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NEGATIVE IMPACT OF LAMENESS ON FERTILITY OUTCOMES AND EARLY PREGNANCY LOSS IN DAIRY COWS

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Abbreviations

FSH	Follicle Stimulating Hormone
LH	Luteinizing Hormone
AI	Artificial Insemination
DIM	Days In Milk
DMI	Dry Matter Intake
NEB	Negative Energy Balance
GnRH	Gonadotropin hormone-Releasing Hormone
CL	Corpus Luteum
GC	Glucocorticoid
PGF-2-α	Prostaglandin F2-Alpha
BHB	Beta-Hydroxybutyrate
HT	Hypothalamus
FSCR	First Service Conception Rate
IGF	Insulin Growth Factor
NEFA	Non-Esterified Fatty Acids
CNS	Central Nervous System
APP's	Acute Phase Protein's
IL-2	Interleukin-2
IFN-α	Interferon-Alpha
IFN-γ	Interferon-Gamma
TNF-α	Tumor Necrosis Factor-Alpha
DHEA	Dehydroepiandrosterone
NMDA	N-Methyl-D-Aspartate (Receptor)
AMPA	Alpha-Amino-3-Hydroxy-5-Methyl-4-Isoazolepropionic Acid (Receptor)
KA	Kainate (Receptor)
MIP-1-β	Macrophage Inflammatory Protein -1-Beta
CKCL-10	CXC Motif Chemokine Ligand 10
VLS	Visual Locomotion Score

1 Abstract

Lameness in dairy cows exerts a negative influence upon the capacity for the dairy cow to demonstrate an optimum fertility status. Lameness impedes the prospect for a dairy cow to successfully grow an embryo and ultimately produce a healthy calf. Therefore, efforts to reduce the levels of lameness within the herd represent a primary consideration for farmers as they strive to secure economic viability. There are different modes of action of lameness which; acting together serve as a detrimental force against the health and well-being of the dairy cow. Lameness causes behavioural changes such as reduced feeding times which subsequently diminishes energy levels and leads to poor reproductive outcomes. Lameness represents a chronic stressor for the dairy cow and is at the core of inflammatory processes which in turn are not compatible with a healthy reproductive status. For this systematic review I have explored studies and papers about lameness in dairy cows and I have compiled my findings according to the negative influence of this lameness on different aspects of reproductive performance.

2 Introduction

Lameness due to injury or disease in the foot or leg is a major animal welfare problem facing the dairy cattle herd today. Causes of lameness can be *infectious* or *non-infectious*. Well-known *infectious* causes of lameness are Foot Rot, Interdigital Dermatitis and Heel Horn Erosion. The most common *non-infectious* causes of lameness in dairy cattle are Sole Ulcers, Sole Haemorrhages, White Line Disease and Toe Ulcers. Of course; the type, frequency and distribution of hoof lesions varies greatly across different countries.

Most studies on lameness are based on two very distinct management systems; namely dairy herds managed under zero-grazing systems and herds with access to grazing for long periods of the year. The prevalence of lameness will vary according to the housing system which considers whether the cows are kept in individual cubicles, tie stalls or in bedded packs. Further variables include the milking system (conventional or robotic) and the lying surface which can take various forms such as rubber matting, straw, sand, wood shavings or access to outdoor areas.

Estimates of lameness prevalence across the worldwide dairy industry range from 8% in pasture-based systems compared to 15-30% for animals in confined housing.

Lameness is a multifactorial condition with various risk factors ranging from sub-optimal managerial practices to environmental factors and of course individual cow factors are unquestionably relevant. Poor herd management is seen when there is evidence of inappropriate stocking densities, inadequate treatment of lameness cases upon diagnosis, inadequate hoof-trimming practices and neglect of proper hygiene protocols. Environmental risk factors are concerned with the stall features, flooring surface and feed alley dimensions. Risk factors revolving around the individual cow include such things as the body condition, history of lameness events in the past, breed and parity.

Lameness amongst dairy cows serves as a platform for many negative consequences, not least the detrimental impact on milk production which can threaten economic viability for the farmer. In the following I would like to focus on the negative impact of lameness on the reproductive status of dairy cows.

Firstly, it is essential to have a good grasp of the events unfolding during the normal reproductive cycle of a dairy cow. In this way it will be easier to appreciate the impact which lameness can bestow upon individual stages of this reproductive cycle. The ovary produces the oocyte by a process known as oogenesis. The oocyte is the female gametocyte or germ cell. It is an immature egg that can eventually be fertilised by sperm to produce offspring. The oestrous cycle is the name given to the cycle of oocyte development in cattle. The oestrous cycle lasts on average 21 days.
The ovary contains thousands of tiny primary follicles. Each follicle consists of a germ cell surrounded by a layer of cells. This germ cell has the potential to mature into an egg if the follicle completes development. In fact, relatively few primary follicles develop completely, through a series of phases. Once the follicle reaches a mature state it is known as a Graafian follicle.

