EFFECT OF LAMENESS ON OVARIAN ACTIVITY IN POST-PARTUM HOLSTEIN COWS

By

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In memory of my father, Eduardo José Garbarino

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An observational cohort study was conducted to examine the relationship between lameness and delayed ovarian cyclicity in post partum Holstein cows. We used 253 cows from a 600-cow dairy herd that calved during a 12-month period. Cows were classified into one of six categories of lameness during the first 35 days post partum using a locomotion scoring system. Cows were blood sampled weekly for detection of plasma P_4 concentrations during the first 60 days post partum. Cows with a delayed resumption of ovarian cyclicity were those with consistent P_4 concentrations < 1 ng/mL during the first 60 days post partum. The null hypothesis that risk of delayed cyclicity is the same in cows classified as non-lame, moderately lame, or lame (after adjusting for potential modifying or confounding effects of loss of body condition and other variables related with delayed cyclicity) was tested using logistic regression. Results of the study support the hypothesis that lameness has a detrimental effect on ovarian activity in Holstein cows

during the early post partum period. Cows classified as lame were 3.5 times at higher risk of delayed cyclicity, compared to cows classified as non-lame

(OR = 3.5; 95% CI = 1.0 – 12.2; P = 0.04). Attributable proportion analysis indicated that delayed ovarian cyclicity in lame cows would be reduced by 71% if lameness had been prevented. In addition, cows classified as moderately lame were 2.1 times at higher risk of delayed cyclicity, compared to non-lame cows

(OR = 2.1; 95% CI = 0.7 – 6.1; P = 0.15).

CHAPTER 1
INTRODUCTION

Lameness is one of the top three health problems that cause premature culling of dairy cows in the United States. The National Animal Health Monitoring System Dairy 2002 Study (NAHMS 2002) reported that lameness was the reason for culling 16% of dairy cows sent to slaughter. Overall, 10% of cows were reported affected with lameness in the previous 12 months. The economic importance of lameness is attributed to cost of treatment and control methods (Shearer and Elliot, 1998; Shearer et al., 1998; Hernandez et al., 1999, 2000; Moore et al., 2001), impaired reproductive performance (Lucey et al., 1986; Lee et al., 1989; Sprecher et al., 1997; Hernandez et al., 2001; Melendez et al., 2003), decreased milk yield (Warnick et al., 2001; Green et al., 2002; Hernandez et al., 2002), increased risk of culling (Sprecher et al., 1997; Collick et al., 1989), and decreased carcass value of culled cows (Van Arendonk et al., 1984). In addition, because of the pain, discomfort, and high incidence of lameness in dairy cows, this disorder should be considered an animal welfare issue.

Delayed ovarian cyclicity in the preservice postpartum period is a common ovarian dysfunction in dairy cows (Opsomer et al., 1998). Late resumption of ovarian activity post partum has a detrimental effect on reproductive performance in dairy cows (Thatcher and Wilcox, 1973; Stevenson and Call, 1983; Lucy et al., 1992). Cows ovulating earlier post partum have fewer services per conception and a shorter calving-to-conception interval (Lucy et al., 1992). Minimizing the interval from calving

to first ovulation provides ample time for completion of multiple ovarian cycles before to insemination, which in turn improves conception rates (Butler and Smith, 1989). Losses in body condition, puerperal disturbances, and ketosis have been identified as risk factors significantly associated with delayed ovarian cyclicity in dairy cows (Opsomer et al., 2000).

While previous studies have shown that lameness has a detrimental effect on reproductive performance (i.e., a prolonged calving-to-conception interval) (Lucey et al., 1986; Collick et al., 1989; Sprecher et al., 1997; Hernandez et al., 2001), the relationship between lameness and ovarian activity in dairy cows has not been investigated using objective research methods. Results of previous studies in Florida suggest that as cows experience increasing positive energy status, there is increased ovarian follicular activity leading to return to ovulation (Staples et al., 1990; Lucy et al., 1992). As energy status becomes more positive for cows early post partum, diameter of the largest follicle increases, the number of double ovulations increases, and time for detection of the first corpus luteum decreases (Lucy et al., 1991). These changes in follicle size and numbers and the number of ovulations, are thought to be caused by increases in luteinizing hormone, follicle-stimulating hormone, insulin, BST, insulin-like growth factor-1, and possibly other yet-to-be determined compounds that are activated by an improved energy status (Beam and Butler, 1998).

Clinical observations by veterinarians and dairy farmers in Florida suggest that lameness has a detrimental effect on ovarian activity in lactating dairy cows. We hypothesized that as lame cows experience a more pronounced loss in body condition (hence a prolonged state of negative energy balance) during the early postpartum period,

lame cows are at higher risk of delayed ovarian cyclicity than non-lame cows. Under field conditions, evidence of corpus luteum function can be determined by monitoring plasma P_4 concentrations weekly during lactation, before and after diagnosis of lameness in dairy cows. The objective of this study was to examine the relationship between lameness and delayed resumption of ovarian cyclicity in Holstein cows during the first 60 days post partum.

Knowledge of the epidemiologic aspects of diseases and lesions that cause lameness is essential to further develop control and prevention methods for lameness in dairy cows. Risk of lameness during lactation, severity and duration of lameness, and the relationship between lameness and ovarian activity in lame cows have not been investigated under US dairy farming conditions. Our prospective research study will allow us to better characterize lameness in dairy cows under commercial farming conditions, and examine the relationship between lameness and ovarian activity. An understanding of the reasons for individual-cow differences in lost revenues will aid producers in making management decisions at the cow and herd levels to manage lameness, reproductive performance, and animal welfare.

CHAPTER 2
LITERATURE REVIEW

Lameness is one of the top three health problems that cause premature culling of dairy cows in the United States. Several studies have reported that the incidence of lameness in dairy cattle operations varies from 7% to 54.6% (Harris et al., 1988; Clarkson et al., 1996; Collick et al., 1989; Barkema et al., 1994; Green et al., 2002; Deluyker et al., 1991), with the highest proportion of cases occurring in the first 100 days in milk (Collick et al., 1989; Barkema et al., 1994; Green et al., 2002).

The economic importance of lameness is reportedly attributable to cost of treatment and control methods (Moore et al., 2001; Hernandez et al., 1999, 2000; Shearer et al., 1998), impaired reproductive performance (Lee et al., 1989; Tranter and Morris, 1991; Sprecher et al., 1997; Hernandez et al., 2001; Melendez et al., 2003), decreased milk yield (Tranter and Morris, 1991; Coulon et al., 1996; Warnick et al., 2001; Hernandez et al., 2002), increased risk of culling (Sprecher et al., 1997; Collick et al., 1989), and decreased carcass value of culled cows (Van Arendonk et al., 1984). In addition, because of the pain, discomfort, and high incidence of lameness in dairy cows, this disorder should be considered an animal-welfare issue.

It is difficult to describe the incidence and prevalence of the problem of lameness in dairy cows, because of the wide variation that exists in published reports. This variation is due to differences in geographic locations and production systems (pasture-based vs. confinement) of study herds, that may alter the frequency distribution of lameness. In addition, the method of gathering data varies among studies (some

collecting data from veterinary practices, and others gathering data directly from the farmer, or by researchers). Whitaker (1983) reported an average incidence of lameness of 25% (range = 2% to 55%) in 21,000 dairy cows in 185 herds in England and Wales. In the study by Whitaker (1983), data were collected by a survey of the number of treatments for lameness either by the farmer or veterinary surgeon. Another study conducted in 37 dairy farms in the United Kingdom and Wales, Clarkson (1996) reported an annual incidence of lameness of 54.6% (new cases/100 cows) with a range from 10.7% to 170.1%. This same study (Clarkson et al., 1996) reported the prevalence of lameness to be 20.6%. Diagnosis of lameness was performed either by the farmer, student, hoof trimmer, or a veterinarian. All treatments were recorded and were used to calculate the prevalence and incidence of lameness. Collick (1989) performed a study in 17 dairies in the UK, and reported an incidence of lameness of 17% during a 6- month period, with a range of 8% to 28%, with 65% of cases occurring in the first 100 days in milk. In this case, diagnosis of lameness was made by the attending veterinarian.

A study conducted in 80 dairies in France (Faye and Lescourret, 1989), the reported annual incidence of lameness was 29.5%. They stated that incidence of lameness was higher than reported incidence rates of mastitis, and that lameness was the most common disease reported in dairies. A study conducted in 101 dairy farms in Sweden (Manske et al., 2002a), found a prevalence of lameness of 5%. In this study (Manske et al., 2002a) lameness examination of cows was done by researchers who looked at every cow on each farm, with the purpose of identifying claw lesions in lame and non-lame cows. In other parts of the world with more extensive production system such as New Zealand, Australia, and Argentina, the incidence/prevalence of lameness differs from

those reported in Europe. Dewes (1978) reported an incidence of lameness of 14%, and more than 85% of the cases of lameness were detected before 90 days in milk. This study (Dewes, 1978) was conducted using data provided by four dairy producers from Waikato, New Zealand. In another study in New Zealand by Tranter and Morris (1991) in three dairy herds, the annual incidence of lameness was 16% with a range from 2% to 38%. In this study the mean days to onset of lameness was 92±54 (mean ±SD). Harris (1988) conducted a study in 73 dairies in Southern Australia and reported and annual incidence of lameness of 7% with a range of 0% to 31%. In this study, diagnosis of lameness was done by the farmer. In a study done in Argentina (Rutter, 1994) involving 4580 dairy cows from 25 farms, the incidence of lameness was 23.4% with the highest incidence occurring in first lactation heifers (45%). In most of these studies, sole ulcers and white line disease were the most predominant lesions observed.

When we examined the incidence and prevalence of lameness studies in the United States, we also found important variations. Reported incidences of lameness were 4.4%, 5.1% and 9.5% (Bartlett et al., 1987; Weigler et al., 1990; Kaneene et al., 1990). In these studies, lameness was diagnosed by the farmers. This motivated Wells (1993a), to design a study on prevalence of lameness, and to compare the diagnosis of lameness performed by researchers and farmers. In both seasons spring and summer, the observed prevalence reported by researchers were 3 times higher than that by the farmers. This study (Wells et al., 1993a) involved 17 dairy farms from Minnesota and Wisconsin and reported a prevalence of lameness of 14% in the summer and 17% in the spring. In another study conducted in 13 dairy farms in Ohio, researchers observed that the incidence of hemorrhages and discoloration of the sole in first calf heifers (from 60 days

pre partum until 100 days post partum) was 62% during this period. In another study by Sprecher (1997) using a locomotion scoring system, the incidence of lame cows (Locomotion Score ≥ 4) was 24.5%, the incidence of moderately lame plus lame cows (LS ≥ 3) was 49.1% at the time of the first service.

Table 2-1. Studies reporting incidence of lameness in dairy cows

Author, year	Country	Incidence of lameness (%)
Rutter, 1994	Argentina	23
Harris et al., 1988	Australia	7 (0 to 31)
Faye and Lescourret, 1989	France	29
Dewes, 1978	New Zealand	14
Tranter et al., 1991	New Zealand	16 (2 to 38)
Manske et al., 2002a	Sweden	5
Whitaker et al., 1983	United Kingdom	25 (2 to 55)
Collick et al., 1989	United Kingdom	17 (8 to 28)
Clarkson et al., 1996	United Kingdom	54
Bartlett et al., 1987	United States	4
Weigler et al., 1990	United States	5
Kaneene et al., 1990	United States	9
Wells et al., 1993	United States	13.7 summer & 16.7 winter
Sprecher et al., 1997	United States	49
Warnick et al., 2001	United States	46

Almost all reports describing lameness in dairy cows considered claw diseases as the most common causes of lameness. Some of the papers listed above (Tranter and Morris, 1991; Deluyker et al., 1991; Murray, 1996) reported claw diseases as responsible for more than 90% of the cases of lameness. When we examined the most common claw disorder, reports are not so much in agreement. Differences maybe due to, different housing types, feeding strategies, environmental challenges and managements systems.

Studies from the United Kingdom (Clarkson et al., 1996; Murray et al., 1996) agreed that sole ulcers, white line disease, laminitis and subsolar abscess are the most common diseases affecting the bovine claw. In another study conducted on 101 dairy farms in Sweden (Manske et al., 2002), sole ulcers and white line lesions were the most common lesions found in lame cows. In New Zealand and Australia (Tranter and Morris, 1991; Dewes, 1978; Harris et al., 1988) where cows are kept in pasture all year around, traumatic pododermatitis (sole bruising), worn soles and interdigital dermatitis the most common diseases of the claw affecting dairy cows. In a study conducted in Argentina (Rutter, 1994), digital dermatitis (39.4%) and interdigital dermatitis (26.3%) were the most common diseases affecting the bovine foot. In the United States, all studies identified claw lesions as the most common lesions affecting dairy cows (Deluyker et al., 1991; Smilie et al., 1996; Hernandez, 2002). These studies, however, were not designed to establish the prevalence of the different diseases of the bovine foot.

With an average incidence of 30 cases per 100 cows per year, a case fatality rate of 2%, involuntary culling of 20% of cases; an average increase of 28 days open, treatment costs including veterinary fees, drugs and farmer labor of $23 per case. The total cost of lameness per 100 cows per year is estimated to be about $9000 (Guard, 1996). This paper is probably underestimating the cost of lameness as it is not taking into account other factors that are affected by lameness. Lameness has been shown to affect milk production and this was not included in the estimation, as well as the decrease of the carcass value of cows sent to slaughter and the increased probability of a cow being culled if experiencing lameness. In another report from the UK, Kossaibati and Esslemont (1997) reported that the average total cost per affected cow of a case of sole

ulcer was £424 (approx $600). This estimation included the cost of treatment, herdsman's time, discarded milk, reduced milk yield, veterinarian's time, increased risk of culling and longer calving interval. Increased risk of culling and a longer calving interval accounted for more than 60% of the cost. Unfortunately, the authors did not explain how they estimated milk loss and increased risk of culling.

Table 2-2. International terminology of digital diseases

International terminology	English	Common terms
Dermatitis interdigitalis	Interdigital dermatitis	Superficial Foot-Rot
Phlegmona interdigitalis	Interdigital phlegmon	Foot-Rot, Foul in the foot
Erosio ungulae	Heel erosion	Underrun heel, Slurry heel
Hyperplasia interdigitalis	Interdigital hyperplasia	Corn, Interdigital fibroma
Dermatitis digitalis	Digital dermatitis	Hairy foot warts, Hairy heels
Pododermatitis aseptica difusa	Diffuse aseptic pododermatitis	Laminitis
Pododermatitis circumscripta	Circumscript pododermatitis	Sole ulcer
Pododermatitis septica traumatica	Traumatic septic pododermatitis	Subsolar, toe and white line abscesses
Fissura ungulae	Hoof wall cracks	Sand cracks: longitudinal and transverse
Pododermatitis locale	Localizedpododermatitis	Bruises
Ungulae deformans	Overgrown hooves	Long toes

To compare results from different studies it is important to standardize the terminology regarding claw lesions. In order to clarify the rest of this manuscript we will follow the terminology proposed by Weaver (1994) (Table 2-2).

Anatomy of the Bovine Foot

Before we start describing the different pathologies affecting the bovine foot, it is important to review it's anatomy and microanatomy.

Foot

The foot includes the entire limb below the fetlock joint. It is comprised of two digits each of which has a horn-covered claw. It should be noted that in cattle the term "claw" is preferable to hoof. The front aspect of the foot is referred to as the dorsal side. The back side of the front foot is referred to as the palmar aspect whereas in the rear foot is referred to as plantar aspect. When referring to an area nearest the longitudinal axis (i.e., toward the center) it is designated as axial, whereas items farther away (away from the center) are designated as abaxial.

Each digit of the foot has 4 bones: phalange 1 (P1), phalange 2 (P2), phalange 3 (P3), and navicular bone; and 2 joints: proximal interphalangeal (PIP) and distal interphalangeal (DIP). The proximal end of P1 articulates with the metacarpus (in the front leg) or metatarsus (in the rear leg) in the fetlock joint, whereas the distal (away from the center of the body) end of P1 articulates with the proximal end of P2. This articulation between P1 and P2 is referred to as the proximal interphalangeal joint (PIP). The distal end of P2 articulates with the proximal end of P3. This joint is referred to as the distal interphalangeal joint (DIP).

P3 is completely enclosed within the claw horn capsule. Its solar surface is concave or arch shaped and marked on the back edge by a bump known as flexor tuberosity. The flexor tuberosity is the site of attachment of the deep flexor tendon. This tuberosity has an important role in the pathogenesis of sole ulcers as it becomes involved in the process of compression of the corium subsequent to laminitis and the displacement of P3 (Toussaint Raven, 1989).

The navicular bone (also referred to as the distal sesamoid bone) is attached to P3 by three small ligaments and also to P2 by collateral ligaments. Between the navicular

bone and the deep flexor tendon is the navicular bursa. The navicular bursa contains joint-fluid which permits movement of the deep flexor tendon over the surface of the navicular bone during extension and flexion of the claw. P3, the DIP joint, navicular bone and navicular bursa all lie within the claw capsule.

Claws

The purpose of the claw horn capsule is to protect the underlying sensitive tissues of the corium and dissipate the concussion forces that occur when the digits impact the ground. It consists of the wall which can be divided into the axial (inside) and the abaxial (outside). The abaxial wall is further subdivided into the dorsal (or front) and lateral (abaxial side) aspects. The wall is demarcated from the heel on the abaxial side of the claw by the abaxial groove. The wall consists of two types of horn: perioplic and coronary. Perioplic horn is the softer horn lying just below the coronet at the skin-horn junction (corresponding to the human cuticule). At the back of the foot the periople gradually widens and eventually becomes the horn of the heel. Coronary horn, the hardest horn within the claw capsule makes up the bulk of the horn of the wall. The wall has faint ridges or rugae, which run horizontally and parallel to each other. Toward the heel these ridges diverge reflecting a more rapid rate of growth in the heel region due to faster rates of wear. In mature Holstein cattle the length of the dorsal wall should be a minimum of 3 inches in length from just below the top of the hairless portion of the wall to the weight-bearing surface. Ideal heel height is 1.5 inches (Toussaint Raven, 1989; Blowey, 1993).

The sole is produced by the solar corium and merges imperceptibly with the horn of the heel at the heel-sole junction. The sole is connected to the wall by means of the white line. White line horn is produced by laminar corium. It courses forward from the

area of the heel on the abaxial side of the claw, around the tip of the toe and about 1/3 of the way back on the axial side of the claw's weight bearing surface. Where the white line leaves the weight bearing surface it courses upward on the axial side of the claw. This white line is a unique and important structure. It is the softest horn within the claw capsule. This permits it to provide a flexible junction between the harder horn of the wall and the softer horn of the sole. On the other hand, because of its softer nature it also represents a weak spot on the weight-bearing surface that is vulnerable to damage.

Suspensory Apparatus and Supporting Structure of the Bovine Digit

Cattle (and all animals with claws or hooves) are suspended in their feet, that is, they stand in their feet, not on them. In other words, the bone within the claw (also known as P3) is suspended within the claw horn capsule by the laminar corium and a series of collagen fibers bundles that stretch from the insertion zone on the surface of P3 to the basement membrane of the epidermis (the line of demarcation between dermis and epidermis). The interface between dermal and epidermal components is the interdigitating dermal and epidermal laminae. The result is that P3 hangs within the claw capsule and weight is transferred as tension onto the wall of the claw capsule.

The suspensory system in cattle differs significantly from that in horses. First, the laminar corium is much less extensive in cattle as compared to horses. Secondly, there are no secondary laminae in the laminar corium of cattle. Therefore, capabilities with respect to mechanical load carried on the claws of cattle vary significantly. In the horse load bearing is primarily on the wall. Cattle, on the other hand, simply cannot handle the same amount of mechanical load on the walls of their claws. Instead, weight-bearing in cattle requires displacement of load to the wall, and support structures within the sole and heel.

The primary structures within the supportive apparatus of the bovine claw are the solar corium and associated connective tissue, and the digital cushion, which consists of loose connective tissue and varying amounts of adipose (fat) tissue. The digital cushions are arranged in a series of three parallel cylinders similar to the design used in the cushion of a running sole. In the cows foot these cushions act like shock absorbers within the claw protecting the corium and permitting limited movement of P3 in the region of the heel.

Horn Formation and Growth

The horn-producing germinal layer of the epidermis and its supporting structure, the corium, consist of four different regions, each producing a structurally different type of horn (Budras et al., 1996). Perioplic horn, overlying the perioplic corium, is found just below the skin-horn junction and extends to the back of the claw to include the heel horn (Budras et al., 1996). Horn of the wall is produced in the area of the coronary corium and it is situated between the perioplic corium and the sensitive laminae. The area overlying the laminar corium produces the horn of the white line, also known as laminar horn. The solar horn overlies the solar corium and is situated between the laminar horn of the white line and the perioplic horn of the heel (Budras et al., 1996; van Amstel and Shearer, 2001).

