thead>
<tr>
<th>VARIABLE</th>
<th>MUCOPURULENT DISCHARGE</th>
<th>COR</th>
<th>AOR</th>
<th>95% CI</th>
<th>P-VALUE</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>n</td>
<td>%</td>
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<td></td>
</tr>
<tr>
<td>Group</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Treated</td>
<td>26</td>
<td>25.7</td>
<td>0.74</td>
<td>0.59</td>
<td>0.30 – 1.18</td>
</tr>
<tr>
<td>Control</td>
<td>34</td>
<td>33.3</td>
<td>1.00</td>
<td>1.00</td>
<td>Referent</td>
</tr>
<tr>
<td>Parity</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Primiparous</td>
<td>20</td>
<td>32.8</td>
<td>1.24</td>
<td>1.37</td>
<td>0.66 – 2.84</td>
</tr>
<tr>
<td>Multiparous</td>
<td>40</td>
<td>28.2</td>
<td>1.00</td>
<td>1.00</td>
<td>Referent</td>
</tr>
<tr>
<td>RFM</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td>28</td>
<td>58.3</td>
<td>5.38</td>
<td>6.13</td>
<td>2.92 – 12.99</td>
</tr>
<tr>
<td>No</td>
<td>32</td>
<td>20.6</td>
<td>1.00</td>
<td>1.00</td>
<td>Referent</td>
</tr>
<tr>
<td>Dystocia</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td>13</td>
<td>61.9</td>
<td>4.66</td>
<td>4.07</td>
<td>1.47 – 11.24</td>
</tr>
<tr>
<td>No</td>
<td>47</td>
<td>25.8</td>
<td>1.00</td>
<td>1.00</td>
<td>Referent</td>
</tr>
</tbody>
</table>
There was no effect of treatment on the presence of purulent or foul-smelling discharge and a cervix >50 mm in diameter at Day 22 post partum (P = 0.913; Table 4-5) adjusting for parity, RFM and dystocia. Cows with retained fetal membranes (AOR: 4.31; 95%CI: 2.10-8.85; P < 0.0001) and cows experiencing dystocia (AOR: 2.93; 95%CI: 1.07-8.00; P = 0.037) were more likely to have a purulent or foul-smelling discharge at Day 22 post partum regardless of treatment. There was no association between parity and a mucopurulent vaginal discharge on Day 22 post partum (P = 0.612; Table 4-5).

Table 4-5. Model 2—Percentage, crude odds ratio (COR), adjusted odds ratio (AOR), 95% confidence interval (CI) and P-value for the risk of finding a mucopurulent vaginal discharge by vaginoscopy and a cervix > 50 mm in diameter by transrectal palpation on Day 22 post partum.

<table>
<thead>
<tr>
<th>VARIABLE</th>
<th>MUCOPURULENT DISCHARGE AND CERVIX &gt; 50 mm</th>
<th>COR</th>
<th>AOR</th>
<th>95% CI</th>
<th>P-VALUE</th>
</tr>
</thead>
<tbody>
<tr>
<td>n</td>
<td>%</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Group</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Treated</td>
<td>26/100</td>
<td>26.0</td>
<td>0.99</td>
<td>0.96</td>
<td>0.48 – 1.91</td>
</tr>
<tr>
<td>Control</td>
<td>26/101</td>
<td>25.7</td>
<td>1.00</td>
<td>1.00</td>
<td>Referent</td>
</tr>
<tr>
<td>Parity</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Primiparous</td>
<td>14/61</td>
<td>23.0</td>
<td>0.80</td>
<td>0.82</td>
<td>0.39 – 1.75</td>
</tr>
<tr>
<td>Multiparous</td>
<td>38/140</td>
<td>27.1</td>
<td>1.00</td>
<td>1.00</td>
<td>Referent</td>
</tr>
<tr>
<td>RFM</td>
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<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td>24/48</td>
<td>50.0</td>
<td>4.46</td>
<td>4.31</td>
<td>2.10 – 8.85</td>
</tr>
<tr>
<td>No</td>
<td>28/153</td>
<td>18.3</td>
<td>1.00</td>
<td>1.00</td>
<td>Referent</td>
</tr>
<tr>
<td>Dystocia</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td>10/21</td>
<td>47.6</td>
<td>2.99</td>
<td>2.93</td>
<td>1.07 – 8.00</td>
</tr>
<tr>
<td>No</td>
<td>42/180</td>
<td>23.3</td>
<td>1.00</td>
<td>1.00</td>
<td>Referent</td>
</tr>
</tbody>
</table>

There was no effect of treatment on the presence of purulent or foul-smelling discharge and a cervix >30 mm in diameter at Day 58 post partum (P = 0.692; Table 4-6) adjusting for parity, RFM, and dystocia. However, multiparous cows (AOR: 0.15; 95%CI: 0.03-0.62; P = 0.010) were less likely to have a purulent or foul-smelling discharge and a cervix > 30 mm in diameter at Day 58 post partum regardless of treatment. Cows with retained fetal membranes (AOR: 6.49; 95%CI: 2.53-16.67; P <
0.0001) and cows that experienced dystocia (AOR: 11.66; 95%CI: 3.22-50.00; P =
0.0003) were more likely to have a purulent or foul-smelling discharge and a cervix > 30
mm in diameter at Day 58 post partum regardless of treatment (Table 4-6).