Hormones have an essential role to play in the sequence of events which culminate with the birth of a new calf. Gonadotropin Releasing Hormone (GnRH) produced in the hypothalamus controls the release of Follicle Stimulating Hormone (FSH) and Luteinizing Hormone (LH) from the pituitary gland at the base of the brain. FSH is needed for proper development of the follicle.

The female hormone Estrogen is produced by the ovary. A high concentration of Estrogen in the blood stimulates the onset of oestrous. Once the level of Estrogen reaches a certain threshold; this facilitates a surge of LH to be released by the pituitary gland. A peak of LH is required for ovulation to occur whereby the follicle is ruptured so that its contents, including the egg, are released.

Following ovulation, the cells that developed within the follicle differentiate to form the Corpus Luteum (CL) which serves a crucial function as it produces Progesterone. Progesterone maintains the proper uterine environment for pregnancy.

The released egg is caught by the infundibulum and moves into the oviduct where fertilization occurs if viable sperm are present. The embryo develops in the uterine horns.

Prostaglandin-F-2-Alpha (PGF2α) produced by the uterus is also of utmost importance. PGF2α is a luteolytic agent which is responsible for the regression of the CL late in the oestrous cycle and thereafter a new oestrous cycle may be initiated in the case of a non-pregnant female. In the case of a pregnant heifer/cow; a signal is sent from the developing embryo to the uterus which prevents the release of PGF2α so that the CL may persist throughout the pregnancy.

Gestation length in dairy cows can vary according to the breed concerned. In the case of Holstein-Friesian dairy cows, the average gestation period is 279 days. Early pregnancy loss can be divided into *early* and *late embryonic death* and the period of *early foetal death*.

The *early embryonic death* happens in the first 16 days. The threshold is 16 days because after this the cow will start a new oestrus cycle. Therefore, if the early embryonic death has occurred; this cow will return to oestrus on time. Pregnancy loss in this earliest stage is very difficult to detect.

The *late embryonic death* occurs in the period between day 16 and the first pregnancy check on day 42. This is a very lethal period whereby 40% of all embryos are lost.

The time after day 42 lasting until day 90 accounts for the period of *early foetal death*. Late embryonic death and early foetal death are both detectable on ultrasound.

The aim of this paper is to highlight some of the key information which I discovered while reviewing literature that was focused on lameness in dairy cows. I dedicated my efforts towards exploring research and literature which had found links between lameness and a compromised ability for dairy cows to grow and produce healthy offspring.

3 Objectives

- Examine the research finding that calving to 1st service interval and calving to conception interval are extended in lame cattle.
- Review the literature to support the hypothesis that lameness can contribute to a prolonged interval between the first service and conception.
- Establish if a link has been found between the presence of lameness and a delayed post-partum resumption of cyclicity.
- Explore the studies which have identified an increased prevalence of early pregnancy loss amongst lame cows compared to those cows not confirmed as lame during the breeding period.
- Considering that lameness alters the normal behaviours and feeding patterns of a cow; a strong objective is to explore how such altered behaviour patterns impede the capacity of the cow to achieve optimal reproductive outcomes.
- Lameness represents a significantly painful experience for the cow; an objective of this literature review is to examine whether any association has been found between poor fertility outcomes and the increased levels of catecholamines and stress hormones in the blood of cows experiencing pain due to lameness.
- Lameness can stimulate inflammatory processes and for this paper it is also important to explore the negative shadow which certain raised inflammatory markers cast upon the reproductive status of dairy cows.

4 Literature Review

4.1 Modes of Action of lameness

4.1.1 Lameness and Behavioural Changes

Lameness is known to erode the time available to cows to fulfil normal activities. The degree to which the altered behaviour patterns are seen depends on the severity of the lameness (Tsousis, Boscos and Praxitelous, 2022). Tsousis et al. highlight that such altered behaviour can impact both directly and indirectly on reproduction.