Horn production and growth are supported by the corium, which corresponds to the dermis. The corium consists of a rich vascular network that terminates in dermal papillae, also called vascular peg (Greenough, 1997). A vascular peg consists of a main arteriole and a venule, which are connected at the tip. Between the arteriole and the venule is an extensive capillary network, and there are also several vascular shunts between such arterioles and venules. These shunts may open under certain circumstances,

cutting of the blood supply to the tip of the vascular peg, which adversely affect horn cell formation. The epidermal layer overlying the vascular pegs produces horn cells in the form of tubules (tubular horn) (Budras et al., 1996; Greenough, 1997). Intertubular horn is produced between the papillae and interconnects the tubular horn. There are approximately 80 vascular pegs or dermal papillae per square millimeter of coronary corium surface (Greenough, 1997), which means that the wall consists of tightly packed tubular horn that is cemented together by intertubular horn. The perioplic corium of the heel horn and the solar corium has fewer vascular pegs per square millimeter. Because tubular horn supplies structural strength to the horn capsule, it follows that the horn of the wall is structurally the strongest, followed by the sole and the heel. Keratin filaments produced by horn cells enhances the rigidity and strength of horn cells as they progress to the exterior.

Laminar horn is immature, nontubular, so it is soft and flexible and has a high turnover rate. Horn cells, whether tubular or nontubular, are cemented by a substance known as membrane-cementing substance (Budras et al., 1998). This substance, a lipoprotein, is permeable and holds water, giving the horn its flexibility (Budras et al., 1998).

Horn quality is dependant on internal and external and factors. Internal factors relates to blood and nutrient supply, whereas external factors relates to environment where the claw is found. Any compromise in blood flow has a negative effect on horn production.

Microanatomy of the Claw: Structure of the Wall

The structure of the claw consists of modified skin that is a continuation of the epidermis of the coronary band. The claw has the same basic structures as the skin. It has

an epidermis (horny wall), a dermis (corium or quick), and a subcutis (fibroelastic heel pad and coronary and digital cushion). The epidermis itself is divided into basement membrane, germinal epithelium (stratum germinativum), stratum spinosum (layers of horn undergoing keratinization) and stratum corneum (the layer of cornified epithelium). The basement membrane is the junction between the epidermis and corium. The stratum germinativum is the germinative layer responsible for horn growth. The stratum corneum is the cornified epithelium forming the claw horn. Cells are arranged into tubular and intertubular horn. The mechanical strength of the bovine claw is a function of the keratinization of cells in the germinal layers of the epidermis (Hendry et al., 1994).

Etiology of Lameness

Lameness in cattle can be caused by a variety of reasons. The purpose of the next section is to describe the different causes of lameness in cattle.

Infectious Diseases of the Digits

Several systemic diseases can be associated with digital lesions potentially leading, as a result of localized pain, to stiffness and lameness. They include Foot-and-Mouth Disease (FMD), Bovine Virus Diarrhea (BVD), Bovine Malignant Catarrh, Bluetongue and Vesicular Stomatitis. This review of lameness is focused on major specific infections of the digits: Interdigital Phlegmon (Foot-Rot), Interdigital Dermatitis and Digital Dermatitis.

Interdigital phlegmon (Foot-rot, Interdigital necrobacillosis)

Interdigital Phlegmon is characterized by fissuring, caseous necrosis of the subcutis in the interdigital space and diffuse digital swelling. Pain, moderate to severe lameness, and pyrexia are also common signs of this disease. A characteristic fetid odor

is usually present because of the presence of *Fusobacterium necrophorum* which if not treated early a common sequela is septic arthritis (Berry, 2001).

Although the pathogenesis of foot rot is not understood completely, bacteria gain entry through abraided skin on the lower part of the foot. Hard surfaces contribute to foot injury, and continuous wetting likely favors abrasions by softening the interdigital skin (Radostits et al., 2000). The greatest economic impact of bovine foot rot is in dairy operations, where milk production may be affected (Hernandez et al., 2002). In this study, cows affected with foot rot produced 10% less milk than normal cows. Also this disease can affect feedlots where antimicrobial treatments require withdrawal times that could delay marketing of products (Radostits et al., 2000). Although spontaneous recovery may occur, lameness may persist for several weeks when infections are left untreated, and complications may cause more severe problems that could eventually lead to death or euthanasia of the animal (Radostits et al., 2000).

Treatment of foot rot can be accomplished with a variety of antimicrobials (Cook et al., 1995; Morck, 1998; Berry, 2001). A recent study looked at the efficacy of Ceftiofur Sodium and Hydrochloride formulation for the treatment of foot rot (Kausche et al., 2003). This was a multilocation study conducted on 11 farms in the US to compare the efficacy of Ceftiofur at a dose of 1.1 mg/kg once a day for 3 consecutive days with a placebo group. Results of this study indicated that cure rate for Ceftiofur was 62.2% versus 14% for the placebo group ($P < 0.003$). These same authors did another study to compare the efficacy of Ceftiofur versus Oxytetracycline at 10 mg/kg. Results of this study indicated that Ceftiofur and Oxytetracycline were comparable in efficacy, with Ceftiofur having excellent injection-site tolerance and a short or no milk discard or

pre-slaughter withdrawal (Kausche et al., 2003). Treatment can also be accomplished by the use of Sulfadimethoxine orally (25 g/lb followed by 12.5 g/lb SID for no more than 5 days) or intravenously (55 mg/kg followed by 27.5 mg/kg SID for 2 days after remission of clinical signs). Good results also can be obtained with Penicillin G intramuscularly for 3 days (Bergsten, 1997).

Prevention and control of foot rot can be accomplisheded by the use of foot baths with 5% to 10% copper sulfate or zinc sulfate (Rebhun, 1982). Formaldehyde solutions of 3 to 5% in water have been reported to be effective in the prevention of foot-rot (Bergesten, 1997). Caution should be emphasized when using formaldehyde due to potential hazards for handlers as well as contamination of the environment. Other measures recommended to prevent foot rot are to maintain clean passageways to reduce the exposure of the feet to feces, maintaining a dry environment and avoiding rough floor surfaces that can traumatize the interdigital skin and allow the entry of bacteria (Blowey, 1994). Efforts to produce vaccines against *Fusobacterium necrophorum* have failed because of the weak immune response to the bacterium (Smith, 1992). There are vaccines available in the US market but there are no peer-reviewed studies to support their use.

Interdigital dermatitis

Interdigital dermatitis occurs as an acute or chronic inflammation of the interdigital skin that does not usually cause lameness (Blowey, 1994; Guard, 1995). The inflammation does not extend to the subcutaneous tissues and in this respect differs from foot-rot, where infection extends to the dermis, leading to fissure formation, infection of deeper structures, and cellulitis of the pastern and fetlock (Blowey, 1994). Some authors implicate *F.necrophorum, Dichelobcater nodosus* and *Bacetroides sp* as the causative agents of interdigital dermatitis (Toussaint Raven, 1989, Peterse, 1982).

Interdigital dermatitis occurs in dairy cattle, especially in wet environments. It is usually an incidental finding when trimming feet because it rarely causes lameness. A study conducted in 17 Danish dairy herds reported that interdigital dermatitis occurred in 4.5% and 7.6% of first and 2+ lactation cows, respectively (Enevoldsen et al., 1991). In this study, severity of disease increased with parity and risk increased with stage of lactation. In a Dutch study on 86 dairy farms, researchers reported that the prevalence of interdigital dermatitis and heel horn erosion was 24% (range = 3 to 92%) (Manske et al., 2002c).

Clinical signs of interdigital dermatitis include hyperemia of the interdigital skin, including the palmar and plantar areas, superficial erosion and ulceration followed by hyperemia with serous or grayish exudates. More aggressive forms interfere with the horn formation in the bulbs, where fissures, hemorrhages and necrosis can arise. The subcutaneous tissue is inflamed secondarily. Swelling and hyperkeratosis may develop in a more chronic stage. Chronic interdigital irritation may cause slight to severe interdigital hyperplasia (Bergsten, 1997). The most common complication of interdigital dermatitis is heel horn erosion. Results of a study conducted by Enevoldsen (1991) support the hypothesis that severe heel erosion and interdigital dermatitis are two manifestations of the same disease with *Dichelobacter nodosus* as an important component and are closely related. In this study (Enevoldsen, 1991) the incidence of heel erosions in 1st and 2+ lactation cows was 43% and 69%, respectively. This study (Enevoldsen, 1991) also reported unhygienic and moist conditions as important risk factors for interdigital dermatitis.

Prevention and treatment is usually accomplished by the use of 5 to 10% copper sulfate footbath or zinc sulfate (10 to 20%), or formalin (3 to 5%) footbaths. Care must be taken to ensure that the footbath remains clean. Interdigital dermatitis can persist in dairies that practice regular footbaths (Guard, 1995). This same report suggested that the causative organisms may survive within deep heel cracks that are not permeated by footbath solutions; hence, heel cracks must be trimmed during hoof trimming to allow for exposure to footbath solutions. Claw trimming causes a mechanical cleansing of affected tissues and an exposure to air that might be beneficial for the curing of dermatitis lesions (Manske et al., 2002b). As reported in this same study, every third hoof affected with a severe dermatitis and concurrent heel-horn erosion had recovered 1 month after trimming.

Digital dermatitis (DD) (Footwarts, Hairy heel warts, Heel warts)

Digital dermatitis is an important cause of lameness in dairy cattle. It was first reported by Cheli and Mortellaro in 1974 (Mortellaro, 1994) as a mysterious cause of epidemic lameness affecting up to 70% of adult cattle in the Po Valley of Italy. Since then, the disease has been reported in other countries such as the Netherlands, France, England, Czechoslovakia, Germany, and Ireland (Bassett et al., 1990; Brizzi, 1993). In the United States, Rebhun (1980) first reported the disease as outbreaks of lameness in New York dairy herds.

Clinically, digital dermatitis typically appears within dairy herds as outbreaks of lameness. It is a superficial skin disease of the bovine digit with variable presentation (i.e., depending upon the stage of the lesion), from painful, moist, strawberry-like lesions to raised, hairy, wart-like lesions (Read and Walker, 1998). These lesions (i.e., usually located on the rear of the foot between the bulbs of the heel) have been referred to by

several names, including: hairy footwarts, strawberry (or raspberry) heelwarts, and papillomatous digital dermatitis. Early lesions produce matting of the hairs, which stand erect in thick, light brown exudates, which have a characteristic pungent odor (Blowey et al., 1994). A study conducted in California described the lesions as being distinctly demarcated, circumscribed, spherical to oval, 0.5 cm to 6 cm across, partially or completely alopecic, moist, painful-to-touch, prone-to-bleed plaques of flat or raised proliferative tissue. Lesion surfaces vary in appearance from being extensively red and granular (31%), often with patches of yellow or gray filiform papillae (41%) to extensively gray, brown or black with papillary outgrowth of the epithelium (28%) (Read and Walker, 1994).

In spite of many studies and specific research, the exact etiology of DD is still unknown. Researchers still believe that DD is a multifactorial disease, even though in some cases high morbidity, apparent contagiousness and response to antimicrobial treatments suggest that an infectious agent is primarily involved (Mortellaro, 1994). In one study the incidence of DD was higher in heifers a few months after they entered the milking herd and may be due to lack of immunity (Read et al., 1992). Initial studies (Rebhun et al., 1980; Cheli and Mortellaro, 1986; Peterse, 1982) were unable to identify any viral pathogens, and results of bacteriology were inconsistent. Peterse (1982) was able occasionally to isolate *Dichelobacter nodosus* from some typical lesions. Bassett (1990) was unsuccessful in isolating a microorganism from DD lesions and also failed to replicate the disease after inoculation of heifers with homogenate from fresh lesions. Read (1992) examined histological lesions and were able to demonstrate the presence of large numbers of spirochetes invading the stratum spinosum and dermal papillae (Blowey

et al., 1994). In a more recent study, Walker (1995) isolated two different spirochetes from cows with DD lesions in California dairy herds. These spirochetes were further categorized based on morphology (intracytoplasmatic tubules), antigenicity and enzymatic activity in the genus *Treponema.* This finding was supported later by another study (Demirkan et al., 1999) where serological evidence suggested that spirochetes are involved in the pathogenesis of DD. Also, this study (Demirkan et al., 1999) supports the hypothesis that *Borrelia burgdorferi* may be involved in the pathogenesis of DD, as first proposed by Blowey (1994). To date, there is still no isolation of the bovine spirochete.

In a study conducted in California dairy herds (Read and Walker, 1994), the prevalence of DD was approximately 90%. Between-herd morbidity varied from 0.5 to 12% per month. Within-herd morbidity was generally greater during spring and summer months. Most lesions occur on the plantar interdigital cleft of the rear foot and less common sites for lesions are the palmar interdigital ridge of a front foot or a dorsal aspect of any foot (Mortellaro, 1994; Read and Walker, 1998). Another study conducted by the National Animal Health Monitoring System involving 83% of US dairy cows in 20 states observed the incidence of digital dermatitis and risk factors (Wells et al., 1999). This study reported, that within the last 12 months of the study, 43.5% of the US dairy herds had cows that showed clinical signs of DD with variation by herd size and region (Table 2-3).

The study by Wells (1999) reported that the average percent of cows affected was 18.9%. A high percentage of digital dermatitis-affected cattle were also reported lame (81.9% of affected cows and 85.9% of bred heifers). This study also looked at risk factors associated with digital dermatitis. Interesting results from this study identified several

factors that contribute to herd incidences > 5%. The percent of cows not born on the dairy operation was strongly associated with high digital-dermatitis incidence, and there was evidence for a dose-response relationship.

Table 2-3. Incidence of Digital Dermatitis in US dairy herds by herd size and region

Variable	Level	Herds with digital dermatitis in the previous 12 months (%)
Herd Size	< 100 cows	36.4
	100 to 199 cows	61.9
	>200 or more cows	80.3
	Total	43.5
Region	Northwest	56.1
	Southwest	70.3
	North Midwest	35.4
	South Midwest	45.5
	Notheast	53.1
	Southeast	20.8

Wells et al., 1999

Rodriguez-Lainz (1996) showed a strong association between introduction of heifers and digital-dermatitis prevalence in southern California dairy herds. These results were in agreement with results from Argáez Rodríguez (1997) who reported that purchased animals were 3.4 times more likely to be affected than animals born on the farm. Farm factors such as type of concrete flooring with concrete abrasive floors or slippery floors were associated with > 5% incidence of DD. Rodriguez-Lainz (1996) reported an association between the incidence of digital-dermatitis and corral moisture in southern California dairy operations with dirt dry lot corrals. Some biosecurity factors identified were hoof trimmers that trim cows on other farms. Herds in which the primary hoof trimmer also trimmed cows hooves on other operations were 2.8 times more likely to have > 5% incidence of digital dermatitis compared to herds where the primary hoof trimmer did not trim hooves on other operations or where cows hooves were not

trimmed. Also, herds in which hoof-trimming equipment was not washed between cows were 1.9 times more likely to have > 5% incidence of digital dermatitis than those where the equipment was washed or where no hooves were trimmed (Wells et al., 1999).

Blowey (1988) reported different treatments when describing one of the first outbreaks of the disease in the UK. Treatment of DD started by the application of parenteral injections of penicillin, streptomycin, tetracyclines, cephalexin, and sulphonamides, but none of these treatments proved effective and most cases recovered on their own. The most effective treatment in this report appeared to be deep scraping of the lesion with a hoof knife, followed by topical oxytetracycline/gentian violet aerosol spray. This treatment led to a reduction of lameness in 6 to 12 hours and complete resolution in 2 days. In this study, footbaths with 5% formalin or 2.5% copper sulfate were used to try to control the outbreak with poor results. Sheldon (1994) further supported treatment with oxytetracycline spray (4 g/L), although this was not a controlled study. Further research conducted by Britt (1996) confirmed the efficacy of Oxytetracycline (100 mg/mL) as a treatment for digital dermatitis applied as a spray. Manske et al., (2002c) reported that Oxytetracycline applied as a bandage was significantly more effective than hoof trimming alone of the affected foot ($P < 0.001$). This study was done to try to find an alternative treatment to antibiotics and Glutaraldehyde bandage was tested as the alternative non- antibiotic treatment. Results of this study did not support the use of this product. Treatment of DD with topical oxytetracycline does not require any milk withdrawal as shown by Britt (1999) who was unable to demonstrate antibiotic residues in milk using standard routines after cows were treated topically with this antibiotic. The most common treatments for digital dermatitis

involve the use of topical antibiotics (Blowey, 1988; Blowey, 1994; Guard, 1995; Berry et al., 1996; Britt et al., 1996; Britt et al., 1998; Read et al., 1998; Berry et al., 1999a; Berry et al., 1999b, Hernandez et al., 1999; Shearer et al., 2000; Manske et al., 2002c). Some of the antibiotics used in these studies were Oxytetracycline, Tetracycline, Lincomycin/Spectinomycin, and Erythromycin.

There also are reports of non-antibiotic products being effective in the treatment of DD (Shearer and Hernandez, 2000; Hernandez et al., 1999). Hernandez (1999) used a topical spray with four different non-antibiotic products in the lame cow with DD. Treatment with an Oxytetracycline solution, or a soluble copper, peroxide compound and a cationic agent appeared to be effective for the treatment of DD, compared to a 5% copper sulfate ($CuSO_4$) solution, or acidified copper solution, or hydrogen peroxide-peroxyacetic acid (HPPA) solution. In another study (Shearer et al., 2000) non-antibiotic compounds were used to treat DD in 78 cows affected with lameness. In this study (Shearer et al., 2000), a previously tested product (Hernandez et al., 1999) was modified to improve its handling and storage characteristics. There were four treatment groups in this study: cows in group A were treated with oxytetracycline solution, cows in group B were treated with the original formulation also containing soluble copper, peroxide compound and a cationic agent, cows in group C were treated with a modified formulation with reduced soluble copper, peroxide compound but increased levels of cationic agent and cows in group D were treated with a modified formulation containing levels of soluble copper and cationic agent similar to the original formulation but reduced concentrations of peroxide compounds. Results of this study (Shearer et al., 2000) indicated that the modified non-antibiotic formulation used on cows in group C appeared

to be the most effective treatment of papillomatous digital dermatitis compared to the other formulations as the proportion of cows with signs of pain was significantly lower in this group of cows (group C). Also this study reported an unexpected low efficacy of the oxytetracycline treatment suggesting the possible development of resistance in dairy cows affected with DD. This study supports the use of non-antibiotic products as an efficacious tool for the treatment of DD, thus minimizing the potential risk for residues in milk and meat.

Control measures of biosecurity, as stated in a previous study (Wells et al., 1999), includes examination of animals entering the herd, cleaning hoof trimming equipment between cows, and to have a farm-set of trimming tools available for the hoof trimmer to avoid contamination incoming from other farms. Footbaths can be used as a part of control measures of DD on infected herds (formalin 5%; copper sulfate 2.5%; oxytetracycline 1 to 4 g/L; zinc sulfate 20%) (Blowey et al., 1988; Brizzi, 1993; Mortellaro, 1994; Guard, 1995). If no preventive herd measures are taken, a relapse may be expected within 5 to 7 weeks after a successful single topical treatment of DD (Berry et al., 1999a) (Guard and Williams, 1995). Although the efficacy of footbaths remains controversial, individual treatment of affected cows combined with the use of footbaths for the herd represent the most effective method of prevention (Mortellaro, 1994).

Metabolic Hoof Horn Disease: Claw Horn Disruption

Hoof horn of low quality is a frequent cause of lameness in cattle. Studies in the UK reported that claw disorders accounted for 70 to 90% of diagnosed cases of lameness in dairy cattle (Whitaker et al., 1983; Clarkson et al., 1996; Murray et al., 1996). These researchers identified subclinical laminitis related disorders, such as sole ulcers and white line disease, as the most important conditions affecting dairy cattle in the UK. Laminitis

was regarded by some authors (Bradley et al., 1989; Greenough and Vermunt, 1991) as an important predisposing factor in lameness caused by claw disorders such as sole ulcers, white line disease, and abscesses in the subsole. Subclinical laminitis has been identified as the underlying cause of abnormalities of hoof horn formation which results in claw disorders (Hoblet et al., 2001). To better understand the pathophysiology of lameness, it is important to review the anatomy of the bovine foot.

Laminitis

Laminitis, or *pododermatitis aseptica diffusa,* is an aseptic inflammation of the dermal layers of the claw (Nielsson, 1963) characterized by defective claw horn production with thrombosis and hemorrhages in the digital corium (Mortensen, 1994). It was originally described as consisting of three clinical forms (acute, subacute, chronic), but Peterse (1979) later described a fourth form of laminitis, "subclinical laminitis". Because the disease process finally affects horn formation at the cellular level, regardless of whether an initial primary inflammatory response had occurred, the term claw horn disruption has been proposed (Logue et al., 1998). Although many different terms have been proposed for this disorder, I will refer to it as laminitis for the purpose of this discussion.

