RECTAL TEMPERATURE, CALVING-RELATED FACTORS, AND THE INCIDENCE OF PUERPERAL METRITIS IN POSTPARTUM DAIRY COWS

By

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To my parents, Marta and Alberto who gave me the gift of life, of love and education.
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The objectives of my study were to evaluate the association of calving status, parity and season on the incidence of puerperal metritis (PM) and clinical endometritis (CE) in lactating dairy cows; and examine the role of rectal temperature as a predictor for puerperal metritis, and document the effect of puerperal metritis on subsequent reproductive performance. This study was a prospective cohort design. Cows were classified as abnormal calving status (Ac), cows calving with dystocia, RFM with or without dystocia or twins and cows with a normal calving status (Nc), those without any calving related problems. Daily rectal temperature (RT) of all cows was taken early in the morning from days 3 to 13 postpartum, and health examinations were performed by the on-farm veterinarian. We evaluated a total of 450 calvings. Cows with Nc had a lower incidence of PM compared to cows with an Ac status (13% vs. 41%, respectively; P < 0.001). During the cool season, primiparous cows had the highest incidence of PM compared to primiparous cows in warm season or multiparous cows in either season (P <
Cows with Ac were more frequently diagnosed with CE than those with Nc (AOR = 2.8, 95% CI 1.7-4.9, P < 0.001). A higher incidence (38.2%) of CE was found in cows diagnosed with PM (AOR = 2.2, 95% CI 1.1-3.9, P < 0.005). The RT in cows diagnosed with PM increased between 48 to 24 h before diagnosis of PM and continued to increase until reaching a maximum RT of 39.2°C ± 0.05 on Day 0 (day of diagnosis). In cows with PM and fever at diagnosis the RT started to increase between 72 to 48 h before the diagnosis of PM, and continued to increase until reaching a maximum RT of 39.7°C ± 0.09 on the Day 0 (day of diagnosis). In cows with PM and no fever at diagnosis there was not a significant daily increment of RT before the diagnosis of PM. Cows without metritis did not show any variation in RT during the first 13 days postpartum. There were no detected differences in accumulated pregnancy rate by 150 days post partum (mean=50%) among normal cows and cows experiencing PM. A season effect was detected (Cool season [40 %] > than warm season [28 %; P < 0.02]).

Occurrence of PM was higher in cows experiencing an Ac. Primiparous cows had a greater incidence of PM in the cool season for both normal and abnormal calvings. In contrast, multiparous cows showed no seasonality in the occurrence of PM. Evaluation of daily RT was able to distinguished PM with fever and PM without fever. Sequential increases in RT on two consecutive days prior to the actual diagnosis can serve as a predictor of PM with fever. Abnormal calving, and PM were risk factors for clinical endometritis. Pregnancy rates were comparable between cows with normal or abnormal calving status cows, regardless of the occurrence of PM.
CHAPTER 1
INTRODUCTION

Puerperal metritis has multiple factors contributing to its etiology, severity, and duration. It occurs during the period from calving to when the anterior pituitary gland becomes responsive to gonadotrophin releasing hormone (GnRH) approximately 7 to 14 days postpartum (Olson et al., 1986). In puerperal metritis there is inflammation of all layers of the uterus and it is characterized by the presence of a fetid, watery reddish-brown vulvar discharge (Lewis, 1997). Sheldom et al. (2006) attempted to standardize the clinical definition of puerperal metritis. Their definition includes clinical symptoms such as decreased milk production, dullness, or other signs of toxemia with fever (> 39.5°C) within the first 21 days postpartum.

Factors that predispose cows to puerperal metritis have been reviewed previously (Curtis et al., 1985; Correa et al., 1993; Markusfeld 1984; Bartlett et al., 1986). However, in some cases puerperal metritis was classified as a disease complex without distinguishing clinical severity or presentation, making studies difficult to compare (Lewis, 1997).

Prevention and early treatment of puerperal metritis is more economical than let this condition progress to a stage where treatment is not beneficial on cost effective (Bartlett et al., 1986). Thus the need to identify puerperal metritis early post partum and provide treatment, monitoring of attitude and fever during the first 10 days postpartum (Upham, 1996). Rectal temperature is an indicator of the core body temperature, and is used as a diagnostic method to determine whether the cow has fever. Fever is the result of
a complex communication between the peripheral immune system and the brain in response to infection, inflammation and/or trauma, and is clinically characterized by a rise in body temperature (Leon, 2002). Fever can be initiated by bacterial lipopolysaccharides (LPS) acting directly as exogenous pyrogens; or indirectly by activating liver macrophages (Steiner et al., 2006) inducing the production of endogenous pyrogens such as interleukin (IL)-6, IL-1, and TNF-α (Luheshi, 1998).

It has been difficult to establish a minimum rectal temperature to define fever in postpartum-health monitoring protocols because of the broad range of rectal temperature described in the literature (Smith et al., 1998; Upham, 1996; Drillich et al., 2001; Zhou et al., 2001; Sheldon et al., 2004), and the multiple factors that affect rectal temperature values (Rebhun, 1995; Rosenberger, 1979). Kristula et al. (2001) evaluated postpartum rectal temperature: 48% of cows that calved normally had at least one daily temperature above 39.1°C compared to 93, 83, 100 and 78% for cows with retained placenta, mastitis, puerperal metritis and dystocia, respectively. Kristula et al. (2001) concluded that rectal temperature (per se) is not enough to determine whether antibiotic treatment is needed for postpartum cows.

Despite the common use of rectal temperature of postpartum cows to evaluate health, there is a lack of research on the value or significance of rectal temperature and calving status as tools to diagnose puerperal metritis in dairy cows. Understanding the factors that predispose cows to puerperal metritis, and understands rectal temperature responses in cows at risk of developing puerperal metritis should aid in the formulation of appropriate postpartum health monitoring strategies. In addition, early diagnosis and
treatment of puerperal metritis could improve later reproductive performance and productivity.

Our study objectives were to evaluate the effect of abnormal calving, parity, season, and rectal temperature on the incidence of puerperal metritis within the first 13 days post partum: and to report the effect (if any) of puerperal metritis and clinical endometritis on reproductive performance.
CHAPTER 2
LITERATURE REVIEW

The Bovine Uterus

The uterus consists of a tubular structure formed by the cervix, uterine body, and the uterine horns. The non gravid uterus is located, depending on factors such as age, breed, and parity of the cow, on the pelvic cavity dorsal to the urinary bladder and ventral to the rectum. The main function of the uterus is to accept a fertilized ovum which becomes implanted into the endometrium, and derives nourishment from blood vessels developed exclusively for this purpose.

The uterus is derived from the embryonic müllerian or paramesonephric ducts, the tubes will develop into the gonaductal system, giving rise, in the case of the female, to the oviduct, uterus, cervix and cranial vagina. In mammals the müllerian ducts fuse from cranial to caudal. The female müllerian ducts start to fuse from the most cranial part, caudally to form the oviducts, uterus, the cervix and the anterior part of the vagina. The fusion of the ducts happens in the medial walls, forming the paramesonephric septum. This partition tends to disappear in time, fusing and forming a single tube. In the cow, the ducts fuse forming a septum that separates the two horns giving the characterization of a bipartite type uterus.

Both sides of the uterus are attached to the pelvic and abdominal walls by the broad ligaments, from were the uterus receives its blood and nerve supply. The middle uterine artery, a branch of either the internal iliac artery or the external iliac artery, provides the blood supply to the uterus in the region were the fetus develops. The cranial uterine
artery, a branch of the utero-ovarian artery, supplies blood to the ovary by the ovarian artery and to the anterior extreme of the uterine horns by the cranial uterine artery. The utero-ovarian artery runs closely along the surface of the corresponding veins. The artery terminates by giving rise to a small branch to the tip of the uterine horn and oviduct. All lymphatics of the uterus are in communication, the cervix drains towards sacral nodes while the body of the uterus and oviducts drain towards the external iliac nodes, also there is some drainage towards internal iliac nodes.

The uterus can be divided in three layers. From the lumen to the abdominal cavity can be divided in endometrium, myometrium and perimetrum or serosa. The inner portion of the uterus is composed of the mucosa and submucosa. Both compose the endometrium, characterized by having many endometrial folds. Histologically the mucosa is characterized by a simple pseudostratified cylindrical cell surface during most stages of the cycle; few ciliated cells are present, especially in the multiparous cow, less than 1%. The submucosa is predominantly connective tissue and houses the uterine glands. The uterine glands develop in the mucosa, projecting to the submucosa where they coil. Their basic structure is similar to those of a mucosal epithelial surface (Marinov and Lovell, 1968). The surface of the endometrium of the cow is covered by non glandular areas denominated caruncles. The caruncles are highly vascularized areas that give rise to the maternal portion were the cotyledons will attach.

The endometrium is covered by two smooth muscular layers that constitute the myometrium. In contact with the endometrium a circular smooth muscle layer is present, it is in this portion where the blood vessels penetrate the submucosa stratum. Up on this portion, the longitudinal layer of smooth muscle is present, easily recognizable for it
creases, or small ridges, palpable during the early post partum of the cow. The outer layer of the uterus is the serosa or perimetrum, quite thin and almost transparent covering the entire uterus and continues dorsally and covers the mesosalpinx. This portion of the uterus will be in contact with the pelvic and abdominal cavity of the cow.

**Uterine Involution**

In the pregnant cow, during the time period 1 month before and 1 month after parturition, several metabolic and endocrine events take place. As the cow enters the transition period, the dam has to be prepared for the impending parturition, and the uterus and ovaries must return to a certain stage to be prepared for a new pregnancy (Kindahl, et al., 2004). Most of these processes are due to or reflected in endocrine changes.

The uterine lumen holds approximately 70 kg at the time of parturition, including fluids, fetal membranes, and the fetus. Approximately half of this weight contains fluids and the other half includes the fetus and the fetal membranes (Mortimer et al., 1997). However, after parturition the uterus of the cow, is a large, flabby sac, nearly a meter long and 9Kg in weight (Gier and Marion, 1968). The rapid re-organization along with a fast reduction in the diameter of the organ constitute probably a protective mechanism against ascending infections (Mortimer et al., 1997).

Involution of the uterus involves loss of intraluminal fluids, reduction in size, and endometrial repair. During the first two days after calving, the expelled fluids are serosanguineous, and change in character after caruncular dissolution begins (Olson et al., 1986; Rasbeck, 1950) describes the elimination of decidual detritus of caruncular tissue starting 3 to 4 days after parturition and increasing until the 9th day, and gradually becomes mixed with blood originating from hemorrhages on the surface of the caruncles. Gier and Marion (1968) found a considerable quantity of blood in the uterus after
parturition, becoming mixed with sloughed caruncular material after day four, which changed by day 12 post partum to a more lymph like fluid which decreases in quantity by day 23 postpartum (Figure 2-1) (Gier and Marion, 1968). This discharge is named uterine lochia, which consist of mucus, tissue, detritus, and blood, that commenced 3 to 4 days and decreasing until the ninth day post partum (Roberts, 1986). Lochia may assume different appearances from white, yellow- white or a grey mucopurulent character toward the latter part of the puerperal period. This discharge is considered a normal process of uterine involution (Roberts, 1986).

![Figure 2-1](image)

**Figure 2-1.** Changes in lochia volume during the first 20 days postpartum in dairy cows (Gier and Marion, 1968).

During the post partum period, re-organization and involution of the uterus occurs and a small opening in the cervix remains for elimination of uterine contents (Wehrend et al., 2003). Reduction in the uterine weight, diameter and length occurs in a decreasing logarithmic scale (Figure 2-2) (Gier and Marion, 1968). This reduction in size may be explained partially by peristaltic contractions at intervals of 3 to 4 minutes during the first
day postpartum and continuing through the second day (Gier and Marion, 1968). Bajcsy et al. (2005) evaluated the contractility of the postpartum uterus by recording and quantifying the frequency, amplitude and duration of intrauterine pressure changes between 12 hours to 48 hours after parturition.

Figure 2-2. Uterine changes in ▲-Weight; ■-Diameter and ●-Length during the first 20 days postpartum in cows (Gier and Marion, 1968).

In that study (Bajcsy et al., 2005) it was reported that mean frequency of uterine contractions were 8.9 contractions per hour at 12 h post partum with a range of 6 to 11 contractions every hour. The mean contraction frequency decreased to 1.8 contractions per hour at 48 h post partum. The largest drop in mean values occurred between 12 and 24 h post partum, and the frequency decreased by 46% of the initial mean value. Results from amplitude showed an initial individual mean value of 40 mmHg at 12 h. Changes in mean amplitude showed a similar pattern as frequency, with highest initial mean values at
12 h postpartum (19.6 mmHg) and a reduction of 16% of the starting values by 48 h postpartum (3.2 mmHg). The most marked drop (42%) in this response also occurred between 12 and 24 h post partum. Mean duration at 12 h was 89.8 seconds and varied between 102.5 at 36 h postpartum to 67.9 seconds at 48 h. In that study (Bajcsy et al., 2005), the relationship between uterine activity and blood calcium levels also was investigated. Neither frequency, amplitude and duration showed a significant relationship to blood Ca2+ levels in cows at any of the four recording times.

Diameter of the cervix at a given time after parturition is influenced by the involution process of the reproductive tract (Oltenacu et al., 1983). Gier and Marion (1968) reported that involution of the cervix measured in slaughtered cows. The diameter of the cervix at day 2 after parturition was about 15 cm; 9 to 11 cm at 10 days, 7 to 8 centimeters by 30 days, and 5 to 6 cm by 60 days. Wehrend et al. (2003) reported results of cervical involution in vivo by measuring the cervix canal from the time after expulsion of the calf up to the tenth day post partum. They showed that the cervical folds were in constant formation and were detected at the third day postpartum. In addition, the organization of cervical folds started from cranial and continued caudally. After the expulsion of the calf, a reduction of the opening from 26.9 ± 1.3 cm to 1.9 ± 0.3 cm on the seventh day post partum was observed (Figure 2-3 and 2-4). Furthermore, up to the third day post partum, the lumen of the cervix was detected in all cows, disappeared from the fourth to the seventh day, and re-appeared at the tenth day after parturition. The authors concluded that this opening was related to allow further elimination of uterine fluid contents (Wehrend et al., 2003).
Figure 2-3. Reduction of the cervix opening during the first 24 h post partum (Wehrend et al., 2003).

Figure 2-4. Reduction of the cervix opening during the first 10 days post partum (Wehrend et al., 2003).
Reduction of the size of the uterus seemed to be produced by early constriction between 5 to 10 days and a secondary cervix opening relaxation produced at 10 days postpartum, at the time of final sloughing of the caruncular masses (Gier and Marion, 1968). Morrow et al. (1966), reported that slow regression of the uterus occurred during the first 4 to 9 days after parturition followed by an accelerated regression during the period 10 to 14 days after calving. In addition, the maternal portion of the placentome (caruncle) after the removal of chronic villi, remained as a loose mass of tissue approximately 70 mm long, 35 mm wide, and 25 mm thick (Gier and Marion, 1968). Caruncular blood vessels constricted rapidly and were nearly occluded within 2 days postpartum. However, blood continued to flow from protruding arterioles and contributed to the luminal fluids for a least 10 days (Gier and Marion, 1968).

Archbald et al. (1972) described the histological involution of the uterus during the first 60 days post partum. During the first day postpartum, the caruncular epithelium was degenerated, as well as the intercaruncular epithelium. The myometrium was edematous with degenerated muscle fibers, and fetal chorionic cells were still present but some were necrotic. By the fifth day post partum, the caruncular epithelium was regenerated, but started to be lost given the sloughing of the superficial layer of the caruncles. The intercaruncular epithelium was regenerated with the exemption of the basilar areas of the cells. The endometrial glands were degenerated in the basilar area with some ducts dilated. Vacuolization of the myometrium was almost generalized. However, the nuclei of the muscle cells were normal and the fetal chorionic cells were necrotic, mineralized and surrounded by macrophages (Archbald et al., 1972).
At visual examination, most of the necrotic layer is removed by day 10 post partum and by 15 days post partum all of the caruncular mass that had been involved in the placentome are sloughed, leaving only stubs of blood vessels extending beyond the surface of the stratum compactum (Gier and Marion, 1968). By 19 days post partum, the arterioles within and beyond the stratum compactum disappeared (Gier and Marion, 1968). The histology showed by day 15 post partum that the caruncular epithelium was absent in some areas, but a cuboidal or flat epithelium was present in other areas. The intercaruncular epithelium was regenerated with few areas of degeneration in the basilar area of the cell and the myometrium was shrunken in size, with vacuolation of the muscle fibers and fetal chorionic cells were not present. By day 19 post partum, the caruncular epithelium was covered by a columnar to cuboidal epithelium over the entire caruncle. A cuboidal epithelium was also present over the entire intercaruncular area. Endometrial glands were normal and non-secretory. The myometrium had few necrotic fibers and continued to shrink in size. By day 19 postpartum fetal chorionic cells were not present (Archbald et al., 1972).

The caruncles already reduced their size at 19 days postpartum to 15 to 20 mm in diameter, and by day 39 post partum, are reduced to smooth knobs of 10 to 15 mm in diameter composed of circular cones of 8 to 10 mm across the base and 4 to 6 mm across the crown by 50 to 60 days postpartum (Gier and Marion, 1968).

The final gross involution of the uterus has been reported to be completed in the previously pregnant horn by day 25 post partum in normal cows, and 30 days post partum in cows with postpartum disease (Morrow et al., 1966). In contrast, the average interval for completion of gross uterine involution for multiparous cows was 40 days, and the
interval was affected by parity, season, and stress factors (Marion et al., 1968). The histological characteristics of the uterus by day 31 to 45 days postpartum showed that the caruncular and the intercaruncular tissue had epithelium present over the entire structures (Archbald et al., 1972). The endometrial glands were still normal and non-secretory. The myometrium was normal, and regained the normal shape and sarcoplasm. By day 60 post partum the uterus regained it’s normal histology. The caruncular area was covered by a columnar type epithelium, somewhat pseudostratified. Many pigment-bearing histocytes were present in the stratum compactum and neutrophils were not observed. There were many plasma cells distributed through the stratum compactum and spongiosum. The intercaruncular area was covered by a single layer of epithelium and consisted of cuboidal and columnar cells. Numerous mast cells and histocytes containing hemosiderin were in the stratum compactum. Numerous mast cells were in the stratum spongiosum, and the blood vessels of this layer appeared normal. The endometrial glands appeared normal and non-secretory. The myometrium and the subserosa layer appeared normal, but were infiltrated by numerous mast cells (Archbald et al., 1972).