4.1.1.1 Reduced Feeding Time

Lameness reduces feeding time which indirectly contributes negatively to reproduction. Moderately lame cows spent twenty minutes less time eating while the severely lame cows were eating forty minutes less than the healthy cows according to Hut, Hostens, Beijaard, van Eerdenburg, Hulsen, Hooijer, Stassen & Nielen, (2021). It was also found that cows which are chronically lame during the dry period engaged in feeding for shorter time periods compared to healthy animals and subsequently these chronically lame cows were more susceptible to transition diseases (Daros, Eriksson, Weary, & von Keyserlingk, (2020)). Tsousis et al. suggest that lame cows are indeed more vulnerable in terms of experiencing a more prolonged and significant Negative Energy Balance (NEB). However, Tsousis et al. also acknowledge that many studies have actually concluded that a NEB is a significant risk factor for lameness as opposed to the other way around. Several studies serve to strengthen this argument such as the study by Lim, Huxley, Willshire, Green, Othman & Kaler (2015) which found that those healthy cows becoming lame were more often those cows demonstrating a low body condition score and lameness was also more likely to occur in cows having a greater decline in body condition score after calving. The study by Bicalho, Machado & Caixeta (2009) also affirms the negative impact of a low body condition score, finding a direct link between it and thinning of the digital cushion and subsequent increased threat of lameness.

4.1.1.2 Negative Energy Balance (NEB)

Praxitelous et al., (2022) conducted a study to provide clarity on the degree to which the negative impact of lameness on reproduction is due to NEB. This study examined lameness, metabolic and ovarian status of only multiparous dairy cows. The initial screening took place at 28 days in milk (DIM) with lesion identification and initiation of a pre-synchronization protocol then occurring at 37 DIM and then inspection again at timed AI (67-74 days in milk).

The sample size was small consisting of 52 lame cows and 27 non-lame cows. Making up the 52 lameness cases were 36 cows with damage to the claw while 15 cows had an infectious disease in the background and there was one mixed case. The study found that the lame cows had higher overall levels of Non-Esterified Fatty Acids (NEFA's) than the non-lame cows.

NEFA's are biomarkers indicative of excessive lipid mobilizations often seen in high-producing cows. Cows, during high production, may experience a NEB and in order to satisfy their increased energy requirements; lipid mobilization is seen in these cows. This causes a shift from anabolic to catabolic metabolism. Triglycerides are the main lipid molecules stored as an energy reserve in adipose tissue. In NEB, triglycerides enter a hydrolysis pathway mediated by lipases. In this catabolic process high energy substrates are released into the bloodstream. Triglycerides release one Glycerol and three NEFA molecules which are the major source of energy of tissues during periods of NEB. The excessive rise of NEFA's and Beta-Hydroxy-Butyrate (BHB) indicates a poor adaptive response to NEB and has a direct negative effect on the health of the animal. In this way cows are increasingly vulnerable to developing pathologies such as ketosis, abomasal dislocation, metritis and mastitis. (Tessari, Berlanda, Morgante, Badon, Gianesella, Mazzotta, Contiero & Fiore, 2020)

The study by Praxitelous et al., (2022) also found that at the initiation of the experiment, the lame cows were cyclic in smaller proportion compared to the non-lame cows based on ultrasonography of the ovaries and progesterone values. Lame cows also had lower first service conception rate (FSCR) at timed AI. Overall the study concluded that lame cows do indeed have suppressed reproductive performance and metabolic status. However the study did not find that these conditions are actually related. To this end it was not found that elevated BHB or NEFA levels impacted on cyclicity status and FSCR and no link to lameness was found.

4.1.1.3 Decreased Mobility

Decreased mobility represents a behaviour change as a consequence of lameness which may have a *direct* negative impact on reproduction. The expression and detection of oestrous rely on adequate cow mobility and farmers will observe their herd in this regard. The only exception is in cases whereby the dairy farmer does not use oestrous detection for any aspect of reproductive management. In the article by Tsousis et al. the authors refer to other studies by Hut et al., (2021) and Weigele et al., (2018) in which mobility sensors measured a 5-15% reduction in the average daily step-count of lame cows as against their non-lame counterparts. In both these studies, Hut et al., (2021) and Weigele et al., (2018) also found that the lying time

was 30-40 minutes longer per day amongst lame cows. It stands to reason that with reduced movement and increased lying time, these cows were less inclined to socialise and their ability to express oestrous behaviour may have been impeded (Sood & Nanda, (2006)).

Tsousis et al., (2022) reveal that many studies such as those by Sood & Nanda, (2006) and those by Walker et al., (2008) and Walker et al., (2010) have established that oestrous intensity is compromised in lame cows. These studies, refer particularly to weakened intensity of the oestrous behaviour and reduced mounting behaviour. Sood & Nanda, (2006) also found that shorter oestrous duration was more prevalent amongst lame cows (30%) versus the non-lame controls (18%) in their study.