Laminitis has a multifactorial etiology and is thought to be associated with several, largely interdependent factors such as genetic predisposition, claw size, body weight, architecture of limb angles, claw hardness, pigmentation of the claw and the quality of the surface over which the animal walks (Mortensen, 1994; Nordlund and Garret, 1994). Nutritional management has been identified as a key component in the development of laminitis, particularly the feeding of increased fermentable carbohydrates which result in an acidotic state. There seems to be no doubt that the disease is related to

high energy intake, frequency and quantity of consumption. Factors such as body condition, body weight, and feet and leg structure, can unnaturally increase the weight load and stress on feet and exacerbate the internal mechanical damage that is associated with laminitis (Nocek, 1997).

Nocek (1997) has described the mechanisms causing development of laminitis in detail on a recent review of the topic. The mechanistic phases of laminitic development were described as alternating stages of disturbances relating to metabolic and subsequent mechanical degradation of the internal foot structure (Mortensen, 1994; Nordlund and Garret, 1994). The process can be segmented into various phases (Nocek, 1997).

Phase 1. The initial activation phase of laminitis, phase 1, is associated with a systemic metabolic insult. This phase is a result of ruminal acidosis, and subsequently an altered systemic pH. The reduction in systemic pH activates a vasoactive mechanism that increases digital pulse and total blood flow. Depending upon the insult that initiates the process, endotoxins, histamine and lactate can be released, which create increased vascular constriction and dilation and, in turn, cause the development of several unphysiological arteriovenous (AV) shunts, further increasing blood pressure. The increased blood pressure causes seepage of serum through vessel walls, which ultimately are damaged. Damaged vessels then exude serum, which results in edema, internal hemorrhaging of the solar corium from thrombosis, and ultimately expansion of the corium, causing severe pain.

Phase 2. As a result of the initial insult, there is mechanical damage, phase 2, which is associated with the vascular system. Once vascular edema has occurred, ischemia results in hypoxemia of the local internal digital tissue causing tissue hypoxia

which results in fewer nutrients and less oxygen reaching the epidermal cells. Ischemia itself can trigger a further increase in AV shunting. Trauma and stress can increase AV shunting. As a result of previous events, increased blood pressure further increases vascular seepage in the lower part of the digit as well as edema and ischemia. This cycle continues as long as the initial insult continues.

Phase 3. In phase 3, as a result of the mechanical damage associated with microvasculature and fewer nutrients provided to the epidermal cells, the stratum germinativum in the epidermis breaks down. These events ultimately cause corium degeneration and breakdown of the laminar region associated with the dermal-epidermal junction.

Phase 4. Ultimately, in phase 4, local mechanical damage occurs. A situation develops in which the epidermal junction is broken down which results in the separation of the strata germinativum and corium. This separation results in a breakdown between the dorsal and lateral laminar supports of the hoof tissue. Ultimately, the laminar layer separates, and P3 takes on a different configuration in relationship to its position in the corium and dorsal wall. As the bone shifts in position it causes a compression of the soft tissue between the bone and sole which is extremely susceptible to damage. The compression of this soft tissue results in hemorrhage, thrombosis, and further enhancement of edema and ischemia which result in a necrotic area within the solar region of the foot. Small areas of scar tissue accumulate because of the necrotic process. Once this process is triggered, continued potential for tissue degeneration persists because cellular debris is incorporated into the cellular matrix and the production and integrity of new horn tissue layers are hindered. Ultimately, a variety of processes can

occur as a result of the incorporation of scar tissue intervention, which includes double sole phenomenon, sole hemorrhages (red blood patches), bruises, white line lesions, and sole ulcers (Vermunt, 1994).

Forms of laminitis

Acute and subacute laminitis. In the acute and subacute stage of the disease, an aseptic inflammation of the corium coincides with a systemically sick animal. At this stage, the claw horn shows few, if any, visible changes. Vessel seepege, edema of capillary beds, and AV shunting are all initiated. Vascular congestion is present. The major clinical sign in addition to pain includes swelling and temperatures that are slightly warmer than normal above the coronary band in the soft tissue (Nocek, 1997). These forms of laminitis are prone to recurrence at varying intervals and often progress to the chronic form (Vermunt, 1994).

Chronic laminitis. Chronic laminitis has no systemic symptoms and changes are localized to the claw. A disturbed horn growth pattern and an alteration in the shape of the claw with an elongated flattened and broadened sole are characteristic (Vermunt, 1994; Greenough, 1997). Internally P3 has separated from the dorsal aspect of the wall. Continued ischemia results in destruction of the capillary beds and development of AV shunts. Cellular destruction results in separation of the dermal-epidermal junction, and internal foot destruction (Nocek, 1997). Grooves and ridges caused by irregular episodes of horn growth can be seen in the claw wall. This deformation of the claw shape often predisposes to sole ulcers, white line disease or subsolar abscesses (Greenough and Vermunt, 1991; Mortensen, 1994).

Subclinical laminitis. Subclinical laminitis was first described in 1979 by Peterse and later by others (Bradley et al., 1989; Greenough and Vermunt, 1991). Lameness is

usually not observed with this form of laminitis, but changes in the hooves can lead to chronic laminitis. The horn becomes softer, discolored, and waxy in appearance. It often stains yellow and hemorrhages can be seen in the weight-bearing surface of the claw, in particular the white zone, apex of the sole and the axial side of the sole-bulb junction (Bradley et al., 1989; Greenough and Vermunt, 1991). Internally, ischemia, hypoxia and epidermal damage are key aspects associated with this stage (Nocek, 1997). The concept of subclinical lameness is now universally accepted (Mortensen, 1994).

Claw Lesions Associated with Laminitis

Hemorrhages of the sole and sole ulcer

Hemorrhages in the sole are the major and characteristic indication of past laminitic insults. The hemorrhages can take the form of a slight pink tinge, a pronounced brush stroke of red coloration, or a dark solid red stain. Hemorrhages of the sole are considered part of the same pathologic process as sole ulcers and are represented by a continuum that ranges, from barely perceptible hemorrhages to severe ulceration of the sole with exposed corium (Leach et al., 1997). Sole hemorrhages have a particularly high incidence in first lactation heifers managed in confinement in the interval from 60 to 100 days after calving (Greenough and Vermunt, 1991; Smilie et al., 1996). The most common spot for sole hemorrhages and sole ulcers is the so-called typical spot of the lateral claw of the hind limb under the flexor tuberosity (axial prominence) of P3 (Smilie et al., 1996).

Softening of the horn of the sole

Although objective evidence is not available, it has been proposed that sole horn produced after an episode of laminitis is softer than normal (Mortensen, 1994).

White line disease

There are a number of names for the lesions in the White Line (WL): WL disease, WL abscess, WL separation, WL fissure, WL lesions, widening of the WL, WL hemorrhages (Bergsten, 2000; Blowey, 1993; Kempson and Logue, 1993; Leach, 1997). These names describe clinical signs or morphological changes in the WL. Different names are descriptions of different stages of development and degree of diseases whose pathogenesis has a common origin (Mülling, 2002). This condition is also considered to be associated with laminitis (Mortensen, 1994).

Heel erosion

Heel erosion has been described as one of the infectious diseases affecting the bovine foot in this manuscript, but there are some authors that also relate this disease with laminitis (Mortensen, 1994).

Diagnosis of Lameness

Several locomotion scoring systems have been developed to standardize gait analysis in cattle. Manson and Leaver (1988) devised a system of locomotion scoring which is a subjective assessment based on observation of cows walking away from the observer on a level concrete surface (Table 2-4). Using this scoring system, cows classified with a score of 3 or higher were counted as lame for calculation of prevalence of lameness. Two problems with this system were its subjectivity and complexity (Ward, 1998). Collick (1989) used this system and factored percentage reduction of weight bearing for the affected foot, foot structure affected and duration of lameness, which made this system more complicated.

Table 2-4. Locomotion scoring developed by Manson and Leaver (1988)

Score	Description
1.0	Minimal abduction/adduction, no unevenness of gait, no tenderness
1.5	Slight abduction/adduction, no unevenness or tenderness
2.0	Abduction/adduction present, uneven gait, perhaps tender
2.5	Abduction/adduction present, uneven gait, tenderness of feet
3.0	Slight lameness, not affecting behavior
3.5	Obvious lameness, some difficulty in turning, not affecting behavior
4.0	Obvious lameness, difficulty in turning, behavior pattern affected
4.5	Some difficulty in rising, difficulty in walking, behavior affected
5.0	Extreme difficulty rising, difficulty walking, adverse effect on behavior

Wells (1993) described a similar but different locomotion scoring system (Table 2-5) arguing that simplicity was necessary when using this type of scoring. This system was used to estimate the prevalence of lameness in 17 dairies in Wisconsin and Minnesota and cows with a score of 2 or higher were classified as lame

Table 2-5. Locomotion scoring used described by Wells (1993)

Score	Gait abnormality	Description
0	None	Gait abnormality not visible at walk; not reluctant to walk.
1	Mild	Mild variation from normal gait at walk; includes intermittent mild gait asymmetry or mild bilateral or quadrilateral restriction in free movement.
2	Moderate	Moderate and consistent gait asymmetry or symmetric gait abnormality, but able to walk
3	Sever	Marked gait asymmetry or severe symmetric abnormality
4	Nonambulatory	Recumbent

This system was used to estimate the prevalence of lameness in 17 dairies in Wisconsin and Minnesota and cows with a score of 2 or higher were classified as lame. In 1997, Boelling (1998) used the locomotion system developed by Manson and Leaver (1988) and described it using a 9 point-scale, from 1 (perfect walk) to 9 (near inability to walk). A score of 1 to 4 was considered sound locomotion, while a score of 5 or higher was regarded as clinical lameness. They used this to estimate the heritability of locomotion, which they found to be 0.06 to 0.11. Sprecher (1997) based on an

observation of Morrow in 1966, where an arched-back posture was associated with acute and chronic laminitis, developed a simple locomotion scoring system. He developed this method of classification of lameness to try to predict future reproductive performance and culling risk of cows classified within different scores. This locomotion scoring system was based on gait and back posture (Table 2-6).

If used consistently, all these scoring systems are useful to screen cows and identify early lesions associated with lameness. Early intervention prevents the more serious stages of a claw disorder (Toussaint Raven, 1989).

Table 2-6. Locomotion scoring system developed by Sprecher et al., 1997

Locomotion score	Clinical description	Assessment criteria
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
3	Moderately lame	An 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
5	Severely lame	The cow additionally demonstrates an inability or extreme reluctance to bear weight on one or more of her limbs/feet

If used consistently, all these scoring systems are useful to screen cows and identify early lesions associated with lameness. Early intervention prevents the more serious stages of a claw disorder (Toussaint Raven, 1989).

On the basis of differences in anatomic location and morphologic characteristics, it is possible to make a clinical diagnosis of interdigital phlegmon, digital dermatitis, or claw lesions in cows affected with lameness. Lame cows with interdigital phlegmon will be cows characterized by fissuring, caseous necrosis of the subcutis in the interdigital

space, and swelling of the entire foot above the dewclaws and separation of the digits. Pain and moderate to severe lameness are often seen with this disease. A characteristic fetid odor is usually present (Berry, 2001). Lame cows with digital dermatitis will have demarcated lesions, circumscribed, spherical to oval, 0.5cm to 6cm across, partially or completely alopecic, moist, painful-to-touch, prone-to-bleed plaques of flat or raised proliferative tissue on the interdigital cleft, heels, or dewclaw (Read and Walker, 1994). Lame cows with claw lesions will be cows that have white line lesions, abscess, or sole ulcers and will be treated by use of corrective foot trimming techniques (Shearer and van Amstel, 2001).

Lameness and Animal Welfare

Because of the pain, discomfort, and high incidence of lameness in dairy cows, lameness is an animal welfare issue. Disturbed claw health is an unequivocal source of suffering for cows, because the disorder is usually long term and painful (Alban, 1995). Some countries are already setting acceptable levels of clinical lameness. As an example, the Dutch Advisory Board for Animal Affairs (RDA) has considered the actual levels of clinical and subclinical claw disorders (30% cow cases per year) in The Netherlands as unacceptable from an animal welfare point of view (Somers et al., 2003). In other countries as well, the prevalence of lameness has also stated as not acceptable and have give rise to a growing concern about animal welfare.

Welfare can be defined as the state of animals regarding their attempts to cope with their environment (Broom, 1988). A lame cow is less able to cope with her environment, as pain might seriously affect walking and other movements (Hassall et al., 1993). The secondary effects of a reduced ability to walk may impact important physiological activities such as reduction in the time feeding (Hassall et al., 1993;

Galindo et al., 2002), also affecting their behavior as suggested by Peeler and Esslemont (1994), were lame cows experienced similar inhibitions as cows on poor footing and would less likely be observed in estrus. These welfare factors are important to consider when evaluating the effects of lameness disease on production.

In most US dairies, incidence of lameness is underestimated because only cows affected with severe signs of lameness are detected and treated; cows with mild or moderate signs of lameness are often not diagnosed. In a study by Wells (1993) the prevalence of lameness diagnosed by farmers in 17 dairies was compared to that by researchers in the same farms. The prevalence of lameness reported by researchers was three times higher than that by farmers. The underestimation of prevalence of lameness keeps farmers and the industry unaware of the importance of this disease.

Lameness and Milk Production

Several studies have been carried out around the world to test the effect of lameness on milk production. Results of these studies are conflicting. Some authors reported a decrease in milk yield after diagnosis of lameness (Whitaker et al., 1983; Tranter and Morris, 1991; Rajala-Schultz et al., 1999; Warnick et al., 2001), a decrease in milk yield before and after treatment (Lucey et al., 1986; Green et al., 2002) or no change in milk yield (Cobo-Abreu et al., 1979). Another study (Barkema et al., 1994) reported an increase in milk yield from 100 to 270 DIM during the same lactation in lame cows with sole ulcers. Green (2002) reported an increase in 100-day cumulative milk volume in the previous lactation for cows with any cause of lameness. Culling bias may in part account for these results because cows with both lameness and low production would be expected to be culled more often than cows with lameness and high production. Argáez-Rodríguez (1997) did a retrospective study a in Mexico to examine the effects of digital dermatitis in

milk production. The authors (Argáez-Rodríguez et al., 1997) reported a statistically non-significant difference between milk production of lame cows due to DD and healthy cows, with cows experiencing DD producing less milk. In this study lame cows due to DD were compared with healthy cows and lame cows for other reasons were included as healthy which can mask the effects of DD on milk production.

Three studies have examined the relationship between lameness and milk yield in US dairy herds. In a study conducted on a 500-cow dairy in California (Deluyker et al., 1991), cows diagnosed as lame during the first 49 days postpartum coincided with higher milk yield. The positive association of lameness and high milk yield during early lactation found in this study suggested that high yield was a risk factor for lameness. Although diseases or foot lesions associated with lameness were not investigated, white line and sole lesions were the most common lesions in this study. In another study conducted on two dairy herds in New York (Warnick et al., 2001), lame cows with claw lesions or interdigital phlegmon produced less milk than healthy cows. Lameness was more common in early lactation and more likely to occur in older cows. Finally, in a study conducted in Florida, lame cows with interdigital phlegmon produced 10% less milk, compared to non-lame cows (Hernandez et al., 2002). The authors (Hernandez et al., 2002) estimated that such a decrease in milk yield of 1,885 lb/cow represented a loss of $301/cow (assuming a milk value of $16.00/100 lb). In that study, most lame cows with interdigital phlegmon were affected during early lactation (within 100 days postpartum), when cows reach peak yields and 60% were culled during lactation. These findings may suggest that lame cows affected with interdigital phlegmon during early

lactation may be sufficiently compromised to adversely affect a cow's ability to achieve its own milk yield potential during the current lactation (Hernandez et al., 2002).

Lameness and Reproductive Performance

A relationship between lameness and reproductive performance has been established in several studies in the United States and in other parts of the world. In 1985, Weaver postulated lameness as a possible cause of reduced fertility because animals spend more time lying down, are less willing to demonstrate standing heat, and are less able to compete for available feed. Some of these suggestions were later confirmed by different studies. Varner (1994) looking at pedometer readings, reported that cows in estrus move and interact with other cows significantly more than the rest of the herd.

Britt (1986) found that excellent footing greatly increases the duration of estrus activity in dairy cows. Peeler and Esslemont (1994) reported that lame cows experience similar inhibitions as cows on poor footing and would less likely be observed in estrus. These findings may in part explain results of studies indicating that lameness has a detrimental effect on reproductive performance. In a study conducted on five dairy farms in the UK, Lucey (1986) found that lame cows affected with sole ulcer and white line disease between 36 and 70 days after calving were associated with longer calving to conception intervals (17 and 30 days, respectively). Collick (1989) did a larger study on 17 dairy farms in the UK involving 427 cases of lameness. The authors reported that lameness happening before 120 days after calving was associated with significantly increased intervals from calving to conception. The largest increase in the intervals from calving to conception were associated with sole ulceration (40 days, $P < 0.01$). In a retrospective study of digital dermatitis in a commercial dairy in Mexico Argáez-Rodríguez (1997) reported that healthy cows conceived 93 days after calving (median),

compared to affected cows with digital dermatitis which conceived 113 days after calving ($P < 0.01$).

Three previous studies have examined the relationship between lameness and reproductive performance in US dairy herds. In one study conducted in five dairy herds in Pennsylvania, cows affected with lameness had a 28-day-longer calving to conception interval, compared to healthy cows (Lee et al., 1989). In another study, a scoring system was developed to identify lameness and predict future reproductive performance in dairy cows, lame cows were 15.6 times more likely to require an interval greater than the mean for days open compared to healthy cows (Sprecher et al., 1997). In a study conducted on a 500-cow dairy in Florida (Hernandez et al., 2001), claw lesions were the most important cause of lameness and impaired reproductive performance in dairy cows, as indicated by a higher incidence of affected cows, a greater time from calving to conception (median, 140 days), and a higher number of services required per conception (median, 5), compared to non-lame cows (100 days and 3 services, respectively). In this study herd, cows were synchronized and time-inseminated; thus the authors were not able to assess the calving to first breeding interval nor to draw any conclusion that lameness has an effect on estrus behavior. Conversely, the significantly higher number of services required per conception and the longer time from calving to conception in lame cows with claw lesions, compared to healthy cows, may suggest that lameness has an effect on conception.

Melendez (2003) examined the association between lameness, ovarian cysts, and fertility on a 3000-cow dairy in Florida. Results of this study showed that cows that became lame within the first 30 days postpartum were associated with higher incidence

of ovarian cysts, a lower likelihood of pregnancy, and a lower fertility than non-lame cows. Although all these studies found a significant association between lameness and reproductive performance, the relationship between lameness and ovarian activity was not investigated.

Resumption of Ovarian Activity Postpartum

Early resumption of ovarian cyclicity postpartum is important for high reproductive efficiency in dairy cows. Delays in the commencement of ovarian cyclicity and estrous expression are associated with reduced conception rates, pregnancy rates and an increased interval from calving to conception (Thatcher and Wilcox, 1973; Stevenson and Call, 1983; Lucy et al., 1992; Senatore et al., 1996). The study done by Thatcher and Wilcox, (1973) reported that cows exhibiting 0 or 1 heat postpartum required significantly more services per conception than cows exhibiting 2 to 4 heats. These authors reported that non-return rates improved as frequency of postpartum heats increased (0 and 1 heat, 37%; 2 to 4 heats 44%; $P < 0.05$). In a study conducted to identify the influence of early estrus, ovulation and insemination on fertility in postpartum dairy cows, Stevenson and Call (1983) reported that the interval to first detected heat had a significant influence on first service intervals and days open. When estrus was not expressed before 60 days postpartum, average days to first service were 18 days longer and days open were 19 days longer than in cows that expressed heat before 60 days postpartum ($P < 0.05$). These authors concluded that more expressed heats early postpartum were associated with positive effects on fertility.

A study conducted by Lucy (1992) who monitored the interval to first ovulation to clarify the importance of milk production, dry-matter intake and energy balance in the interval to first ovulation, reported that cows having first ovulation before 42 days tended

to have a shorter interval from calving to detected estrus, required fewer services per conception and had a shorter interval from calving to conception compared with cows having first ovulation after 42 days postpartum. More recently, Darwash (1997) reported that the interval to postpartum commencement of luteal activity was correlated favorably with measures of fertility such that for every day delay in the interval to commencement of ovarian activity, there was an average delay of 0.24 and 0.41 ($P < 0.001$) days in the interval to first service and conception respectively. These studies pointed out the importance of early resumption of ovarian cyclicity postpartum as a factor contributing to high reproductive efficiency on dairy cows.