Table 4-6. Model 2—Percentage, crude odds ratio (COR), adjusted odds ratio (AOR),
95% confidence interval (CI) and P-value for the risk of finding a
mucopurulent vaginal discharge by vaginoscopy and a cervix > 30 mm in
diameter by transrectal palpation on Day 58 post partum.

<table>
<thead>
<tr>
<th>VARIABLE</th>
<th>MUCOPURULENT DISCHARGE AND CERVIX &gt; 30 mm</th>
<th>COR</th>
<th>AOR</th>
<th>95% CI</th>
<th>P-VALUE</th>
</tr>
</thead>
<tbody>
<tr>
<td>n</td>
<td>%</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Group</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Treated</td>
<td>12/92</td>
<td>0.71</td>
<td>0.83</td>
<td>0.32 – 2.11</td>
<td>0.692</td>
</tr>
<tr>
<td>Control</td>
<td>17/98</td>
<td>1.00</td>
<td>1.00</td>
<td>Referent</td>
<td>NA</td>
</tr>
<tr>
<td>Parity</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Primiparous</td>
<td>3/56</td>
<td>0.23</td>
<td>0.15</td>
<td>0.03 – 0.62</td>
<td>0.010</td>
</tr>
<tr>
<td>Multiparous</td>
<td>26/134</td>
<td>1.00</td>
<td>1.00</td>
<td>Referent</td>
<td>NA</td>
</tr>
<tr>
<td>RFM</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td>16/42</td>
<td>6.39</td>
<td>6.49</td>
<td>2.53 – 16.67</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>No</td>
<td>13/148</td>
<td>1.00</td>
<td>1.00</td>
<td>Referent</td>
<td>NA</td>
</tr>
<tr>
<td>Dystocia</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td>9/19</td>
<td>6.79</td>
<td>12.66</td>
<td>3.22 – 50.00</td>
<td>0.0003</td>
</tr>
<tr>
<td>No</td>
<td>20/171</td>
<td>1.00</td>
<td>1.00</td>
<td>Referent</td>
<td>NA</td>
</tr>
</tbody>
</table>

Model 3

In Model 3, the effect of treatment on the presence of purulent or foul-smelling
discharge, cervix > 50mm and the presence of a uterine horn ≥ 30 mm in diameter on
Day 22 post partum and the presence of purulent or foul-smelling discharge, cervix >
30mm and the presence of a uterine horn ≥ 30 mm in diameter on Day 58 post partum
were evaluated.

Progesterone concentrations (<1.0 ng/ml/≥1.0 ng/ml) on Days 8 and 15 post partum had no association with the presence of purulent or foul-smelling discharge,
cervix > 50mm and the presence of a uterine horn ≥ 30 mm in diameter at Day 22 post partum (Chi-square: P = 0.42 and P = 0.79, respectively).

There was no effect of treatment on the presence of purulent or foul-smelling discharge, a cervix >50 mm in diameter and previously pregnant horn ≥ 30 mm in diameter at Day 22 post partum (P = 0.508; Table 4-7) adjusting for parity, RFM and dystocia. There was a tendency for primiparous cows (AOR: 0.38; 95%CI: 0.14 – 1.06; P = 0.065) to be less likely to have a purulent or foul-smelling discharge, a cervix > 50 mm in diameter and the previously pregnant horn ≥ 30 mm at Day 22 post partum regardless of treatment (Table 4-7). Cows with retained fetal membranes (AOR: 7.58; 95%CI: 3.34 – 13.89; P < 0.0001) and cows experiencing dystocia (AOR: 4.22; 95%CI: 1.28 – 13.89; P = 0.018) were more likely to have a purulent or foul-smelling discharge, a cervix > 50 mm in diameter and the previously pregnant horn ≥ 30 mm at Day 22 post partum regardless of treatment (Table 4-7).

There was a tendency for P₄ concentrations (<1.0 ng/ml/≥1.0 ng/ml) on Days 22 post partum to be associated with the presence of purulent or foul-smelling discharge, cervix > 30mm and the presence of a uterine horn ≥ 30 mm in diameter at Day 58 post partum (Chi-square: P = 0.07) while P₄ concentrations (<1.0 ng/ml/≥1.0 ng/ml) on Day 36 post partum were not associated with the criteria for Model 3 (Chi-square: P = 0.59).
Table 4-7. Model 3—Percentage, crude odds ratio (COR), adjusted odds ratio (AOR), 95% confidence interval (CI) and P-value for the risk of finding a mucopurulent vaginal discharge by vaginoscopy, cervix > 50 mm in diameter and previously pregnant horn (PPH) ≥ 30 mm by transrectal palpation on Day 22 post partum.