**Endocrine Changes During Early Post Partum**

A balanced, coordinated endocrine system is important for normal reproductive function. A graphical representation of this complex process is presented in (Figure 2-5). These changes involve the gonadotrophin releasing hormone (GnRH) from the hypothalamus, follicle stimulating hormone (FSH), luteinizing hormone (LH), prolactin (PRL) from the adenohypophysis, prostaglandin F\textsubscript{2α} from the uterus, progesterone (P\textsubscript{4}) from the corpus luteum, and estrone sulfate (E\textsubscript{1}SO\textsubscript{4}), and estrone (E\textsubscript{1}), from McDonald (1980).
Cortisol is recognized as a stress hormone and is also responsible for the regulation of prostaglandin synthesis (Hafez, 1993). In the fetus prior to parturition, adrenocorticotropic hormone (ACTH) is released from the pituitary gland, stimulating the adrenal glands to release cortisol. The fetal cortisol is central to the survival of the neonate, as well as for induction of lactation and parturition in the cow (Nathanielsz 1993).

Figure 2-5. Pooled within cows least squares regressions of plasma progesterone (P₄), estrone sulfate (E₁SO₄), estrone (E₁), prolactin (Prl), and 13, 14-dihydro, 15-keto prostaglandin F₂α (PGFM) during the periparturient period (Eley et al., 1981).

As parturition approaches, the fetal adrenal cortex becomes increasingly sensitive to adrenocorticotropic (ACTH). Mean fetal levels of corticosteroids are within 5.0 ng/ml at 20 days to 9.3 ng/ml at 10, and 25 ng/ml 4 days pre partum, and the level progressively increases to a mean of 74 ng/ml on the day of calving (Hunter, et al., 1977). At 30 days pre partum, only 50-60% of the corticosteroids fraction is cortisol, whereas in the last 10
days of gestation the proportion of cortisol rise to over 90% (Hunter, et al., 1977). Dystocia may affect levels of cortisol. Severe dystocia resulted in lower calf rectal temperature, reduced serum cortisol and increased serum glucose (Bellows and Lammoglia 2000). Close to parturition the release of cortisol induces the 17α-hydroxylase enzyme in the fetal membranes to start the conversion of progesterone to estrogen compounds (Hoedermarker et al., 1990).

Progesterone is secreted by luteal cells of the corpus luteum and by the placenta, and it secretion is primarily induced by LH (Hafez, 1993). The function of progesterone is to prepare the endometrium for the implantation of the embryo and for the maintenance of pregnancy. In contrast to sheep and horses that exhibit placental progesterone production, the corpus luteum of the cows maintains pregnancy by luteal cells P₄ production which fluctuates between 6 to 15 ng/ml through gestation (Knickerbocker et al., 1986). During the peripartum period there is approximately a 20% reduction of the levels of progesterone within 4 to 1 week before parturition (Edqvist et al. 1978). A second phase of reduction can be distinguished with a more abrupt decrease of progesterone during the last 2–3 days prior to parturition, and this is interpreted as pre-partal luteolysis (Edqvist et al. 1978). Maternal blood progesterone falls towards term, decreases rapidly over the last 48 to 36 h post partum to levels of less than 1 ng/ml (Hunter et al., 1977; Eley et al., 1981), and it is not until day 16 post partum that cows increase their P₄ levels over 1 ng/ml (Eley et al., 1981). The rise in fetal corticosteroids during the last month of gestation is reflected by the increment of estrogens levels (Knickerbocker et al., 1986).
Cotyledonary tissue is the main source of placental estrogens (Hoedermarker et al., 1990). Characterization of E₁ and pooled 17-α/β estradiol sulfate (E₂SO₄), reflect similar patterns to estrone sulfate (E₁SO₄). Thus, placental steroid activity is most commonly determined by concentrations of plasma estrone sulfate (E₁SO₄) (Knickerbocker et al., 1986).

Maternal plasma concentration of E₁SO₄ increases gradually from a baseline of 30 to 60 pg/ml before day 60 to approximately 500 pg/ml by day 100 of pregnancy (Eley et al., 1979). A rapid elevation then occurs until day 150 when estrone sulfate concentrations approach 3000 pg/ml. After 150 days of pregnancy E₁SO₄ remains constant until approximately day 240 when estrone sulfate increases rapidly (Thatcher et al., 1982). Concentrations of estrogens decline abruptly in association with delivery of the conceptus (calf and placenta). Basal concentrations of P₄, E₁ and E₁SO₄, are low by 24h after parturition and remains low for approximately 14 day after parturition (Eley et al., 1981).

Prostanoids (prostaglandins and thromboxanes) are forms from arachidonic acid by cyclooxygenases (COX). At least two different COX enzymes, which are isoenzymes, have been found: COX-1 mainly a constitutive and COX-2 an inducible enzyme COX-2 is involved in both physiological (luteolysis, parturition) and pathological (inflammatory) processes (Kindahl et al., 2004). The most important product linked to reproduction is PGF₂α. The metabolism of PGF₂α is very rapid to its metabolite 15-keto-13,14-dihydro-PGF₂α (referred to 15-ketodihydro- PGF₂α or PGFM) (Granstrom and Kindahl 1982). The uterus is the primary source of PGF₂α during the early postpartum period, and the
caruncles also contribute to the synthesis and metabolism of PGF2α (Guilbault, et al., 1984).

Utero ovarian venous prostaglandin F2α levels remained relatively constant at around 500 pg/ml by 48 to 36 h before calving when they increased rapidly especially over the last 24 h gestation, reaching peak levels of around 5 ng/ml during labor (Hunter, et al., 1977).

During the prepartum period levels of PGFM are negatively correlated to P4 (Eley et al., 1981). A final abrupt increase in PGFM is accomplish by a decline in progesterone associated with CL regression just prior parturition (Eley et al., 1981). Increases in PGFM occurs during the postpartum period (0 to 11 days) after luteolysis and delivery of the calf (Eley et al., 1981).

The major increase in concentration of PGFM in plasma during the periparturient period occurs 1 to 4 days postpartum with concentration returning to base level by day 15 (Eley et al., 1981). Prolactin acts on the central nervous system to induce maternal behavior (Hafez, 1993). Prolactin concentration begins to rise from a variable baseline of approximately 80ng/ml 2 at weeks prepartum and reach peak values of 200 to 400 ng/ml just prior calving (Eley et al., 1981). The levels of prolactin are maintained elevated up to the third day post partum, when they return to baseline levels of 80ng/ml (Eley et al., 1981).

**Uterine Defense Mechanisms**

The immune system functions to defend the host against infections. Host defense requires different recognition systems and a wide variety of effector mechanisms to seek out and destroy the wide variety of pathogens in their particular habitats within the body and it’s external and internal surfaces (Janeway, 2005).
Innate immunity serves as a first line of defense. Once body surfaces (skin, mucosa), secretions and anatomical barriers are breached, macrophages and neutrophils of the innate immunity system provide a first line of defense against many common microorganisms. However, they do not always eliminate the infection. Consequently, the adaptive immune system has evolved to provide a more versatile and specific means of defense, and increased protection against subsequent reinfection (Janeway, 2005).

The inner layers of the uterus are part of the mucosal immune system with structural and functional similarities, and common lymphocyte trafficking network with the intestinal, bronchial, nasal, ocular, salivary and mammary gland tissue (Ogra et al., 1999). The most prominent difference between the uterus and the other mucosal surfaces is the lack of organized secondary lymphoid nodules analogous to Peyer’s patches and bronchus-associated lymphoid tissue (BALT) (Head and Billingham, 1986). Furthermore, in the majority of domestic species the uterus is exceptional among mucosal tissues because the ovarian steroid hormones have considerable effects on immune events (Lewis, 2004).

To the complexity of this specialized immunological system a production stressor factor is added, and the modern dairy cow is unique in her experience of repeated lifetime cycles of pregnancy and parturition followed by lengthy lactations producing high volumes of milk (Mallard et al. 1998). In addition, the onset of lactation imposes tremendous physiological challenges to the homeostatic mechanisms of the cow which influences immunological responses (Goff and Horst, 1997).

**Innate Uterine Defense**

Innate immune mechanisms act immediately, and are followed by early induced responses which can be activated by infection without generating an immunological
response (Janeway et al., 2005). The uterus has its own innate immunological system which includes anatomical barriers, uterine and cervical secretions and bacterial antagonism. Once this defense mechanism is breached, such as in the case of parturition, the most important line of defense is the innate cell defenses composed of phagocytic cells such as neutrophils, macrophages, effectors cells (basophils, mast cells, and eosinophils) which have the capacity to induce an influx of other immunological factors (BonDurant, 1999).

**Anatomical barriers**

The uterine environment is protected by anatomical characteristics that act as barriers to the external environment of the cow. These physical barriers consist of the vulva, vaginal vestibule and cervix which are all covered by a mucosal layer that produces and secretes specific and nonspecific immunological factors (Senger, 1999).

The cranial vagina as well and the fornix vaginae are characterized by columnar epithelium which is highly secretory under the influence of estrogen. The cranial vagina is characterized by some ciliated columnar epithelium that participates in the process of mucus elimination (Senger, 1999). The caudal vagina is characterized by a stratified squamous epithelium and the dermal epithelium which have an exfoliative process that eliminates microbes adhered to epithelial cells. The secretory function of the vagina varies according to the endocrine status. During estrus, under the influence of estrogen, the stratified epithelium becomes thickened, protects the vagina during copulation, and reduces access of microorganisms to the vasculature of the submucosa (Senger, 1999). Mucus secretion acts as a mechanical flushing system for pathogens and antigens that are captured by mucoproteins (Senger., 1999).
The two labia of the vulva are in close contact by the action of the constrictor vulvae muscle which minimizes entrance of foreign material to the vulva. The skin of the labia is part of the integument and has numerous sebaceous and sweat glands which produces antimicrobial factors (Senger, 1999). The cervix isolates the uterus from the external environment by forming an anatomical barrier trough of multiple folds and three rings which protrude into the cervical canal (Senger, 1999).

During parturition there is dilation of the cervix which allows expulsion of the fetus. This event removes the cervical seal, allowing the interior of the uterus to be in contact with the environment of the cow, and does not return to it’s normal anatomical form in cows with a normal puerperium until 7 to 10 days post partum (Wehrend et al., 2003).

**Uterine and cervical secretions**

The internal epithelium of the cervix and the uterus contain cells that secrete mucus, which is composed of glycoproteins (mucins). Mucus traps microorganisms and prevents them from reaching and colonizing the mucosal epithelium. Mucus also contains lysozymes that help degrade bacteria, antibodies that prevent microbes from attaching to mucosal cells, lactoferrin that binds iron making it unavailable to microbes, and lactoperoxidase that generates toxic superoxide radicals that kill bacteria (Ogra et al., 1999).

The complement system is made up of many distinct plasma proteins that react with one another to opsonize pathogens and induce a series of inflammatory responses that help prevent infections (Janeway et al., 2005). The function of complement is to recruit mononuclear phagocytes, initially circulating neutrophils and tissue macrophages, that remove the microbes (phagocytosis) and release additional, different mediators (e.g.,
cytokines, prostaglandins, leukotrienes, etc.) that enhance the inflammatory process (Janeway et al., 2005). Complement is an important component of the innate immunological reaction in the reproductive tract of the cow, opsonizing and lysing bacteria (Corbeil., 2002). Moreover, it’s activation seems to be due to the interaction of immunocomplexes formed by antigens and immunoglobulin, specifically IgG2 (Corbeil., 2002). Increased concentrations of complement C3 are found in vaginal and uterine secretions of infected cattle (Kania et al., 2001). Although the exact source of C3 in the uterus and vagina of cattle is still unknown, it has been proposed that increased concentration of C3 in vaginal and uterine secretions result from serum-derived complement (Kania et al., 2001). In rats, C3 production is positively influenced by estrogen and inhibited by progesterone (Hasty et al., 1994).

Lactoferrin is a ferric protein found in exocrine secretions of the mucosal surfaces of the cow (Dixon and Gibbons, 1979). It is produced and secreted by the exocrine glands of the uterus, epithelial cells of the cervix and ampulla of the uterine tube (Inoue et al., 1993). Lactoferrin acts as a powerful bacteriostatic, bactericidal, fungicidal and virucidal agent (Ogra et al., 1999).

Another serum protein that is secreted in the reproductive tract is plasminogen which is converted to plasmin by a series of specific serine proteases called plasminogen activators (Lijnen and Collen, 1985). Through the generation of nonspecific, protease plasmin, plasminogen activators influence numerous physiopathological processes, including fibrinolysis thrombolysis, and invasiveness, metastasis, and cell migration at sites of inflammation; this process also involves degradation of the injured tissue and the plasminogen activators released by macrophages and granulocytes may contribute to this
process by degrading extracellular proteins (Dano et al., 1995). Two types of plasminogen activators, urokinase (u-PA) and tissue type (t-PA), are present in endometrial tissues and uterine fluids of the cow, and are thought to be involved in the resolution of endometritis in cows (Moraitis et al., 2004).

Peroxidase activity has been measured in many mucosal secretions (Ogra et al., 1999), including the uterus. The activity of peroxidase is derived from enzymes synthesized by exocrine glands and secreted onto mucosal surfaces by the glands (Ogra et al., 1999). It produces toxic oxygen-derived products that act as a bactericidal agent. In addition, leukocytes present in the uterus can also produce large amounts of hydrogen peroxide (Hansen et al., 1987) which enhance lactoferrin activity (Ogra et al., 1999). In addition, nitric oxide is another exocrine product of the uterine mucosa (Lapointe et al., 2000), and produces its bactericidal activity by producing toxic nitrogen oxides on the surface of the mucosa (Janeway et al., 2005).

**Bacterial antagonism**

The normal microbial flora of the bovine urogenital tract is made up of a dynamic mixture of aerobic, facultative anaerobic and strict anaerobic micro-organisms (Hafez 1993). The normal flora act as an inhibitory flora to help prevent infections by reproductive pathogens (Corbeil and BonDurant, 2001). The normal microbial flora of the reproductive tract is composed of bacteria of the genus *Staphylococcus, Streptococcus* and the coliform group (Hafez 1993). Contrary to what it is reported in humans and rats (Reid et al. 1985), the number of lactobacilli appears to be lower in cervix and vaginal fluids of the cows (Otero et al., 2000). In contrast, *Coagulase-negative Staphylococcus* and *α-haemolytic Streptococcus* bacteria are predominant in the vagina of the cow (Otero et al., 2000). This normal flora of nonpathogenic bacteria compete with pathogenic
microorganisms for nutrients and for attachment sites on the epithelial cells and produce antimicrobial substances, such as lactic acid (Janeway et al., 2005).

**Innate cell component**

The main phagocytic barrier in the uterus is provided by the invasion of neutrophils in response to bacteria (Sheldon and Dobson, 2004) that are present on the surface of the endometrium and into the lumen (BonDurant, 1999). Consequently, neutrophils are the earliest and most important phagocytic cells to be recruited from the peripheral circulation to the uterine lumen, killing internalized bacteria and contributing to the formation of pus when the phagocytes die (Sheldon and Dobson, 2004). Experimental approaches with bacteria and bacterial components to induce an influx of neutrophils have been reported (Zerbe et al., 2001), However, when a non specific inflammation was induced, such as in the case of LPS inflammation, intracellular killing by uterine neutrophils was reduced compared to circulating neutrophils (BonDurant, 1999). This suggests the importance of cytokines in uterine infections. In addition, the experimental infection with *E. coli* and *A. pyogenes*, the predominant bacteria in cases of bovine uterine infections, resulted in high concentrations of viable neutrophils in uterine secretions. However, the large numbers of neutrophils were not able to eliminate the bacterial infection present (Zerbe et al., 2001). Consequently, other unknown factors may influence leukocyte activity (Mallard et al., 1998).

Macrophages are also important in the uterine immune response. Macrophages sense bacteria or endotoxins through toll-like receptors (TLRs) which are the principal signaling molecules through which mammals sense infection (Beutler et al., 2003). The activation of the macrophages leads to the production of cytokines such as tumor necrosis factor-alpha (TNF-α), and interleukins (IL-1, IL-6, IL-8). These cytokines alert other
immune cells such as neutrophils or lymphocytes, by supporting the development of an adaptive immune response (Beutler et al., 2003). In addition, macrophages induce the transmigration of leukocytes to inflamed tissue by inducing the expression of selectins, by TNFα. Selectins cause the rolling of leukocytes on endothelial cells which are attracted to the site of infection by a concentration gradient produced by the above mentioned cytokines, especially IL-8 (Janeway et al., 2005). In addition to neutrophil and macrophage migration, mast cells and eosinophils are present on the surface of the uterine mucosa. Both eosinophils and mast cells have high affinity receptors that bind IgE antibody (Janeway et al., 2005). The eosinophils also release inflammatory mediators and antimicrobial factors such as peroxidase and lytic enzymes (BonDurant 1999). The number of mast cells and eosinophils are known to vary with the stage of the estrous cycle (Likar et al., 1964; Matsuda et al., 1983). Little is known about bovine uterine mast cells, but they secrete some of the usual mast cell mediators, such as histamine, leukotrienes, prostaglandins, heparin, and proteinases, as well as proinflammatory cytokines and Th2 related cytokines. Mast cell and eosinophil mediators increase vascular permeability which results in the subsequent influx of other immune cells and serum immunoglobulins to the uterine lumen (Corbeil et al., 2005; BonDurant, 1999).