Following calving, the reproductive strategy of the cow is transformed from delivering and nourishing a healthy calf to reestablishing pregnancy. The dormancy of ovarian follicular development that prevailed during late pregnancy must now be replaced by a sequence of events culminating with behavioral estrus, ovulation of healthy follicles and normal luteal function. These are the requirements for successful reproductive performance in any type of cattle production system (Rhodes et al., 2003).

After regression of the corpus luteum of pregnancy, there is a variable anovulatory period before first ovulation takes place (Savio et al., 1990a). This period is characterized by an absence of estrus behaviour and lack of progesterone secretion by the ovary and a return to basal concentrations of estradiol during the first week postpartum (Echternkamp and Hansel, 1973; Stevenson and Britt, 1979; Webb et al., 1980; Humphrey et al., 1983; Peters, 1984; Savio et al., 1990a; Rhodes et al., 2002). Following parturition, a wave of follicular development occurs in 5 to 7 days regardless of negative energy balance and in response to an elevation in plasma FSH concentrations (Beam and

Butler, 1997). As reported by Savio (1990a), ovarian follicular turnover starts early after calving and is similar to that observed during normal estrous cycles. In this study Savio (1990a) reported that the postpartum interval to the detection of the first dominant follicle was 11.6 ± 8.9 days and the interval to first ovulation was 27.4 ± 23 days. Beam and Butler (1997) reported that the emergence of the first follicular wave postpartum occurred after a peak in mean peripheral FSH levels and rather synchronously with the clearance of gestational estradiol from blood. Subsequent, mean levels of FSH increased in the first 5 days postpartum and decreased from 5 to 11 days. This is in accordance with previous work (Butler et al., 1983; Price and Webb, 1988) that indicated that removal of estradiol negative feedback inhibition of FSH release would account for the increase in mean plasma FSH observed between days 2 and 9 postpartum. After returning to basal levels, estradiol concentrations fluctuate or remain low until 2 to 3 days before estrus when they peak (Echternkamp and Hansel, 1973; Stevenson and Britt, 1979). Estradiol levels declined from the 1st day postpartum until day 7, when estradiol levels slowly increase with the concurrent development of a dominant ovarian follicle (Beam and Butler, 1997). Plasma estradiol concentration is related with the degree of the peak of LH release in response to GnRH as reported by Zolman (1974); Kesler (1977); and Fernandes (1978). These studies agreed with that of Moss (1985) who reported that pituitary responsiveness to GnRH is not restored until approximately 8 to 10 days post-partum. As described by Savio (1990a), follicular growth is accompanied by episodic LH secretion of variable amplitude and frequency. The use of ultrasound techniques allowed Savio (1990a) to relate the stage of follicular development and the pulsatile secretion of LH. Within a 6-hour period, 2 to3 LH pulses occurred when concentrations of estradiol were low (< 5

pg/mL), and the frequency of LH pulses increased to 6 when estradiol concentrations increased (> 10 pg/mL) coinciding with a dominant follicle. Stevenson and Britt (1979) reported that the interval from calving to first postpartum ovulation was associated inversely with the number of episodic LH surges and magnitude of the largest LH surge. This high frequency mode of pulsatile LH secretion has been identified as necessary for the final phase of maturation of ovarian follicles and thus induction of estrus and ovulation (Webb et al., 1980; Humphrey et al., 1983; Randel, 1990). Canfield and Butler (1990a) reported a high correlation between the interval from parturition to the highest LH pulse frequencies and first ovulation, emphasizes the importance of achieving this pattern of secretion for the stimulation of first ovulation, support this.

These series of events can lead to three different outcomes of follicular development as described by Beam and Butler (1997):

a. Ovulation of the first dominant follicle;
b. Non-ovulation of the first dominant follicle followed by turnover and a new follicular wave;
c. The dominant follicle fails to ovulate and becomes cystic.

The development of non-ovulatory dominant or cystic follicles prolongs the interval from calving to first ovulation. In dairy cattle, the interval from calving to first ovulation has been reported to be between 17 and 34 days (Table 2-7) The variation observed between some of these studies may be due to differences in study populations such as animal breeds, production systems, level of production, feeding systems, or it might be as concluded by Opsomer (2000) that the first postpartum ovulation in modern high-yielding dairy cows tends to occur later than it did a decade ago. Results of these studies are difficult to compare since mean days to first ovulation were calculated only

for normal cows in some of these studies, which excluded cows that had follicular cysts or those who did not ovulate early postpartum; while in other studies, all cows were in the calculations.

Table 2-7. Days from calving to 1st ovulation reported in the literature

Author, year	Days to 1st postpartum ovulation (d)
Schams (1978)	17
Stevenson (1979)	18
Webb (1980)	17
Butler (1981)	36
Stevenson (1983)	19
Fonseca (1983)	20
Meisterling (1987)	33 Short luteal phase 25 Normal luteal phase
Butler (1989)	30
Canfield (1990)	19
Harrison (1990)	29
Staples (1990)	22
Savio (1990)	22
Spicer (1990)	24
Canfield (1990)	29
Etherington (1991)	24
Nakao (1992)	30
Zurek (1995)	24
Darwash (1997)	22
Opsomer (2000)	32
Reist (2000)	34

If ovulation occurs then plasma progesterone concentrations increase to greater than 1ng/mL within 2 to 3 days after ovulation (Schams et al., 1978; Stevenson and Britt, 1979). An elevation of plasma Progesterone concentration above 1 ng/mL has been used as an indication of resumption of postpartum ovarian cyclicity in several studies (Butler et al., 1981; Harrison et al., 1990; Staples et al., 1990; Canfield et al., 1991; Zurek et al., 1995; Beam and Butler, 1997; Beam et al., 1998)

The duration of the first postpartum estrous cycle can be variable, being shorter than normal or normal, and it can occur with or without estrus behavior. Stevenson and

Britt (1979) reported 32% of first estrous cycles were shorter (16 d vs. 20 d) than subsequent estrous cycles. These observations have been supported by different studies (Schams et al., 1978; Webb et al., 1980; Stevenson and Call, 1983; Fonseca et al., 1983; Savio et al., 1990b; Staples et al., 1990; Senatore et al., 1996).

Among dairy cows, those that have not ovulated by 60 days post partum have been defined as having a delayed resumption of ovarian cyclicity (Humboldt and Thibier, 1980; Stevenson and Call, 1983; Staples et al., 1990; Moreira et al., 2001). Incidence rates of delayed cyclicity reported in the literature are summarized in Table 2-8.

Table 2-8. Incidence rates of delayed cyclicity reported in the literature

Author, year	Delayed cyclicity	Milk/Plasma	Incidence of delayed cyclicity (%)
Humboldt (1980)	P4 ≥ 1.5 ng/mL 60d	Plasma	29
Bartlett (1987)	Palpation 70d		23
Miesterling (1987)	P4 ≥ 4 ng/mL 65d	Milk	21
Archbald (1990)	Presence of CL and P4 > 1ng/mL 1st month PP	Plasma	30
Nakao (1990)	P4 < 1 ng/mL 50d	Milk	25
Staples (1990)	P4 < 1 ng/mL 63d	Plasma	28
Etherington (1991)	P4 ≥ 2 ng/mL 50d	Milk	33
Lamming (1998)	P4 > 3 ng/mL 45d	Milk	11
Opsomer (2000)	P4 < 15 ng/mL 50d	Milk	21
Moreira (2001)	P4 ≤ 1 ng/mL 63d	Plasma	23

Several factors can affect the interval between calving and first postpartum ovulation, the incidence rate of delayed cyclicity, and the reproductive performance of affected cows. Some of theses factors cited in the literature are energy balance, season, parity, and periparturient diseases (Butler et al., 1981; Fonseca et al., 1983; Canfield et al., 1990b; Lucy et al., 1992; Senatore et al., 1996; Beam and Butler, 1997; Darwash at al., 1997; Jonsson et al., 1997; Opsomer et al., 2000; Moreira et al., 2001).

Several studies have found associations relating energy balance to ovarian activity postpartum, and some of these studies have proposed a casual path to this association. However, the effects of energy balance affecting postpartum ovarian activity are not completely elucidated. Milk production and dry matter intake increase after calving but at different rates with the maximum feed intake occurring some weeks after maximum milk production. The result of this delay is negative energy balance that persists for 4 to 12 weeks of lactation (Butler at al., 1981), when most dairy cows must mobilize body reserves to support milk production (Bauman and Currie, 1980). Negative energy balance is usually maximal during the first 3 weeks of lactation (Canfield et al., 1990b). Butler (1981) reported that first ovulation occurred 10 days after the negative energy balance nadir was reached, and energy balance was still negative at ovulation, but was returning towards zero. The authors (Canfield et al., 1990b) concluded that there was an inverse relationship between the interval from parturition to first normal ovulation and the average energy balance during the first 20 days postpartum. These results were further supported by other studies (Staples et al., 1990; Canfield and Butler, 1990a; Lucy et al., 1992; Senatore et al., 1996).

The greater the average energy deficit incurred, the longer the delay to ovulation. Canfield and Butler (1990b) showed a high correlation between days to negative energy balance nadir and days to first ovulation. This relationship suggested that first ovulation does not occur in an individual animal until energy balance progresses beyond its most negative value and is returning toward balance. In this study, 1st ovulation also occurred 10 and 13 days following negative energy balance nadir in non-lactating and lactating cows respectively. It was proposed from this study that energy status could be acting to

slow an increasing LH pulse frequency until the cow has begun to return to a positive energy balance. Cows that reached the energy balance nadir earlier postpartum (4 d vs. 14 d) ovulated earlier (14 d vs. 27 d) and had higher concentrations of insulin. These results, together with the finding that the pulse frequency of LH was not different for both groups, made the authors propose that insulin levels may act permissively on the ovary to enhance follicular responsiveness. These same authors (Canfield and Butler, 1990a) examined the effect of energy balance and changes in plasma concentrations of glucose, insulin, non-esterified fatty acids (NEFA), and ketones on pulsatile LH secretion in early postpartum period. They reported a direct relationship between postpartum energy balance and first ovulation, and between negative energy balance nadir and changes in pulsatile LH secretion, suggesting that as negative energy balance reaches it's nadir and starts returning towards balance, LH secretion is disinhibited and first ovulation occurs.

The relationship between energy balance and first ovulation was further supported by different studies. A study by Staples (1990) examined the relationship between ovarian activity and energy status during the early postpartum period. In this study, cows were classified according to the time of first ovulation as early responders (resume ovarian activity within 40 days postpartum), late responders (resume ovarian activity between 40 and 60 days postpartum), and no responders (resumption of ovarian activity after 63 days PP). These authors concluded that the early and late responders cows were in less negative energy balance than non-responders and were able to restore ovarian activity during the first 63 days postpartum. On the other hand, the non-responder cows did not have the capability to consume as much dietary energy, produced less milk and were more dependent on energy from body reserves to produce milk. As a consequence,

metabolic status inhibited the initiation of postpartum ovarian activity in cows during the first 63 days postpartum. In addition, an increased loss of body weight during the first 2 weeks of lactation coincided with decreased ovarian activity; early responder cows losing the least weight and non-responder cows losing the most weight.

Lucy (1992) examined the influence of diet composition, dry-matter intake, milk production, and energy balance on time to post-partum ovulation. Results of this study confirmed those of Staples (1990) where the interval to first ovulation was shorter in cows that consumed more dry-matter and produced more milk. In contrast, low milk producing cows consumed less dry-matter and were more likely to be classified as late responders. These two studies suggested that the fact that higher producing cows ovulating earlier than low producing cows did not contradict the effects of energy balance on the interval to first ovulation, as higher producing cows can be in a less negative energy balance and thus ovulate earlier. It was also reported that cows ovulating earlier (before day 42 postpartum) had a superior reproductive performance. Also results from the study done by Lucy (1992) are in agreement with those of Fonseca (1983) where Jersey cows producing more milk ovulated earlier than lower producing herdmates.

These two studies concluded that the data do not support linear relationships between days to first estrus, days to first insemination, and days open with increasing milk yield as suggested by others. They proposed that this contradiction occurred because lowest producing cows in commercial herds are culled before they have the opportunity to express their reproductive potential. As stated by Staples (1990) and supported by several studies (Fonseca et al., 1983; Harrison et al., 1990; Lucy et al., 1992), if energy status of the cow is more important than milk yield in determining return to estrus, then

milk yield alone may be either positively or negatively correlated with days to first ovulation. Although an association between energy balance and postpartum ovarian activity has been clearly established, the causal path remains unclear. Detrimental effects of negative energy balance on ovarian activity could be due to effects on any of the components of the reproductive endocrine axis.

Physiological Factors Involved in Ovarian Activity Potentially Affected by Energy Balance

Effects of negative energy balance in LH secretion

It has been already mentioned the importance of a high frequency mode of pulsatile LH secretion in the final phase of maturation and ovulation of ovarian follicles. Although pituitary content of gonadotropins increase rapidly after calving and are capable of supporting ovulation by day 8 to 10 postpartum (Kesler et al., 1977; Fernandes et al., 1978; Moss et al., 1985), however, pulsatile LH secretion capable of inducing ovulation generally occurs near 1st ovulation which usually takes place at 17 to 34 days (see table 2.4). There is strong evidence that secretion of LH is impaired in cows not recovering from negative energy balance. Peters (1985) reported that very few lactating cows ovulated in response to pulsatile administration of LHRH when delivered at a time when animals were in a negative energy balance.

Canfield (1988) compared LH secretion at 2 weeks postpartum and again at the energy balance nadir (during the return towards EB), and reported an increase in LH frequency and a decrease in pulse amplitude. These authors concluded that energy balance plays an important role in the control of first ovulation by suppressing LH pulse frequency following calving. Canfield and Butler (1990a) demonstrated a direct relationship between postpartum energy balance and 1st ovulation, and between negative

energy balance nadir and changes in pulsatile LH secretion. Therefore, it was concluded that as negative energy balance reaches it nadir and starts returning towards balance, LH secretion is disinhibited and 1st ovulation occurs. Canfield and Butler (1991) reported that dairy cows in a negative energy balance had similar LH patterns but ovulated later than cows in positive energy balance. In another study, increased energy balance was associated with increase pulse amplitude of LH secretion (Lucy et al., 1991). Schillo (1992), in a review of the topic proposed that, the reduction on the LH pulse frequency observed during negative energy balance, represents one of the most important means by which energy balance impairs reproductive activity in cattle. The above results are consistent with findings of Beam and Butler (1997) which reported that follicles emerging after the negative energy balance nadir, rather than before, exhibited greater growth and diameter, enhanced estradiol production, and were more likely to ovulate.

Metabolic hormones

There is evidence that metabolic hormones such as growth hormone, insulin, IGF-I, and leptin have important roles in the control of ovarian follicular development and are likely to be important mediators of the effects of dietary intake and energy balance on cow fertility.

Insulin –like growth factor-I. IGF-I and insulin are effectors of follicle cell function in vitro with stimulation of steroidogenesis and cell proliferation in granulosa and thecal cells (Monniaux et al., 1992; Spicer et al., 1993; Magoffin et al., 1993; Spicer et al., 1996). Insulin-like growth factor-1 (IGF-I) is decreased in postpartum cows when experiencing negative energy balance (Spicer et al., 1990; Beam and Butler, 1999) Cows in poor body condition or cows not recovering body condition during lactation have also been identified as having low blood IGF-I concentrations. Beam and Butler (1997)

reported that levels of plasma IGF-I averaged approximately 40% higher during the first 2 weeks postpartum in cows ovulating during the first follicular wave postpartum than in cows not ovulating. Plasma IGF-I in cows ovulating the 1st follicular wave was higher at day 1 postpartum, before the establishment of follicular dominance and subsequent increases in peripheral estradiol. Therefore, the authors suggested that higher IGF-I in ovulatory cows did not result from greater dominant follicle estradiol production, but preceded and possibly contributed to differences in follicular function (Beam and Butler, 1997). Results of this study also suggest that low concentrations of circulating IGF-I are related to low steroidogenic output of dominant ovarian follicles early post partum. Results of another study (Cohick et al., 1996) also suggested that changes in systemic levels of IGF-I and IGFBP affect their concentrations in follicular fluid and follicular development. Finally, Beam and Butler (1999) proposed that during the negative energy balance period, the ability of follicles to produce sufficient estradiol for ovulation seems to depend on the availability of insulin and IGF-I in serum and the changing energy balance profile.

Insulin. There is significant evidence that dietary restriction and negative energy balance reduce circulating concentrations of insulin (Vizcarra et al., 1998; Mackey et al., 2000). In vitro studies (Stewart et al., 1995) showed that insulin at physiological levels affected proliferation of bovine thecal cells, and acted synergistically with luteinizing hormone in stimulating steroidogenesis. Spicer (2001) reported that insulin by itself was a more effective stimulator of aromatase activity than FSH in vitro. Beam and Butler (1997) reported a greater insulin:GH ratio during the first week postpartum in cows ovulating during the first follicular wave than those that did not, suggesting that levels of

insulin and GH during the very early stages of follicular recruitment may be important to later follicular function. Gong (2002) showed that dairy cows fed a diet that increased circulating concentrations of insulin during the first days 50 postpartum had shorter postpartum anestrus intervals, and an increased conception rate to first service independent of any effects on LH or FSH and without affecting milk yield or energy balance. The authors proposed that the increase in insulin concentrations promoted differentiation and maturation of dominant follicles during early lactation, thereby increasing the chance of these dominant follicles of ovulating in response to the LH surge. These results suggest that insulin may have a direct effect at the ovarian level.

Thyroid hormones (T_3 and T_4). Reist (2003) examining associations between postpartum reproductive function and metabolic status in high yielding cows, reported that cows with higher plasma levels of thyroid hormones (T_3 and T_4), were associated with early start of ovarian cycle; proposing that Thyroid hormones can also play an important role in the resumption of ovarian activity postpartum. These results are supported by previous findings of Spicer (2001) who provided evidence for a role of T_3 and T_4 in regulating steroidogenesis of bovine follicles. The author proposed that T_3 and T_4 may have a minor positive impact on FSH-induced progesterone production by bovine granulosa cells, and a major positive impact on LH-induced androstenedione production by bovine thecal cells, both of which would result in a net increase in estrogen production by the follicle, however, T_3 and T_4 have little or no direct effect on aromatase activity. This evidence supports a role of Thyroid hormones as part of a multihormonal complex regulating ovarian activity in cattle.

Leptin. Leptin is a protein hormone secreted by adipocytes (Bradley et al., 2000) and acts on the central nervous system to reduce voluntary feed intake (Schwartz et al., 2000). Block (2001) reported that postparturient cows undergoing negative energy balance had significantly lower plasma concentrations of leptin compared with postparturient cows in positive energy balance. In addition, at the first week of lactation the plasma concentration of leptin was correlated positively with plasma concentrations of glucose and insulin and negatively correlated with plasma concentrations of GH and NEFA. The author concluded that these correlations could represent a co-regulation by energy balance and these factors in mediating the effect of energy balance on leptin synthesis.

Leptin has diverse effects on the neuroendocrine axis in addition to appetite and body weight regulation (Ahima and Flier, 2000). Leptin stimulated gonadotropin release and inhibited insulin-like growth factor-mediated release of estradiol in ovarian follicular cells in rat ovarian granulosa cells (Zachow and Magoffin, 1997). Another study (Chehab et al., 1996) reported that correction of the sterility defect in homozygous (ob/ob) obese female mice could be accomplished by repeated administration of human recombinant leptin, resulting in ovulation, pregnancy and parturition. Williams (2002) reported that short term fasting of growing prepubertal heifers causes marked reductions in circulating leptin, concomitant with declines in LH pulse frequency, and serum concentrations of insulin and IGF-I. In this same study results could not be repeated for mature cows under the same short term fasting. Altogether, these results may indicate that leptin signals the adequacy of energy stores for reproduction, by interacting with different target organs in the hypothalamic-pituitary-gonadal axis in cattle and other species. It has been proposed

that the effects of leptin might be mediated in part by NPY, which in turn has been shown to regulate gonadotrophin release by inhibiting LH secretion in ewes (McShane et al., 1992).

NPY is a potent inhibitor of LH release and unlike leptin, is a potent stimulator of food intake (Houseknecht et al., 1998) Concentrations of NPY increase in cerebrospinal fluid during undernutrition and can negatively modulate the secretion of LH when centrally infused in cattle (Gazal et al., 1998). These results may stimulate future research to explore the role and potential interactions between hormones, neuropeptides and resumption of ovarian cyclicity under negative energy balance in postpartum dairy cattle.

It has been well established in cattle that ovarian function is controlled primarily by an integrated GnRH-gonadotrophin-ovarian axis. Recent work has shown that factors classically thought to be mainly involved in the regulation of metabolic processes, such as GH, insulin and IGF-I, thyroid hormones, leptin,and neuropeptide-Y may play an important role in the control of ovarian activity in the postpartum dairy cow (Spicer at al., 1995; Reist et al., 2003; Williams et al., 2002). Metabolic hormones can act either directly to control gonadotrophin –independent stages of follicle development (Gong et al., 1996), or in synergy with gonadotrophins to modulate follicular recruitment and final development and maturation of preovulatory follicles (Spicer et al., 1995; Armstrong et al., 2002). These effects could represent at least part of the mechanism underlying well documented but not completely understood nutritional influence on reproductive function in cattle.