<table>
<thead>
<tr>
<th>VARIABLE</th>
<th>MUCOPURULENT DISCHARGE, CERVIX &gt; 50 mm and PPH ≥ 30 mm</th>
<th>COR</th>
<th>AOR</th>
<th>95% CI</th>
<th>P-VALUE</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>N</strong></td>
<td><strong>%</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Group</td>
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<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Treated</td>
<td>20/99</td>
<td>20.2</td>
<td>1.33</td>
<td>1.33</td>
<td>0.57 – 3.06</td>
</tr>
<tr>
<td>Control</td>
<td>16/100</td>
<td>16.0</td>
<td>1.00</td>
<td>1.00</td>
<td>Referent</td>
</tr>
<tr>
<td>Parity</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Primiparous</td>
<td>6/60</td>
<td>10.0</td>
<td>0.40</td>
<td>0.38</td>
<td>0.14 – 1.06</td>
</tr>
<tr>
<td>Multiparous</td>
<td>30/139</td>
<td>21.6</td>
<td>1.00</td>
<td>1.00</td>
<td>Referent</td>
</tr>
<tr>
<td>RFM</td>
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<td></td>
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</tr>
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<td>45.8</td>
<td>8.28</td>
<td>7.58</td>
<td>3.34 – 13.89</td>
</tr>
<tr>
<td>No</td>
<td>14/151</td>
<td>9.3</td>
<td>1.00</td>
<td>1.00</td>
<td>Referent</td>
</tr>
<tr>
<td>Dystocia</td>
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<td></td>
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<td></td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td>8/21</td>
<td>38.1</td>
<td>3.30</td>
<td>4.22</td>
<td>1.28 – 13.89</td>
</tr>
<tr>
<td>No</td>
<td>28/178</td>
<td>15.7</td>
<td>1.00</td>
<td>1.00</td>
<td>Referent</td>
</tr>
</tbody>
</table>

There was an effect of treatment for the variables in Model 3 on Day 58 post partum. Cows in Group 1 (treated) were less likely to have a purulent or foul-smelling discharge, a cervix >30 mm in diameter and the previously pregnant horn ≥ 30 mm in diameter on Day 58 post partum (AOR: 0.30; 95%CI: 0.09 – 0.98; P = 0.047; adjusting for parity, RFM and dystocia).

Cows with retained fetal membranes (AOR: 8.47; 95%CI: 2.84 – 25.00; P = 0.0001) were more likely to have a purulent or foul-smelling discharge, a cervix > 30 mm in diameter and the previously pregnant horn ≥ 30 mm in diameter at Day 58 post partum regardless of treatment (Table 4-8). There was a tendency for cows that experienced dystocia (AOR: 3.92; 95%CI: 0.98 – 15.63; P = 0.054) to be more likely to have a purulent or foul-smelling discharge, a cervix > 30 mm in diameter and the previously
pregnant horn ≥ 30 mm in diameter at Day 58 post partum regardless of treatment (Table 4-8).

Table 4-8. Model 3—Percentage, crude odds ratio (COR), adjusted odds ratio (AOR), 95% confidence interval (CI) and P-value for the risk of finding a mucopurulent vaginal discharge by vaginoscopy, cervix > 30 mm in diameter and previously pregnant horn (PPH) ≥ 30 mm by transrectal palpation on Day 58 post partum.

<table>
<thead>
<tr>
<th>VARIABLE</th>
<th>MUCOPURULENT DISCHARGE, CERVIX &gt; 30 mm and PPH ≥ 30 mm</th>
<th>COR</th>
<th>AOR</th>
<th>95% CI</th>
<th>P-VALUE</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group</td>
<td></td>
<td>n</td>
<td>%</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Treated</td>
<td>5/92</td>
<td>5.4</td>
<td>0.34</td>
<td>0.30</td>
<td>0.09 – 0.98</td>
</tr>
<tr>
<td>Control</td>
<td>14/97</td>
<td>14.4</td>
<td>1.00</td>
<td>1.00</td>
<td>Referent</td>
</tr>
<tr>
<td>Parity</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Primiparous</td>
<td></td>
<td>2/55</td>
<td>3.6</td>
<td>0.26</td>
<td>0.24</td>
</tr>
<tr>
<td>Multiparous</td>
<td></td>
<td>17/134</td>
<td>12.7</td>
<td>1.00</td>
<td>1.00</td>
</tr>
<tr>
<td>RFM</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td>12/42</td>
<td>28.6</td>
<td>8.00</td>
<td>8.47</td>
<td>2.84 – 25.00</td>
</tr>
<tr>
<td>No</td>
<td>7/147</td>
<td>4.8</td>
<td>1.00</td>
<td>1.00</td>
<td>Referent</td>
</tr>
<tr>
<td>Dystocia</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td>5/19</td>
<td>26.3</td>
<td>3.98</td>
<td>3.92</td>
<td>0.98 – 15.63</td>
</tr>
<tr>
<td>No</td>
<td>14/170</td>
<td>8.2</td>
<td>1.00</td>
<td>1.00</td>
<td>Referent</td>
</tr>
</tbody>
</table>