**Humoral-mediated immunity**

Antibodies are thought to be the most protective arm of the immune response process in defense against extracellular pathogens (Corbeil, 2002). The distribution of immunoglobulins in external secretions is vastly different from that found in serum. Plasma cells secrete different immunoglobulin after a period of differentiation and isotype switching (Janeway et al., 2005). Different isotypes and allotypes of immunoglobulins are present in different compartments of the body, and the isotype, and
quantity may vary depending on the antigen stimulation (Janeway et al., 2005). Immunoglobulin-A (IgA) is the immunoglobulin mainly found on mucosal surfaces (Ogra et al., 2002). However, in the vascular system, secretory IgA (sIgA) is the predominant form, and is principally found as a dimer (Butler, 1972). It is the predominant immunoglobulin in nasal secretions, tears and saliva. In cervico-vaginal secretions, the relative concentration is lower than IgG, but still much higher proportionally to serum (Duncan et al., 1972). The majority of IgA found in external secretions is derived from local synthesis by plasma cells rather than selective transport from blood (Duncan et al., 1972). Immunoglobulin A is the major immunoglobulin class in the superficial portion of the reproductive tract and immunoglobulin-G (IgG) is the major class in secretions of the uterus, oviduct and follicular fluid (Corbeil et al., 1976; Whitmore and Archbald 1977). Antigenic stimulation of the bovine uterus results in specific antibody response to IgG class, whereas vaginal stimulation leads to an IgA response in vaginal secretions. Two subclasses of IgG are predominantly found in the serum of cattle, IgG1 and IgG2 (Butler, 1973). The rate of transport of IgG1 into vaginal mucus is more rapid than that of IgG2 (Curtain et al., 1971). In contrast to IgG2, IgG1 is present in higher concentration in the uterus and vagina (Curtain et al., 1971). However, mechanisms by which serum IgG reaches the uterus or vagina are still unknown, because unlike IgA, IgG does not have a secretory piece to mediate transport across epithelial cell (Corbeil et al., 2005) In addition, the bovine cervix is capable of local antibody production (Corbeil et al., 1976), where IgG predominates. Immunoglobulin-G levels in vaginal mucus exceed those for IgM. Perhaps the lack of detectable IgM in vaginal
mucus can be related to the relative inefficiency of IgM transudation since it is restricted predominantly to the vascular space (Wilkie et al., 1972).

**Cell-Mediated Immunity**

All lymphocytes are programmed during their development to specific migration pathways through the body that enable antigen specific immune response to be concentrated at certain sites (Janeway et al., 2005). Directed migration, or homing, to mucosal tissues is controlled by expression of distinct patterns of adhesion molecules on the lymphocyte cell surface which mediate differential recognition and adherence to the endometrium in mucosal sites (Janeway et al., 2005). Intraepithelial lymphocytes are generally present in the stratum compactum of the endometrium, and their number fluctuates with the stage of the estrous cycle (Vander-Wielen and King, 1984). In addition, the main lymphocyte population is formed by CD8 type (Cobb and Watson 1995). The induction of local immune responses in the female genital tract of any species is poorly understood (Corbeil et al., 2005). However, during uterine infections with *Trichomonas foetus*, accumulation of immunocytes, lymphoid nodules, and follicles (some with germinal centers) were detected under the epithelium and adjacent to infected glands (Anderson et al., 1996). The kinetics of isotype-specific antibody responses, mast cell degranulation and clearance of infection demonstrate that immune defense of the uterus is related to increasing antibody levels and decreasing detectable subepithelial mast cells (Corbeil et al., 1974)

**Fever Pathway During Infections**

The term fever specifically defines elevation of body core temperature that occurs in defensive response to the entry into the body of pathogenic agents (IUPS Glossary,
2002). Functionally, the onset of fever is manifested by an increase in metabolic heat production and cutaneous vasoconstriction to reduce heat loss from the skin.

The generation of fever is accomplished by the interaction of multiple endogenous mediators induced by pyrogens, such as lipopolysaccharides (LPS). Kupffer cells, splenic macrophages, and neutrophils are reported to contribute to the intravascular clearance of LPS and produce cytokines (Scapini et al., 2000). Presumably fever is mediated by LPS which stimulates the Kupffer cells located in the liver (Blatteis, 2006), but to a lesser degree in the spleen. Kupffer cells detect bacterial components such as endotoxins (LPS) and peptidoglycans through the toll-like receptors on the cell surface, specific for LPS (TLR-4) (Beuttler et al., 2003). This stimulation induces the production and secretion of cytokines such as tumor necrosis factor (TNF-α), and IL-1, IL-6, IL-8 (Beuttler et al., 2003).

Two types of cytokines are responsible for the generation of fever. Pyrogenic cytokines that induce fever include interleukins IL-1, IL-6, IL-8, PGE$_2$, macrophage-inflammatory protein-$1\beta$ (MIP-$1\beta$), and interferon-$\gamma$. The other types of cytokines are endogenous antipyretics which limit the magnitude and duration of fever such as IL-10, arginine vasopressin (AVP), $\alpha$-melanocyte-stimulating hormone ($\alpha$-MSH), and glucocorticoids (Figure 2-6). Although AVP, $\alpha$-MSH, and glucocorticoids are not true cytokines, they still possess endogenous antipyretic properties. Other substances such as tumor necrosis factor-$\alpha$ have pyrogenic and antipyretic properties, depending on the experimental conditions (Leon, 2002).

Pyrogenic cytokines in the bloodstream are transported to the preoptic-anterior hypothalamic area (POA), which is the primary brain site for thermoregulation. The
ventromedial preoptic nucleus (VMPO) is thought to be the fever-producing locus (Boulant, 2000) where cytokines act. Prostaglandin-E (PGE$_2$) is considered to be the final fever mediator in the POA. The synthesis of PGE$_2$ in the POA/VMPO is effected through catalysis of arachidonic acid by cyclooxygenase (COX)-2 and microsomal PGE synthase (mPGES)-1 selectively upregulated by the pyrogenic cytokines (Blatteis, 2006). In addition, it is hypothesized that the febrile response to peripheral LPS is not initiated by pyrogenic cytokines released by LPS-stimulated leukocytes generally, but by PGE$_2$ specifically generated by Kupffer cells activated by LPS (Blatteis, 2006).

Fig. 2-6. Pathway of fever development in response to infection, inflammation, or trauma. (Leon, 2002)
The interactions of endogenous pyrogens and antipyretics are responsible for the magnitude of fever reaction. Interleukin-1 and other inhibitory cytokines stimulate the production of IL-1 receptor agonist which prevents further binding of IL-1, and decreases the effective concentration of IL-1. Interleukin-10 is one of the principal interleukins that down-regulate the pyrogenic process. IL-10 is a product of T helper-2 subset, and is induced by pyrogenic cytokines. It also inhibits the LPS-induced production of many cytokines implicated in fever, including IL-1β, IL-6 and TNF-α (Leon, 2002). In addition the physiological control of the febrile response may prevent extreme elevation in body temperature. This regulation seems to be dose related, in which a high dose of LPS during sepsis functions to lower the temperature, thus attenuating fever or producing hypothermia (Leon, 2002).

**General Review of Uterine Infections**

The uterus of postpartum cows is usually contaminated with a wide spectrum of bacteria. However, this is not consistently associated with clinical disease. In the majority of cows 1 to 4 weeks post partum, species of microorganisms such as *α-hemolytic Streptococcus*, *Arcanobacterium pyogenes*, *Enterobacteria*, *Bacillus spp*, *Staphylococcus epidermidis*, *Staphylococcus aureus*, *Fusobacterium*, *Bacteroides spp*, *Clostridium spp* and *Proteus* can colonize the uterus (Griffin et al., 1974; Olson et al., 1986). This bacterial content is negatively correlated with days postpartum. Within the first 15 days post partum, 90% of uterine samples have a positive bacteriological culture. However, by 60 days post partum, the percentage of positive bacteriological cultures are reduced to 10%. (Elliot et al., 1968). Figure 2-7 represent the percentage of uteri that have positive bacteria culture during the first 60 days post partum (Elliott et al., 1968).
In addition, the common contamination of the uterus by these bacteria is not usually associated with clinical signs. Moreover, contamination does not imply infection, as reflected by the adhesion of pathogenic organisms to the mucosa, colonization or penetration into the epithelium, and/or release of bacterial toxins that result in the establishment of uterine disease (Janeway et al., 2005).

![Graph](image.png)

Fig 2-7. Percentage of uteruses from postpartum cows in which bacteria were recovered in the first 60 days post partum. (Elliott et al., 1968)

The development of uterine disease depends on the immune response of the cow, and the species and number (load or challenge) of bacteria. The number of pathogenic bacteria in the uterus of postpartum cows may be large enough to overwhelm uterine defense mechanisms and cause life-threatening infections, although these are relatively uncommon (Sheldon, and Dobson 2004).

**Endometritis**

**Pathogenesis**

Endometritis is defined as an inflammation of the endometrial lining of the uterus. Studies by Griffin et al. (1974) and Elliott et al. (1968) have shown that uterine infections and endometritis are commonly present during the early post partum. In fact, during the
first week post partum 90 % of cows experience some degree of endometritis (Olson et al., 1986). Inflammation of the uterus (endometrium) and degree of this process is correlated with the type of bacteria cultured (Studer and Morrow, 1978). Bacteria in the uterus such as Coliforms, Streptococcus, and Arcanobacter are associated highly with endometrial inflammation (Studer and Morrow, 1978). Griffin et al. (1974) found a direct correlation between Corynebacterium pyogenes infection and degree of endometritis. However, during the early stages of Corynebacterium pyogenes infections, endometritis usually was classified as mild or moderate, and if the infection persisted for more than a week, the degree of endometritis changed to severe (Griffin, 1974). In most instances, the infection is eliminated within 3 weeks post partum. However, in cows that are unable to clear the infection, their reproductive performance was compromised (Griffin et al., 1974). Consequently, endometritis is a normal process of uterine involution of the uterus and classifying a cow as having clinical endometritis less than 21 days post partum will include a high proportion of cows that are spontaneously resolving the bacterial uterine infection (Sheldon et al., 2006). Figure 2-8 represents the incidence and degree of endometritis in uterine biopsy samples during the first seven weeks post partum.

**Histopathological disease definition**

Endometritis consists for the most part of a diffuse but light infiltration of inflammatory cells with slight desquamation of the superficial epithelium without significant vascular changes and with minimal involvement of the uterine glands. The significance of leukocytes found in the stroma is equivocal in cattle 2 to 3 days after parturition (Jubb and Kennedy 1992).
The best indication of endometritis in all species consists of the accumulation of plasma cells and lymphocytic foci in the stroma (Jubb and Kennedy 1992). Changes in the degree of inflammation depends on the duration and severity of inflammation but generally consists of fibrosis in which leukocytes, lymphocytes and plasma cells predominate. The endometrium can become thickened by inflammatory tissue where endometrial glands may become atrophic, flattened, attenuated, or cystic, due to the periglandular fibrosis (Jubb and Kennedy 1992).
Clinical disease definition

Studer and Morrow (1978) diagnosed endometritis using uterine biopsy and found a positive correlation with rectal palpation findings and uterine discharge characteristics. Consequently, they suggested that use of rectal palpation can be utilized to identify cows with endometritis (Studer and Morrow, 1978).

Sheldon et al. (2006), defined clinical endometritis as those cows with a purulent uterine discharge detectable in the vagina 21 days or more post partum, or mucopurulent discharge detectable in the vagina after 26 days post partum. This definition was based on the finding from a study conducted by LeBlanc et al. (2002), who reported that presence of purulent vaginal mucus or a cervical diameter >7.5 cm 21 days or more post partum; had a negative effect on reproductive performance. These results agree with those of Oltenacu et al., (1983), who concluded that the diameter of the cervix estimated by rectal palpation 12 to 26 days post partum (i.e., diameter > 5.5 cm primiparous and 6.0 cm multiparous) was the best indicator of subsequent poor reproductive performance. However, Oltenacu et al., (1983) did not find a direct effect of uterine discharge on reproductive performance.

Puerperal Metritis

Pathogenesis

Puerperal metritis has been described as a life-threatening infection, characterized by a fetid vulvar discharge that may be associated with clostridial infections (Roberts, 1986). The presence of a fetid discharge appears to be an unequivocal sign of uterine infections which reflects the level of bacterial contamination in the uterus. Mateus et al. (2003) using cows that were within 6 weeks post partum, evaluated the bacteriological
content of the uterus, uterine horn size and fluid content by ultrasonography. In this study (Mateus et al., 2003), uterine discharge by vaginoscopy was classified as normal lochia, mild endometritis (purulent lochia), and severe endometritis (heavy, fetid purulent lochia) associated with or without systemic symptoms. Results indicated that uterine involution of cows with a fetid discharge was delayed. Furthermore, uteri that contained fetid purulent lochia present at examination had a greater horn diameter (i.e., at 3 to 4 weeks postpartum) and a greater amount of uterine fluid (i.e., at 2 to 6 weeks postpartum) than the normal puerperium group, respectively (Mateus et al., 2002). No differences in uterine involution were observed between the group classified as normal and the group classified as purulent lochia. In addition, the bacterial cultures showed that *Arcanobacter pyogenes*, *E. coli*, *Fusobacterium* sp. and *Bacteroides* sp. were more frequently isolated from cows with mild endometritis or severe endometritis than in cows without these conditions. In cows with a normal puerperium *A. pyogenes* was isolated in 74% of the cases, and gram negative anaerobes only occurred through the second week post partum, whereas cows with mild or severe endometritis, these response were evident until the fourth to 6 week post partum, respectively. These results agreed with Hirvonen et al. (1999) in which cows with a fetid discharge showed higher bacterial growth of *E. coli* and *A. pyogenes* compared to cows without a purulent discharge. Furthermore, if the cows showed systemic signs then *Bacteroides* sp. and *Fusobacterium necrophorum* also were isolated. In addition to the previous results (Mateus et al., 2003; Hirvonen et al., 1999), Dohmen et al., (2000) showed that cows experiencing dystocia or retained fetal membranes with a fetid discharge showed a high growth rate of *E. coli*, black pigmented
G- anaerobes and *Clostridium spp.*, from uterine cultures, compared to cows that calved without dystocia or retained fetal membranes and did not have a fetid vulvar discharge.

General signs of toxemia can be found in cows with puerperal metritis (Rebhun et al., 1995). Endotoxins or lipopolysaccharides (LPS) are among the most important virulent factors of coliform bacteria. Lipopolysaccharides are somatic antigens of bacteria composed of polysaccharides, phospholipids and a small amount of protein (Lohuis et al., 1988). High LPS concentrations in lochia were found to be positively correlated with the presence of a fetid discharge and presence of *E. coli* bacteria (Dohmen et al., 2000). However, LPS concentration in the uterus was not correlated with LPS concentrations in blood. In contrast, Mateus et al. (2003) positively correlated the presence of a fetid uterine discharge with LPS concentration in blood. Furthermore, Peter et al. (1990) reported increments of LPS concentrations in blood after intrauterine infusion of endotoxins in postpartum cows and suggested that LPS are absorbed from the uterus. Mechanisms by which LPS are absorbed from the uterus were hypothesized as: direct absorption from the uterus, passive diffusion and/or transmural leakage, or escape through the oviducts and fimbria into the peritoneal cavity (Peter et al., 1990). In addition, Peter et al. (1990) demonstrated that absorption of LPS by the uterus decreased as days postpartum increased. Intrauterine infusion of LPS in cows 20 days post partum did not show an incremental increase in LPS concentration in blood compared to cows infused at 5 days post partum (Peter et al., 1990).

Signs of endotoxemia include depression, respiratory distress, vasomotor disturbance, shock, fever, sometimes followed by hypothermia, disturbance of gastrointestinal tract motility and metabolic disturbances (Lohuis et al., 1988).
Immune cells detect bacterial components such as LPS and peptidoglycan via toll-like receptors that are present in macrophages (TLR-4) (Beuttler et al., 2003) to stimulate production of the cytokines (i.e., TNF-α, IL-1, IL-6, IL-8). These cytokines act as internal pyrogens to increase core body temperature. Dohmen et al. (2000), found a positive correlation between a fetid uterine discharge, LPS and rectal temperature. Cows with a fetid uterine discharge had higher rectal temperatures (mean = 39.3°C). However, fever is not associated always with this type of uterine discharge. Hirvonen et al. (1999) found that only 8 (42%) of 19 cows that were diagnosed with puerperal metritis (acute puerperal metritis, putrid vulvar discharge) between 4 to 11 days postpartum, developed systemic clinical signs of fever (39.5°C – 41.0°C) and poor appetite during the acute phase of the infection. These results agree with those of Pugh et al (1994), in which only 42.3% 78 cases of puerperal metritis evaluated within 14 days post partum had fever (RT > 39.4°C) at diagnosis. However, in this later study the type of uterine discharge was not described for cows with puerperal metritis.

Tissue injury and inflammation induce the release of interleukin-6 (Hirano 1992). This interleukin is one of the cytokines involved in the development of fever (Beuttler et al., 2003), as well as inducing the synthesis of acute phase proteins such as haptoglobin and alpha-1-acid glycoprotein by hepatocytes (MacKay and Lester 1992). Systemic responses that result in fever also increase the levels of acute phase proteins. However, Smith et al. (1998), reported no significant correlation between level of haptoglobin in blood with rectal temperature in cases of puerperal metritis (foul smelling vulvar discharge with a rectal temperature > 102.5). Nevertheless, concentration of haptoglobin in cows with puerperal metritis were between 13 to 20mg/dl on the day of puerperal
metritis diagnosis, which agrees with the ranges (>10 mg/dl) reported by Skinner et al. (1991) in cows with puerperal metritis (foul-smelling vulvar discharge, with or without fever). Williams et al. (2005) compared levels of alpha-1-acid glycoprotein in cows having purulent fetid discharge with cows having a purulent but no fetid discharge at 21 or 28 days postpartum. Cows with a fetid uterine discharge had higher concentrations of alpha-1-acid glycoprotein (1.5 mg/dl) than those cows without a fetid discharge (1.03 mg/dl). However, the levels of alpha-1-acid glycoprotein found by Williams et al. (2005) during days 21 or 28 post partum were within the normal levels (1.2-1.4 mg/dl) in normal cows within 10 days post partum reported by Sheldon et al. (2001). In contrast, Sheldon et al. (2004), found that cows with one or more events of fever during the first 10 days post partum had higher levels of alpha-1-acid glycoprotein than cows without fever during the same time period.

**Histopathological disease definition**

Puerperal metritis is defined as the inflammation of the mucosa, submucosa, muscular and the serosal layers of the uterus. It is described as a purulent inflammation, where the sub-serosal connective tissues are edematous and infiltrated with leukocytes, with the same process observed surrounding blood vessels of the myometrum that extend to muscle fibers which undergo granular degeneration. The leukocyte mass on the mucosal surface is associated with extensive hemorrhage, necrosis, and sloughing (Jubb, et al. 1992) induced by bacteria toxins produced by bacteria. The hemorrhage and necrosis, along with bacterial products characterize the clinical findings (Jubb, et al. 1992).
The histopathological definition of puerperal metritis is straightforward (Sheldon et al., 2006). However, this is not a common practice because of logistical reasons and the sampling per-se poses a risk to the health of the animal more than the disease itself (Etherington et al., 1988). Consequently, as well as in other diseases, a clinical definition is needed to diagnose uterine infections. These definitions should characterize clinical findings to conclude whether or not the disease is life threatening and whether treatment should be applied.