Other factors

Season. The effect of season has been reported as having an effect on resumption of ovarian activity postpartum. In a study conducted in North Carolina, Fonseca (1983)

reported that cows calving in the winter had 6.5 more days to first ovulation compared with cows calving in the fall. Peters (1984) reported that cows calving in the spring underwent longer periods between calving and first ovulation than autumn calvers. Savio (1990a) reported that the postpartum interval to detection of the first dominant follicle was shorter in autumn than in the spring. When only normal dominant follicles were considered, the cows that calved in autumn tended to have a shorter, and less variable, intervals from calving to first ovulation. In Australia, cows calving in summer had significantly longer intervals from calving to first postpartum ovulation than those calving in winter (23d vs. 18d) (Jonsson et al., 1997). In addition, cows losing more bodyweight had longer intervals from calving to first ovulation. In Belgium, cows calving in the winter (stable housing) were more prone to delayed ovarian function compared to cows calving in the spring (pasture housing) (Opsomer et al., 2000). None of these studies proposed a path as to how season affects ovarian cyclicity. In Switzerland, Reist (2003) examined the relationship between reproductive function and metabolic and endocrine status in dairy cows. Results of this study showed a significant effect of season in resumption of ovarian activity. Cows calving in the fall were more likely to start ovarian cyclicity earlier postpartum than cows calving in the spring.

Parity. The data on the effect of parity on the interval to first ovulation is contradictory and does not support an effect of parity on resumption of ovarian cyclicity. Stevenson and Britt (1979) reported that interval to first ovulation tended to be longer for pluriparous than for primiparous cows (18.7 vs 16.3 days), but the interval to first estrus was not different between parity groups (26.1 vs 27.7 days). Fonseca (1983) reported that in cows between 33 and 60 months of age, younger cows had their first ovulation earlier

than older cows. In this study, there were very few observations in older cows. In contrast, Lucy (1992) reported that ovarian activity was delayed in primiparous cows compared with multiparous cows. However, primiparous cows were similar to multiparous cows with respect to first service, first service conception rate, services per conception, and days open. Moreira (2001) reported that the incidence of anestrus at 63 days was greater in primiparus than in multiparous cows.

Periparturient diseases. The effects of periparturient diseases in resumption of ovarian cyclicity in dairy cows have been reported. Fonseca (1983) reported that cows with abnormalities after calving had 8.8 more days to first ovulation than cows without abnormalities after calving. In this study the main abnormalities at parturition were retained placenta and milk fever in Holstein cows and Jersey cows, respectively. During the postpartum period, uterine infection was the most frequent clinical abnormality in Holsteins, while ovarian cysts ranked first (followed by injury or disease and uterine infection) in Jerseys. Opsomer (2000) conducted a study to identify risk factors associated with postpartum ovarian dysfunction in dairy cows. In this study, cows suffering from clinical diseases such as mastitis, severe lameness, or pneumonia during the first month of lactation, were 5 times more at risk of developing delayed resumption of ovarian activity than healthy cows. In addition, cows developing clinical symptoms of ketosis with a positive prussiate test were 11 times more at risk of delayed resumption of ovarian cyclicity than normal cows. Cows with abnormal calvings and abnormal vaginal discharges were 3.6 and 4.5 times more likely to develop delayed ovulation compared to cows with normal vaginal discharges respectively. Similar results were reported by

Etherington (1991) where cows with retained placenta were associated with longer intervals to first ovulation.

Loss of body condition early in the postpartum period was another factor increasing the risk of delayed cyclicity. Cows losing more in body condition were 19 and 11 times more at risk of delayed cyclicity at 30 days and 2 months after calving. Cows with normal progesterone profiles lost on average 0.26 points during the first month after calving and 0.29 points during the first 2 months after calving, while cows with delayed ovarian function lost 0.39 and 0.49 points, respectively (Opsomer et al., 2000). These results were in agreement with those of Moreira (2001), where an effect of body condition at 63 days was associated with the frequency of cows classified as anestrus. As body condition increases the incidence of anestrus decreases. In another study conducted by Mateus (2003) a relationship between endotoxin concentrations in blood from cows with endometritis and a prolonged anestrus period was observed. This result is in agreement with a previous study from Peter (1990) where intrauterine infusions of endotoxins reduced the preovulatory LH surge in cows. This could have been mediated by high cortisol levels and resulted in ovulation failure. Ketosis is another periparturient disorder related to delayed resumption of ovarian activity. Reist (2000) examined the relationship between ketone body concentration and first ovulation in dairy cows. Cows classified as late responders (cows with a first ovulation between 31 and 87 days postpartum) had higher blood and milk ketone bodies concentrations compared to early responders (cows with first ovulation within 30 days postpartum), with no significant differences in body condition scores between groups.

Lameness and Ovarian Activity

While several studies have shown a relationship between lameness and reproductive performance, the relationship between lameness and ovarian activity in dairy cows has not been investigated using objective research methods. To our knowledge, there is only one study that looked at the relationship between lameness and ovarian activity in dairy cows. This study was conducted in 335 dairy cows on six high producing dairy herds in Belgium. Cows diagnosed with clinical mastitis, severe lameness, or pneumonia by farmers were at higher risk of delayed cyclicity, compared to cows classified as clinically healthy (Opsomer et al., 2000); however, the actual number of cows affected with clinical mastitis, severe lameness, or pneumonia was not reported.

In Florida, clinical observations by veterinarians and dairy farmers suggest that cycle cows affected with lameness experience anestrus, and the duration of anestrus is associated with severity, duration, and diseases or lesions associated with lameness (eg, interdigital phlegmon, papillomatous digital dermatitis, claw lesions). We hypothesized that as lame cows experience a more pronounced loss in body condition (hence a prolonged state of negative energy balance) during the early post partum period, lame cows are at higher risk of delayed ovarian cyclicity than non-lame cows. Lame cows eat less and go into a negative energy state. In order to meet energy deficit, body reserves are mobilized resulting in body weight loss. The output of energy in lame cows (body maintenance and milk yield) may exceed the energy input in the form of feed. It is possible that cows that become lame are in a progressive negative energy state, go into anestrus and experience long delays to restore ovulation.

Results of previous studies suggest that as cows experience increasing positive energy status, there is increased ovarian follicle activity leading to early return to

ovulation (Butler et al., 1981, Staples et al., 1990; Lucy et al., 1991). As energy status becomes more positive for cows in early postpartum, diameter of the largest follicle increases, the number of double ovulations increases, and time for detection of the first corpus luteum decreases. (Lucy et al., 1991).These changes in follicle size and numbers and the number of ovulations are thought to be aroused by increases in luteinizing hormone (LH), follicle stimulating hormone (FSH), insulin, BST, insulin-like growth hormone-1 (IGF-1), and possibly other yet-to-be determined compounds as activated by improving energy status (Beam and Butler, 1998). Therefore, lameness may have an effect on feed intake and energy status leading to changes in concentrations of reproductive hormones. Under field conditions, evidence of corpus luteum function can be determined by monitoring plasma progesterone (P_4) concentrations weekly during lactation, before and after diagnosis of lameness in dairy cows.

CHAPTER 3
MATERIALS AND METHODS

Cows and Herd Management

Cows in this study were from a high-producing dairy herd (rolling herd average milk production, approx 12,000 kg) of approximately 600 Holstein cows located in Florida. Cows were milked and fed a TMR ration three times per day. Cows were housed in lots equipped with sprinklers, fans and shade cloth over the feed bunks to reduce the effects of heat stress. This herd was selected for study on the basis of a history of lameness, quality of veterinary records, and willingness of the owner to participate in the study.

Study Design

This study was designed as an observational cohort study. Sample size calculations were made on the basis of our estimate of the number of cows affected with delayed ovarian cyclicity increasing from 10% in non-lame cows to 30% in lame cows (type I error = 0.05; type II error = 0.20). A total of 253 (45%) of 563 Holstein cows identified with an even numbered ear-tag that calved from June 1, 2002 until May 31, 2003 was used in the study. Cows with an even numbered ear-tag were enrolled in the study as they calved (instead of cows randomly selected) to overcome logistical identification procedures and to reduce disruption of routine veterinary medical and management procedures at the study farm. Cows were classified into one of six categories of lameness during the first 35 days post partum using a modification of the locomotion scoring developed by Sprecher et al., 1997. Blood samples were obtained for weekly for

detection of plasma progesterone (P_4) concentrations during the first 60 days post partum. Risk of delayed cyclicity was compared between cows classified as non-lame, moderately lame, or lame.

Data Collection

Using farm records, the following data were collected for each cow: lactation number, calving date, calving season (winter months: Jan to Apr and Oct to Dec; summer months: May to Sep), dystocia (yes, no), retained placenta (yes, no), metritis (yes, no), mastitis (yes, no), ketosis (yes, no), body condition score at calving (Edmonson et al., 1989), change in body condition score in the first 50 days post partum, use of $PGF_{2\alpha}$ (Lutalyse, Pharmacia, Kalamazoo, MI) prior to resumption of ovarian activity (yes, no), and 305-day mature equivalent milk yield. From Dairy Herd Improvement Association (DHIA) records, projected 305 day ME milk yield data were collected based upon production at 60 days post partum. Levels of milk yield were defined as low (5,530 to 10,619 kg), medium (10,620 to 12,978 kg) and high (12,979 to 15,137 kg) on the basis of the frequency of distribution (first, second and third, and fourth quartiles, respectively).

Diagnosis of Lameness

During the first 35 days post partum, study cows were examined weekly (Tuesday) for diagnosis of lameness using a locomotion scoring system described by Sprecher (1997) with modifications (Table 1). Cows were observed and scored by the same veterinarian (EJG) as they walked-out of the wash pen to the holding area prior to milking. Cows with a locomotion score of 4 or 5 were further examined on a tilt table for diagnosis and treatment of lameness, noting lesions observed and date of occurrence. Lame cows with claw lesions had white line lesions or sole ulcers and were treated by corrective foot trimming techniques (Shearer and van Amstel, 2001). Lame cows with

subacute laminitis were those with yellow and red discoloration of the sole and white line, and in most cases they had thin soles and were sensitive at examination with hoof testers (Mortensen, 1994; Toussaint Raven, 1989). Lame cows with interdigital dermatitis were those with inflammation confined to the epidermis and in some cases hyperkeratosis, which creates a roughened appearance to the interdigital skin (Blowey, 1994); a fetid serous exudate could be present, and there was mild sensitivity to pressure. This condition was frequently accompanied by cracks in the heel, heel horn erosion, with potential under-running of the heel horn (Berry, 2001).

Collection of Blood Samples and Detection of Plasma P_4 Concentrations

Cows were blood-sampled and scored for body condition (Edmonson et al., 1989) weekly (Thursday) for detection of plasma progesterone concentrations during the first 60 days post partum. Cows were blood-sampled via coccygeal venipuncture using vacutainer collection tubes containing K_3 EDTA (Becton, Dickinson, and Company, Franklin Lakes, NJ). Blood samples were refrigerated until and during transportation to a laboratory at the University of Florida where they were centrifuged for 20 min at 3000 RPM at room temperature for plasma harvest. Plasma samples were frozen at – 20 C until tested for P_4 concentrations using the Coat-A-Count® Progesterone Kit (DPC® Diagnostics Products Corporation) radioimmunoassay. The Coat-A-Count® Progesterone procedure is a solid-phase radioimmunoassay where ^{125}I-labeled progesterone competes for a fixed time with the progesterone content of the cow's plasma sample. Because antibody is bound to the wall of the polypropylene tube, simply decanting the supernatant is enough to terminate the competition and to isolate the antibody-bound fraction of the radiolabeled progesterone. A standard curve dilution was prepared using coated tubes and non-coated tubes were used for total counts and non-specific binding. A 100 µl volume of

increasing concentrations of progesterone calibrators, (0, 0.1, 0.25, 0.5, 2, 5, 10, 20, and 40 ng/mL) were placed in the tubes. Plasma samples (100 µl) were added to coated tubes and 1 mL of ^{125}I-labeled progesterone (25000 cpm) to all tubes. Every 6th plasma sample was evaluated in duplicate. After an incubation period of 3 hours, the supernatant was discarded and tubes were dried for 15 minutes. They were then placed in a gamma counter. Calculation of the progesterone concentration in the plasma sample was made by computerized data process using a spline fitting curve.

Accuracy of assay procedures was determined by measuring known quantities of exogenous progesterone (0.625, 1.25, 2.5, and 5.0 ng/mL) in plasma in seven different assays. Recovery of added (x) versus measured (Y) P_4 concentrations was described by linear regression ($Y= 0.57 + 0.93x$; $R^2 = 0.89$). The regression intercept value (0.57 ng/mL) represented original P_4 concentrations measured in a plasma pool prior to addition of exogenous masses.

Parallelism of logit plots between the displacement curves for different volumes of a plasma pool containing 8 ng/mL of P_4 (i.e., 25, 50 and 100 µl) and standard P_4 amounts (i.e., 0.1, 0.25, 0.5, 1.0, 2.0, 5.0, 10.0 and 20.0 ng/mL) was tested for homogeneity using regression analysis (Wilcox et al., 1990). The linear regression curves for plasma and P_4 standards were parallel ($Yp= 0.60 - 1.44x$, $R^2 = 0.99$; $Ys = 0.21 - 1.61x$, $R^2 = 0.99$; where Yp and Ys = ln B/F, x = $\log_{10}$ of assay volume or $\log_{10}$ standard P_4 concentrations, respectively). The slopes were not different.

Coefficients of variation were calculated from a reference sample (luteal phase) and duplicate samples obtained from all assays. Duplicate plasma concentrations of P_4 were categorized into high (≥ 3.0 ng/mL; n = 359), medium (≥1.0 and <3.0 ng/mL; n = 128),

and low (≥ 0.3 and <1.0 ng/mL; n = 52), and the coefficients of variation were 12.4%, 12.4%, and 14.2%, respectively. Inter-and intraassay coefficients of variation for the luteal phase reference sample were 8.9% and 8.34%, respectively.

Resumption of Ovarian Cyclicity

Cows with evidence of normal ovarian cyclicity during the first 60 days post partum were those with: i) weekly plasma P_4 concentrations > 1 ng/mL for 2 or 3 consecutive samples followed by a decline in P_4; or ii) if P_4 concentration > 1 ng/mL was followed by a marked decrease after $PGF_{2\alpha}$ injection and this followed by an increase in P_4 concentration. Cows with a delayed resumption of ovarian cyclicity were those with consistently low P_4 concentrations ≤ 1 ng/mL during the first 60 days post partum (Staples et al., 1990). For the purpose of this study, cows with P4 values above 1 ng/mL for 4 or more consecutive samples were classified as cows with extended luteal phases (Opsomer et al., 1999). First luteal phase was defined as the first rise in P_4 above 1 ng/mL.

Reproductive and Health Management

All cows were subjected to a pre-synchronization program. After days 30 to 35 postpartum cows received an injection of prostaglandin F2α and then again at 44 to 49 days. Cows observed in heat after the second injection of prostaglandin F2α were inseminated, and those that did not demonstrate behavioral signs of estrus were enrolled in a timed insemination program. This program involved the use of GnRH on Day 0, 7 days later prostaglandin F2α, a second GnRH on Day 9, and timed inseminated 16 to 18 hours later. All cows were examined for pregnancy at 42 to 49 days post insemination by palpation of the uterus and its contents by the attending veterinarian.

Farm personnel examined cows for the detection of health problems several times post partum following a pre-established protocol (Table 3-1). Cows were grouped by days in milk and a group of fresh cows (less than 30 DIM) were kept together. Cows were examined after each milking. On Day one cows were checked for retained fetal membranes (fetal membranes visible at the vulva for more than 24hs after calving), udder edema (by observation and palpation of the udder), and mastitis (by stripping all four quarters). On Day 4, cows were checked for production (using Afimilk® computerized system), udder edema, metritis (Table 3-2) and rectal temperatures were taken. On day 7 cows were checked for Ketonuria (using Ketostixs®), metritis, body temperature, and using a stethoscope, rumen movements, and displaced abomasum (DA) (tympanic sound on the left side at simultaneous auscultation-percussion of the left paralumbar fossa). This 7-day check was repeated at day 10 and 15. In all checks, daily milk yield was monitored for every cow for deviations in production.

Table 3-1. Protocol for examination of cows postpartum

Check day	Health checks
1	Retained fetal membranes (RFM), udder edema, mastitis
4	Temp, udder edema, metritis, production, mastitis, manure consistency
7	Temp, ketosis, DA, rumen, metritis, mastitis, manure consistency
10	Temp, ketosis, DA, rumen, metritis, mastitis, manure consistency
15	Temp, ketosis, DA, rumen, metritis, mastitis, manure consistency

Table 3-2. Definitions of metritis done by farm personnel based on discharge and palpation findings

Metritis code	Definition
U1	Normal size and abnormal discharge.
U2	Abnormal size and abnormal discharge (pus discharge)
U3	Abnormal size and abnormal discharge (watery and foul smelling)
U4	Abnormal size, abnormal discharge and the cow looks sick

Cows with U1 and some with U2 were treated with an injection of prostaglandin F2α. Some cows with U2 and all with U3 were treated with an intrauterine infusion of tetracycline (100 mg/mL) solution. Cows with U4 were treated with systemic antibiotics. Other health codes recorded were calving outcomes (See table 3-3)

Table 3-3. Definition of calving outcomes

Calving code	Definition
Pull 1	Easy pull, 1 person.
Pull 2	Difficult pull, 1 person
Pull 3	Easy for 2 persons
Pull 4	Difficult pull for 2 persons
Pull 5	Extreme pull.

Cows with pulls 4 and 5 were started with systemic antibiotics after calving.

All cows in the herd were monitored daily for deviations in milk (Afimilk®) production, and milk conductivity for detection of mastitis following a pre-established criteria. (Table 3.4). All health events and treatments were recorded on a cow-side computer program (Visi-Cow®) used by farm personnel.

Table 3-4. Criteria for monitoring production health and mastitis using Afimilk® system

Population	% Decrease in daily milk production (%)	Increase in daily conductivity (%)	Increase daily milk production (%)
1 to 15 DIM	----	----	< 10
1 to 40 DIM	15	12	---
41 to 100 DIM	20	15	---
> 100 DIM	25	18	---

Statistical Analyses

The null hypothesis that risk of delayed ovarian cyclicity is the same in cows classified as non-lame, moderately lame, or lame was tested using logistic regression. In the analysis, non-lame cows were those with a score of 3 for one week only, or scores of ≤ 2. Cows classified as moderately lame were those with a score of 3 on two consecutive

weeks. Lame cows were those classified at least once with a locomotion score of 4 or 5. Additional independent variables (lactation number, calving season, milk yield, dystocia, retained placenta, metritis, ketosis, body condition score, use of $PGF_{2\alpha}$) were included in the analysis to address possible modifying or confounding effects of these factors on risk of delayed ovarian cyclicity. Stepwise forward regression was used, and a variable had to be significant at the 0.20 level before it could enter the model. A variable remained in the model when its significance level was < 0.10. Variables for lactation number and calving season were forced into the model.

In the final model, adjusted odds ratios (OR) and 95% confidence intervals (CI) were reported. The OR was used as an epidemiologic measure of association between a variable (i.e., lameness) and the outcome of interest (i.e., delayed ovarian cyclicity). In each variable, the reference category had an OR = 1. An assessed OR > 1.0 indicates that the probability of delayed ovarian cyclicity increased, compared with cows in the reference category. The attributable proportion was estimated as (OR – 1)/OR, and interpreted to represent the proportion of lame cows that experienced delayed ovarian cyclicity because of lameness (Martin et al., 1987).

The null hypothesis that number of days post partum to first luteal phase did not differ among groups of cows classified as non-lame, moderately lame, or lame was tested by use of the Kruskal-Wallis nonparametric test (because the dependent variable failed to meet assumptions of parametric testing), and multiple ANOVA for the dependent variable of days to first luteal phase (ranked data) while simultaneously adjusting for variables related to ovarian cyclicity (i.e., lactation number, calving season, ketosis, milk yield). Significance was set at $P \leq 0.05$.