First service pregnancy rate

A total of 33 cows was either bred and culled prior to examination for pregnancy, or not bred for various management and reproductive reasons. The median day to first service was 131.80 ± 1.19 days for cows in Group 1 (treated) and 132.52 ± 1.04 days for cows in Group 2 (control; P>0.05). Data for first service pregnancy rate were available for 170/203 (83.7%) cows, and this included 86 and 84 cows in Groups 1 and 2, respectively. The first service pregnancy rate was 23.3% (20/86) and 22.6% (19/84) for cows in Groups 1 and 2, respectively. There was no effect of treatment on first service pregnancy rate (AOR: 1.10; 95%CI: 0.52-2.30; P = 0.81), and there was no association
between parity (P = 0.24), dystocia (P = 0.61), RFM (P = 0.09) or days to first service (P = 0.20) on the outcome of pregnancy regardless of treatment (Table 4-9).

Table 4-9. Percentage, crude odds ratio (COR), adjusted odds ratio (AOR), 95% confidence interval (CI) and P-value for the risk of pregnancy in dairy cows treated sequentially with PGF2α in the early postpartum period to first service post partum.

<table>
<thead>
<tr>
<th>VARIABLE</th>
<th>PREGNANCY</th>
<th>COR</th>
<th>AOR</th>
<th>95% CI</th>
<th>P-VALUE</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>n</td>
<td>%</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Group</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Treated</td>
<td>20/86</td>
<td>23.3</td>
<td>1.04</td>
<td>1.10</td>
<td>0.52 – 2.30</td>
</tr>
<tr>
<td>Control</td>
<td>19/84</td>
<td>22.6</td>
<td>1.00</td>
<td>1.00</td>
<td>Referent</td>
</tr>
<tr>
<td>Parity</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Multiparous</td>
<td>29/114</td>
<td>25.4</td>
<td>1.57</td>
<td>1.65</td>
<td>0.72 – 3.79</td>
</tr>
<tr>
<td>Primiparous</td>
<td>10/56</td>
<td>17.9</td>
<td>1.00</td>
<td>1.00</td>
<td>Referent</td>
</tr>
<tr>
<td>Dystocia</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td>3/19</td>
<td>15.8</td>
<td>0.60</td>
<td>0.71</td>
<td>0.19 – 2.67</td>
</tr>
<tr>
<td>No</td>
<td>36/151</td>
<td>23.8</td>
<td>1.00</td>
<td>1.00</td>
<td>Referent</td>
</tr>
<tr>
<td>RFM</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td>5/38</td>
<td>13.2</td>
<td>0.44</td>
<td>0.41</td>
<td>0.15 – 1.15</td>
</tr>
<tr>
<td>No</td>
<td>34/132</td>
<td>25.8</td>
<td>1.00</td>
<td>1.00</td>
<td>Referent</td>
</tr>
<tr>
<td>Days to First Service</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>≤130 days</td>
<td>15/80</td>
<td>18.8</td>
<td>0.63</td>
<td>0.61</td>
<td>0.29 – 1.29</td>
</tr>
<tr>
<td>&gt;130 days</td>
<td>24/90</td>
<td>26.7</td>
<td>1.00</td>
<td>1.00</td>
<td>Referent</td>
</tr>
</tbody>
</table>

Experiment 2 – Part B : Conception Rate to First Service

One hundred and sixteen (116) cows were removed from the study. Sixty (60) cows were not bred and culled for various management and reproductive reasons prior to completion of the experiment, 13 cows were bred to the incorrect date, and 43 cows were removed from the experiment for failing to complete treatment and/or Presynch-Ovsynch protocol. A total of 302 animals successfully completed Experiment 2, Part B (Group 1; n=145 and Group 2; n=157). All cows were enrolled at 7 days in milk (DIM or 7 days post partum). Table 4-10 represents the baseline comparison of Group 1 and 2. There was no difference in parity (2, 3+), retained fetal membranes (yes/no), dystocia (yes/no) and abnormal calving (RFM and/or dystocia) between the groups.
Table 4-10. Baseline data for cows within Group 1 and Group 2 based on parity (2, 3+), dystocia (yes/no), retained fetal membranes (yes/no) and abnormal calving (RFM and/or dystocia) that successfully completed the study.

<table>
<thead>
<tr>
<th>VARIABLE</th>
<th>GROUP 1 (Treated)</th>
<th>GROUP 2 (Control)</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>N</td>
<td>%</td>
<td>N</td>
</tr>
<tr>
<td>Parity</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>67/145</td>
<td>46.2</td>
<td>61/157</td>
</tr>
<tr>
<td>3+</td>
<td>78/145</td>
<td>53.8</td>
<td>96/157</td>
</tr>
<tr>
<td>Dystocia</td>
<td></td>
<td></td>
<td>0.76</td>
</tr>
<tr>
<td>No</td>
<td>120/145</td>
<td>82.8</td>
<td>132/157</td>
</tr>
<tr>
<td>Yes</td>
<td>25/145</td>
<td>17.2</td>
<td>25/157</td>
</tr>
<tr>
<td>Retained Fetal Membranes</td>
<td></td>
<td></td>
<td>0.14</td>
</tr>
<tr>
<td>No</td>
<td>128/145</td>
<td>88.3</td>
<td>129/157</td>
</tr>
<tr>
<td>Yes</td>
<td>17/145</td>
<td>11.7</td>
<td>28/157</td>
</tr>
<tr>
<td>Abnormal Calving</td>
<td></td>
<td></td>
<td>0.33</td>
</tr>
<tr>
<td>No</td>
<td>108/145</td>
<td>74.5</td>
<td>109/157</td>
</tr>
<tr>
<td>Yes</td>
<td>37/145</td>
<td>25.5</td>
<td>48/157</td>
</tr>
</tbody>
</table>