**Clinical Disease Definition**

Highly pathogenic types of bacteria are present in the uterus and along with their toxins are absorbed into the circulation producing symptoms associated with septicemia, endotoxemia, and pyemia (Roberts, 1986). Diagnosis of puerperal metritis has been performed by rectal palpation of the uterus (Markusfeld, 1984; Pugh et al., 1994; Risco, and Hernandez, 2003). However, vaginoscopy has also been used (Hirvonen et al., 1999).

Based on clinical experience Rebhun (1995) described puerperal metritis (septic or toxic metritis) as those cows with a fetid watery uterine discharge from the vulva that varied in color from brown, amber to gray or red, but fluid are low in mucus content and contained purulent material. Cows become ill within the first 7 to 10 days post partum and had fever (40.0°C to 41.39°C), tachycardia, inappetence, decreased production, rumen stasis, and toxemia. Dehydration, diarrhea, and depression of varying severity are also observed. Extremely severe infection may cause recumbency secondary to toxemia, weakness, and metabolic disorders.
Literature disease definitions

Terminology used to classify uterine infections, specially for puerperal metritis has been vague and inconsistent (Lewis 1997). Many studies have been published that describe the epidemiology of puerperal metritis, it’s effect on reproduction performance and milk production, and treatment and prevention of this disease. However, differences in disease definition contributed to conflicting results on effect on reproductive performance, milk production, and disease incidence.

Bartlett et al. (1986) described the epidemiology of metritis and estimated the economic impact of metritis in Michigan Holstein-Friesian dairy cows. Metritis was defined according to the thickness of the uterine wall and the fluid content of the uterine cavity in relation to the number of days post partum. The study involved 22 herds and information about disease was recovered from a dairy herd health computer network. In over 3773 lactations were studied with an lactational incidence of 18% within 10 to 30 days post partum was found, that varies from 3 to 45%. In addition, metritits was most commonly diagnosed between 11 to 20 days post partum. After including the effect on reproductive efficiency, milk production, cost of medication and losses due to culling, the total cost estimate was $106.00 for a lactation with metritis.

Beaudeau et al. (1995) assessed the effect of health disorders on length of reproductive life in 47 French Holstein commercial dairies during 4 years. Metritis was one of the explanatory variables and was further divided as early metritis and late metritis. Early metritis included vulvitis, vuvlvovaginitis, endometritis, vaginal discharge, metritis or pyometritis diagnosed from 22 to 49 days postpartum. Late metritis included vulvitis, vulvovaginitis, endometritis, vaginal discharge, metritis or pyometritis diagnosed
beyond 50 days post partum. The overall incidence for early metritis was 6.4%, however the incidence ranged form 5.2 % when calculated in cows with less than 90 days postpartum or 8.1 % if calculated in cows with more than 210 days postpartum. The overall incidence of late metritis was 5.6 % and ranged from 1.4 % in cows with less than 90 day postpartum and 14.1 % in cows with more than 210 days in milk. It was found that cows with late metritis or early abortion had poor survival, thus higher culling rate.

Bruun et al. (2002) identified risk factors for metritis in 102,060 Danish dairy cows. Information was recovered from the Danish cattle database. Metritis was not defined. However, diagnoses were made within 1 to 30 days post partum . In this study, the incidence risk ranged from 1 to 21 % in 391 herds observed.

Callahan and Horstman (1987) reported a retrospective analysis of treatment alternative in dairy cows affected with postpartum metritis in the Purdue University Dairy center. The criteria for diagnosis of metritis consisted of ballottement of uterine fluid, possible crepitant feel of the uterine content, lack of myometrial tone, retarded involution and the presence of abnormal discharge. Discharge characteristics ranged from thin, watery and fetid to purulent or mucopurulent. The study was conducted in a 5 year period and 1108 lactations were evaluated. An incidence of 33.8 % was reported within 14 days postpartum. In this study no effect on reproduction performance was found in cows with puerperal metritis. Furthermore, they stated that the early diagnosis of metritis may reduce the impact on the reproductive performance.

Chenault et al. (2004) evaluated the efficacy of ceftioufur hydrochloride for the treatment of postpartum metritis. Metritis cases were defined as cows with a rectal temperature \(\geq 39.5^\circ\text{C}\), with a fetid vaginal discharge that was red or pink to chocolate
brown in color and serous with or without pieces of necrotic tissue, within 1 to 14 days post partum. Given that only metritis cows were reported as an outcome, incidences were not reported. However they concluded that ceftiofur hydrochloride administered at a dosage of 2.2 mg of CE/kg, subcutaneously or intramuscularly once daily for 5 days was efficacious for treatment of acute puerperal metritis in dairy cows.

Cobo-Abreu et al. (1979) observed the association between disease, production and culling in a Holstein dairy herd in Ontario, Canada. This study was conducted during seven years. Metritis definition, incidence and days in milk to diagnosis were not defined.

Correa et al. (1993) modeled a path analysis with logistic regression for seven postpartum clinical diseases in cows and also observed the factors related to the calf. Data were from 7761 lactations from 34 commercial dairy herds close to Cornell University. Metritis was defined as an enlarged uterus found at rectal palpation in cows with or without other clinical signs within 30 days post partum. However a metritis event included cases of endometritis, pyometras and metritis as defined above. A lactational incidence of 7.2% was observed. It was found that stillbirth increased the odds of developing metritis and retained placenta, cows that twinned had increased odds of developing dystocia and retained placenta. Dystocia was related to an increase in the odds of retained placenta. Milk fever, dystocia, and ketosis each increased the odds of developing left-displaced abomasum. Postpartum periods with dystocia, retained placenta, or ketosis had increased odds of metritis.

Curtis et al. (1985) used path analysis and logistic regression to model direct and indirect relationships among clinical periparturient (within 30 days after calving) diseases. Data were obtained from 1374 lactations of multiparous Holstein cows in 31
commercial herds near the Cornell University area during a period of one year. The definition of metritis was not given. However, an incidence of 7.8 % was reported, within 30 days post partum. Retained placenta, left displaced abomasum, and parturient paresis directly increased risk of complicated ketosis. The study suggested that feeding higher intakes (relative to National Research Council recommendations) of protein and energy in the last 3 week of the dry period may reduce the incidence of metabolic and reproductive disorders.

Drillich et al. (2001) evaluated the efficacy and financial viability of systemic treatment of toxic puerperal metritis in dairy cows with ceftiofur. Toxic puerperal metritis was defined as the presence of a fetid, reddish-brown vaginal discharge and a rectal temperature $\geq 39.5^\circ\text{C}$, within 4 to 6 days postpartum. During the study period, a total of 1756 calvings were observed and an incidence of 18.5 % was reported. There were no significant differences among the treatment groups regarding clinical efficacy at d 6 after first treatment (group 1 received 600 mg of ceftiofur intramuscularly on 3 consecutive days; group 2 received an intrauterine treatment with antibiotic pills consisting of 2500 mg of ampicillin and 2500 mg of cloxacillin and an additional 6000 mg IM of ampicillin on 3 consecutive days and group 3 received the same intrauterine treatment as in group 2, in addition to 600 mg of ceftiofur IM on 3 consecutive days. The cure rates based on rectal temperatures declining to below 39.5 degrees C on d 6 after treatment were 82.9, 84.8, and 84.6% for groups 1, 2, and 3, respectively. Reproductive performance did not differ significantly between group 1 and groups 2 and 3 for any of the measures tested. A financial analysis with 87 different cost scenarios demonstrated that a systemic treatment
of toxic puerperal metritis in cattle with ceftiofur is an effective alternative to the combination of local and systemic treatments.

Etherington et al. (1985) used a path analysis to determine the interrelationship between ambient temperature, age at calving, postpartum reproductive events and reproductive performance in dairy cows. Within the reproductive event metritis was included. A cow was considered to have metritis if she exhibited decreased milk production, decreased feed intake, pyrexia and had foul smelling vaginal discharge. An incidence of 23% was reported. However days in milk of the diagnosis was not reported. In this study there was an increase in the incidence of retained placenta, in the percentage of cows with abnormal vaginal discharge in the early postpartum period as well as a delay in uterine involution during the winter months. In addition, cows calving during the winter had prolonged intervals to first estrus, first service and conception compared to cows calving during the summer. Cows calving during the warmest months, on average, were seen in estrus 24 days sooner, received first service 42 days sooner and conceived 27 days sooner than cows calving during the coldest months of the year.

Erb and Martin (1978), studied age, breed and seasonal patterns in the occurrence of ten diseases on which metritis was one of them. In this study, information of the disease was retrieved from a University central database. The definition of metritis included endometritis, metritis and pyometra. However, none of these diseases were defined. An incidence of 14% was observed. No description of the time frame of the diagnoses was made. Using the log-odds method, trends were noted for the youngest cows to be at increased risk of the reproductive diseases such metritis and for the
Guernsey cows to be at increased risk of the uterine diseases. There was a tendency for peaks in disease occurrences in the winter (as opposed to summer) months.

Erb et al. (1985) observed the relationship between occurrence of metritis with other diseases. A total of 2960 lactations of Holstein dairy cows were included in the study. Metritis was not defined. However, the incidence was reported to be a 9.9%. No description of the time frame of the diagnoses was reported. It was shown that heifers that were older, of lighter weight, or who had lower estimated transmitting ability for milk had more problems, less milk, and poorer survival. Dystocia in heifers had several detrimental consequences including 2.9 to 4 times more retained placenta, metritis, and culling and +7.4 d more to first service. Cystic ovaries were associated directly with 376 kg greater milk yield and with a 16.5-d delay in first service. Failure to conceive at first service and mastitis increased risk of culling 5.2 to 10 times. In multiparous cows, milk fever increased risk of reproductive disorders by 1.6 to 4.2 times and indirectly contributed to poor breeding performance and increased culling. Risk of culling was increased 2.1 to 3.7 times directly by mastitis and dystocia and by poor breeding performance.

Harman et al. (1996) quantified the effect of season of parturition, parity, and diseases on time to conception in 44450 Holstein dairy cows. Metritis was divided into early (less than 42 days postpartum) and late metritis (more than 42 days post partum). However no definition of disease was given. The lactational incidences were 2.0% for early with median day to diagnosis of 18 days after parturition, and 1.1% for late metritis with median day to the diagnosis of 108 days after parturition. For multiparous cows, parturition in the spring or summer and being of parity 2 or 3-4 (vs older) increased the
chance of conceiving; 10 diseases or disorders decreased this probability. In primiparous, parturition in spring or summer increased the probability of conception, and 6 disorders decreased it. Disorders that were found to be detrimental in both models were anestrus, ovulatory dysfunction, other infertility, late metritis, and clinical ketosis.

Hirvonen et al. (1999) examined the role of systemic acute phase proteins regarding diagnostic values of Haptoglobin, alpha1- acid glycoprotein, and plasma N-acetyl-beta-D-glucosaminidase activity in clinical and bacteriologically defined acute postpartum metritis in dairy cows. Acute metritis was defined as a putrid, reddish-brown, watery, foul smelling vaginal discharge, within 4 to 11 days post partum. No incidence were reported given that puerperal metritis was the principal outcome. Results showed that plasma haptoglobin concentration remained low in most cows with acute postpartum metritis. Only the 3 most severely affected cows exhibited a strong haptoglobin response. These were later culled due to poor condition and reduced fertility. It was suggested that in acute uterine infection a highly increased haptoglobin concentration indicated a poor prognosis for repeat conception. Plasma alpha1-acid glycoprotein concentration increased in acute postpartum metritis, the response pattern being less prominent than that for haptoglobin. The alpha1-acid glycoprotein concentrations did not correlate with severity of disease, and, consequently, the capacity of alpha1-acid glycoprotein in differentiating genital infections was relatively poor. Highest alpha1-acid glycoprotein concentrations were detected in cows with retained placenta and/or dystocia. In addition, plasma N-acetyl-beta-D-glucosaminidase activity levels did not differ between the cows with acute postpartum metritis and healthy control cows.
Lee et al. (1989) described the use of survival analysis to quantify the days open for different diseases during the early post partum period. Metritis was used as an explanatory variable and this disease was further divided into metritis, non systemic and systemic. However incidences and the definition of metritis and day in milk to diagnosis was not provided. It was found that retained placenta, nonsystemic metritis, systemic metritis, ovarian cysts, and lameness were associated with a decrease in conception rate and an increase in median days open. The hazard ratios for conception were .66, .83, .70, .70, and .69 and the increase in median days open 5, 15, 13, 22, and 28 d for the five diseases, respectively.

Markusfeld, O (1984) observed the factors associated with retained fetal membranes and postpartum metritis in 2017 Holstein dairy cows. Metritis was defined as any purulent foul smelling discharge. The incidence observed was 37.3 % and ranged between 31.2 to 43.8 %. The diagnosis were made within 14 days post partum. In this study risk factors associated with metritis include declining parity, long gestations, induction of parturition, stillbirth, multiple births, low milk yield before drying off, left displacement of the abomasum, ketosis and winter calvings.

Markusfeld and Ezra (1993) observed the effect of herd, sire, season, body height, body weight, age at calving, and metritis on performance of first lactation cows. Metritis was defined as described in Markusfeld (1987). Of a total of 621 first lactation heifers, an incidence of 48.6 % was reported, within 5 to 12 days postpartum. This study reported that short, heavy first lactation cows had an odds ratio of 3.1 of incidence of metritis at calving compared with all others; 648 first lactation cows were measured at wk 1 postpartum. Sire, herd, age, height, season, and BW contributed to peak milk yield.
Metritis did not affect peak yield. Herd, sire, height, and age contributed to mature equivalent corrected 305-d milk yield. No effect was found for BW, season, or metritis. Herd was the only variable contributing to month of peak yield and rate of monthly drop in yield. Interactions between BW, height, and incidence of metritis were significant. Tall, heavy first lactation cows with metritis peaked higher and yielded more than those without metritis. Short, light first lactation cows with metritis yielded less and peaked lower than their healthy counterparts. Metritis did not affect future fertility, but season and the interaction between BW and height did. Tall, heavy first lactation cows had a lower pregnancy rate from first AI, independent of milk yield. The relative importance of height as a predictor of future milk yield is underestimated. The interaction between height and BW may have an antagonistic effect on yield and fertility.

Melendez et al., (2004) evaluated the effect of 2 doses of PGF$_2$α injected early postpartum on uterine involution, serum concentration of alpha1- acid glycoprotein and fertility in Holstein cows with acute puerperal metritis. Acute puerperal metritis was diagnosed by per rectum palpation of the uterus at 8 d post partum. Criteria for diagnosis was an enlarged and flaccid uterus with a foul-smelling uterine discharge, without fever (>39.5°C). During the study period, 1536 cows calved; an incidence of 15.3% was reported. However only cows diagnosed with retained fetal membranes and metritis were included and those cows with metritis and systemic signs were excluded. In this study postpartum, primiparous, treated cows had smaller uterine diameters and lower uterine scores than controls. Cows with a uterine diameter <5.1 cm at 12 d postpartum were 5.5 times more likely to conceive at first service than cows with larger uterine horn diameter. Treatment significantly reduced the concentrations of serum alpha1-acid glycoprotein.
Within primiparous cows, treatment also increased conception at first service by 17%. It was concluded that 2 doses of PGF$_{2\alpha}$ 8 h apart at 8 d postpartum in primiparous cows with acute puerperal metritis decreased the diameter of uterine horns and serum concentration of alpha1-acid glycoprotein at 12 d postpartum and increased the conception rate at first service.

Overton et al. (2003) studied the effect of a prophylactic treatment with estradiol cypionate (ECP) in cows at a high risk to develop metritis. Metritis was defined as any combination of fever $\geq 39.7^\circ$C with a watery and or fetid vulvar discharge within 1 to 10 days postpartum. Metritis were further divided into mild or severe. Metritis was mild when the cow never had a rectal temperature $\geq 39.7^\circ$C and severe when the rectal temperature was $\geq 39.7^\circ$C. An incidence of 10% was observed in 1284 calvings observed. They concluded that prophylactic administration of ECP to dairy cows at high risk for metritis did not reduce risk for metritis.

Pugh et al. (1994) described 78 cases of postpartum metritis in dairy cows. Forty two and 36 cases of postpartum metritis were recovered from records of the large animal hospital from Auburn University and Tuskegee University, respectively. The definition was not reported. However, it was stated that the diagnoses were based on the vaginal-uterine discharge obtained by rectal palpation of the uterus. Older cows and those with hyperthermia were less likely to recover from puerperal metritis. Furthermore, only 42% of the treated cows were with hyperthermia at the moment of the physical exam.

Rajala and Gröhn (1998) evaluated the effects of dystocia, retained placenta, and metritis on milk yield using repeated, monthly test day milk yields, on 37,776 Finnish Ayrshire dairy cows in 2337 herds, recovered from the national Finnish health recording
system. Metritis was not defined, however diagnosis was further divided in early and late metritis. The lactational incidence of early metritis ranged from 1.6 to 2.6 % within 28 days post partum and the incidence of late metritis, ranged from 1.2 to 1.5 % after 28 days postpartum. Dystocia, retained placenta, and early metritis significantly affected milk yield, as indicated by monthly test day milk yields. Late metritis was not associated with milk loss. The impact of the diseases differed across parities and also across different levels of milk yield. Using 305-d milk yield as the milk measure, no diseases were associated with reduced milk yield.

Risco and Hernandez (2003) compared the administration of ceftiofur hydrochloride and ECP® on the prevention of puerperal metritis. In this study the definition of metritis was not stated. However, diagnoses were made within the first 30 days post partum. Incidences were not reported given that cows with metritis were the experimental units. Results showed that the proportion of cows that developed metritis was significantly different in cows treated with ceftiofur hydrochloride (13%), compared with cows treated with ECP (42%) or cows that received no treatment (42%). Uterine involution patterns (i.e. median time to complete retraction of the uterus and mean diameter measure of cervix and uterine horns) were not significantly different between groups. Cows treated with ECP were 0.40 times as likely to conceive as control cows (P=0.05); median time to conception in cows treated with ECP (192 days) was longer, compared to control cows (124 days). It was concluded that systemic administration of ceftiofur hydrochloride is beneficial for prevention of metritis, but its effect on reproductive performance was not significantly different to that of ECP or no treatment.
In addition, administration of ECP did not have beneficial effects on metritis prevention and reproductive performance.