Contrarily then, the study by Walker et al., (2010) and the study by Sood & Nanda, (2006) revealed that secondary oestrous signs such as restlessness or sniffing the vulva nor the incidence of oestrous; are not influenced by lameness providing that ovarian activity has already commenced. However mounting is the most important sign and often the only sign used by farmers for oestrous detection and so reproductive management is profoundly disturbed when such mounting behaviour cannot proceed in the usual manner. Tsousis et al., (2022) acknowledge that while activities such as sniffing are not painful for lame cows; on the other hand, mounting does demand physical soundness and therefore cows with hoof lesions for example, will engage in reduced mounting which can be attributed at least partly to physical constraints.

4.1.2 Lameness as a Chronic Stressor

Tsousis et al., (2022) discussed that a hormonal background could also be responsible for behavioural changes in dairy cows, especially regarding oestrous expression. Oestrous behaviour in cows is dependent upon a hormonal cascade of events.

The sequence of events begins with GnRH secretion form the Hypothalamus (HT) which initiates Luteinizing Hormone (LH) pulses. This is followed by a stimulation of Estradiol production from the dominant follicle which induces the pre-ovulatory LH surge and the subsequent expression of oestrous. (Lyimo, Nielen, Ouweltjes, Kruip & Eerdenburg (2000))

However, this sequence of events can be disrupted at various levels for cows experiencing some chronic stressor such as lameness. In addition, Lyimo et al., (2000) found that there is correlation between proper oestrous intensity and healthy Estradiol levels.

Stress and the reproductive axis share endocrine glands in the HT and pituitary and so it is hypothesized that interactions can occur (Crowe & Williams, (2012)).

During a stress response, Glucocorticoids (GC's), mainly Cortisol and Catecholamines (Adrenaline and Noradrenaline) are raised. These GC's participate in alteration of carbohydrate and protein metabolism, growth, reproduction and immune function. The initiation of a stress response coincides with a nutritionally demanding immune response for the animal. In cattle, correlation has been found between Cortisol and reduced rates of reproduction, suppressed milk production and suppression of the immune system rendering such animals more vulnerable for developing disease. Short-term exposure to a stressor leads to a sharp increase in Cortisol secretion while long term exposure to a stressor means that the animal suffers from a chronic stress. Chronic stress is often accompanied by reduced Cortisol levels in the blood and/or reduced responsiveness of cells to Cortisol which may in turn increase the risk of chronic inflammation. (Villamediana, P., (2022))

Cortisol is continually released during a stress response and acts in the liver to synthesize more glucose. The pancreas then begins to release more insulin as it attempts to keep up with the changes in metabolism. This cycle caused by stress leads to insulin resistance and decreased Glucose uptake by muscle and fat tissues. Therefore, reproductive efficiency and high levels of milk production both require optimum responsiveness of the cows cells to insulin. (Villamediana, P., (2022))

Dobson et al., (2000) found that heifers exposed to Adrenocorticotrophic hormone experienced diminished pulsatile secretion of LH while Maciel et al., (2001) also found suppression of luteal function related to lower plasma IGF-1 and IGF-2 concentrations in dairy cows after a dexamethasone challenge. Woelders et al., (2014) again emphasised that inadequate progesterone priming and prolonged luteal phases in ruminants can be deleterious to the expression and intensity of oestrous. Tsousis et al., (2022) highlight that there are disruptions to the HT-Pituitary-Ovarian Axis in lame cows, pointing to the finding by Almeida et al., (2008) that lame cows have both decreased Dehydroepiandrosterone (DHEA) and increased Cortisol:DHEA ratio compared to non-lame cows. Tsousis et al., say that this is suggestive that foot lesions do reach the CNS.

The article by Tsousis et al., (2022) also uncovers the findings of a study by O'Driscoll et al., (2015) who made the same finding that in lame cows there is an increased Cortisol:DHEA ratio. O' Driscoll et al., (2015) also found that serum levels of lame cows contained increased

cortisol levels. They also found an increase in the Neutrophil:Lymphocyte ratio of lame cows with sole ulcers as opposed to non-lame cows, affirming that this is indicative of long-term stress.

The findings of the study by Walker et al., (2008) did not correlate with those of O' Driscoll et al., (2015) as they found no marked differences in the hormonal profile of lame cows versus their non-lame counterparts.