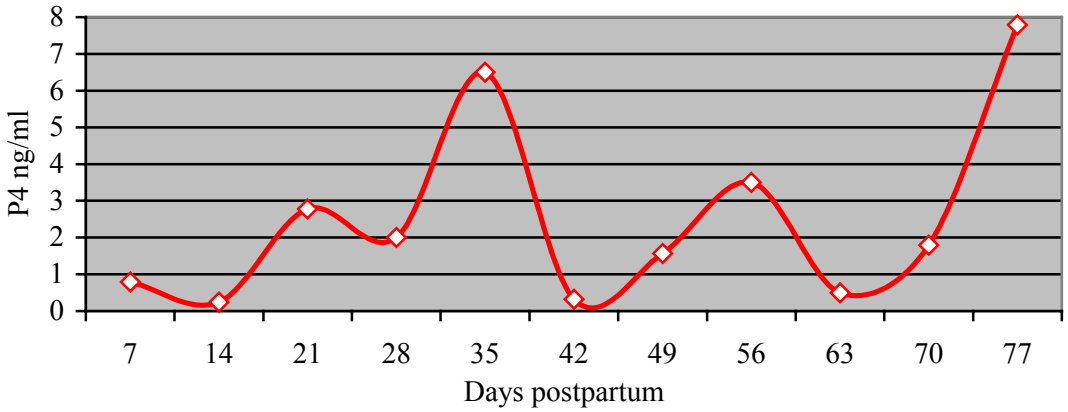

Figure 1. Normal ovarian cyclicity

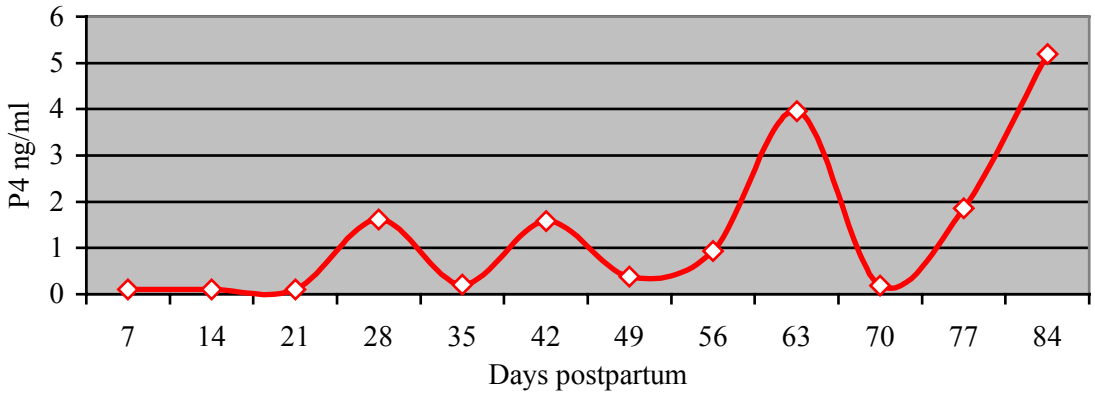

Figure 2. Normal ovarian cyclicity for cows treated with PGF2

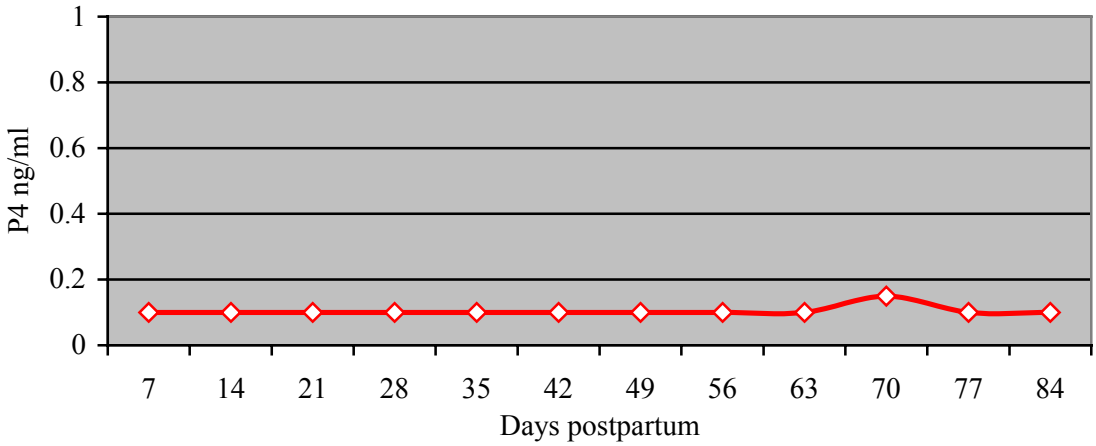

Figure 3. Delayed resumption of ovarian cyclicity

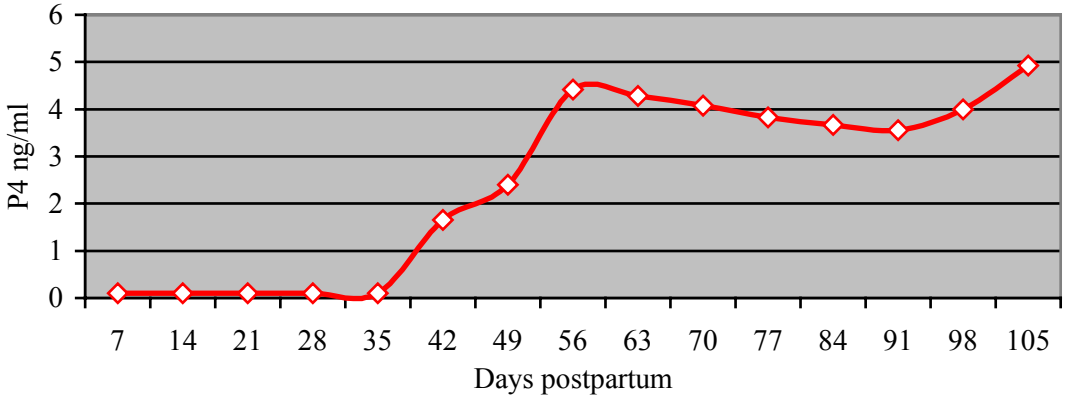

Figure 4. Extended luteal phase

CHAPTER 4
RESULTS

All 253 cows enrolled in the study were followed-up successfully during the 60-day study period. Two hundred and thirty-eight (94%) cows met the criteria for ovarian delayed cyclicity used in this study. A visual examination of plasma P_4 concentration patterns revealed that 15 cows (6%) experienced an extended luteal phase. A total of 101 of 238 (42%) cows were classified as moderately lame (locomotion score = 3) and 41 (17%) as lame (score = 4) (Table 4-1). The mean number of days post partum when cows were classified as lame was 15 days (1 – 34 days). The most common lesions observed were subacute laminitis (26/41 = 63%), and claw lesions such as sole ulcers and white line disease (9/41 = 22%).

The overall incidence of delayed ovarian cyclicity was 11%. The incidence of delayed ovarian cyclicity was higher in cows classified as moderately lame (14/101; 14%) or lame (7/41; 17%), compared to non-lame cows (6/96; 6%). In the univariable analysis, cows classified as moderately lame were 2.4 times at higher risk of delayed ovarian cyclicity compared to non-lame cows (OR = 2.4; 95% CI = 0.9 – 6.7; $P = 0.07$) (Table 4-2). Cows classified as lame were 3.1 times at higher risk of delayed ovarian cyclicity compared to non-lame cows (OR = 3.1; 95% CI = 0.9 – 9.9; $P = 0.05$).

In the multivariable analysis, lameness, lactation number, season, ketosis and milk yield were retained in the final modeling process (Table 4-3). Addition of two-way interaction terms did not contribute to the final model for risk of delayed ovarian cyclicity, and these terms were removed from the model. Cows classified as moderately

lame were 2.1 times at higher risk of delayed ovarian cyclicity, compared to non-lame cows (OR = 2.1; 95% CI = 0.7 – 6.1; $P = 0.15$). Cows classified as lame were 3.5 times at higher risk of delayed ovarian cyclicity compared to non-lame cows (OR = 3.5; 95% CI = 1.0 – 12.2; $P = 0.04$). The attributable proportions of cows that experienced delayed ovarian cyclicity associated with moderate lameness and lameness were 0.52 and 0.71, respectively (Table 4-4).

Overall, the time interval (median) from calving to first luteal activity in the study population was 31 days. This time period was more prolonged in cows classified as lame (median = 36; range = 17 to 97) or moderately lame (median = 32; range = 4 to 146), compared with non-lame cows (median = 29; range = 2-172) ($P \leq 0.05$).

Table 4-1. Frequency distribution of cows classified as lame or non-lame using a modification of the locomotion scoring system developed by Sprecher, 1997

Locomotion score	Clinical description	Assessment criteria	Cows n = 238 No. of cows (%)
0	Normal	The cow stands and walks with a level-back posture. Gait is normal.	3 (1)
1	Barely lame	The cow stands with a level-back posture but develops an arched back posture while walking. Gait remains normal.	17 (7)
2	Mildly lame	An arched-back posture is evident both while standing and walking. Normal gait.	76 (32)
3	Moderately lame	An arched-back posture is evident both while standing and walking. Gait is affected and best described as short strides with one or more limbs.	101 (42)
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.	41 (17)
5	Severely lame	In addition to criteria in LS4, the cow demonstrates an inability or extreme reluctance to bear weight on one or more of her limbs/feet.	0 (0)

Table 4-2. Descriptive statistics and unadjusted odds ratios for risk of delayed ovarian cyclicity in post-partum Holstein cows

Variable	Delayed cyclicity Yes n = 27	Delayed cyclicity No n = 211	OR	95% CI	*P*
	No. of cows (%)	No. of cows (%)			
Lameness group					
Locomotion score ≤ 2	6 (22)	90 (43)	1.0	Reference	NA
3	14 (52)	87 (41)	2.4	0.9 – 6.7	0.07
4	7 (26)	34 (16)	3.1	0.9 – 9.9	0.05
Lactation number					
1	10 (37)	77 (36)	1.0	Reference	NA
≥ 2	17 (63)	134 (64)	0.9	0.4 – 2.2	0.95
Season					
Winter	18 (67)	123 (58)	1.0	Reference	NA
Summer	9 (33)	88 (42)	0.7	0.3 – 1.6	0.40
Milk yield					
Low	9 (33)	50 (24)	1.0	0.4 – 2.5	0.91
Medium	16 (59)	102 (48)	1.0	Reference	NA
High	2 (8)	56 (27)	0.2	0.05 – 0.9	0.04
Dystocia					
No	20 (74)	163 (77)	1.0	Reference	NA
Yes	3 (11)	17 (8)	1.4	0.3 – 5.3	0.58
Retained placenta					
No	23 (85)	181 (86)	1.0	Reference	NA
Yes	4 (15)	30 (14)	1.0	0.3 – 3.2	0.93
Metritis					
No	16 (59)	132 (63)	1.0	Reference	NA
Yes	11 (41)	79 (37)	1.1	0.5 – 2.5	0.73
Mastitis					
No	25 (93)	174 (82)	1.0	Reference	NA
Yes	2 (7)	37 (18)	0.3	0.09 – 1.6	0.19
Ketosis					
No	18 (67)	177 (84)	1.0	Reference	NA
Yes	9 (33)	34 (16)	2.6	1.0 – 6.2	0.03
BCS at calving					
< 2.75	3 (11)	34 (16)	0.6	0.1 – 2.3	0.52
2.75 – 3.5	20 (74)	151 (71)	1.0	Reference	NA
> 3.5	4 (15)	26 (12)	1.1	0.3 – 3.6	0.79
BCS change (0.75)					
No	20 (74)	173 (82)	1.0	Reference	NA
Yes	7 (26)	38 (18)	1.5	0.6 – 4.0	0.32
Use of $PGF_{2\alpha}$					
No	17 (63)	125 (59)	1.0	Reference	NA
Yes	10 (37)	86 (41)	0.8	0.3 – 1.9	0.70

Table 4-3. Final logistic regression model for risk of delayed ovarian cyclicity in post- partum Holstein cows

Variable	Adjusted odds ratio	95% confidence interval	*P* value
Lameness group			
Locomotion score ≤ 2	1.0	Reference	NA
3	2.1	0.7 – 6.1	0.15
4	3.5	1.0 – 12.2	0.04
Lactation number			
1	1.0	Reference	NA
≥ 2	1.2	0.5 – 2.3	0.65
Season			
Winter	1.0	Reference	NA
Summer	0.9	0.3 – 2.3	0.90
Ketosis			
No	1.0	Reference	NA
Yes	2.7	1.0 – 7.0	0.03
Milk yield			
Low	0.9	0.3 – 2.5	0.98
Medium	1.0	Reference	NA
High	0.2	0.05 – 0.9	0.04

NA = Not applicable

Table 4-4. Attributable proportion of cows that experienced delayed resumption of ovarian cyclicity

Locomotion score	N	OR	Attributable proportion
≤ 2	6/96	1	NA
3	14/101	2.1	0.52
4	7/41	3.5	0.71

CHAPTER 5
DISCUSSION

The results of the study reported here support the hypothesis that lameness has a detrimental effect on ovarian activity in Holstein cows during the early post partum period. Cows classified as lame were 3.5 times at higher risk of delayed cyclicity, compared to non-lame cows. Attributable proportion analysis indicated that delayed ovarian cyclicity in lame cows would be reduced by 71% if lameness had been prevented. In addition, cows classified as moderately lame were 2.1 times at higher risk of delayed ovarian cyclicity compared to non-lame cows (OR = 2.1; 95% CI = 0.7 – 6.1; P = 0.15). Even though this association was not statistically significant, the OR and the position of the confidence interval (Szklo and Nieto, 2000) suggest that cows classified as moderately lame were at high risk of delayed ovarian cyclicity. This observation is further supported by the fact that the interval from calving to first luteal phase was more prolonged in both lame cows (median = 36 days) or moderately lame cows (32 days) compared with non-lame cows (29 days) ($P \leq 0.05$). Thus preventive measures (such as examination of cows feet and, if necessary, use of corrective foot trimming techniques) should be targeted at the group of moderately lame cows since as they represented 42% of the study population. We examined a second logistic regression model which included the 15 cows that experienced an extended luteal phase (in addition to the 238 cows that met the criteria for ovarian cyclicity used in this study), and the effect of lameness on delayed cyclicity did not disappear. Cows classified as moderately lame and lame were 2 (OR = 2.0; 95% CI = 0.7, 5.9; P = 0.17) and 3 times (OR = 3.0; 95% CI = 0.9, 10.3; P =

0.07) at higher risk of delayed cyclicity, respectively, compared to non-lame cows. To our knowledge, only one previous study has examined the relationship between lameness and ovarian activity. In a study conducted in 335 dairy cows on six high producing dairy herds in Belgium, cows diagnosed with clinical mastitis, severe lameness, or pneumonia by farmers were at higher risk of delayed ovarian cyclicity compared to cows classified as clinically healthy (Opsomer et al., 2000). However, the actual number of cows affected with clinical mastitis, severe lameness, or pneumonia was not reported.

The incidence of cows classified as moderately lame and lame during the first 35 days post partum was 42% and 17%, respectively. In a previous study involving 66 dairy cows on a farm in Michigan (Sprecher et al., 1997), a locomotion scoring system similar to that in our study was used for diagnosis of lameness. In the Michigan study, 27 (49%) cows and 14 (24%) cows were classified as moderately lame and lame, respectively. Results from that study are difficult to compare with results of the present study because of differences in the scoring system. After testing the locomotion scoring system (Sprecher et al., 1997) weekly for two months in the study herd, a new category was added to include cows that were observed with an arched-back posture that was evident both while standing and walking, but their gait seemed normal (score = 2, mildly lame); 76 (32%) cows were included in this category. In our analysis, this group of cows was classified as non-lame. Assuming that this group of cows was misclassified as non-lame, the incidence of cows classified as moderately lame (score = 3) would have been higher (76 + 101 = 177 cows or 74%).

However, study results support our clinical observations and locomotion scoring system for diagnosis of lameness. The possibility that cows classified as mildly lame

were misclassified is unlikely since the incidence of delayed ovarian cyclicity was lower in mildly lame cows (3/76 or 4%) compared to moderately lame cows (14/101 or 14%). If mildly lame cows were misclassified, there would have been an expected an incidence of delayed ovarian cyclicity similar to that observed in cows classified as moderately lame.

Although we established an association between lameness and delayed ovarian cyclicity, we failed to identify loss of body condition (or a modifying effect of lameness and loss of body condition) as a significant risk factor associated with delayed ovarian cyclicity. The risk of delayed ovarian cyclicity was 1.5 times higher in cows that had a change in BCS ≥ 0.75 in the first 50 days post partum compared to cows with a change in BCS < 0.75. Therefore, this association was not significant (OR = 1.5; 95% CI = 0.6 - 4.0; $P = 0.32$). The observed incidence of delayed ovarian cyclicity in the study population was low (11%) compared to other studies (23 to 29%), (Humboldt and Thibier, 1980; Bartlett et al., 1987; Staples et al., 1990), creating a sample size limitation. In the previous study conducted in 335 dairy cows in Belgium, cows losing more body condition during the first and second month after calving were at higher risk of delayed ovarian cyclicity (Opsomer et al., 2000).

In our study, ketosis was, by itself, a risk factor for delayed resumption of ovarian cyclicity. This result is in agreement with a study conducted in 84 dairy cows on 8 farms in Switzerland (Reist et al., 2000) where blood serum and milk ketone body concentrations during the first 6 weeks post partum were higher in cows classified as late responders (i.e., cows started post partum ovarian cyclicity after 30 days) than in early responders, with no significant differences in body condition scores between groups. It is possible that lameness and ketosis may additionally interact with each other to affect the

risk of delayed ovarian cyclicity, but the small sample size was too small in the present study did not allow detection of such an interaction.

Lameness can depress dry matter intake (Hassall et al., 1993; Galindo and Broom, 2002) and result in negative energy balance. It has been reported that negative energy balance contributes to increased ketone body formation and delays the onset of ovarian activity (Reist et al., 2000). A negative energy balance post partum not only contributes to increased ketogenesis, but also delays the onset of ovarian cyclicity, especially if energy deficiency is prolonged (Butler and Smith, 1989; Staples et al., 1990; Lucy et al., 1992). Furthermore, results of the study reported here revealed that the risk of delayed ovarian cyclicity was lower in high milk producing cows, compared to medium or low producing cows. The results of previous studies suggested that low producing cows have reduced inferior dry matter intake, a more negative energy balance, and are less likely to restore ovarian activity during the first 63 days post partum compared to high producing cows (Staples et al., 1990; Lucy et al., 1992).

Although is clear that lameness has an effect on resumption of ovarian cyclicity in postpartum cows, we could not establish the cause of this effect. We hypothesized that energy balance would be responsible for the delayed in resumption of ovarian cyclicity, but our study could not support such pathway. Even though there are no studies reporting a pathway to explain the effect of lameness on ovarian activity, we cannot ignore other proposed mechanisms by which lameness may affect the hypothalamus-pituitary-ovarian axis.

Dobson (2000) proposed that activation of the hypothalamus-pituitary-adrenal axis by stressors reduces the pulasatility of GnRH/LH by actions at both the hypothalamus

and pituitary gland, depriving the ovarian follicle of adequate LH support. This will lead to reduced estradiol production by slower growing follicles. A combination of a reduced GnRH/LH pulsatility with a reduced production of estradiol, contributes to the delay and reduced magnitude of the LH surge and a delayed or absence of ovulation (Dobson et al., 1999; Dobson et al., 2000). Phogat (1997; 1999) provided evidence that the effect of increased concentrations of ACTH either exogenously, or after transport, reduced the amounts of LH released after challenges with small doses of GnRH, providing support for additional effects at the pituitary level. Dobson (2000) proposed that in situations such as during chronic stress of severe lameness or fever, the pulse GnRH/LH frequency will be so slow that initial follicular growth will occur but will be unable to continue into the later stages that depend on faster pulse frequencies. Thus the animal fails to maintain an estrus cycle developing anestrous.

Another hypothesis is that the effect of lameness on cyclicity could be driven by endotoxins released after an event of ruminal acidosis (Nocek, 1997). As shown by Peter (1990) increases in cortisol concentrations after the infusion of endotoxin might block the synthesis of estradiol at ovarian level resulting in failure of a preovulatory LH surge. This may lead to anovulation or delayed ovarian cyclicity. Supporting the effects of endotoxins, Bataglia (1997) found that the suppressive effects of endotoxins on the reproductive axis can be mediated centrally through an inhibition of GnRH and thus LH pulsatile secretion. Ruminal acidosis has been identified as risk factor for lameness (Nocek, 1997; Hoblet et al., 2001). So if lame cows are suffering from acidosis with endotoxin released by gram-negative bacteria, endotoxins can mediate the effect of

lameness in delayed resumption of ovarian cyclicity. At this point these hypotheses are only speculative and need further research to be proved.

CHAPTER 6
CONCLUSION

Analysis of results of the study reported here support the hypothesis that lameness has a detrimental effect on ovarian activity in Holstein cows during the early post partum period. The locomotion scoring system used in this study is a useful management tool that veterinarians and dairy farmers can adopt for early detection of lameness in dairy cows. The use of corrective foot trimming techniques in moderately lame cows may help reduce the risk of delayed ovarian cyclicity associated with the more severe forms of lameness (i.e., score = 4 and 5) in dairy cows.