Schnier et al. (2002) compared the incidence of diseases in 5000 Finland dairy cows kept in cold or loose-housing systems. Information was recovered from the Finish health data recording system. Metritis included cases of acute and chronic metritis, pyometra, vaginitis and disturbed involution within 0 to 44 days post partum. However none of these conditions were defined. The overall incidence for both housing system was 3.3%. They found that cows in a cold loose-housing system were at lower odds for developing late mastitis (15-305 days in milk), and metritis (Friesian breed); of the same odds for ketosis and early mastitis (0-14 days in milk); but at higher odds for developing parturient paresis and metritis (Ayrshire breed).

Smith et al. (1998) compared procaine penicillin, intrauterine infusion of oxytetracycline or ceftiofur in dairy cows for the treatment of toxic postpartum metritis. Toxic postpartum metritis was defined as any cow with a rectal temperature >39.2°C, a flaccid, non retractable uterus that was located in the abdomen, a cervical diameter >75 mm, and a watery, fetid vulvar discharge. Other criteria used to diagnose toxic puerperal metritis was depressed milk yield (<7.4 kg at the morning milking), within 3 to 20 DIM. Given that only cows with puerperal metritis were used no incidence was reported. No difference was observed among groups for milk yield on d 1 and 12 or for temperature on d 1 and 5. Serum haptoglobin was elevated to > 10 mg/dl for cows in all groups; however, no difference was observed among groups on d 1 and 5. Because all groups showed a favorable response, this study suggests that there is no difference in treatment efficacy among antibiotics used to treat cows affected with toxic puerperal metritis.
Urton et al. (2005) related cows with high or low feeding behavior during the prepartum transition phase with increased risk of developing metritis after calving. Two metritis classification were used. Animals were classified as having metritis if they showed a mucopurulent and foul smelling and fever (rectal temperature > 39.5°C) or acutely metritis if they showed a reddish brown, watery, foul smelling vaginal discharge and fever. The incidence of metritis ranged between 38% to 27% for metritis and acute metritis respectively, within 3 to 15 days post partum, however the total calvings were not reported. Cows suffering from metritis, exhibit reduced milk yield and reproductive performance. These cows spent on average 22 min/d less time at the feed alley during the transition period than did non-metritic cows. For every 10-min decrease in average daily feeding time, cows were twice as likely to be diagnosed with metritis. A threshold of 75 min of average daily feeding time was 89% sensitive and 62% specific for detection of acute metritis. It was concluded that reduced time at the feeder can be used to identify dairy cows at risk for metritis.

**Current research definition**

Given the various definitions used in the literature to describe uterine infections, Sheldon et al. (2006) proposed a series of definitions. In this review, a distinction between puerperal metritis and clinical metritis was made. Puerperal metritis is defined as those cows with an abnormally enlarged uterus and a fetid watery red-brown uterine discharge associated with signs of systemic illness (decreased milk yield, dullness or other signs of toxemia) and fever >39.5 °C within 21 days post partum. In addition, clinical metritis was defined as those cows that do not appear sick, but had an abnormally enlarged uterus and a purulent uterine discharge present in the vagina, within 21 days.
after parturition. The definitions proposed by Sheldon et al. (2006) were based from previous studies related to metritis treatments (Drillich, et al., 2001), pyrexia in postpartum cows (Sheldon et al., 2004), and risk factors for puerperal metritis (Markusfeld, 1984). However, none of the cited studies used to define uterine infections related clinical signs to histopathology and possible pathophysiology of this disease. Furthermore, they did not include any controlled studies that may relate clinical signs with risk factors to systemic illness or impairment of cow performance.

**Risk Factors for Puerperal Metritis**

Puerperal metritis is often associated with retained fetal membranes (RFM), dystocia, stillbirth or twins and usually occurs during the first two weeks post partum (Olson et al., 1986). However, puerperal metritis may also occur in cows without calving related disorders (Olson et al., 1986).

Studies using path analysis and risk assessment have consistently indicated that dystocia, retained fetal membranes, and metabolic conditions increased the likelihood that a cow will develop metritis. However, in some cases puerperal metritis was classified as a disease complex without distinguishing the clinical presentation or severity, making comparison between studies difficult (Lewis, 1997). Many of these studies used odds ratios (OR) as a measurement of risk factor, but relative risk (RR) another measurement of association, also has been used.

**Retain fetal membranes**

Retained fetal membrane (RFM) is a major predisposing cause of metritis. The third stage of parturition involves the expulsion of the fetal membranes, and is completed within 8 hours after parturition. A persistence of the third stage of parturition, that is,
failure to expel the fetal membranes is considered abnormal (Roberts., 1986). Fetal membranes are considered retained when the fetal cotyledonary villi fail to separate from the crypts of the maternal caruncles within 12 to 24 hours of parturition (Roberts S. J, 1986) and mechanisms why this process fails has been described (Gunnink 1984; Kimura et al., 2002).

The condition of retained placenta occurs in 4 to 18% of calvings (Markusfeld, 1985; Esslemont and Kossainbati, 1996; Erb, et al., 1985). Various risk factors for the development of retained placenta have been reported. Calving problems including dystocia (OR = 4.0) (Correa, et al., 1993; Markusfeld O. 1984; Erb, et al., 1985), stillbirths (Correa, et al., 1993; Markusfeld, 1984), and multiple births (Correa, et al., 1993; Markusfeld, 1984), parturient paresis, low prepartum protein and age of cows (Curtis et al., 1985) have been found to be risk factors for RFM. Dohmen et al. (2000), reported that immediately after calving, RFM cows had high concentrations of LPS/endotoxin in lochia and were more often infected with E. coli, Clostridium spp. and G- anaerobes (prevalence rates up to 97%) bacteria than cows without periparturient disorders. The principal deleterious effect of retained fetal membranes on the dairy cow is impaired fertility by delaying involution of the uterus, thereby facilitating the development of uterine infections (Sandals et al., 1979).

Retention of fetal membranes is one of the major factor predisposing cattle to metritis (Bartlett et al., 1986; Correa et al., 1993). Several studies have related the incidence of metritis with RFM. Sandals et al. (1979) reported an incidence of 54.8 percent of metritis following retained fetal membranes. Erb et al. (1985) reported an important biological causal association between RFM and metritis. It was reported by Erb
et al. (1981) that cows with RFM were almost six times more likely to developed metritis compared to cows without RFM.

Various studies have associated RFM and metritis OR = 4.4 (Gröhn et al., 1990); OR = 6.0 (Correa et al., 1993); OR = 2.5 (Bruun et al., 2002); OR = 5.8 (Erb et al., 1985).

**Stillbirth, multiple birth and dystocia**

Stillbirth, multiple birth and dystocia are events that occur at parturition and are risk factors for metritis and are related to one another. When the first, or the second stages of parturition are prolonged, it becomes difficult or impossible for the dam to give birth without artificial aid and then this condition is termed dystocia (Roberts, 1986). The incidence of dystocia ranges from 6% to 25% (Roberts, 1986; Adamec et al., 2006).

Prepartum dietary energy and parturient paresis have direct effects leading to veterinary assisted dystocia (Curtis et al., 1985). However, prepartum energy does not seem to affect the incidence of dystocia (Markusfeld, 1985). Dystocia is greater in pregnancies that terminate early due to uterine disease, fetal death, and twinning (Roberts, 1986). Dystocia can increase the risk of trauma to the uterine wall and thereby increase the odds of metritis (Bruun et al., 2002). In addition, assistance during calving may increase the risk of uterine contamination (Bruun et al., 2002). Erb et al. (1985) differentiated the incidence of metritis by parity and whether or not the cow had dystocia using path analysis. For primiparous and multiparous cows, dystocia was a risk factor for metritis (OR=3.0; OR = 3.5; respectively). Dohoo et al. (1984), and Curtis et al (1985), also found a positive association between dystocia and metritis (OR = 2.5) and (OR = 4.9), respectively. In the study reported by Curtis et al. (1985), dystocia were only those calvings assisted by a veterinarian. However, this assistance was not a risk for RFM.
These results are in agreement with studies by Correa et al. (1993) who reported that cows requiring assistance at calving were 2.1 (OR) times more likely to develop metritis.

Stillbirth is the expulsion of a dead fetus at parturition (Roberts, 1986). Calving difficulty has been implicated as the major cause of stillbirth, yet about 50% of stillborn calves are from unassisted births (Philipsson, 1996). Martinez et al. (1983) reported that the stillbirth rate in U.S. Holsteins is around 10.5% for first lactations, 5.5% for second lactation and 5.7% for third lactation. Markusfeld (1984), reported that heifers and cows which gave birth to dead calf had a higher rate of RFM or to develop metritis compared to those which gave birth to live calves (OR = 2.19) These results agree with those of Correa et al. (1993), who reported that cows delivering dead calves were 1.5 (OR) times more likely to develop metritis than those than did not have stillbirth at parturition. In contrast, Emanuelson et al. (1993) found that stillbirths had a direct effect on the risk of retained placenta but not on metritis.

When an uniparous animal aborts two or more or gives birth to fetuses are called twins (Roberts, 1986). The incidence of twinning in dairy cattle has increased dramatically over the past two decades (Nielen et al., 1989; Kinsel at al., 1998). Risk factors for twin calvings such as parity, season, herd and previous twinning have been described ((Nielen et al., 1989). The incidence of twinning has been reported to range from 1.04% to 9% (Roberts, 1986; Kinsel et al., 1998). Twin calving increased the risk of dystocia (OR = 10.5), RFM (OR = 3.4; Correa et al., 1993) and reduces milk production and increased culling rate than single calvings (Nielen et al., 1989). Twinning often results in decreased gestation length and increased dystocia and mortality rates. Markusfeld (1987) found that cows delivering twins were 12 times (OR) more likely to
RFM and 2.3 times (OR) more likely to develop metritis than cows without RFM.

Correa et al. (1993), did not find an effect of twins on the incidence of metritis

**Parity**

The effect of parity on metritis has been reported previously with conflicting results. Gröhn et al., 1990 found no association between parity and metritis. However, Markusfeld (1984; 1987) found an association. As parity increased, the incidence of RFM increased, but the incidence of metritis decreased (Markusfeld, 1984). In contrast, primiparous cows were more likely (OR = 2.7) than second or greater parity cows to develop metritis (Markusfeld, 1984). This finding is in agreement with Erb and Martin (1978), were first lactation cows compared to other lactation cows were more likely (OR = 1.48) to develop puerperal metritis.

Bruum et al. (2002) suggested that there is an u-shaped association between parity and metritis. Primiparous cows are more likely (OR = 1.6) to develop metritis than second-parity cows because damage to the uterus is more common in heifers given the high incidence of dystocia (Bruum et al., 2002). In contrast, third-parity or greater cows were more likely (OR = 1.58) to develop metritis compared to second-parity cows related to a delay in uterine involution which increases the risk to develop uterine infections (Bruum et al., 2002). Tendency for an u-shaped relationship between parity and incidence of metritis was also reported by Rajala and Gröhn (1998). However, this relationship was not significant. In addition Smith et al. (1998), found a relationship between parity and dystocia. Primiparous cows were more likely (OR = 2.04) than multiparous cows to have dystocia. However, Pugh et al. (1994) did not find any association between cases of metritis and parity.
Season

Season has been reported to be a risk factor for metritis (Sandals et al. 1979; Markusfeld, 1984; Erb and Martin, 1985; Etherington et al., 1985). Markusfeld (1984) found that cows that calved in the summer had a greater risk (RR = 1.64) of RFM and a lower risk of developing metritis (RR = 0.64) compared to those that calved in winter. Bruun et al., 2002 found that the odds ratio for metritis in cows calving during the cold season were 1.2 times higher than those cows calving during the warm season. Bruun et al. (2002) stated that during the winter months the general health of cows is lowered making them more prone to infections. In a study that involved 3773 lactations, Bartlett et al., 1986 found that metritis was less common during summer months, although their findings were not statistically significant. Etherington et al. (1985), described a higher incidence of dystocia during the summer months, contrary to the incidence of RFM which was higher during the winter months. Markusfeld O (1984) reported that cows experiencing dystocia were more likely to develop endometritis, pyometra and RFM was directly associated with metritis.

Schnier et al. (2002) compared disease incidence of dairy cows kept in cold or warm loosing house, in Ayrshire and Fresian dairy cows. The odds of contracting metritis during the first 44 days in milk of cows calving in a cool loose house compared to cows calving in a warm loose housing during the indoor period were lower for cows of the Fresian compared to the Ayrshire breed, OR = 0.2 and OR = 1, respectively. In addition, Gröhn et al. (1990) found that cows calving during January – April (OR = 1.6) and September – December (OR = 1.7) were more likely to develop early metritis than those
calving during the month of May-August. A later report found same results (Gröhn et al., 2000).

**Hypocalcemia**

Uterine inertia and a decrease in uterine involution predispose cows to puerperal metritis (Roberts, 1986). Cows with postpartum hypocalcemia have been found to have increased incidence of postpartum metritis compared to cows without this condition (Boseberry and Dobson, 1989). Erb and Martin (1985) found that cattle suffering from hypocalcemia were (OR = 4.2) more likely to have a veterinary assisted dystocia, and more likely (OR = 2.0) to have RFM. Both dystocia and RFM have been found to be predisposing factors for metritis (Erb et al., 1985; Markusfeld, 1987). Curtis et al. (1983) did not find an association between metritis and milk fever, in agreement with Markusfeld (1987) who found no association between metritis and milk fever. However, Gröhn et al. (1990) found that cows with parturient paresis were more likely (OR = 1.5) to develop puerperal metritis compared to cows without parturient paresis.

**Postpartum Health Monitoring**

An important concept of a dairy herd health program is early disease diagnosis and treatment of sick cows. A delay in treating a sick cow not only reduces her chances for a full recovery, but results in milk production loss that may impair reproductive performance. Because the early postpartum period of the dairy cow determines productive and reproductive responses during lactation, it is a pivotal time in the production cycle of the cow. During this period, dairy cows are at risk of developing calving related diseases, such as hypocalcemia, puerperal metritis, ketosis and displacement of the abomasum (Curtis et al, 1983). These are costly disorders with estimated economic losses ranging from 200 to 400 dollars per case per lactation (Bartlett
et al, 1986). Monitoring the postpartum health of dairy cows allows the opportunity to identify sick cows early and provide appropriate therapy. Furthermore, it can help prevent diseases.

Diagnostic tests are used to classify or confirm a disease process and to provide appropriate treatment. A test is a device or process designed to detect, or quantify a clinical sign, substance, tissue change, or body response in an animal. Tests are used for screening and to identify the proportion of diseased or sick animals correctly (sensitivity). After a positive result is obtained an in depth diagnostic work-up is performed, in order to correctly answer whether or not the animal in question is sick or not (specificity).

Sensitivity and specificity are the most important characteristic of a test, however they do not directly tell how useful is the test in detecting a disease. Consequently, predictive values are used to estimate the probability that an animal with a positive test result for a particular disease is truly positive (has the disease; positive predictive value) or is truly negative (does not have the disease; negative predicted value).

A postpartum health monitoring program consists of evaluation of rectal temperature, attitude, milk production, urine for the presence of ketone bodies and characterization of uterine discharge. To some extent these procedures are objective, however the majority are subjective and related to the experience of the technician who performs them.

**Attitude**

Attitude is a subjective parameter that describes the anatomical impression of the patient (Rosenberger, 1979). Attitude and posture are synonyms, although posture relates mainly to the disposition of the limbs, attitude is used as a behavioral indicator. For the assessment of attitude, the evaluator has to assess by external visual inspection the
position or relation of the lips, ears, head, neck, forelimbs and tail, in relation to the body of the animal (Rebhum, 1995). A healthy cow is one that it is aware of her environment and displays the common curious behavior of cattle. There are some attitudes or postures that suggest a specific diagnosis or a specific system disorder (Rebhum, 1995). Changes in the shape of the vertebral column and a tense abdomen are characteristics of an abdominal illness such as peritonitis which can be related to different causes, like traumatic reticuloperitonitis, as well as septic metritis (Rosenberger, 1979). Holding the tail up usually indicates a painful process in the anus, rectum or genital tract, accompanied by fractious compressions of the abdominal wall (tenesmus). Animals with an extended neck and head usually suffer from pharyngeal and esophageal obstruction or from severe respiratory diseases (Rosenberger, 1979).

Certain attitudes displayed by cows are grouped and measured in a scoring system to add objectivity to this subjective approach. One of the most common scoring systems used in the dairy industry is lameness evaluation in order to assess the severity of lameness in the cow. This scoring system consists in the observation of the stationary as well as the walking attitude of the cow (Sprecher, et al., 1997). The scoring system is based on a five point scale were: 1) normal: the cow stands and walks with a level-back posture, her gait is normal; 2) mildly lame: the cow stands with a level-back posture but develops an arched-back posture while walking, her gait remains normal; 3) moderately lame: arched-back posture is evident both while standing and walking, her gait is affected and is best described as short striding with one or more limbs; 4) lame: an arched-back posture is always evident and gait is best described as one deliberate step at a time, the cow favors one or more limbs/feet and 5) severely lame: the cow additionally
demonstrates an inability or extreme reluctance to bear weight on one or more of her limbs/feet.

Positioning of the cow’s ears is also a good indicator of a cow’s attitude. Sick cows usually have ears that droop down due to depression, pain, or fever. Healthy cows on the other hand, appear bright, alert and are curious about their environment. Positioning and appearance of the eyes within the socket have also been used to assess the level of dehydration. A scoring system such as 1 (minimal), 2 (mild), 3 (moderate), or 4 (severe) has been proposed to assess dehydrated state (Smith and Risco, 2005). A cow with a score of 1 usually will have bright eyes that are positioned normally within the eye socket. A cow with a score of 2 will have dull eyes that are slightly sunken (1-2mm) within the eye socket. A cow with a score of 3 will have glazed eyes that are moderately sunken (2-4 mm) where a cow with a score of 4 will have dry eyes that are severely sunken (>5mm) within the eye socket. However, this method has not been compared with the actual degree of dehydration.