However, Walker et al., (2010) did find evidence which coincided with the frequent finding of compromised luteal function in lame cows. Compromised luteal function is expressed by Progesterone levels and Walker et al., (2010) found that Progesterone levels of lame cows were lowered in the six days preceding oestrous compared to non-lame cows and of course this directly diminishes oestrous expression.

Lower Progesterone levels were also observed in the study by Melendez et al. (2018) which found these decreased values in cows between thirty and forty days in milk.

The article by Tsousis et al., (2022) also reveals details of a study by Morris et al., (2011) in which even those lame cows which do respond to a GnRH stimulation programme (70% of the cows in their study); still demonstrate sub-optimal pre-ovulatory progesterone levels. This is supporting the common finding of the demeaning influence of lameness upon normal progesterone levels.

In their own study, Praxitelous et al., (2022) randomly allocated lame cows to a pre-synchronization protocol with progesterone or the randomly allocated cows received two injections, 14 days apart of PGF-2-α.

Non-lame cows in their study acted as controls. All of the cows received OV Synch and timed AI 12 days after the pre-synchronization. Praxitelous et al., (2022) found no difference in the pre-synchronization and synchronization success in the proportion of cows showing incomplete luteolysis before insemination and ovulating but they did find a tendency for lower First Service Conception Rate (FSCR). This agreed with the findings of a study by McNally et al., (2014) in which a synchronization protocol in lame cows resulted in 80% of cows exhibiting oestrous but only 22.7% proceeded to successfully conceive. Therefore Tsousis et al., (2022) concluded that lame cows can obviously respond at least partly to hormonal manipulations, yet conception rates well below 30% should be expected.

4.1.3 Lameness and Inflammatory Processes

4.1.3.1 Raised Haptoglobin Levels

Tsousis et al., (2022), in their article; unveiled findings from studies about the raised inflammatory markers in lame cows and importantly the subsequent influence upon reproductive performance. They refer to the findings of O' Driscoll et al., (2015) that there is increased Haptoglobin levels and increased cytokine relative gene expression in leukocytes of cows with sole ulcers.

Haptoglobin belongs in the category of Acute Phase Proteins (APP's). APP's are a group of blood proteins which will demonstrate changes in their respective concentrations in the case of animals subjected to a particular internal or external challenge such as infection, inflammation, surgical trauma or stress (Eckersall, PD., 2004).

Also finding increased Haptoglobin levels were Smith et al., (2010) but their study found this increase more so in severe lameness cases such as Septic Pododermatitis and Foot Rot. Smith et al., (2010) found that increased Haptoglobin levels occurred to a lesser degree in the case of milder lameness presentations such as sole ulcers and digital dermatitis.

Tadich et al., (2013) also found that Haptoglobin levels are gradually increasing in lame cows which is indicative of the presence of inflammation. Levels of APP's such as Haptoglobin are low or undetectable in healthy cows by comparison (Tadich et al., 2013).

Tsousis et al., (2022) qualify the relevance of the findings in these studies as they conclude their discussion of Haptoglobin levels by pointing to research by Cheong et al., (2017) which found that increased Haptoglobin levels have been related to post-partum anovulation in dairy cows. A study by Krause et al., (2014) serves to further confirm the relevance of raised Haptoglobin levels as they found that such raised Haptoglobin levels can still be in the background of post-partum anovulation in dairy cows even if these levels of Haptoglobin were raised in the pre-partum period.

4.1.3.2 Raised Inflammatory Cytokines

Following an injury, immune cells are responsible for the release of pro-inflammatory cytokines at the site of injury (Sommer, C. & Kress, M. (2004)). Nazifi et al., (2012) conducted a study which found that lame cows diagnosed with Interdigital Dermatitis had higher plasma levels of IFN-γ and TNF-α.

Further exploring the territory of raised inflammatory markers in lame cows, Tsousis et al., (2022) revealed findings from a study by Herzberg et al., (2020) which involved researching the cytokine profile in the spinal cord of both lame dairy cows and a non-lame control group. The study by Herzberg et al., (2020) found higher concentrations of TNF- α, raised Interferon-γ and raised Interleukin-1-α levels in the lame cows.

4.1.3.3 Insulin and Insulin Growth Factor-1 (IGF-1)

The release of pro-inflammatory mediators means that the immune system will be activated. This is a process which demands alot of energy. In order to meet such increased energy requirements by the immune system; there must be a reallocation of available nutrients and energy. This comes at the expense of certain functions within the body of the cow which are suddenly regarded as dispensable when inflammation occurs. Growth, production and reproduction are some of the functions which receive less nutritional support in the presence of inflammation as more resources must be dedicated to the immune system to aid its fight against the inflammation. Hence when inflammation is present; proinflammatory mediators induce insulin and IGF-1 resistance. Immune cells such as neutrophils and macrophages have a great reliance on circulating glucose to meet their metabolic needs and hence this takes up large amounts of nutrients which ordinarily could be used for normal physiological functions.