LIST OF REFERENCES

Ahima R.S., Flier J.S. 2000. Leptin. Annu. Rev. Physiol. 62:413-437.

Alban L. 1995. Lamaness in Danish dairy cows; frequency and possible risk factors. Prev. Vet. Med. 22:213-225.

Archbald L.F., Norman S.N., Bliss E.L., Tran T., Lyle S., Thomas P.G.A., Rathwell A.C. 1990. Incidence and treatment of abnormal postpartum ovarian function in dairy cows. Theriogenology 34:283-290.

Argáez-Rodríguez F.J., Hird D.W., Hernandez J., Read D.H., Roríguez-Lainz A. 1997. Papillomatous digital dermatitis in a commercial dairy farm in Mexicali, Mexico: Incidence and effect on reproduction and milk production. Prev. Vet. Med. 32:275-286.

Armstrong D.G., Gong J.G., Gardner J.O., Baxter G., Hogg C.O., Webb. 2002. Steroidogenesis in bovine granulosa cells: the effect of short-term changes in dietary intake. Reproduction 123:371-378.

Barkema H.W., Westrik J.D., van Keulen K.A.S., Schukken Y.H., Brand A. 1994. The effect of lameness on reproductive performance, milk production and culling in Dutch dairy farms. Prev. Vet. Med. 20:249-259.

Bassett H.F., Monaghan M.L., Lenhan P., Doherty M.L., Carter M.E. 1990. Bovine Digital Dermatitis. Vet. Rec. 126:164-165.

Bartlett P.C., Kirk J., Coe P., Marteniuk J., Mather E.C. 1987. Descriptive epidemiology of anestrus in Michigan Holstein-Fresian cattle. Theriogenology 27:459-476.

Bauman D.E., Currie W.B. 1980. Partitioning of nutrients during pregnancy and lactation: a review of mechanisms involving homeostasis and homeorhesis. J. Dairy Sci. 63:1514-1529.

Beam S.W., Butler W.R. 1997. Energy balance and ovarian follicule development prior to the first ovulation postpartum in dairy cows receiving three levels of dietary fat. Biol. Reprod. 56:133-142.

Beam S.W., Butler W.R. 1998. Energy balance, metabolic hormones, and early postpartum follicular development in dairy cows fed prilled lipid. J. Dairy Sci. 81:121-131.

Bergsten C. 1997. Infectious diseases of the digits. In: Lameness in cattle. 3rd Ed. W.B Saunders, Philadelphia, Pennsylvania, 89-93.

Bergsten C. 2000. Descriptive nomenclature and scoring of foot lesions at hoof trimming. Proceedings of the XI International Symposium on Disorders of the Ruminant Digit, Parma, Italy.

Berry S.L. 2001. Diseases of the digital soft tissues. The Veterinary Clinics of North America. Food Animal Practice. Lameness 17:1,136-139.

Berry S.L., Graham T.W., Mongini A. 1999b. The efficacy of *Serpens spp* bacterin combined with topical administration of lincomycin hidrochloride for treatment of papillomatous digital dermatitis (footwarts) in cows on a dairy in California. The Bovine Practitioner 33:6-11.

Berry S.L. Read D.H., Walker R.L. 1999a. Recurrence of papillomatous digital dermatitis (footwarts) in dairy cows after treatment with lincomycin HCL or oxytetracycline HCl. J. Dairy Sci. 82 (suppl 1):34.

Berry S.L., Mass J., Reed B.A. 1996. The efficacy of 5 topical spray treatments for control of Papillomatous digital dermatitis in dairy herds. Proc. American Association of Bovine Practitioners 29:188.

Block S.S., Butler W.R., Ehrhardt R.A., Bell A.W., Van Amburgh M.E., Boisclair Y.R. 2001. Decreased concentration of plasma leptin in periparturient dairy cows is caused by negative energy balance. J. Endocrinology 171:339-348.

Blowey R.W., Ossent P., Watson C.L., Hedges V., Green L.E., Packington A.J. 2000. Possible distinction between sole ulcers and heel ulcers as a cause of bovine lameness. Vet. Rec. 147:110-112.

Blowey R.W., Weaver A.D. 1997. Color Atlas of diseases and disorders of cattle. Ames, Iowa, University Press 96-97.

Blowey R.W.,Carter S.W., White A.G., Barnes A. 1994. *Borrelia burgdorferi* infections in UK cattle: a possible association with digital dermatitis. Vet. Rec. 135:577-578.

Blowey R.W. 1994. Interdigital causes of lameness. In: Proceedings 8th. International Symposium on disorders of the ruminant digit. Banff, Canada 142-154.

Blowey R.W., Done S. H., Cooley W. 1994. Observations on the pathogenesis of digital dermatitis in cattle. Vet. Rec. 115-117.

Blowey R.W. 1993. Cattle lameness and hoofcare: An Illustrated Guide. Ipswich, UK, Farming Press 20.

Blowey R.W., Sharp M.W. 1988. Digital dermatitis in dairy cattle. Vet. Rec. 122:505-508.

Boelling D., Pollott G.E. 1998. Locomotion, lameness, and hoof and leg traits in cattle. I. Phenotypic influences and relationships. Livest. Prod. Sci. 54:193-203.

Bradley H.K., Shannon D., Nielson D.R. 1989. Subclinical laminitis in dairy heifers. Vet. Rec. 125:177-179.

Bradley R.L., Kokkotou E.G., Maratos-Flier E., Cheatham B. 2000. Melanin-concentrating hormone regulated Leptin synthesis and secretion in rat adipocytes. Diabetes 49:1073.

Britt J.S., Carson M.C., von Bredow J.D., Condon R.J. 1999. Antibiotic residues from milk samples obtained from cows after treatment for papillomatous digital dermatitis. J. Am. Vet. Med. Assoc. 215:833-836.

Britt J.S., McClure J. 1998. Field trials with antibiotic and non-antibiotic treatments for Papillomatous digital dermatitis. The Bovine Practitioner 32:25-28.

Britt J.S., Gaska J., Garrett E.F. 1996. Comparison of topical application of three products for the treatment of Papillomatous digital dermatitis in dairy cattle. J. Am. Vet. Med. Assoc. 209:1134-1136.

Britt J.S., Scott R., Armstrong J., Whitacre M. 1986. Determinants of estrous behaviour in lactating Holstein cows. J. Dairy Sci. 69:2195-2202.

Brizzi A. 1993. Bovine Digital Dermatitis. Proc. 26th Ann. Conf. Am. Assoc. Bov. Pract., Albuquerque, NM.

Broom D.M. 1988. The scientific assessment of animal welfare. Applied Animal Behaviour Science 20:5-19.

Budras K.D., Geyer H., Maier J. 1998. Anatomy and structure of hoof horn. Proceedings of the 10th International symposium on lameness in ruminants, Lucerne, Switzerland 176-188.

Budras K.D., Habil D.R., Mülling C.H. 1996. Rate of keratinization of the wall segment of the hoof and its relation to width and structure of the zona alba (white line) with respect to claw disease in cattle. Am. J. Vet. Res. 57:444-455.

Butler W.R., Smith R.D. 1989. Interrelationships between energy balance and postpartum reproductive function in dairy cattle. J. Dairy Sci. 72:767-783.

Butler W.R., Everett R.W., Coppock C.E. 1981. The relationship between energy balance, milk production and ovulation in postpartum Holstein cows. J. Anim. Sci. 53:742-748.

Canfield R.W., Butler W.R. 1991. Energy balance, first ovulation and the effects of naloxone on LH secretion in early postpartum dairy cows. J. Anim. Sci. 69:740-746.

Canfield R.W., Butler W.R. 1990a. Energy balance and pulsatile LH secretion in early postpartum dairy cattle. Domestic Animal Endocrinology 7:323-330.

Canfield R.W., Sniffen C.J., Butler W.R. 1990b. Effects of excess degradable protein on postpartum reproduction and energy balance in dairy cattle. J. Dairy Sci. 73:2342-2349.

Canfield R.W., Sniffen C.J., Butler W.R. 1988. Effects of excess degradable protein on postpartum reproduction and energy balance in dairy cattle. J. Dairy Sci. 73:2342-2349.

Chehab F., Lim M., Lu R. 1996. Correction of the sterility defect in homozygous obese female mice by treatment with the human recombinant leptin. Nat. Genet. 12:318-320.

Clarkson M., Downham D., Faull W., Hughes J., Manson F., Murray R., Russell W., Sutherst J., Ward W. 1996. Incidence and prevalence of lameness in dairy cattle. Vet. Rec. 138:563-567.

Cohick W.S., Armstrong J.D., Whitcare M.D., Lucy M.C., Harvey M.W., Campbell R.M. 1996. Ovarian expression of insulin-like growth factor-I (IGF-I), IFG binding proteins, and growth hormone (GH) receptor in heifers actively immunized against GH-releasing factor. Endocrinology 137:1670-1677.

Collick D.W., Ward W.R., Dobson H. 1989. Associations between types of lameness and fertility. Vet. Rec. 125:103-106.

Cook N.B., Cutler K.L. 1995. Treatment and outcome of a severe form of foul-in-the-foot. Vet. Rec. 136:19-20.

Coulon J.B., Lescourret F., Fonty A. 1996. Effect of foot lesions on milk production by dairy cows. J. Dairy Sci. 79:44-49.

Darwash A.O., Lamming G.E.,Woolliams, J.A. 1997. The phenotypic association between the interval to post-partum ovulation and traditional measures of fertility in dairy cattle. Animal Science 65:9-16.

Deluyker H.A., Gay J.M., Weaver L.D., Azari, A.S. 1991.Change of milk yield with clinical diseases for a high producing dairy herd. J. Dairy Sci.74:436-445.

Demirkan I., Walker R.L., Murray R.D., Blowey R.W.,Crater S.D. 1999. Serological evidence of Spirochaetal infections associated with digital dermatitis in dairy cattle. Vet. J. 157:69-77.

Dewes H.F. Some aspects of lameness in dairy herds. 1978. New Zealand Vet. J. 26:147-159.

Dobson H., Ribadu A.Y., Noble K.M., Tebble J.E., Ward W.R. 2000. Ultrasound and hormone profiles of ACTH-induced persistent ovarian follicles (cysts) in cattle. J Reprod Fert 120:405-410.

Dobson H., Tebble J.E., Phogat J.B., Smith R.F. 1999. Effect of transport on pulsatile and surge secretion of LH in ewes in the breeding season. J. Reprod. Fert. 116:1-8.

Echternkamp S.E. and Hansel W. 1973. Concurrent changes in bovine plasma hormone levels prior to and during the first postpartum estrous cycle. J. Anim. Sci. 37:1362-1370.

Edmonson A.J., Lean I.J., Weaver L.D., Farver T., Webster G. 1989. A body condition scoring chart for Holstein dairy cows. J. Dairy Sci. 72:68-78.

Enevoldsen C., Grohn Y. T., Thysen I. 1991. Heel erosion and other interdigital disorders in dairy cows: Associations with season, cow characteristics, disease, and production. J. Dairy Sci. 74:1299-1309.

Etherington W.G., Christie K.A., Walton J.S., Leslie K.E., Wickstrom S., Johnson W.H. 1991. Progesterone profiles in postpartum Holstein dairy cows as an aid in the study of retained fetal membranes, pyometra and anestrus. Theriogenology 35:731-746.

Faye B., Lescourret F. 1989. Environmental factors associated with lameness in dairy cattle. Prev. Vet. Med. 7:267-287.

Fernandes L.C., Thatcher W.W., Wilcox W.W., Call E.P. 1978. LH release in response to GnRH during the postpartum period of dairy cows. J. Anim. Sci. 46:443-448.

Fonseca F.A., Britt J.H., McDaniel B.T., Wilk J.C., Rakes A.H. 1983. Reproductive traits of Holsteins and Jerseys. Effects of age, milk yield, and clinical abnormalities on involution of cervix and uterus, ovulation, estrous cycles, detection of estrus, conception rate, and days open. J. Dairy Sci. 66:1128-1147.

Galindo F., Broom D.M. 2002 .The effects of lameness on social and individual behavior. Journal of Applied Animal Welfare Science, 5:193-201.

Gazal O.S., Leshin L.S., Stanko R.L., Thomas M.G., Keisler D.H., Anderson L.L., Williams G.L. 1998. Gonadotropin-releasing hormone secretion into third-ventricle cerebrospinal fluid of cattle: correspondence with the tonic and surge release of luteinizing hormone and its tonic inhibition by suckling and neuropeptide-Y. Biol. Repro. 59:676-683.

Gong J.G., Lee W.J., Garnsworthy P.C., Webb R. 2002. Effect of dietary-induced increases in circulating insulin concentrations during the early postpartum period on reproductive function in dairy cows. Reproduction 123:419-427.

Gong J.G., Campbell B.K., Bramley T.A., Gutierrez C.G., Peters A.R., Webb R. 1996. Suppression in the secretion of follicle-stimulation hormone and luteinizing hormone, and ovarian follicle development in heifers continuously infused with a gonadotrophin-releasing hormone agonist. Biol. Reprod. 55:68-74.

Green L.E., Hedges V.J., Schukken Y.H., Blowey R.W., Packington A.J. 2002. The impact of clinical lameness on the milk yield of dairy cows. J. Dairy Sci. 85:2250-2256.

Greenough, P.R., and Vermunt J.J. 1991. Evaluation of subclinical laminitis in a dairy herd and observations on associated nutritional and management factors. Vet. Rec. 128:11-17.

Greenough P.R., Weaver A.D. 1997. Lameness in cattle 3rd edition. Ed. W.B. Saunders, Philadelphia, 277.

Guard C. 1996. Laminitis in dairy cattle: Recognition of the disorder and management of the causative factors. In: Proc. 28th Annual Convention of the American Association of Bovine Practitioners. San Antonio, Texas 71-74.

Guard C. and Williams E.I. 1995. Recognizing and managing infectious causes of lameness in cattle. In: Proceedings of the 27th Annual Convention of the American Association of Bovine Practitioners 27:80-82.

Hahn M.V., Mc Daniel B.T., Wilk J.C. 1986. Rates of hoof growth and wear in Holstein cattle. J. Dairy Sci. 69:2148.

Harris D., Hibburt C., Anderson G., Younis P., Fitspatrick D., Dunn A., Parson I., McBeath N. 1988. The incidence, cost and factors associated with foot lameness in dairy cattle in south-western Victoria. Aust. Vet. J. 65:171-176.

Harrison R.O., Ford S.P., Young J.W., Conley A.J., Freeman A.E. 1990. Increased milk production versus reproductive and energy status of high producing dairy cows. J. Dairy Sci. 73:2749-2758.

Hassall S.A., Ward W.R., Murray R.D. 1993. Effects of lameness on the behaviour of cows during the summer. Vet. Rec. 132:578-580.

Hendry K.A.K., Lancelott M.J., Knight C.H. 1994. Bovine Lameness: A cell culture approach. 8th Symposium on Disorders of the Ruminant Digit. Banff, Canada, 53.

Hernandez J., Shearer J.K., Webb W.D. 2002. Effect of lameness on milk yield in dairy cows. J. Am. Vet. Med. Assoc. 220:640-644.

Hernandez J., Shearer J.K., Webb D.W. 2001. Effect of lameness on the calving-to-conception interval in dairy cows. J. Am. Vet. Med. Assoc. 218:1611-1614.

Hernandez J., Shearer J.K. 2000. Efficacy of oxytetracycline for treatment of papillomatous digital dermatitis lesions on various anatomic locations in dairy cows. J. Am. Vet. Med. Assoc. 216:1288-1290.

Hernandez J., Shearer J.K., Elliott J.B. 1999. Comparison of topical application of oxytetracycline and four nonantibiotic solutions for treatment of papillomatous digital dermatitis in dairy cows. J. Am. Vet. Med. Assoc. 214:688-690.

Hobelet K.H., Weiss W. 2001. Metabolic hoof horn disease: Claw horn disruption. In: The Veterinary Clinics of North America, 17:111-127.

Houseknecht K.L., Baile C.A., Matteri R.L., Spurlock M.E. 1998. The biology of leptin; a review. J. Anim Sci. 76:1405-1420.

Humboldt P., Thibier M. 1980. Progesterone monitoring of anestrous dairy cows and subsequent treatment with prostaglandin f2α analog or gonadotropin-releasing hormone. Am. J. Vet. Res. 41:1762-1766.

Humphrey W.D., Kaltenbach C.C., Dunn T.G., Koritnik D.R. 1983. Characterization of hormonal patterns in the beef cow during postpartum anestrus. J. Anim. Sci. 56:445-453.

Jonsson N.N., McGowan M.R., McGuigan K., Davison T.M., Hussain A.M., Kafi M., Matschoss A. 1997. Relationships among calving season, heat load, energy balance and postpartum ovulation of dairy cows in a subtropical environment. Animal Reprod. Sci. 47:315-326.

Kaneene J.B., Hurd H.S. 1990. The National Animal Health Monitoring System in Michigan. I. Design, and data frequencies of selected dairy cattle diseases. Prev. Vet. Med. 8:103-114.

Kausche F.M., Robb E.J. 2003. A comprehensive review of Ceftiofur Sodium and Hydrochloride formulations for the treatment of acute bovine foot rot. Veterinary Therapeutics 4: 83-93.

Kempson S.A. and Logue D.N. 1993. Ultrastructural observations of hoof horn from dairy cows: changes in the white line during the first lactation. Vet. Rec. 132:524-527.

Kesler D.J., Garverick H.A., Youngquist R.S., Elmore R.G., Bierschwal C.J. 1977. Effect of days postpartum and endogenous reproductive hormones on GnRH-induced LH release in dairy cows. J. Anim. Sci. 46:797-803.

Kossaibati M.A., Esslemont R.J. 1997. The cost of production diseases in dairy herds in England. Vet. J. 154:41-51.

Lamming G. E., Darwash A. O. 1998. The use of milk progesterone profiles to characterize components of subfertility in milked dairy cows. Anim. Repro. Sci. 52:175-190.

Leach K.A., Logue D.N., Kempson S.A., Offer J.E., Ternent H.E., Randall J.M. 1997. Claw lesions in dairy cattle: Development of sole and white line hemorrhages during the first lactation. Vet. J 154:215.

Lee L.A., Ferguson J.D., Galligan D.T. 1989. Effect of disease on days open assessed by survival analysis. J. Dairy Sci. 72:1020-1026.

Logue D.N., Kempson S.A., Leach K.A. 1998. Pathology of the white line. Proceedings of the 10th International Symposium on Lameness in Ruminants, Lucerne, Switzerland, 142.

Lucey S., Rowlands G.J., Russell A.M. 1986. The associations between lameness and fertility in dairy cows. Vet. Rec. 118:628-631.

Lucy M.C., Staples C.R., Thatchet W.W., Erickson P.S., Cleale R.M. Firkins J.L. Clark J.H., Murphy M.R., Brodie B.O. 1992. Influence of diet composition, dry-matter intake, milk production and energy balance on time of post-partum ovulation and fertility in dairy cows. Anim. Repro. Sci. 54:323-331.

Lucy M.C., Staples C.R., Michel F.M., Thatcher W.W. 1991a. Energy balance and size and number of ovarian follicles detected by ultrasonography in early postpartum dairy cows. J. Dairy Sci. 74:473-482.

Lucy M.C., Staples C.R., Michel F.M., Thatcher W.W. 1991b. Effect of feeding calcium soaps to early postpartum dairy cows on plasma prostaglandin $F_{2\alpha}$, luteinizing hormone, and follicular growth. J. Dairy Sci. 74:483-489.

Mackey D.R., Wylie A.R.G., Sreenan J.M., Roche J.F., Diskin M.G. 2000. The effect of acute nutritional change on follicle wave turnover, gonadotrophin, steroid concentration in beef heifers. J. Anim. Sci. 78:429-442.

Magoffin D.A., and Weitsman S.R. 1993. Insulin-like growth factor-I stimulates the expression of 3ß-hydroxysteroid dehydrogenase messenger ribonucleic acid in ovarian theca-interstitial cells. Biol. Repro. 48:1166-1173.

Manske T., Hultgren J., Bergsten C. 2002a. Prevalence and interrelationships of hoof lesions and lameness in Swedish dairy cows. Prev. Vet. Med. 54:247-263.

Manske T., Hultgren J., Bergsten C. 2002b. The effect of claw trimming on the hoof health of Swedish dairy cattle. Prev. Vet. Med. 54:113–129.

Manske T., Hultgren J., Bergsten C. 2002c. Topical treatments of digital dermatitis associated with severe heel-horn erosion in a Swedish dairy herd. Prev. Vet. Med. 53:215-231.

Manson F.J., Leaver J.D. 1989. The effect of concentrate: silage ratio and of hoof trimming on lameness on dairy cattle. Anim. Prod. 49:15-22.

Manson F.J., Leaver J.D. 1988. The influence of concentrate amount and clinical lameness in dairy cattle. Anim. Prod. 47:185-190.