Changes in feeding behavior can also be used as an indicator of health. In those farms that have locking stanchions, the attitude of the cow can be observed after feeding to evaluate appetite. A cow that is sick will not eat, conversely a healthy cow aggressively consumes her feed. Smith and Risco (2005) have proposed the following scoring system to evaluate appetite; 1) cows that lock and eat 2) cows that lock appear dull and do not eat and 3) cows that do not lock to eat and appear dull or sick. Cows that fall in categories 2 or 3 should be monitored or examined carefully for illness.

Urton et al., (2005) evaluated if depression in feeding behavior was a good indicator of metritis (mucopurulent, foul smelling discharge and fever > 39.5°C) or acute
metritis (reddish brown, watery, foul smelling discharge with fever > 39.5°C), in dairy cows. Both prepartum and postpartum feeding behavior were observed in healthy cows and in those that developed metritis or acute metritis postpartum. Cows diagnosed with either metritis or acute metritis spent less time feeding pre and post partum than did non-metritic cows. These cows also spent significantly less time feeding over the post-calving period than did their healthy counterparts. However, only those cows diagnosed with acute metritis showed significantly lower feeding time during the precalving period. Figure 2-9 represents the feeding times for cows with metritis and cows with acute metritis during pre and post partum period.

Figure 2-9. Daily mean feeding time (min/d) of 9 Holstein cows with acute metritis (▲) and 17 Holstein cows without acute metritis (□) (±SE) from 12 d before calving until 19 d after calving.
Furthermore, the risk of acute metritis related to prepartum feeding behavior was evaluated. It was found that for every 10 minutes in reduction of feeding behavior during the prepartum, the odds of developing acute metritis increased by 1.57 when compared to cows without acute metritis. In addition in the final model parity was significant, and was shown that primiparous cows were at a higher risk.

**Milk Production**

The evaluation of daily milk production is an objective parameter that can be used to identify sick cows. It has been suggested that an unexpected decrease in milk production may be reflecting the inappetence of the cow, and thus it may be used as a monitoring parameter to identify sick cows (Smith and Risco, 2005). The application of this method has been extended with the use of computerized milk-meters which identify and record the production of individual cows on a daily basis. Results in lactation performance have been published in diseases such as ketosis, metritis, or displacement of the abomasum. Rajala-Schultz et al. (1999) found that milk yield decreased before the diagnosis of clinical ketosis, and the loss of milk continued for at least 2 wk after diagnosis. The effect of metritis, as one diagnosis, did not have any effect on milk yield (Rajala-Schultz and Gröhn 1998). Metritis in the cited study (Rajala-Schultz and Gröhn 1998) was defined as early &le; 28 days postpartum and late metritis &gt; 28 days postpartum. Once these categories were separated, the importance of the timing of the disease on milk loss became apparent. Late metritis did not have a significant effect on milk yield, but early metritis significantly reduced milk yield. This result could be explained by the possibility that the cases of early metritis were more severe and included systematic symptoms as high temperature and inappetence, possibly resulting from difficult calving or retained placenta. Deluyker et al. (1991) found milk losses that varied from 250 to 800 kg during a
305-day in cows with left displaced abomasums. However, few studies have been conducted to determine the effect of postpartum diseases on daily milk production and its association to postpartum health as a diagnostic tool.

Daily milk production can be recorded by milking machine software in which cows are identified if there is a deviation from an expected milk production. Changes in daily milk production can be used to identify those cows that have a drop in milk production, or are not producing at an expected level (Smith and Risco, 2005). Also, milk production can be measured as a rate of milk produced per hour between consecutive milking sessions (in kg/h). This parameter may be more accurate than daily milk yield parameter since it reflects the actual milk production changes during milking session intervals. It provides the opportunity to monitor more frequently (2-3 times a day) the performance of an individual cow (Moallem, et al., 2002).

Edwards and Tozer (2004) investigated the possibility of predicting an occurrence of a disease before clinical diagnosis based on changes in daily walking activity (measured with activity meters) and milk production of cows with left displaced abomasum (LDA), ketosis, and digestive problems. Walking activity in cows with these disorders gradually decreased from 8 days to 1 days before clinical diagnosis. In addition, milk yield began to decline 6 days before diagnosis of cows with ketosis, 7 days for cows with an LDA, and 5 days for cows with digestive disorders. Overall activity started to decline before milk production. Figure 2-10 represents the difference in activity and daily milk yield for cows with an occurrence of ketosis, left displaced abomasum, and general digestive disorders during days −10 to 10 relative to the day of diagnosis (day 0).
Consequently, using both activity and milk production together would be more sensitive in detecting cows with LDA, ketosis and digestive problems.

Figure 2-10. Difference in activity (-----) and daily milk yield (-----) for cows with an occurrence of ketosis (a), left displaced abomasum LDA (b), and general digestive disorders (c), compared with cows without an incidence of a disease in the prebreeding stage during day −10 to 10, relative to the day of diagnosis (day 0).
Rectal Temperature

Body temperature affects tissue function and is the result of chemical and physiological processes. The cow as a homothermous mammal has the capacity to maintain a constant temperature range under different environmental conditions, through the inputs and output of heat regulation. An increase in body temperature beyond the normal range can be characterized as fever (pyrexia), or hyperthermia. Fever is defined as a controlled elevation of core body temperature by supportive changes in thermo-effector activities. It is commonly regarded as beneficial, that is, having survival value (Kluger, 1986). Fever is the result of communication between the peripheral immune system and the brain in response to infection, inflammation and/or trauma, and is clinically characterized by a rise in the body temperature (Cunningham, 2002). In contrast, hyperthermia is a rise in core temperature resulting from the inability to lose sufficient heat to balance the total of endogenous plus exogenous heat loads and can be potentially lethal (Hales et al., 1996).

The rectal temperature (RT) is obtained by introducing a glass or a digital thermometer through the anal canal into the rectum and placed in close contact to the rectal mucosa. The blood supply to the rectal mucosa is derived from the cranial artery of the caudal mesenteric artery, and by several short middle rectal branches from the caudal branch of the urogenital artery (Getty, 1975). Because of the close contact of the rectal mucosa to its blood supply, temperature in blood is transferred to the rectal mucosa. Consequently, rectal temperature is a useful indicator of the core temperature of the animal. However, rectal temperature is lower than the core temperature of the animal, and changes in rectal temperature may lag behind changes in core temperature (Cunningham, 2002).
Monitoring of rectal temperature during the first 10 days post partum has been used to identify cows with fever (Upham, 1996). Normal RT has been reported to range between 38.0°C to 39.1°C (Rebhun, 1995) or 38.0°C to 39.0°C (Rosenberger, 1979). Cows with RT above the upper limit are defined as febrile or abnormal. However, because of the multiple factors that affect RT, a cut-off value to define fever is difficult to define (Rebhun, 1995; Rosenberger, 1979). Different cut-off values have been reported to define fever in cows with uterine infections; 39.2°C (Smith et al., 1998), 39.3°C (Kristula et al., 2001) greater than or equal to 39.4°C (Upham, 2001), and 39.5°C (Drillich et al., 2001). Sheldon et al. (2004) described a cut off point for defining fever by using the maximum RT between days 1 and 10 post partum: mean of 39.3°C, and upper quartile equal to ≥39.7°C, which was defined as a febrile level. Kristula et al. (2001) have suggested that cows with RT between 38.8°C and 39.4°C should be monitored carefully to determine whether or not they require systemic antibiotic therapy. Furthermore, because cows with a RT of 39.7°C experienced a reduction in RT of 0.6°C after treatment, a cut-off value of RT of 39.4°C or 39.7°C was proposed to be used in the decision of whether or not to initiate antibiotic treatment.

The protocol for monitoring daily RT in postpartum cows has been readily accepted by dairy producers and veterinarians because it is an objective response that can be used to evaluate health. However, this protocol is time consuming because all postpartum cows are monitored including those cows that calved normally that may be at a lower risk to develop puerperal metritis. Therefore, the benefit of evaluating all cows is unknown. In a study that evaluated postpartum RT, 48% of cows that calved normally had at least one daily temperature above 39.1 °C compared to 93, 83, 100 and 78 % for
cows with RFM, mastitis, metritis and dystocia respectively (Kristula et al, 2001). In addition, cows with an abnormal parturition (RFM, dystocia with or without RFM) had rectal temperatures greater than 39.0 °C for significantly more days (2.9) than cows that calved normally (1.9) (Kristula et al, 2001). However, they conclude that two consecutives day with a RT greater than 39.1 °C will not be a sufficient indicator to treat the cows with an antibiotic.

**Ketones Bodies**

Ketosis is a disorder of carbohydrate and fat metabolism characterized by increased concentrations of ketone bodies in blood (ketonemia), urine (ketonuria), milk (ketolactia), and other body fluids (Geishauser, et al., 1998). The metabolic state of ketonemia has a negative effect on milk production (Rajala-Schultz et al., 1999; Detilleux et al., 1994) and reproduction (Andersson et al., 1991).

The major ketone bodies are β-hydroxybutyrate (BHBA), acetoacetate (AcAc), and acetone (Ac) (Andersson, 1988). During early lactation, the amount of energy that is required for maintenance of body tissues and milk production exceeds the amount of energy the cow can obtain from dietary sources (Baird, 1982). As a result, the cow must utilize body fat as a source of energy. However there is a limited amount of fatty acid that can be oxidized to completion by the tricarboxylic acid cycle in the liver or exported from the liver as very low density lipoprotein. When this limit is reached, triglycerides accumulate within the hepatocytes, impair their function, and the acetyl-coenzyme A that is not incorporated into the tricarboxylic acid cycle is converted to acetoacetate and β-hydroxybutyrate. The appearance of these ketone bodies in blood, milk, and urine is diagnostic of ketosis and usually becomes clinically evident from 10 d to 3 wk after calving (Goff and Horst 1997). In addition, ketosis may also occur secondary in cows
with diseases that affect feed intake (Kronfeld, 1982). Risk factors for subclinical ketosis include metritis, displaced abomasum, RFM and hypocalcemia (Curtis et al., 1985; Correa et al., 1993). However a cause-effect relationship has not been clearly defined (Duffield, 2000).

Analyses for the presence of ketone bodies in urine and milk are commonly used to diagnose ketosis in cattle. Strips, tablets or powders that contain nitroprussic acid are used semiquantitatively to measure the concentration of ketone bodies in urine or milk (Larsen and Nielsen, et al., 2005). However, these tests vary in sensitivity and specificity (Table 2-1) (Duffield, 2000).

Table 2-1 Ketosis threshold, sensitivity and specificity for different ketosis test.

<table>
<thead>
<tr>
<th>Test and body fluid</th>
<th>Subclinical Ketosis Threshold (Serum BHB µmol/L)</th>
<th>Sensitivity (%)</th>
<th>Specificity (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Utrecht nitroprusside-milk</td>
<td>1400</td>
<td>90</td>
<td>96</td>
</tr>
<tr>
<td>Bioketone (urine)</td>
<td>1200</td>
<td>33</td>
<td>100</td>
</tr>
<tr>
<td>Ketocheck (urine)</td>
<td>1200</td>
<td>28</td>
<td>100</td>
</tr>
<tr>
<td>Utrecht powder (urine)</td>
<td>1200</td>
<td>42</td>
<td>100</td>
</tr>
<tr>
<td>Ketostix (urine)</td>
<td>1200</td>
<td>5</td>
<td>100</td>
</tr>
<tr>
<td>Ketolact-50 µmol/L (Milk)</td>
<td>1200</td>
<td>92</td>
<td>55</td>
</tr>
<tr>
<td>Ketolact-100 µmol/L(Milk)</td>
<td>1200</td>
<td>72</td>
<td>89</td>
</tr>
<tr>
<td>Ketolact-200 µmol/L(Milk)</td>
<td>1200</td>
<td>45</td>
<td>97</td>
</tr>
<tr>
<td>Ketolact-500 µmol/L(Milk)</td>
<td>1200</td>
<td>17</td>
<td>100</td>
</tr>
<tr>
<td>Ketocheck (Milk)</td>
<td>1400</td>
<td>38</td>
<td>99</td>
</tr>
<tr>
<td>Ketocheck (Milk)</td>
<td>2000</td>
<td>61</td>
<td>98</td>
</tr>
</tbody>
</table>


Given the high incidence of ketosis (29 to 35%) from primary and secondary causes (Emery et al., 1964; Duffield et al., 1998) during the first 2 weeks post partum, monitoring postpartum cows for this condition may be of limited value if applied only to
identify sick cows. In addition, the utility of cow side ketone tests to identify sick animals with a primary disease such as displaced abomasum, mastitis or metritis is not known.

**Evaluation of Uterine Discharge**

A major component of a postpartum health monitoring program is the evaluation of uterine discharge by rectal palpation in order to screen cows that have uterine infections (Griffin et al., 1974; Studer and Morrow, 1978; Roberts, 1986, Upham, 1996). The ability to diagnose uterine infections by rectal palpation varies among veterinarians (Lewis, et al., 1997). Typically, evaluation of the uterus by rectal palpation is performed between 25 to 50 days postpartum to evaluate uterine health (Studer and Morrow, 1978). Evaluation of uterine health by rectal palpation has been directed to the first two weeks postpartum (Uphan, 1996) for early identification and treatment of puerperal metritis to reduce the adverse effect of metritis on fertility (Callahan and Horstman, 1987).

The use of rectal palpation to evaluate uterine infection is used commonly by veterinarians. However, examination of the vagina with a speculum provides a more accurate diagnosis of endometritis that rectal examination. A vaginal examination in combination with rectal palpation of the uterus 25 to 50 days post partum resulted in twice as many cows with positive cultures for pathogenic bacteria compared to cows classified as infected using rectal palpation alone (59% vs 22%, respectively) (Miller et al., 1980). Studer and Morrow (1978) found gross palpation of uterine size was associated weakly with bacterial culture and histology. In a study by LeBlanc et al (2002), a cervical diameter at rectal palpation of a >7.4 cm either at 20 to 26 or 27 to 33 days post partum by rectal palpation and presence of a mucopurulent, purulent or foul uterine discharge by vaginoscopy inspection at 27 to 33 days post partum were predictors of reproductive performance. For the palpation-based case definition of clinical
endometritis, ignoring vaginoscopy, the sensitivity and specificity were 17 and 88%, respectively, for non-pregnancy beyond either 120 or 150 DIM. However, between 27 and 33 days post partum, diagnosis of endometritis with vaginoscopy was more sensitive than palpation-based diagnosis (21 vs. 12% sensitivity for classification of non-pregnancy beyond 150 day in milk, respectively). However, during this period, specificity of rectal palpation was higher than vaginoscopy (89 and 94%, respectively). Consequently, because of the changes in size and discharges that the uterus undergoes early post partum, the criteria for assessing uterine health change according to days post partum at examination (Ferguson, 1995). In the study by Callahan and Horstman (1987) a metritis (fluid, crepitus, lack of myometrial tone, and vaginal discharges) incidence of 33% at 1-3 weeks was reported. However, if the first examination had not been performed until 2 weeks later, the incidence of metritis would have been 7.8%.

Based on the review by Sheldon et al. (2006) in which the proposed definitions for the different uterine infections, it can be inferred that the studies previously published that evaluated rectal palpation as a diagnostic tool (Studer and Morrow, 1978; Miller et al., 1980; Callahan and Horstman, 1987) referred to endometritis and not puerperal metritis or metritis. Consequently, it remains unclear and more research is needed on the test characteristics of rectal palpation to evaluate uterine discharge as it relates to puerperal metritis.
Cows and Herd Management

The study was conducted between August 2002 and April 2003 in a commercial dairy farm located in north east Florida (30° 18’ N, 81° 56’ W). One thousand lactating cows were milked with a yearly rolling herd milk production average of 9,165Kg. The herd was milked 3 times per day. The farm was a member of DHIA (Dairy Records Management Systems, Raleigh, NC) and used an on-farm computer based record system for maintaining production and health data.

Prepartum transition cows that were within 3 weeks of calving were maintained on dry lots, fed a cationic diet and monitored for signs of calving by farm employees trained to assist with parturition. Calving events such as dystocia and retained fetal membranes (RFM) were recorded by farm personnel. Dystocia was defined and recorded based on a five point scale as follows: (1) no assistance (2) slight problem (assistance for < 15 minutes) (3) needed assistance (assistance for > 15 minutes with moderate difficulty for extraction) (4) considerable force used (5) extreme difficulty or veterinary assistance (DHIA, 1997). Cows that did not expel the placenta within 24 hrs after calving were considered to have RFM (Risco and Hernandez, 2003). Cows with dystocia delivered by cesarean section or fetotomy were not included in the study.

After parturition cows were sent for 2 days to a hospital herd housed in a concrete floor open-sided barn with stanchions that provided free access to a dry lot. At the hospital barn, cows were treated according to standard operation procedures of the farm
which consisted of administration of an oral calcium paste (Balance®, Bayer, Shawnee Mission KS) and a single intrauterine infusion of 6 g of oxytetracycline dissolved in 75 ml of sterile water to multiparous cows with RFM. At three days post partum, cows were moved to a lactating herd kept in an open barn with dry compost bedding, and fed four times a day. Diets for both pre-and postpartum transition cows were a total mixed ration formulated to meet the requirements of lactating dairy cows according to guidelines established by the National Research Council (NRC, 2001).

Reproductive management consisted of a voluntary waiting period of 60 days. After that, cows were identified in estrus by visual observation with the aid of tail chalk or neck activity meters (WestfaliaSurge, Inc.1880 Country Farm Drive 60563 Naperville IL) Cows not artificially inseminated by 80 days post partum were examined for cyclicity, and cows with a corpus luteum (CL) were treated with 25 mg of PGF\textsubscript{2\alpha} intramuscularly (IM) (Lutalyse®, Pfizer Animal Health, Kalamazoo, MI.). Cows were artificially inseminated at detected estrus. Non-cyclic cows with inactive ovaries were treated with 100 µg of GnRH IM (Cystorelin®, Meriel Limited, Iselin, NJ.) followed by 25 mg of PGF\textsubscript{2\alpha} IM 7 days later and AI at detected estrus. Cows not seen in estrus 14 days after PGF\textsubscript{2\alpha} treatment were re-examined for cycling status and treated with PGF\textsubscript{2\alpha} only if a CL were present. Cows not AI by 120 days post partum were enrolled in the OvSynch program (Pursley et al., 1995). Cows not pregnant > 150 days post partum were also enrolled in the OvSynch program.