A total of 116/302 (38.4%) cows was pregnant to first service. This included 50/145 (40.0%) in Group 1, and 58/157 (36.9%) in Group 2. There was no significant difference between the conception rates to first service between the groups (Chi-square: P = 0.59; Logistic regression: AOR: 1.13; 95% CI: 0.71 – 1.81; P = 0.60; Table 4-11). There was no association between parity, dystocia or retained fetal membranes on the conception rate to first service (Table 4-11).
Table 4-11. Percentage, crude odds ratio (COR), adjusted odds ratio (AOR), 95% confidence interval (CI) and P-value for the risk of pregnancy in dairy cows treated sequentially with PGF2α in the early postpartum period and subjected to the Presynch-Ovsynch protocol.

<table>
<thead>
<tr>
<th>VARIABLE</th>
<th>PREGNANT</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>N</td>
<td>%</td>
</tr>
<tr>
<td>Group</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1 (Treated)</td>
<td>58/145</td>
<td>40.0</td>
</tr>
<tr>
<td>2 (Control)</td>
<td>58/157</td>
<td>36.9</td>
</tr>
<tr>
<td>Parity</td>
<td></td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>51/128</td>
<td>39.8</td>
</tr>
<tr>
<td>3+</td>
<td>65/174</td>
<td>37.4</td>
</tr>
<tr>
<td>Dystocia</td>
<td></td>
<td></td>
</tr>
<tr>
<td>No</td>
<td>95/252</td>
<td>37.7</td>
</tr>
<tr>
<td>Yes</td>
<td>21/50</td>
<td>42.0</td>
</tr>
<tr>
<td>Retained Fetal Membranes</td>
<td></td>
<td></td>
</tr>
<tr>
<td>No</td>
<td>98/257</td>
<td>38.1</td>
</tr>
<tr>
<td>Yes</td>
<td>18/45</td>
<td>40.0</td>
</tr>
</tbody>
</table>

Discussion

The hypothesis of Experiment 2, Part A, was that sequential administration of PGF2α in the immediate postpartum period would decrease the prevalence of mucopurulent discharge, size of the cervix and previously pregnant uterine horn (PPH), and increase first-service conception rate in postpartum dairy cows. In Experiment 2, Part A, PGF2α treatment had no effect on Model 1 (presence of a mucopurulent discharge) on Day 22 or Day 58 post partum, Model 2 (presence of a mucopurulent discharge and a cervix >50 cm on Day 22 post partum and presence of a mucopurulent discharge and a cervix >30 cm on Day 58 post partum), or Model 3 (presence of a mucopurulent discharge, cervix >50 cm and previously pregnant horn ≥ 30 mm) on Day 22 post partum. However, PGF2α treatment had an effect on the prevalence of the variables in Model 3 on Day 58 post partum. Cows treated with PGF2α in the early postpartum period were less likely to have a mucopurulent discharge, cervix >30 mm and previously pregnant horn.
horn ≥ 30 mm on Day 58 post partum. However, treatment was not associated with an increase in risk of pregnancy in Experiment 2 Part A.

The rationale behind the administration of PGF2α was based on studies which showed that the administration of prostaglandin F2α decreased the time for complete involution of the uterus as detected by rectal palpation (Lindell and Kindahl, 1983). This may be due to a direct effect of PGF2α on the bovine myometrium by increasing uterine tone and motility (Patil et al., 1980) with reduction in size of the previously pregnant uterus and the evacuation of uterine content. The results of these studies substantiate the findings in Experiment 2, Part A, which suggested that PGF2α reduced the size of the previously pregnant horn by Day 58 post partum in Model 3.

There was no effect of treatment on the variables of Model 3 at Day 22 post partum. There is a massive release of PGF2α post partum in both normal and abnormal cows and the blood level of PGF2α remains high for 2 to 3 weeks post partum (Lindell et al., 1982). Cows that had a shorter interval from parturition to uterine involution had a longer period of postpartum PGF2α release (Lindell et al., 1982). It is possible that the high endogenous levels of PGF2α in Group 2 (control) were sufficient to cause the uterus to involute at a similar rate as cows in Group 1 (treated).