By contrast, in a more desirable situation whereby inflammation is absent; then milk production is promoted, and stages of reproduction may flourish. In such a scenario insulin can function efficiently so that the nutrients are diverted away from the peripheral adipose tissue and muscle to the mammary gland which will aid in more copious milk synthesis and a more esteemed fertility status. (Bradford et al., (2015))

4.1.3.4 Inflammation of the Central Nervous system

The findings of Herzberg et al., (2020) are hugely significant as here the researchers could conclude that hoof lesions have a more severe impact than just causing local inflammation because inflammation of the CNS is also pertinent.

Herzberg et al., (2020) shed light on the mechanism by which such a state of neuroinflammation is maintained as they refer to the explanation from Raghavendra et al., (2004) that glial cells in the CNS react to signals associated with nociceptive transmission with the result being a morphological change of the glial cells to a reactive phenotype. Watkins et al., (2001) elaborate that reactive microglia and cytokines act in an autocrine and paracrine

manner through their facilitation of neuron to glia communication and glia to glia communication. In this way inflammation of the CNS is facilitated.

4.1.3.4.1 *Hyperalgesia*

Tadich et al., (2013) also highlighted the phenomenon of *hyperalgesia,* emphasising its increased prevalence in lame cows. Hyperalgesia is a phenomenon whereby the pain experienced is unusually severe. For example, concerning lame cows; of course, it is expected that such cows will be liable to suffer from pain as a symptom but if hyperalgesia prevails then the pain experienced is much greater than it should be and does not correlate with the severity of the condition. (Mainau et al., (2022)) Cows suffering from mild to severe lameness develop mechanical hyperalgesia of the dorsal aspect of the metatarsus (Whay et al., (2008)).

4.1.3.5 The perception of pain and the interplay between pro-inflammatory cytokines and chronic pain

An article by Anderson, D. & Muir, WW., (2005) aids understanding of the process of the perception of pain in cattle. Physiological pain uses normal sensory pathways and has a vital role in protecting the animal from tissue damage (Scholz, J. & Woolf, CJ., (2002).

The nociceptors are high-threshold pain receptors which are located on nerve endings of afferent myelinated or unmyelinated fibers. These nociceptors encode noxious stimuli depending on several variables related to the stimulus such as the modality, intensity, duration and location of the stimulus. The noxious stimulus is translated into electrical impulses that are transmitted to the dorsal horn of the spinal cord. The result is the release of Glutamate from the pre-synaptic nerve terminals. This Glutamate in turn activates post-synaptic AMPA and KA receptors. The AMPA and KA receptors are primarily responsible for mediating fast excitatory pain transmission. Pain is considered to be 'physiologic' when it is present in the absence of tissue damage and such pain serves to warn the animal of potentially harmful stimuli. (Craig, A.D., 2003)

On the other hand, 'pathologic' pain is present when tissue or nerve damage occurs. Pathologic pain often involves the development of peripheral sensitization and central sensitization. During tissue damage and the associated inflammatory response, various chemicals are released and these function as nociceptor activators or sensitizers. The various chemicals include potassium and hydrogen ions, prostaglandins, histamine, bradykinin, nerve growth factor, cytokines and chemokines. (Muir, WW. & Woolf, CJ., (2001))

4.1.3.5.1 *Central & Peripheral Sensitization and Hyperalgesia*

Spinal cord cytokines can increase synaptic transmission towards supraspinal levels and this enhances the sensation of pathological pain and also facilitates central sensitization. These phenomena in turn play an integral role in the development and maintenance of chronic pain. (Kawasaki et al., (2008)

These factors acting together are responsible for the conversion of high-threshold nociceptors to low-threshold nociceptors. In addition, the release of such chemicals is behind the activation of quiescent nociceptors which in turn is responsible for the development of peripheral sensitization and a zone of *primary hyperalgesia*. Escalation of the inflammatory process through local vasodilation and plasma extravasation provides a platform for the development of *secondary hyperalgesia* which happens when the hypersensitivity is seen spreading to surrounding tissues. (Muir, WW. & Woolf, CJ., (2001))