**Study Design**

This study followed a prospective cohort design. All cows underwent a postpartum health monitoring program consisting of daily evaluation of rectal temperature (RT) and attitude from day 3 to 13 post partum. All cows were examined for clinical endometritis
between 20 to 30 days post partum. Rectal temperature was determined with the use of a
digital thermometer (GLA, Agricultural Electronics, San Luis Obispo, CA) between 0700
to 0900 h immediately after milking. Cows that either appeared sick (depressed, sunken
and/or tented eyes) or had a RT ≥ 39.4°C were examined for puerperal metritis. The
criterion for diagnosis of puerperal metritis were the presence of a watery, brown-
colored, fetid discharge from the vulva (noted after rectal palpation of the uterus), with or
without a RT ≥ 39.4°C. All information concerning rectal temperature and incidence of
puerperal metritis were stored daily in a separate database belonging to the principal
investigator. Cows diagnosed with puerperal metritis were treated daily with ceftiofur
hydrochloride (2.2 mg/kg IM. Excenel®, Pfizer Animal Health, Kalamazoo, MI) IM for
three days. In addition, supportive therapy consisting of anti-inflammatory agents,
calcium and energy supplements were administered. Cows that did not respond to the
three day ceftiofur treatment, based on the persistence of a fetid discharge, received an
intrauterine infusion of 3 g of oxytetracycline diluted in 75 ml of sterile water.

The criterion used to diagnose clinical endometritis between 20 to 30 days post
partum were one of the following condition associated or not with each other: cervical
diameter greater than 6.0 cm; asymmetry of the uterine horns with fluid content and/or
pus present at the vulva following rectal manipulation of the uterus. All cows diagnosed
with clinical endometritis were treated with a single injection of 25 mg of PGF$_{2\alpha}$
(Lutalyse®, Pfizer Animal Health, Kalamazoo, MI) IM. Pregnancy diagnosis was
determined by trasrectal palpating of the uterus and its contents 42 to 49 days after
artificial insemination (Zemjanis,1970).
**Data Management**

Data for parity and calving status: (dystocia: calving score $\geq 3$, RFM, and twins) were recovered from the database, and two groups of cows were established based on calving status. Cows with a normal calving (NC) status were those without calving related problems, and cows with an abnormal calving status (AC) were those with dystocia, RFM with or without dystocia or twins. Cows were classified as having puerperal metritis (M+) or without puerperal metritis (M-) in a two level variable classified as metritis. Cows with and without metritis were also classified in a 3 level variable defined as Mettemp (MT) according to whether or not they had fever (RT $\geq 39.4^\circ C$) when puerperal metritis was diagnosed. Cows with puerperal metritis and fever were classified as MT-1, cows with puerperal metritis without fever at the time of diagnosis were classified as MT-2, and cows without puerperal metritis as MT-3.

Cows were classified as primiparous or multiparous in a two level variable classified as parity. The different seasons during the study were defined based on the thermal heat index (THI = td – (.55 - .55RH) (td – 58)) (West,1994). This index was calculated using daily ambient temperature (td) and percent relative humidity (RH) recorded at the closest weather station at Macclenny, FL (30º 24’ N, 82º 11’ W). Based on a previous report (West, 1994) a cut-off of 76.2 was used to define two seasons. A cool season was defined as those months with an average THI of < 76.2 (October to April), and a warm season were those months with an average THI $\geq 76.2$ (May to September).

**Statistical Analyses**

All outcome variables and the incidence of puerperal metritis and clinical endometritis were analyzed using logistic regression PROC GENMOD of SAS 9.1; (SAS, 2003) with a binomial distribution and logit link.
Modeling was performed using manual backward elimination starting from the more complex model with a third order interaction and the exclusion criteria were determined at \( P > 0.30 \). The model fit statistics were performed by comparing the difference in the deviances by the likelihood-ratio statistic test (Agresti, 1986). Main effects were forced all models. Adjusted odds ratios (AOR) and 95% confidence intervals (95% CI) were determined.

The model used to analyze the incidence of puerperal metritis included the main effects of calving status, parity, and season at calving. A second model was used to analyze the incidence of clinical endometritis and included calving status, parity, season at calving, and puerperal metritis. A third model was analyzed the incidence of clinical endometritis and the variable puerperal metritis was substituted by the MT variable.

Reproductive performance was evaluated by analyzing first service pregnancy rate (%), cumulative pregnancy rate by 150 days in milk (%) and, number of inseminations for pregnant cows (mean ± S.E.M). Number of inseminations for pregnant cows was also analyzed by logistic regression (PROC GENMOD, SAS) with a Poisson distribution and logit link. All models for reproductive performance included the main effects of calving status, parity, season at first service, puerperal metritis and clinical endometritis as explanatory variables. Time to pregnancy was analyzed with survival analysis using Cox’s proportional hazards regression (PROC PHREG, SAS). Adjusted hazard ratio (HR) was obtain from the proportional hazards regression model and crude median days open and the survival function estimates for the cumulative pregnancy up to 150 days postpartum graph were obtained from the Kaplan-Meier analysis (PROC LIFETEST, SAS).
Data for daily RT from days 3 to 13 post partum were used to compare the RT of cows with or without puerperal metritis, and descriptive statistics were determined. Rectal temperature of cows with puerperal metritis 5 days prior and 5 days after diagnosis were compared to the rectal temperature of cows without puerperal metritis (control cows). Control cows, without puerperal metritis, were assigned to cows with puerperal metritis, by controlling for group, parity and season, using a random number generator procedure. In cows with puerperal metritis, days post partum when puerperal metritis was diagnosed were reclassified as follow: Day 0 day postpartum when puerperal metritis was diagnosed: days -1, -2, -3, -4, -5 before diagnosis, and days 1, 2, 3, 4, 5 after diagnosis. Days post partum of control cows were similarly re-classified and matched to cows with puerperal metritis in a rate of 3.7 controls cows per each puerperal metritis case. Therefore, day 0 for control cows (without puerperal metritis) corresponded with the same day post partum when puerperal metritis was diagnosed. This rearrangement of the data was done to control for the rectal temperature by days post partum of cows with or without puerperal metritis.

The analysis of RT between cows with and without puerperal metritis by day post partum was performed using PROC MIXED procedure for repeated measures of SAS 9.1(SAS, 2003). The model was subjected to 4 covariate structures: compound symmetry, compound symmetry-heterogeneous, autoregressive order-1 and autoregressive order-1 heterogeneous matrix. The autoregressive order-1 covariance structure was found to have the smallest Akaike’s information criterion and Schwarz’s Bayesian criterion (Littell et al., 2002). Consequently the covariate structure that specified a correlation structure within cows that decreased with increasing lag between measurements was used in the
model. Two models were analyzed. The first model included the effects of calving status, puerperal metritis, parity, season at calving and day as main effects as well as the second and third order interactions between the main effects. The second model was analyzed by including the variable Mettemp as a replacement for the variable Metritis. This variable (Mettemp) differentiates the daily rectal temperature of cows with puerperal metritis and fever, and cows with puerperal metritis without fever at the day post partum of puerperal metritis diagnosis, and cows without puerperal metritis. For both models, repeated measurements of RT were also analyzed by testing homogeneity of regression curves for day trends. A single polynomial regression for day was fitted for RT, and the differences from fitting individual regressions for the effect of calving status, metritis, parity, season and their interactions were tested. Given that not all puerperal metritis cases were diagnosed on the same day post partum, the actual post partum day at diagnosis was included in the model as a covariable for both models.

The mixed model for repeated measures was:

\[ Y_{ijklmfg} = \mu + T_i + \text{Cow}(T_i) + \text{Day}_k + \text{Par}_1 + \text{Seas}_m + \text{Stat}_f + \text{Dmet}_g (\text{Day} \times T)_{ki} + e_{ijklmfg} \]

Where:
- \( Y_{ijklmfg} \) = daily rectal temperature
- \( T_i \) = fixed effect of group (normal or abnormal calving status)
- \( \text{Cow}(T_i) \) = random effect of cow nested in group
- \( \text{Day}_k \) = fixed effect of time
- \( \text{Par}_1 \) = fixed effect of parity
- \( \text{Seas}_m \) = fixed effect of season
- \( \text{Stat}_f \) = fixed effect of metritis (yes, no or MT-1, MT-2, MT-3)
- \( \text{Dmet}_g \) = regression coefficient for days in milk to metritis
- \( (\text{Day} \times T)_{ki} \) = fixed effect of interaction time and treatment
- \( e_{ijklmfg} \) = random error term

Differences among rectal temperature for the different days were determined by the use of PDFF option of SAS. Least squares means (± SEM) of RT were determined and illustrated in the graphics.
CHAPTER 4
RESULTS

Final Sample
A total of 488 cows calved during the study period and thirty-eight (38) cows were not included in the study because they received antibiotic treatment beyond 3 days postpartum at the hospital barn and did not complete 10 days of health evaluation. Therefore, a total of 450 calvings was evaluated, of which 327 (73%) were classified as normal, and 123 (27%) as abnormal. Ninety four (94) cows were diagnosed with puerperal metritis of which 55 (58.5%) had no fever, and 39 (41.4%) had fever.

Incidence of Diseases
In the logistic regression multivariate analysis, cows with normal calving status had a lower incidence of puerperal metritis compared to cows with an abnormal calving status (43/327 (13%) vs. 51/123 (41%), respectively; P < 0.001). There was a significant interaction (P < 0.01) between parity and season. During the cool season, primiparous cows had the highest incidence of puerperal metritis compared to primiparous cows in warm season or multiparous cows in either season: Cool season: 39.4% > 11.0% for primiparous and multiparous, respectively; and for primiparous 39.4% > 12.7%, for Cool vs. Warm, respectively (Table 4-1).

The overall incidence of clinical endometritis was 24%; Cows with abnormal calvings were more frequently diagnosed with clinical endometritis than those with normal calving status (AOR = 2.8, 95% CI 1.7-4.9, P < 0.001). A higher incidence (38.2%) of clinical endometritis was found to be significant for those cows diagnosed with
Table 4-1. Incidence and risk factors of puerperal metritis in the first 13 days post partum of lactating dairy cattle.

<table>
<thead>
<tr>
<th>Variable</th>
<th>Level</th>
<th>Incidence of Puerperal Metritis</th>
<th>Risk of Puerperal Metritis</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>%</td>
<td>N</td>
</tr>
<tr>
<td>Metritis</td>
<td>Yes</td>
<td>21.1</td>
<td>94</td>
</tr>
<tr>
<td></td>
<td>No</td>
<td>79.1</td>
<td>356</td>
</tr>
<tr>
<td>Calving Status</td>
<td>Normal</td>
<td>13.1</td>
<td>43/327</td>
</tr>
<tr>
<td></td>
<td>Abnormal</td>
<td>41.4</td>
<td>51/123</td>
</tr>
<tr>
<td>Lactation X Season</td>
<td>Cold</td>
<td>Multiparous</td>
<td>11.0</td>
</tr>
<tr>
<td></td>
<td>Primiparous</td>
<td>39.4</td>
<td>54/137</td>
</tr>
<tr>
<td></td>
<td>Warm</td>
<td>Multiparous</td>
<td>18.1</td>
</tr>
<tr>
<td></td>
<td>Primiparous</td>
<td>12.7</td>
<td>6/47</td>
</tr>
<tr>
<td>Primiparous</td>
<td>Cold</td>
<td>39.4</td>
<td>54/137</td>
</tr>
<tr>
<td></td>
<td>Warm</td>
<td>12.7</td>
<td>6/47</td>
</tr>
<tr>
<td>Multiparous</td>
<td>Cold</td>
<td>11.0</td>
<td>22/200</td>
</tr>
<tr>
<td></td>
<td>Warm</td>
<td>18.0</td>
<td>12/66</td>
</tr>
</tbody>
</table>

Puerperal metritis (AOR = 2.2, 95% CI 1.1-3.9, P < 0.005) compared to cows without puerperal metritis. The incidence of clinical endometritis of cows diagnosed with puerperal metritis and fever (38.1%) and puerperal metritis without fever (38.4%) at the day of diagnosis was greater than cows without puerperal metritis (20.2%) (puerperal Metritis with Fever (AOR = 2.2, 95% CI 1.07 - 4.6, P < 0.02); puerperal Metritis without Fever (AOR = 2.1, 95% CI 1.09 - 4.19, P < 0.02). Non significant difference was found between fever and no fever puerperal metritis cows on the incidence of clinical endometritis (P < 0.9); Table 4-2.
Table 4-2. Incidence and risk factors of clinical endometritis at 20 to 30 days post partum of lactating dairy cattle.

<table>
<thead>
<tr>
<th>Variable</th>
<th>Level</th>
<th>Incidence of Clinical Endometritis</th>
<th>Risk of Clinical Endometritis</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>%</td>
<td>N</td>
</tr>
<tr>
<td>Endometritis</td>
<td>Yes</td>
<td>24.0</td>
<td>108</td>
</tr>
<tr>
<td></td>
<td>No</td>
<td>76.0</td>
<td>342</td>
</tr>
<tr>
<td>Calving Status</td>
<td>Normal</td>
<td>17.7</td>
<td>58/327</td>
</tr>
<tr>
<td></td>
<td>Abnormal</td>
<td>40.6</td>
<td>50/123</td>
</tr>
<tr>
<td>Parity</td>
<td>Multiparous</td>
<td>26.0</td>
<td>69/266</td>
</tr>
<tr>
<td></td>
<td>Primiparous</td>
<td>23.3</td>
<td>43/184</td>
</tr>
<tr>
<td>Puerperal Metritis</td>
<td>No Metritis</td>
<td>20.2</td>
<td>72/356</td>
</tr>
<tr>
<td></td>
<td>Metritis</td>
<td>38.2</td>
<td>36/94</td>
</tr>
<tr>
<td></td>
<td>No Fever</td>
<td>38.4</td>
<td>21/55</td>
</tr>
<tr>
<td></td>
<td>Fever</td>
<td>38.1</td>
<td>15/39</td>
</tr>
</tbody>
</table>

**Rectal Temperature**

The mean (± SEM), median, upper and lower quartiles and the 95% confidence interval for RT from day 3 through day 13 post partum are shown in Table 4-3. In both models the effect of day to diagnosis was significant (P < 0.001). The analysis of model one showed a significant interaction between DAY and METRITIS (P < 0.001). Curves of daily rectal temperature during this period described a polynomial of second order for puerperal metritis cows and first order for cows without puerperal metritis. Rectal temperature from cows with puerperal metritis was significantly higher in cows without puerperal metritis 72 h before the diagnosis of puerperal metritis (DAY -3: 38.7°C ± 0.05 > 38.6°C ± 0.03; P < 0.009) and daily comparisons between groups continued to be different until the fourth day after the diagnosis and treatment of puerperal metritis (DAY 4: 38.7°C ± 0.07 > 38.5°C ± 0.04; P < 0.001).
Table 4-3. LSM ± (SE), 25th quartile, median, 75th quartile, and population 95% confidence intervals rectal temperatures of cows with and without puerperal metritis.

<table>
<thead>
<tr>
<th></th>
<th>LSM ± SE</th>
<th>25th Q</th>
<th>Median</th>
<th>75th Q</th>
<th>LSM ± 1.96 SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>No Metritis Cows</td>
<td>38.6 ± 0.01</td>
<td>38.3</td>
<td>38.5</td>
<td>38.8</td>
<td>37.8 - 39.3</td>
</tr>
<tr>
<td>Primiparous</td>
<td>38.5 ± 0.02</td>
<td>38.2</td>
<td>38.5</td>
<td>38.8</td>
<td>37.5 - 39.6</td>
</tr>
<tr>
<td>Multiparous</td>
<td>38.6 ± 0.01</td>
<td>38.3</td>
<td>38.5</td>
<td>38.8</td>
<td>37.8 - 39.4</td>
</tr>
<tr>
<td>Metritis Cows</td>
<td>38.9 ± 0.03</td>
<td>38.4</td>
<td>38.7</td>
<td>39.1</td>
<td>37.4 - 40.3</td>
</tr>
<tr>
<td>Primiparous</td>
<td>38.9 ± 0.04</td>
<td>38.4</td>
<td>38.7</td>
<td>39.1</td>
<td>37.7 - 40.6</td>
</tr>
<tr>
<td>Multiparous</td>
<td>38.8 ± 0.05</td>
<td>38.3</td>
<td>38.7</td>
<td>39.2</td>
<td>36.7 - 40.9</td>
</tr>
<tr>
<td>Mettemp-1 (n=55)</td>
<td>38.7 ± 0.04</td>
<td>38.3</td>
<td>38.6</td>
<td>39.0</td>
<td>36.7 - 40.7</td>
</tr>
<tr>
<td>Mettemp-2 (n=39)</td>
<td>39.2 ± 0.05</td>
<td>38.5</td>
<td>39.0</td>
<td>39.4</td>
<td>37.1 - 41.3</td>
</tr>
</tbody>
</table>

Rectal temperature in cows that were diagnosed with puerperal metritis increased between 48 h (DAY -2: 38.8°C ± 0.07) to 24 h (DAY -1: 38.9°C ± 0.06) before diagnosis of puerperal metritis (difference 0.13°C ± 0.08 C, P < 0.09), and continued to increase until reaching a maximum RT of 39.2°C ± 0.05 on DAY 0 (day of diagnosis). Twenty-four hours before the diagnosis of puerperal metritis, daily increments in RT were found to be significant (difference, 0.28°C ± 0.07 C; P < 0.001). After treatment (DAY 1), RT of cows with puerperal metritis showed a significant reduction (0.33°C ± 0.07 C, P < 0.001), and subsequent daily reductions in RT were not significantly different (Figure 4-1). Curves of RT between cows with puerperal metritis and cows without puerperal metritis tested by homogeneity of regression showed a significantly difference linear and quadratic effect (P < 0.001).

In model 2 there was a significant interaction DAY and METTEMP (P < 0.001). Curves of daily rectal temperature 5 days prior and 5 days after the diagnosis of puerperal metritis described a polynomial of second order for MT-1 and first order for MT-2 and MT-3. Rectal temperature from MT-1 cows had higher RT 72 h before the day of
diagnosis of puerperal metritis compared to MT-3 cows (DAY -3: 38.8°C ± 0.10 > 38.6°C ± 0.04; P < 0.04).