Martin S.W., Meek A.H., Willeberg P. 1987. Disease Causation. In: Veterinary Epidemiology. Ames, Iowa: Iowa State University Press, 121-148.

Mateus L., Lopes da Costa L., Diniz P., Ziecik A.J. 2003. Relationship between endotoxin and prostaglandin (PGE2 and PGFM) concentrations and ovarian function in dairy cows with puerperal endometritis. Anim. Reprod. Sci. 76:143-154.

McShane T.M., May T., miner J.L., Keisler D.H. 1992. Central actions of neuropeptide-Y may provide neuromodulatory link between nutrition and reproduction. Biol. Repro. 46:1151-1157.

Meisterling E.M., Dailey R.A. 1987. Use of concentrations of Progesterone and Estradiol-17ß in milk in monitoring postpartum ovarian function in dairy cows. J. Dairy Sci. 70:2154-2161.

Melendez P., Bartolome J., Archbald L. F., Donovan A. 2003. The association between lameness, ovarian cysts and fertility in lactating dairy cows. Theriogenology 59:927-937.

Monniaux D., Pisselet C. 1992. Control pf proliferation and differentiation of ovine granulosa cells by insulin-like growth factor-I and follicle-stimulating hormone in vitro. Biol. Repro. 46:109-119.

Moore D.A., Berry S.L., Truscott M.L. 2001. Efficacy of a nonantimicrobial cream administered topically for treatment of digital dermatitis in dairy cattle. J. Am. Vet. Med. Assoc. 219:1435-1438.

Morck D.W., Olson M.E., Louie T.J. 1998. Comparison of ceftiofur sodium and oxytetracycline for treatment of acute interdigital Phlegmon (foot rot) in feedlot cattle. J. Am. Vet. Med. Assoc. 212:254-257.

Moreira F., Orlandi C., Risco C.A., Mattos R., Lopes F., Thatcher W.W. Effects of presynchronization and bovine somatotropin on pregnancy rates to a timed artificial insemination protocol in lactating dairy cows. J. Dairy Sci. 84:1646-1659.

Mortellaro C.M. 1994. Digital Dermatitis. Proceedings of the 8th International symposium on disorders of the ruminant digit. Banff, Canada 137-141.

Mortensen K. 1994. Bovine laminitis (diffuse aseptic pododermatitis): clinical and pathological findings. Proceedings of the 8th International Symposium on Disorders of the Ruminant Digit 210-226.

Moss G.E. Parfet J.R., Marvin C.A., Allrich R.D. 1985. Pituitary concentrations of gonadotropins and receptors for the GnRH in suckled beef cows at various intervals after calving. J. Anim. Sci. 60:285-293.

Mülling C.K.W. 2002. Theories on the pathogenesis of white line disease – An anatomical perspective. Proceedings of the 12th International Symposium on Lameness in Ruminants. Orlando, Florida 91-96.

Murray R.D., Downham D.Y., Clarkson M.J., Faull W.B., Hughes J.W., Manson F.J., Merritt J.B., Russell W.B., Sutherst J.E., Ward W.R. 1996. Epidemiology of lameness in dairy cattle: description and analysis of foot lesions. Vet. Rec. 138:586-591.

National Animal Health Monitoring System (NAMHS) Dairy Part I. Reference of dairy health and management in the United States, 2002. United States Department of Agriculture: Animal Plant Health Inspection Service: Veterinary Services, Fort Collins. 2002, #N341.0701.

Nakao T., Moriyoshi M., Kawata K. 1992. The effect of postpartum ovarian dysfunction and endometritis on subsequent reproductive performance in high and medium producing dairy cows. Theriogenology 37:341-349.

Nielsson S.A. 1963. Clinical, morphological and experimental studies of laminitis in cattle. Acta Vet. Scand. 4(suppl 1):7.

Nocek J.E. 1997. Bovine Acidosis: Implications on Laminitis. J. Dairy Sci. 80:1005-1028.

Nordlund K.V., Garret E.F. 1994. Rumenocentesis: a technique for collecting rumen fluid for the diagnosis of subacute rumen acidosis in dairy herds. Bovine Pract. 28:109-112.

Opsomer G., Gröhn Y.T., Hertl J., Coryn M., Deluyker H., de Kruif A. 2000. Risk factors for postpartum ovarian dysfunction in high producing dairy cows in Belgium: A field study. Theriogenology 53:841-857.

Opsomer G., Coryn M., de Kruif A. 1999. Measurement of ovarian cyclicity in the postpartum dairy cow by progesterone analysis. Reprod. Dom. Anim. 34:297-300.

Opsomer G., Coryn M., Deluyker H., de Kruif A. 1998. An analysis of ovarian dysfunction in high yielding dairy cows after calving based on progesterone profiles. Reprod. Dom. Anim. 33:193-204.

Peeler E.J., Otte M.J., Esslemont R.J. 1994. Inter-relationships of periparturient diseases in dairy cows. Vet. Rec. 134:129-132.

Peter A.T., Simon J.E., Lucker and Bosu W.T.K. 1990. Site of action for endotoxin-induced cortisol release in the suppression of preovulatory luteinizing hormone surges. Theriogenology 33:637-643.

Peters A.R. 1984. Reproductive activity of the cow in the post-partum period. I. Factors affecting the length of the post-partum acyclic period. Br. Vet J. 140:76.

Peterse D.J., Cornelisse J.L., Toussaint Raven E. 1982. Dermatitis Digitalis. In: Proc. 12th International Conference on diseases of cattle 830-832.

Phogat J.B., Smith R.F., Dobson H. 1997. Effect of adrenocorticotrophic hormone on gonadotrophin-releasing hormone-induced luteinizing hormone secretion in vitro. Anim Repro. Sci. 48:53-65.

Phogat J.B., Smith R.F., Dobson H. 1999. Effect of transport on pituitary responsiveness to exogenous pulsatile GnRH and estradiol-induced LH release in intact ewes. J. Reprod. Fert. 116:9-18.

Radostits O.M., Gay C.C., Blood D.C., Hinchcliff K.W.2000. Bovine interdigital necrobacillosis, in *Veterinary Medicine*, ed 9. Philadelphia, WB Saunders 948-950.

Rajala-Schultz P.J., Gröhn Y.T., McCulloch C.E. 1999. Effect of milk fever, ketosis and lameness on milk yield in dairy cows. J. Dairy Sci. 82:288-294.

Randel R.D. 1990. Nutrition and postpartum rebreeding in cattle. J. Anim. Sci. 68:853-862.

Read, D.H., Walker, R.L., 1998. Papillomatous digital dermatitis (footwarts) in California dairy cattle: clinical and gross pathologic findings. J. Vet. Diagn. Invest. 10:67-76.

Read D.H., Walker R.L. 1994. Papillomatous digital dermatitis of dairy cattle in California: Clinical Characteristics. 8th International Symposium on disorders of the ruminant digit. Banff, Canada 159-163.

Read D.H., Walker D.L., Castro A.E., Sundberg J.P., Thurmond M.C.1992. An invasive spirochete associated with interdigital papillomatosis of dairy cattle. Vet. Rec. 130:59-60.

Rebhun, W.C., Payne, R.M., King, J.M., Wolfe, M., Begg, S.N., 1980. Interdigital papillomatosis in dairy cattle. J. Am. Vet. Med. Assoc. 177: 437-440.

Rebhun W.C., Pearson E.G. 1982. Clinical management of bovine foot problems. J. Am. Vet. Med. Assoc. 181:572-579.

Reist, M., Erdin D.K., von Euw D., Tschümperlin K.M., Leuenberger H., Hammon H.M., Morel C., Philipona C., Zbinden Y., Künzi N., Blum J.W. 2003. Postpartum reproductive function: association with energy, metabolic and endocrine status in high yielding dairy cows. Theriogenology 59:1707-1723.

Reist M., Koller A., Küpfer U., Blum J. W. 2000. First ovulation and ketone body status in the early postpartum period of dairy cows. Theriogenology 54:685-701.

Rhodes F.M., McDougall S., Burke C.R., Verkerk G.A., Macmillan K.L. 2003. Treatment of cows with extended postpartum anestrous interval. J. Dairy Sci. 86:1876-1894.

Rhodes F.M., Burke C.R., Clark B.A., Day M.L., Macmillan K.L. 2002. Effect of treatment with progesterone and estradiol benzoate on ovarian follicular turnover in postpartum anoestrus cows and cows which have resumed oestrous cycles. Anim. Repro. Sci. 69:139-150.

Rodriguez-Lainz, A., Hird, D.W., Carpenter, T.E., Read, D.H. 1996. Case-control study of papillomatous digital dermatitis in southern California dairy farms. Prev. Vet. Med. 28: 117-131.

Rutter B. 1994. Lameness in dairy cows – Incidence in Argentina. 8th Symposium on disorders of the ruminant digit. Banff, Canada 40.

Savio J.D., Boland M.P., Hynes N., Roche J.F. 1990a. Resumption of follicular activity in the early post-partum period of dairy cows. J. Reprod. Fert. 88:569-579.

Savio J.D., Boland M.P., Roche J.F. 1990b. Development of dominant follicles and length of ovarian cycles in post-partum dairy cows. J. Reprod. Fert. 88:581-591.

Schams, D., Schallenberger, E., Menzer Ch., Stangl, J., Zottmeier K., Hoffman B., Karg H. 1978. Profiles of LH, FSH and Progesterone in postpartum dairy cows and their relationship to the commencement of cyclic functions. Theriogenology 10:453-467.

Schillo K.K. 1992. Effects of dietary energy on control of luteinizing hormone secretion in cattle and sheep. J. Anim. Sci. 70:1271-1282.

Schwartz M.W. Woods S.C., Porte D., Seeley R.J. 2000. Central nervous system control of food intake. Nature 404:661-671.

Senatore E.M., Butler W.R., Oltenacu P.A. 1996. Relationships between energy balance and post-partum ovarian activity and fertility in first lactation dairy cows. Animal Science 62:17-23.

Shearer J.K., van Amstel S.R. 2001. Functional and corrective claw trimming. In: Anderson DA, eds. The Veterinary Clinics of North America. Philadelphia, Pennsylvania: WB Saunders 53-72.

Shearer J.K., Hernandez J. 2000. Efficacy of two modified nonantibiotic formulations (Victory) for treatment of papillomatous digital dermatitis in dairy cows. J. Dairy Sci. 83:741-745.

Shearer J.K., Elliott J.B. 1998. Papillomatous digital dermatitis: treatment and control strategies – Part I. Compend. Contin. Educ. Pract. Vet. 20:158-166.

Shearer J.K., Hernandez J., Elliott J.B. 1998. Papillomatous digital dermatitis: treatment and control strategies – Part II. Compend. Contin. Educ. Pract. Vet. 20:213-223.

Sheldon I.M. 1994. Digital and Interdigital Dermatitis in dairy cattle. Vet. Rec. 559-560.

Singh S. S., Ward W. R., Lautenbach K., Murray R. D. 1993. Behaviour of lame and normal dairy cows in cubicles and in straw yard. Vet. Rec. 133: 204-208.

Smilie R.H., Hoblet K.H., Eastridge M.L., Weiss W.P., Schnitkey G.L., Moeschberger M.L. 1999. Subclinicallaminitis in dairy cows: use of severity of hoof lesions to rank and evaluate herds. Vet. Rec. 144:17-21.

Smilie R.H., Hoblet K.H., Weiss W.P. 1996. Prevalence of lesions associated with subclinical laminitis in first-lactation cows from herd with high milk production. J. Am. Vet. Med. Assoc. 208:1445.

Smith G.A., Wallace L.M. 1992. Further observations on the weak immunugenicity of *Fusobacterium necrophorum*. Res. Vet. Sci. 52:262-263.

Somers J.G.C.J., Frankena K., Noordhuizen-Stassen E.N., Metz J.H.M. 2003. Prevalence of claw disorders in dutch dairy cows exposed to several floor systems. J. Dairy Sci. 86:2082-2093.

Spicer L.J., Alonso J., Chamberlain C.S. 2001. Effects of Thyroid hormones on bovine granulosa and thecal cell function in vitro: Dependence on insulin and gonadotropins. J. Dairy Sci. 84:1069-1076.

Spicer L.J. and Stewart R.E. 1996. Interactions among basic fibroblast growth factor, epidermal growth factor, insulin and insulin-like growth factor-I on cell numbers and steroidogenesis of bovine thecal cells: role of IGF-I receptors. Biol. Repro. 54:255-263.

Spicer L.J., Echternkamp S.E. 1995. The ovarian insulin and insulin-like growth factor system with an emphasis on domestic animals. Domest. Anim. Endocrinol. 12:223-245.

Spicer L.J., Alpizar E., Echternkamp. 1993. Effects of insulin, insulin-like growth factor I, and gonadotropins on bovine granulose cell proliferation, progesterone production, estradiol production, and (or) insulin-like growth factor production in vitro. J. Anim. Sci. 71:1232-1241.

Spicer L.J., Tucker W.B., Adams G.D. 1990. Insulin-Like Growth Factor-I in dairy cows: Relationships among energy balance, body condition, ovarian activity, and estrus behavior. J. Dairy Sci. 73:929-937.

Sprecher D.J., Hostetler D.E., Kaneene J.B. 1997. A lameness scoring system that uses posture and gait to predict dairy cattle reproductive performance. Theriogenology 47:1179-1187.

Staples C.R., Thatcher W.W., Clark J.H. 1990. Relationship between ovarian activity and energy status during the early postpartum period of high producing dairy cows. J. Dairy Sci. 73:938-947.

Stevenson J.S., Call E.P. 1983. Influence of early estrus, ovulation, and insemination on fertility in postpartum Holstein cows. Theriogenology 19:367-375.

Stevenson J.S., Britt J.H. 1979. Relationships among luteinizing hormone, estradiol, progesterone, glococorticoids, milk yield, body weight and postpartum ovarian activity in Holstein cows. J. Anim. Sci. 48:570-577.

Stewart R.E., Spicer L.J., Hamilton T.D., Keefer B.E. 1995. Effects of Insulin-Like Growth Factor I and Insulin on proliferation and basal and luteinizing hormone-induced steroidogenesis of bovine thecal cells: Involvement of glucose receptors for insulin-like growth factor-I and Luteinizing hormone. J. Anim. Sci. 3:3719-3731.

Szklo M. and Nieto C.J. 2000. Epidemiology beyond the basics. Aspen Publishers Inc., Gaithersburg, Maryland 416-419.

Tarlton J.F., Holah D.E., Evans K.M., Jones S., Pearson G.R., Webster J.F. 2002. Biochemical changes in the support structures of bovine hooves around the time of first calving. Vet. J. 163:196-204.

Thatcher W.W., Wilcox C.J. 1973. Postpartum estrus as an indicator of reproductive status in the dairy cow. J. Dairy Sci. 59:608-610.

Toussaint Raven E. 1989. Cattle Footcare and claw trimming. Ipswich, UK, Farming Press.

Tranter W. and Morris R. 1991. A case study of lameness in three dairy herds. NZ. Vet. J. 39:88-96.

Van Arendonk J.A.M., Stokvisch P.E., Korver S.1984. Factors determining the carcass value of culled dairy cows. Livest. Prod. Sci. 11:391-400.

Varner M., Maatje K., Nielen M., Rossing W. 1994. Changes in dairy cow pedometer readings with different number of cows in estrus. Proc. 3rd International Dairy Housing Conference, Orlando, Fl, ASAE:434-442.

Vermunt J.J. 1994. Predisposing causes of laminitis. In: Proceedings of the 8th International Symposium of Disorders of the Ruminant Digits. Banff, Canada 236-258.

Villa-Godoy A., Hughes T.L., Emery R.S., Chapin L.T., Fogwell R.L. 1988. Associations between energy balance and luteal function in lactating dairy cows. J. Dairy Sci. 71:1063-1072.

Vizcarra J.A., Wettemann R.P., Spitzer J.C., Morrison D.G. 1998. Body condition at parturition and postpartum weight gain influence luteal activity and concentrations of glucose, insulin, and non-esterified fatty acids in plasma of primiparous beef cows. J. Anim. Sci. 76:927-936.

Walker R.L., Read D.H., Loretz K.J., Nordhausen R.W. 1995. Spirochetes isolated from dairy cattle with Papillomatous digital dermatitis and interdigital dermatitis. Vet. Microbiol. 47:343-355.

Ward W.R. Standardization of gait analysis in cattle. 1998. In: 10th International Symposium on Lameness in Ruminants. Lucerne, Switzerland 33-35.

Warnick L.D., Jansen D., Guard C.L. 2001. The effect of lameness on milk production in dairy cows. J. Dairy Sci. 84:1988-1997.

Weaver A.D. 1994. International terminology of digital diseases. 8th International Symposium on disorders of the ruminant digit. Banff, Canada. 25-25.

Webb R., Gosden R.G., Telfer E.E., Moor R.M. 1999. Factors affecting folliculogenesis in ruminants. Anim. Sci. 68:257-284.

Webb R., Lamming G.E., Haynes N.B., Foxcroft G.R. 1980. Plasma progesterone and gonadotrophin concentrations and ovarian activity in post-partum dairy cows. J. Reprod. Fert. 59:133-143.

Weigler B.J., Hird D.W., Sischo W.M. 1990. Veterinary and non-veterinary costs of diseases in 29 California dairies participating in the national Animal Health Monitoring System from 1988 to 1989. Prev. Vet. Med. 196:1945-1949.

Wells S.J., Garber L.P., Wagner B.A. 1999. Papillomatous digital dermatitis and associated risk factors in US dairy herds. Prev. Vet. Med. 38:11-24.

Wells S.J., Trent A.M., Marsh W.E., Robinson R.A. 1993a. Prevalence and severity of lameness in lactating dairy cows in a sample of Minnesota and Wisconsin herds. J. Am. Vet. Med. Assoc. 1:78-82.

Wells S.J., Trent A.M., Marsh W.E., Mc Govern P.G., Robinson R.A. 1993b. Individual cow risk factors for clinical lameness in lactating dairy cows. Prev. Vet. Med. 17:95-109.

Whitaker D., Kelly J., Smith E. 1983. Incidence of lameness in dairy cows. Vet. Rec. 113:60-62.

Wilcox, C.J., W.W. Thatcher, F.G. Martin. Statistical analysis of repeated measurements in physiology experiments. 1990. Page 92 in Proc.Final Res. Coord. Mtg. FAO/IAEA/Arreglos Regionales Co-Operativas para la promocion de la Ciencia y la Tecnologia Nucleares en America Latina III, International Atomic Energy Agency, Vienna, Austria, (Abstr.)

Williams G.L., Amstalden M., Garcia M.R., Stanko R.L., Nizielski S.E., Morrison C.D., Keisler D.H. 2002. Leptin and its role in the centrl regulation of reproduction in cattle. Dom. Anim. Endoc. 23:339-349.

Zachow R.J., Magoffin D.A. 1997. Direct intraovarian effects of Leptin: Impairment of the synergistic action of insulin-like growth factor-I on follicle-stimulating hormone-dependent estradiol-17ß production by rat ovarian granulosa cells. Endocrinology 138:847-850.

Zolman J., Convey E.M., Britt J.H. 1974. Relationships between the luteinizing hormone response to gonadotrophin releasing hormone and endogenous steroids. J. Anim. Sci. 39:355-359.

Zurek E., Foxcroft G.R., Kennelly J.J. 1995. Metabolic status and interval to first ovulation in postpartum dairy cows. J. Dairy Sci. 78:1909-1920.

BIOGRAPHICAL SKETCH

Eduardo Jose Garbarino is the son of Marta Pisarenko and Eduardo Jose Garbarino. He was born in Buenos Aires, Argentina, on July the 8th of 1970. He lived in Buenos Aires during his entire childhood and finished his high school studies in 1989. He started veterinary medicine in 1994 at Universidad del Salvador, College of Veterinary Medicine, in Argentina. In 1998, he was honored with the President's Award (from the President of the Argentinean Nation) for the most qualified graduate from all Veterinary Medicine Schools in Argentina. He completed the degree of "Medico Veterinario" (MV) in March 1999 (with Honors Diploma). After graduation, he obtained a scholarship from the Argentinean government to work at the National Institute of Agricultural Research, in the areas of mastitis and subclinical ketosis in dairy cows. In the year 2000, he obtained a 1-year scholarship from the Secretary of Science and Techniques of the Argentinean Nation (Secretaría de Ciencia y Técnica de la Nación Argentina) to work on research in the dairy project of the Agricultural Experimental Station of the National Institute of Agricultural Research (INTA), comparing two different milk-production systems. From December of 2000 to April 2001, he did and externship at a 2300-cow dairy farm in Torreón, Mexico. In May of 2002, he started a 3-year residency program at the University of Florida, College of Veterinary Medicine, in the Food Animal Reproduction and Medicine Service. In 2002, he enrolled in the graduate program at the Department of Large Animal Clinical Sciences, College of Veterinary Medicine, University of Florida, to obtain the degree of Master of Science.