Cows with endometritis and retained fetal membranes have elevated PGF2α for the first five days post partum which return to normal levels by 7 days post partum (Thompson et al., 1987). Continuous administration of PGF2α has been shown to cause down-regulation of the PGF2α receptor. It is questionable if the administration of PGF2α had any effect on the uterus prior to ovulation and subsequent development of a corpus luteum. This theory is substantiated by the results of Experiment 2, Part A which showed
that treatment had no effect on the variables of Models 1, 2 or 3 at Day 22 post partum. This was probably due to the absence of a CL when PGF2\(\alpha\) was administered on Days 8 and 15 post partum.

The positive effect of treatment on prevalence of the variables of Model 3 at Day 58 post partum may be due to the luteolytic effect of PGF2\(\alpha\) on Day 22 and Day 36 post partum. This probably resulted in the emergence of a new follicular wave with a resultant increase in estrogen secretion by the dominant follicle. It has been established that estrogen is beneficial to clearance of bacterial contaminants from the uterus (Black et al., 1953;1954) and is effective in preventing experimental infection (Rowson et al., 1953; Hawk et al., 1960). It is speculated that lysis of the CL reduced the length of time the uterus was under the influence of progesterone, increased the time when estrogen was the dominant hormone and led to clearance of uterine contamination and improvement of uterine involution.

There was no effect of treatment on pregnancy rate to first service in Experiment 2, Part A, and to conception rate to first service following the Presynch-Ovsynch protocol in Part B. This is in contrast to several studies which have indicated that the administration of PGF2\(\alpha\) in the early postpartum period had a positive effect on reproductive performance (White and Dobson, 1990; Pankowski et al. 1995; Nakao et al., 1997; Kristula and Bartholomew, 1998; Sheldon and Noakes, 1998; Schofield et al., 1999; Heuwieser et al., 2000).

In Experiment 2, Part A, the first postpartum insemination of cows in both Group 1 and Group 2 occurred at a mean of 130 to 134 days post partum. Cows were inseminated at detected estrus after the 100-day voluntary waiting period or subjected to
the Ovsynch protocol. The first service occurred at a mean of 100 days post treatment with PGF2α. It is difficult to say that treatment had no effect on the outcome of first service conception rate. The time lapse between treatment and insemination makes evaluation of the effect of treatment speculative. The decision to not inseminate cows earlier was made by management, based on the high number of dystocia and retained fetal membranes experienced in cows calving in the summer.

In Experiment 2, Part B, attempts were made to objectively investigate the effect sequential administration of PGF2α on the first conception rate. There was no effect of treatment on first service conception rate (Group 1: 40% vs Group 2: 36.9%). This is in contrast to earlier work which showed that the use of PGF2α in the early postpartum period increased subsequent fertility (White and Dobson, 1990; Pankowski et al. 1995; Nakao et al., 1997; Kristula and Bartholomew, 1998; Sheldon and Noakes, 1998; Schofield et al., 1999; Heuwieser et al., 2000). In cows calving normally, Pankowski et al. (1995) found that cows receiving two doses of PGF2α 14 days apart, with the first dose administered between 25 to 32 days post partum, had 10% higher rate of pregnancy than cows not receiving PGF2α.

A possible explanation for the lack of difference between pregnancy rate in Group 1 and Group 2 is the use of Presynch-Ovsynch protocol. Pre-synchronization (Presynch) with two injections of PGF2α 14 days apart increases the pregnancy rate to the Ovsynch protocol. In a group of 100 cows with a 20-day estrous cycle, 40% of cows would be in the ideal stage of the estrous cycle (Days 5 to 12) to begin the Ovsynch protocol. However, administration of PGF2α 14 days apart places 90% of cows between Day 5 to 10 of the estrous cycle and an expected pregnancy rate of 45% to the Ovsynch protocol.
(Thatcher et al., 2001). The Presynch-Ovsynch protocol, in which PGF2α is given 14 days apart and the Ovsynch protocol initiated 12 days after the second injection of PGF2α have been shown to increase pregnancy rate by 18 percentage units compared to Ovsynch alone (25% to 43%; Moreira et al., 2000a). The pregnancy rates obtained in the present study are similar to that predicted by Thatcher et al. (2001) and actual percent pregnancy obtained by Moreira et al. (2000a).

From the results of this experiment, it was concluded that sequential administration of PGF2α early post partum only had an effect on the prevalence of a mucopurulent discharge, size of the cervix and size of the previously pregnant horn at Day 58 post partum. In addition, there was no effect on pregnancy either following insemination at estrus, or timed insemination.
CHAPTER 5
SUMMARY AND CONCLUSIONS

There are two physiological factors which influence reproductive success in the postpartum dairy cow. The first is ovarian cyclicity, and the second is uterine health. In this study, 2 experiments were conducted using postpartum dairy cows. In Experiment 1, exogenous hormones were used to initiate normal cyclicity and improve pregnancy rate in cows with ovarian cysts. In Experiment 2, exogenous hormones were used to reduce the incidence of a mucopurulent discharge, the size of the cervix, the size of the previously pregnant uterine horn, and increase pregnancy rate.