Figure 4-1. LSM ± SEM of daily rectal temperatures of cows 5 days prior to and 5 days after the diagnosis of metritis, for cows with puerperal metritis (n=94; ■), and cows without puerperal metritis (n=356; ♦). *P < 0.05 **P < 0.001.

This daily difference continued to be significant until 24 h after the diagnosis and treatment of puerperal metritis (DAY 1: 39.0°C ± 0.09 > 38.5°C ± 0.04; P < 0.001). In this group (MT-1), the RT started to increase 72 hours (DAY -3: 38.8°C ± 0.10) to 48 h (DAY -2: 39.0°C ± 0.09) before the diagnosis of puerperal metritis (difference 0.20°C ± 0.12 C, P < 0.09), and continued to increase until reaching a maximum of 39.7°C ± 0.09 on the day of diagnosis (Day 0).

Twenty four hours before the diagnosis of puerperal metritis, a daily increment in RT of 0.53°C ± 0.11 was found to be significant (P < 0.001). After treatment, cows with puerperal metritis and treated showed a significant reduction in RT in the first 24 h (0.65°C ± 0.11 C, P < 0.001). This reduction continued to be significant between 24
(DAY 1: 39.05°C ± 0.09) to 48 h (DAY 2: 38.7°C ± 0.10) after the diagnosis of puerperal metritis (difference 0.35°C ± 0.12 C, P < 0.01). Daily rectal temperature from MT-2 cows were higher compared to MT-3 24 h before the diagnosed (DAY -1: 38.8°C ± 0.09 > 38.5°C ± 0.04; P < 0.02), and continued to be different until the fifth day after the diagnosis and treatment of puerperal metritis (DAY 5: 38.7°C ± 0.11 > 38.5°C ± 0.04; P < 0.07). In this group, there was not a statistically significant difference between daily increments of RT before the diagnosis of puerperal metritis. Cows diagnosed with puerperal metritis in the absence of fever had a RT of 38.9°C ± 0.08 on the DAY 0 (day of diagnosis). There was not a significant reduction in the RT after the diagnosis and treatment of puerperal metritis (Figure 4-2).

Figure 4-2. LSM ± SEM of daily rectal temperatures of cows 5 days prior to and 5 days after diagnosis, for cows with puerperal metritis and fever (n=39; ■), puerperal metritis without fever (n=55; ▲) and cows without puerperal metritis (n=356; ♦). a, b, c differ with a P< 0.05, * P< 0.005.
Reproductive

There were no detected differences in first-service pregnancy rate (No-Metritis (32%) vs. Metritis (28%); P < 0.3), and accumulated pregnancy rate by 150 days post partum (No-Metritis (59%) vs. Metritis (52%); P < 0.7), or days in postpartum to first service or pregnancy for cows with or without metritis. As expected, a seasonal effect was detected in both models for first service pregnancy rate (Cool season (34 %) > than warm season (16 %); P < 0.005), accumulated pregnancy rate by 150 days (Cool season (64 %) > Warm season (25 %); P < 0.001). Days to first service as well as days to pregnancy was also influenced by the season effect. No significant differences were found in the number of services for pregnant cows between cows with or without puerperal metritis (Mean ± SE, 1.5 ± 0.05 vs. 1.6 ± 0.11, respectively; P < 0.8).

![Figure 4-3. Proportion of pregnant cows during cold or warm season by 150 days postpartum.](image-url)
Figure 4-4. Proportion of pregnant cows with or without puerperal metritis by 150 days postpartum.
TABLE 4-4. Logistic regression model of conception rate to first service and pregnancy rate at 150 day postpartum days postpartum by group, presence or not of metritis, parity, season and presence or not of endometritis.

<table>
<thead>
<tr>
<th>Variables</th>
<th>First Service Conception Rate</th>
<th>Pregnancy 150 Days Postpartum</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Levels</td>
<td>%</td>
</tr>
<tr>
<td>Group</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Normal</td>
<td>0.2</td>
<td></td>
</tr>
<tr>
<td>Abnormal</td>
<td>0.9</td>
<td></td>
</tr>
<tr>
<td>Metritis</td>
<td>0.3</td>
<td></td>
</tr>
<tr>
<td>No</td>
<td>0.7</td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td>0.7</td>
<td></td>
</tr>
<tr>
<td>Lactation</td>
<td>0.6</td>
<td></td>
</tr>
<tr>
<td>Multiparous</td>
<td>0.6</td>
<td></td>
</tr>
<tr>
<td>Primiparous</td>
<td>0.6</td>
<td></td>
</tr>
<tr>
<td>Season</td>
<td>0.005</td>
<td></td>
</tr>
<tr>
<td>Cold</td>
<td>0.001</td>
<td></td>
</tr>
<tr>
<td>Warm</td>
<td>0.001</td>
<td></td>
</tr>
<tr>
<td>Endometritis</td>
<td>0.6</td>
<td></td>
</tr>
<tr>
<td>No</td>
<td>0.7</td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td>0.7</td>
<td></td>
</tr>
</tbody>
</table>
TABLE 4-5. Cox proportional model of days postpartum to first service and pregnancy rate at 150 day postpartum days postpartum by group, presence or not of metritis, parity, season and presence or not of endometritis.

<table>
<thead>
<tr>
<th>Variables</th>
<th>Days Postpartum to First Service</th>
<th>Days Postpartum to pregnancy</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Levels</td>
<td>Median ± S.E</td>
</tr>
<tr>
<td>Group</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Normal</td>
<td>69.0 ± 1.7</td>
</tr>
<tr>
<td></td>
<td>Abnormal</td>
<td>78.0 ± 2.9</td>
</tr>
<tr>
<td>Metritis</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>No</td>
<td>70.0 ± 1.7</td>
</tr>
<tr>
<td></td>
<td>Yes</td>
<td>76.0 ± 3.3</td>
</tr>
<tr>
<td>Lactation</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Multiparous</td>
<td>73.0 ± 2.1</td>
</tr>
<tr>
<td></td>
<td>Primiparous</td>
<td>68.5 ± 2.1</td>
</tr>
<tr>
<td>Season</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Cold</td>
<td>69.0 ± 1.6</td>
</tr>
<tr>
<td></td>
<td>Warm</td>
<td>83.0 ± 4.0</td>
</tr>
<tr>
<td>Endometritis</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>No</td>
<td>70.0 ± 1.8</td>
</tr>
<tr>
<td></td>
<td>Yes</td>
<td>73.0 ± 2.7</td>
</tr>
</tbody>
</table>
CHAPTER 5
DISCUSSION

In this study, cows with twins, dystocia, RFM with or without dystocia were considered to have an abnormal calving. These disorders have been reported to be risk factors for metritis in dairy cows (Markusfeld, 1984; Curtis et al., 1985; Bartlett et al., 1986; Correa et al., 1993). It was not the intent of this study to determine the individual effect of these disorders on the incidence of puerperal metritis. Instead, we considered cows with one or more of these disorders as a high risk group to develop puerperal metritis and retrospectively compare the incidence of puerperal metritis to a low risk group (cows with a normal calving). The clinical approach used to identify cows to be evaluated for puerperal metritis was based on evaluation of attitude (appeared sick) or a RT ≥ 39.4°C. Despite the fact that a systematic examination of the uterus of all cows was not considered, the incidence of puerperal metritis of (42%) in cows with an abnormal calving was similar to previous reports (Markusfeld, 1984; Curtis et al., 1985) and within the range in published studies that evaluated all cows for puerperal metritis regardless of attitude or RT (Markusfeld, 1984).

The definition of puerperal metritis used in the present study was based on the presence of a fetid vulvar discharge with or without fever. Of the cows with puerperal metritis, only 41.4% had fever when puerperal metritis was diagnosed. These results are in agreement with those of Pugh et al (1994), in which 42.3% of 78 cows evaluated for metritis 14 days post partum had fever (RT > 39.4°C). The diagnosis of puerperal metritis in the present study was made by per rectum palpation of the uterus and visual inspection
of the vulva to observe a uterine discharge. This method of diagnosis of puerperal metritis has been previously described (Markusfeld, 1984; Pugh et al., 1994; Risco, and Hernandez, 2003). In contrast to the diagnosis of clinical endometritis (Bonnett et al., 1993; Leblanc et al, 2002), no other previously reported method have been proven to be more or less sensitive and or specific to diagnose puerperal metritis.

Primiparous cows in the present study had a higher incidence of puerperal metritis during the cool season. However, no seasonal effect was observed in multiparous cows. An effect of season and parity on the incidence of postpartum uterine infections have been previously described (Markusfeld, 1984; Bartlett et al., 1986; Gröhn et al., 1990; Smith et al., 1998). However, an interaction between these two factors was not reported. Markusfeld (1984) and Bartlett et al (1986) hypothesized that during the winter months a high concentration of calvings and a wet and dirty environment may increase the challenge of pathogens to the uterine environment. However, in the present study, both primiparous and multiparous cows were equally exposed to these factors. In the present study, season was determined by the thermal heat index, which is an indirect measurement of heat stress in the cow. Previous reports have shown the relationship between heat stress and uterine involution. Heat stress during late gestation increased concentrations of blood prostaglandins and progesterone during the postpartum period (Lewis et al., 1984), and reduced prepartum blood concentrations of estrone sulfate (Collier et al., 1982). These changes in hormonal profiles have been reported to have a positive effect on uterine involution (Lewis et al., 1984; Nakao et al., 1997; Zhang et al., 1999) which may have reduced the incidence of puerperal metritis during the warm season observed in the present study.
The overall incidence of clinical endometritis is in agreement with that observed by Leblanc et al. (2002). The relationship between abnormal parturition and chronic uterine infections has been previously reported (Dohmen et al., 2000). Hirvonen et al. (1999) reported that 58% and 6% of the cows diagnosed with puerperal metritis (putrid smelling, reddish-brown, watery vaginal discharge; rectal temperature 39.5 to 41.0°C; within 4 to 11 days postpartum) had purulent vaginal discharge within 15 to 22 and 32 to 44 days postpartum, respectively. In addition, in the present study, the incidence of clinical endometritis (38.2%) in cows with puerperal metritis treated with ceftiofur is similar to that observed by Drillich et al., (2001). In that study (Drillich et al., 2001), 44.8% of the cows with puerperal metritis (fetid reddish brown vulvar discharge with a RT ≥ 39.5°C) treated with ceftiofur hydrochloride developed clinical endometritis within 32 to 34 days post partum. An interesting finding in the present study was that cows with puerperal metritis developed clinical endometritis whether or not they had fever when puerperal metritis was diagnosed. This may suggest that despite treatment of puerperal metritis, cows without fever were just as likely as those with fever to develop clinical endometritis.

Normal rectal temperature had been reported to be within the range of 38.0°C to 39.1°C (Rebhun, 1995) 38.0°C to 39.0°C (Rosenberger, 1979). Cows with a RT above the upper limit are defined as febrile or abnormal. In the present study, the mean RT for cows without metritis classified as normal was within the above described ranges, and in agreement with that reported by Kristula et al. (2001). Based on a previous report (Upham, 1996), used in the present study definition of fever was determined to be ≥ 39.4°C. Sheldon et al. (2004) described a cut off point for defining fever by using the
maximum RT between day 1 and 10 post partum, obtaining a mean of 39.3°C, and upper quartile equal to ≥ 39.7°C, which was defined as a febrile level. In the present study, values of RT between 3 to 13 days post partum for cows without puerperal metritis followed a normal distribution with a mean (± SEM) of 38.5°C ± 0.01°C. Based on our data, we found that the 95% confidence interval of the RT values within 3 to 13 days post partum were equal to 37.7°C - 39.3°C, after correcting by group, parity, season and presence or not of puerperal metritis. If it were hypothesized that the remaining 5% of values will be considered abnormal or febrile, cows with fever will be those with a RT ≥ 39.4°C.

Daily incremental increases of RT before diagnosis of puerperal metritis have not been previously reported. Rectal temperature of cows with puerperal metritis started increasing two days before the diagnosis of puerperal metritis. However, the RT value did not reach a febrile level of 39.4°C and were similar to those reported in cows with puerperal metritis (Smith et al., 1998). This is related to the fact that a proportion of cows with puerperal metritis on Day 0 (day of puerperal metritis diagnosis) did not have fever. Regardless of whether or not cows with puerperal metritis had fever, an incremental increase of daily temperature was significantly different from cows without puerperal metritis. Following treatment of cows with puerperal metritis with ceftiofur, rectal temperature decreased to a level similar to cows without puerperal metritis that were not treated. A similar response in RT reduction has been observed in cows with puerperal metritis treated with ceftiofur (Simith et al., 1998; Chenault et al., 2004; Wagner and Apley, 2004).
Results from model two showed two different patterns of RT for the different classifications of puerperal metritis. Cows classified as MT-1 showed a marked increase in rectal temperature that was significant 24h before diagnosis, and this high RT was rapidly reduced after diagnosed and treatment. In contrast, this pattern was not observed in cows classified as MT-2. Physiological control of a febrile response is multifactorial with mechanisms to prevent extreme elevation in body temperature (Leon, 2002). Our results showed that RT of cows with puerperal metritis has daily incremental increases of RT to a point were they became febrile. This may occur from the interaction between the host immune system and bacterial endotoxins which trigger the cascade of events that lead to elevated temperature. Dohmen et al. (2000) correlated the presence of a fetid uterine discharge with high concentration of LPS in the uterus and an elevated rectal temperature. However, comparison of LPS concentrations between the uterus to those in plasma were not correlated. In contrast, Peter et al. (1990) and Mateus et al. (2003) demonstrated that the uterus of cows during the early postpartum period is permeable to Escherichia coli LPS, and that these endotoxins provoked the systemic release of prostaglandin F2α (PGF2α) and thromboxane A2a.

The reason why a proportion of cows did not have fever when puerperal metritis was diagnosed or why they did not experience an incremental increase in RT before puerperal metritis diagnosis is not known. However, because all cows with puerperal metritis without fever were treated on the same day of diagnosis, a lack of disease progression may have occurred. It is also possible that these cows were under a septicemic process with higher rate of LPS absorption or concentration than cows with fever. Fever induced by LPS seems to be dose-related in that a high dose of LPS lowers
body temperature, attenuates fever and causes hypothermia. In contrast, lower doses of LPS seem to stimulate the production of TNF with subsequent production of fever (Leon, 2002).

Farm personnel involved in the conduction of the reproductive management of the study farm were blinded to the calving or puerperal metritis status of cows. Abnormal calving, puerperal metritis or clinical endometritis did not have any effect on reproductive performance. Typically, rectal examination of postpartum cows had been performed between 25 to 50 days post partum (Studer and Morrow, 1978), recently this procedure had been also done in the first two weeks post partum (Upham, 1996). In the present study, the intensity of the post partum health monitoring program resulted in an earlier diagnosis and treatment of puerperal metritis and clinical endometritis. Consequently, it can be assumed that earlier identification and treatment of puerperal metritis, leaded to a decreased adverse effect on fertility (Callahan and Horstman, 1987).

A meta-analysis study on the effect of disease on reproduction (Fourichon et al., 2000) showed less effects of puerperal metritis in studies reporting routine examination of cows compared with herds in which the owner reported the disease. In addition Harman et al. (1996) did not find any effect on the risk of conception for those cows with dystocia, retained placenta, or early metritis during 56 to 120 days postpartum. However, other studies showed a negative effect of abnormal parturition or puerperal metritis on reproductive performance (Borsberry and Dobson, 1989; Gröhn and Rajala-Schultz, 2000). All cows with clinical endometritis in the present study were treated with a single injection of 25 mg of PGF$_{2\alpha}$. In addition, cows were treated with PGF$_{2\alpha}$ in cases of non observed estrus. It is possible that cows affected with clinical endometritis may have
recovered sufficiently during the 60-day voluntary waiting period after treatment with PGF2a. The use of PGF$_2\alpha$ during the early postpartum period improved fertility in cows which experienced an abnormal calving (Risco et al., 1994).
CHAPTER 6
CONCLUSIONS

Occurrence of puerperal metritis was higher in cows experiencing an abnormal calving. Primiparous cows had a greater incidence of puerperal metritis in the cool season for both normal and abnormal calving. In contrast, multiparous cows showed no seasonality in the occurrence of puerperal metritis. A high proportion of cows did not have fever at the time of puerperal diagnosis, suggesting that puerperal metritis may not always be accompanied with a fever.

Evaluation of daily RT distinguished both cows with puerperal metritis, and those with or without fever. Prior to diagnosis, sequential increases in RT on two consecutive days prior to the actual diagnosis could serve as a predictor of puerperal metritis and may warrant earlier treatment. Early therapeutic treatment of all cows diagnosed with puerperal metritis resulted in pregnancy rates comparable to cows with normal or abnormal calving status that did not experiencing puerperal metritis.
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BIOGRAPHICAL SKETCH

Mauricio Esteban Benzaquen was born in the Federal Capital of the Argentinean Republic, on November 28, 1975. Mauricio was raised in Escobar, Argentina’s flower capital. There he spent time with family, and enjoyed brotherhood, friendship and studies, until the road of life took him north, still maintaining the principles with which he was raised.

In 1994 he went to the veterinary school at the recently created Veterinary School at Universidad del Salvador in Argentina. Early in his career he was influenced by several professors with doctorate degrees from North American Universities. With them and especially with Dr. Carlos Eddi (professor of Parasitology), he first encountered the passionate world of research. Mauricio’s interest in ruminants grew from his interaction with a great clinician (Dr. Enrique Renner, professor of Buiatrics), at that University. After graduation he accepted a 1-year research position in parasitology. Mauricio’s then took a research position with Dr. Carlos Corbellini at the National Institute of Agricultural Technology where he worked on the dairy industry in projects related to epidemiology and control of mastitis in dairy cows.

In 2002 he was given an opportunity to work and learn in the Florida dairy industry, in the United States. On August 17, 2002, Mauricio arrived at Mecklenburg Farm where he spent a year and a half on daily dairy activities. There he met Dr. Carlos Risco and Dr. Louis Archbald, and a new chapter of Mauricio’s life began.

In late 2002, Mauricio accepted a residency at the Food Animal and Reproduction Service in the College of Veterinary Medicine at the University of Florida.
of residency (and given his strong interest in research), Mauricio was offered (by Dr. Carlos Risco) the possibility of starting a Master of Science degree program.