Muir, WW. & Woolf, CJ., (2001) found that a key factor in the development of central sensitization, *secondary hyperalgesia* and pain amplification is the activation and modulation of NMDA receptors by the excitatory neurotransmitter, Glutamate. These NMDA receptors are located on neurons in the dorsal horn of the spinal cord. There is a fundamental difference between central sensitization and peripheral sensitization because over time in the case of central sensitization; low intensity, non-painful stimuli carried by low threshold Aβ sensory nerve fibers, begin to produce painful sensations. (Muir, WW. & Woolf, CJ., (2001)

Ultimately central sensitization maintains a commanding role when it comes to animals experiencing profound discomfort following severe injury and central sensitization is also responsible for the expansion of receptive fields and for increasing the responsiveness of dorsal horn neurons to sensory inputs. (Muir, WW. & Woolf, CJ., (2001)

The article from Herzberg et al., (2020) makes similar points to those emphasised by the much earlier work of Muir, WW. & Woolf, CJ., (2001) with Herzberg et al., (2020) also explaining that after injury, immune cells release pro-inflammatory cytokines at the site of injury. This decreases the threshold of the nociceptors resulting in *primary hyperalgesia* (Sommer, C. & Kress, M., (2004)).

4.1.3.6 Reproductive Issues due to Raised Inflammatory Cytokines

The article by Tsousis et al., (2022) then proceeds to aid the reader in better understanding how such raised concentrations of Interleukin-1-α, Interferon-γ and TNF-α exert an influence in the

CNS when lameness is present in dairy cows. They do so by uncovering the findings of a study by McCann et al., (2000) which found that Interleukin-1-α acts on Luteinizing Hormone-releasing neurons, which thereby blocks the essential pulses of Luteinizing Hormone.

Tsousis et al., (2022) also refer to the study by Skarzynski et al., (2003) which found that low doses of TNF-α induce lower Progesterone levels and encourage luteolysis. Tsousis et al., (2022) emphasise that the findings of the aforementioned studies are representative of a direct association with the earlier findings which Tsousis et al., (2022) had discussed of low progesterone levels measured in lame cows as found in different studies by Melendez et al., (2018), Morris et al., (2011) and Walker et al., (2010).

The findings discussed above concerning the events which unfold at the level of the CNS, as a consequence of lameness, may also act as evidence of an indirect association with the lower conception rates in cows despite having been successfully synchronized. (McNally et al., (2014) and Praxietelous et al., (2022))

While previously low levels of TNF-α were discussed; Skarzynski et al., 2003 also explored the consequence of high doses of TNF-α, finding that this can stimulate the function of the corpus Luteum, prolonging the oestrus cycle and could subsequently cause both low oestrous intensity and anovulation.

There is an association between the findings above and higher early embryonic losses as discussed in the article by Hansen et al., (2004). Hansen et al., (2004) declare that TNF-α, Nitric Oxide and PGF-2-α can impact embryonic development as these bioactive substances may exert influence upon either the oocyte or the developing embryo. Hansen et al., (2004) point to the study by Soto et al., (2003) which found that TNF-α can have deleterious actions on oocyte maturation. Therefore, the resultant embryo is exposed to a vulnerable situation whereby it's proper development may be compromised. Soto et al., (2003) also found that exposure of fertilized embryos to TNF-α didn't inhibit development to the blastocyst stage. However, the researchers did find that TNF-α does increase the occurrence of apoptosis amongst blastomeres when the exposure to TNF-α was true for embryos ≥ 9 cells. The researchers concluded that such increased blastomere apoptosis could "conceivably compromise subsequent embryo survival". (Soto et al., (2003))

5 Materials and Methods

I searched the databases for English-language studies and articles. Databases which I availed of predominantly were CAB Abstracts and Web of Science. I read and included articles, journals, books and internet sources in order to strengthen my knowledge and accumulate a body of information around which to structure my paper. My major sources for data collection were ACVIM, PubMed, NCBI, Researchgate, Wiley Online Library and Science Direct.

6 Discussion & Results

6.1 Lameness prevalence during the breeding season; the impact on first service and pregnancy outcomes

There is less data available from seasonally breeding, pasture-based herds such as those in Ireland. Therefore, the study by Somers et al., (2015) was focused on uncovering any link between lameness and poor fertility in dairy cow herds in Ireland where cows are housed during the winter months but managed at pasture for the remaining months of the year. The study by Somers et al., (2015) determined the prevalence of lameness in a group of 786 cows through serial locomotion scoring during and after the breeding season. Ten commercial Irish dairy farms located in Co. Kildare and Co. Wicklow were at the centre of the prospective observational study carried out by Somers et al. in 2013. The ten farms concerned were predominantly made up of Holstein-Friesian herds and all the farms used seasonal breeding.