The objective of Experiment 1 was to determine the ovarian response and pregnancy rate of lactating dairy cows with ovarian cysts treated with GnRH and subjected to the Ovsynch protocol 7 days later. A total of 155 cows with ovarian cysts was sequentially allocated to 3 groups at the time of diagnosis (Day 0). Cows in Group 1 (n = 55) were treated with GnRH on Day 0 followed by the Ovsynch protocol on Day 7. Cows in Group 2 (n = 49) were subjected to the Ovsynch protocol on Day 0, and cows in Group 3 (n = 51) were not treated with GnRH on Day 0, but were subjected to the Ovsynch protocol on Day 7. Pregnancy was determined by per rectum palpation of the uterus between 45-50 days after TAI. On both Days 0 and 7, cows in all groups were subjected to ultrasonographic examination of the ovaries and blood samples were obtained from the coccygeal vessels for determination of progesterone concentration (P₄) using a solid-phase, no-extraction RIA. Baseline data for parity, time of year, days in milk (DIM) and P₄ on Day 0 were compared using Chi-square (P ≤ 0.05). Data for
ovarian response on Day 7 were analyzed using logistic regression adjusting for DIM, time of year, parity and P₄ on Day 0. Data for pregnancy rate were analyzed using logistic regression adjusting for DIM, time of year, P₄ on Day 0 and Day 7, and ovarian response on Day 7. There was no significant difference in the baseline data for cows in all groups. Cows in Groups 1 and 2 were more likely to have a CL and high P₄ on Day 7 compared to cows in Group 3. There was no difference in pregnancy rate between cows in all groups. However, cows with a CL on Day 7 were more likely to become pregnant compared to cows without a CL on Day 7. From the results of Experiment 1, it was concluded that administering GnRH to cows with ovarian cysts 7 days prior to the initiation of the Ovsynch protocol did not increase pregnancy rate. However, the presence of a CL on Day 7 had a beneficial effect on pregnancy rate.

There were 2 parts to Experiment 2 (Part A and Part B). The objective of Part A was to determine the effect of sequential administration of PGF₂α in the immediate postpartum period on the incidence of mucopurulent discharge, size of the cervix and previously pregnant uterine horn (PPH) and first-service pregnancy rate in postpartum dairy cows to either insemination at estrus or timed insemination. Postpartum dairy cows between Day 7 to 9 post partum were sequentially allocated to 2 groups. Cows in Group 1 (n=114) were treated with one luteolytic dosage of PGF₂α 8 hours apart on Days 8 and 15 post partum, and one luteolytic dosage of PGF₂α once on Days 22 and 36 post partum. Cows in Group 2 (n=114) were not treated with PGF₂α on Days 8, 15, 22 and 36 post partum and served as untreated controls. Vaginoscopy and transrectal palpation were performed on Days 22 and 58 post partum. Cows in both groups were either inseminated at estrus, or timed inseminated approximately 130 to 134 days post partum.
Pregnancy was determined by per rectum palpation of the uterus between 45-50 days after TAI. Baseline data for parity, days in milk (DIM), dystocia, retained fetal membranes and P₄ on Day 8 were compared using Chi-square (P ≤ 0.05). Data for the incidence of mucopurulent discharge, size of the cervix and previously pregnant uterine horn (PPH) were analyzed using logistic regression adjusting for parity, dystocia and retained fetal membranes. Data for first-service pregnancy rate were analyzed using logistic regression adjusting for parity, dystocia, retained fetal membranes and days to first service. There was no significant difference in the baseline data for cows in both groups. Sequential administration of PGF2α in the immediate postpartum period had no effect on the incidence of mucopurulent discharge, the size of the cervix, and the previously pregnant uterine horn on Day 22 post partum. However, this treatment scheme reduced the incidence of mucopurulent discharge, size of the cervix and previously pregnant uterine horn on Day 58 post partum. However, there was no difference in pregnancy rate between cows in both groups.

The objective of Part B was to determine the effect of sequential administration of PGF2α in the immediate postpartum period on first-service conception rate in postpartum dairy cows subjected to a timed insemination protocol consisting of Presynch-Ovsynch protocol. Postpartum dairy cows at 7 days post partum were sequentially allocated to 2 groups. Cows in Group 1 (n=209) were treated with one luteolytic dosage of PGF2α 8 hours apart on Days 7 and 14 post partum, and a single luteolytic dosage of PGF2α on Days 21 and 35 post partum. Cows in Group 2 (n=209) were not treated with PGF2α on Days 7, 14, 21 and 35 post partum and served as untreated controls. Cows in both groups were subjected to Presynch and Ovsynch protocols on Day 49, and TAI on either Day 85
or Day 86 post partum. Pregnancy was determined by ultrasonography on Days 29 to 32 after TAI. Baseline data for parity, dystocia, and retained fetal membranes were compared using Chi-square (P ≤ 0.05). Data for first-service conception rate were analyzed using Chi-square and logistic regression adjusting for parity, dystocia and retained fetal membranes. There was no significant difference in the baseline data for cows in both groups. There was no significant difference between the conception rates to first service between cows in both groups. From the results of Experiment 2, it was concluded that sequential administration of PGF2α in the early postpartum only had an effect on the incidence of mucopurulent discharge, size of the cervix, and size of the previously pregnant horn at Day 58 post partum. In addition, there was no effect on pregnancy either following insemination at estrus, or timed insemination.
APPENDIX

VAGINOSCOPY AND TRANSRECTAL PALPATION FORM

Date: ______________________
Cow Number: ________________    Group Assignment: Group_____  □ Blood
Calving date: __/__/2003    Season: ________________    Parity: _____
Calving Status:__________  Normal = 0
                      Abnormal (assisted by vet/personnel) = 1
                      Retained Fetal Membranes (failure to pass fetal membranes
                      within 24 hours of calving) = 2

Vaginoscopic Examination:

Cervix  Open = 1  Color of vaginal wall  Pink = 0
        Closed = 0  Red = 1
                      Red and inflamed = 2

Discharge

Lochia = 0

Discharge  Quantity
Normal _______  Slight = 1  Moderate = 2  Copious = 3
Clear _________  Slight = 1  Moderate = 2  Copious = 3
Mucoid_________  Slight = 1  Moderate = 2  Copious = 3
Mucopurulent_______  Slight = 1  Moderate = 2  Copious = 3
Serous mucopurulent_______  Slight = 1  Moderate = 2  Copious = 3
Transrectal Palpation of the Reproductive Tract & Ultrasound

Size of cervix: ________________________________

Previously pregnant horn

<table>
<thead>
<tr>
<th>Horn</th>
<th>Size of Horn</th>
</tr>
</thead>
<tbody>
<tr>
<td>Right</td>
<td>1</td>
</tr>
<tr>
<td>Left</td>
<td>2</td>
</tr>
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</table>

Fluid in horn

<table>
<thead>
<tr>
<th>Horn</th>
<th>Presence</th>
</tr>
</thead>
<tbody>
<tr>
<td>Right</td>
<td>☐ Present</td>
</tr>
<tr>
<td>Left</td>
<td>☐ Absent</td>
</tr>
</tbody>
</table>

Structures on ovary

Left ovary

- NSS = 0
- Follicle(s) = 1 Size(s) _____
- CL present = 2
- CL absent = 3

Right ovary

- NSS = 0
- Follicle(s) = 1 Size(s) _____
- CL present = 2
- CL absent = 3
LIST OF REFERENCES


Alila HW and Hansel W. Origin of different cell types in the bovine corpus luteum as characterized by specific monoclonal antibodies. Biol Reprod 1984;31:1012-1025.


Archbald LF, Tran T, Thomas PGA, Lyle SK. Apparent failure of prostaglandin F2a to improve the reproductive efficiency of postpartum dairy cows that had experienced dystocia and/or retained fetal membranes. Theriogenology 1990;34:1025-1034.


Etherington WG, Martin SW, Bonnet BN, Johnson WH, Miller RB, Savage NC, Walton JS, Montgomery ME. Reproductive performance of dairy cows following treatment with a single or two sequential doses of cloprostenol 26 and/or 40 days postpartum. Theriogenology 1988; 29:565-575.


Fazeli M, Ball L, Olson JD. Comparison of treatment of pyometra with estradiol cypionate or clorprostenol followed by infusion or non-infusion with nitrofurazone. Theriogenology 1980;14:339-343.


Hinckley ST, Mivae RA. Endothelin-1 mediates prostaglandin F2α-induced luteal regression in the ewe. Biol Reprod 2001;63;1619-1623.


McShane TM, May T, Miner JL, Keisler DH. Central actions of NPY may provide a neuromodulatory link between nutrition and reproduction. Biol Reprod 1992;46:1151-1157.


Padmanabhan V, Keech C, Convey EM. Cortisol inhibits and adrenocorticotropin has no effect on luteinizing hormone-releasing hormone-induced release of luteinizing hormone from bovine pituitary cells in vitro. Endocrinology 1983;112:1782-1787.


Peter AT and Bosu WT. Effect of intrauterine infection on the function of the corpora lutea formed after first postpartum ovulation in dairy cows. Theriogenology 1987;27:593-609.


Skarzynski DJ, Okuda K. Sensitivity of bovine corpora lutea to prostaglandin F2α is dependent on progesterone, oxytocin, and prostaglandins. Biol Reprod 1999;60:1292-1298.


Ursely J, Leymarie P. Varying response to luteinizing hormone of two luteal cell types isolated from bovine corpus luteum. J Endocrinol 1979;83:303-310


BIOGRAPHICAL SKETCH

Katherine Elizabeth May Hendricks was born on June 4, 1972, as the first of two daughters to Elsa May Binns and Lloyd Ivanhoe Binns in St. Andrew, Jamaica. In 2002, she received her degree in veterinary medicine from the University of the West Indies, St. Augustine campus, located in the Republic of Trinidad and Tobago. In January of 2003 she started her master’s program at the University of Florida under the supervision of Dr. Louis Archbald. After completion of her program, she plans to continue her education in the animal molecular and cell biology PhD program under the supervision of Dr. Peter Hansen.