University College Dublin oversaw a herd health management programme for each of the herds. A veterinarian from University College Dublin visited each of the farms every 21 days to evaluate such areas as milk yield and body condition score. The veterinarian acquired critical information related to the calving date, calving difficulty and peri-parturient disease events. The veterinarian also conducted the pre-breeding ultrasound examinations and ultrasound-based pregnancy diagnosis at day 30 and day 60.

The Irish breeding system specifies that cows are eligible for breeding from the *mating start date* which only arises once the 42-day *voluntary waiting period* has been completed. The *earliest serve date* is the first day when the cow is eligible for breeding.

The farmers oversaw direct observation of oestrus to detect the cows in heat. In addition, heat detection aids were called upon by the farmers such as vasectomised bulls, pedometers and tail paint. Reproductive performance was monitored at herd level with respect to *submission rate* to first serve within three weeks from the *earliest serve date* and of course the number of pregnancies served as a key marker.

The study found that the prevalence of lameness at all periods of the breeding season compared favourably to results from housed cattle which is a common theme found in other studies carried out among grazing herds. It was necessary to control for such variables as the month of calving, body condition score at calving, body condition score loss after calving and economic breeding. Thereafter a fundamental finding of the study was that cows <u>becoming</u> lame during

the breeding season and cows lame before and during the breeding season; were less likely to become pregnant.

The findings were only significant for cows becoming lame after the *earliest serve date* and cows lame both before and after the start of breeding. Cows lame before the *earliest serve date* but no longer lame during the breeding season were 12% less likely to become pregnant compared to cows which were never observed as lame during the study. In the case of cows becoming lame after the *earliest serve date* and cows identified as lame before and after this date; the study found that these cows were 35% and 38% respectively, less likely to become pregnant.

The study concluded that cows no longer lame during the breeding season had a lower submission rate to first serve within 3 weeks of the *earliest serve date*. However, the pregnancy rate was not significantly lower in these animals compared to animals never diagnosed as lame.

(Somers et al., 2015)

6.2 Increased calving to pregnancy interval in lame cows

Logrono et al., (2021) conducted an observational study on 7156 lactations from highly supplemented grazing dairy cows to assess the associations between the timing of lameness clinical case occurrence in lactation with productive and reproductive performances in grazing Holstein cows. The study took place on a commercial farm in Argentina, milking approximately 2,600 cows. Breeding occurred all year round excluding the hottest months of the summer (January & February).

A veterinarian visited the farm every 14 days. Tail chalking was used after a voluntary waiting period of around 50 days and at this point cows were observed twice daily for signs of oestrus. Insemination was carried out when cows were found to be in oestrus. Ultrasound examination was used for pregnancy diagnosis at day 30-45.

Farm personnel with special training from the veterinarian, were responsible for lameness diagnosis, which was carried out immediately after milking, every two weeks. A five-point locomotion score was used and a cow was considered lame if having a score ≥4. Hoof trimming was applied for lame cows and systematic antibiotics were administered in cases of suspected Foot Rot. Foot bathing with 5% formalin was used for lactating cows at three milkings per week and once weekly for dry cows.

This study found that as well as producing less milk in all cases of lameness; cows becoming lame after the first service had a calving to pregnancy interval of 87 days longer than their healthy herd mates. This study also found that cows becoming lame before the first service had a 38 day longer calving to pregnancy interval compared to their healthy herd mates. It was possible for the researchers to conclude that the impact of lameness really is embedded in the timing of case occurrence. In this regard, especially cows becoming lame after the first service demonstrated the most striking prolongation of the calving to pregnancy interval.

The delay in conception is significant as these cows will have shorter lactations than non-lame cows in seasonally breeding cattle managed on a pasture-based system. Of course, shorter lactations pose a threat to economic viability for the farmer.

Garbarino et al, (2004) also examined the effect of lameness on the resumption of ovarian cyclicity during puerperium. They based their findings around weekly Progesterone measurements and found that lame cows had 3.5 greater odds of delayed cyclicity within the first 60 Days In Milk (DIM) compared to non-lame cows. They also found that lame cows demonstrated a period of 3-7 days longer to the first Luteal Phase versus non-lame cows.

Melendez et al., (2018) also found a lower ovulation rate of 50% within the first 30 DIM, based on ultrasound examination, and this compared to 100% ovulation rate in sound cows.

Omontese et al., (2020) conducted a study amongst Jersey cows and found that there was a 14% decrease in the proportion of cyclic Jersey cows at 41 Days In Milk (DIM) in those cows having had a hoof lesion at 20 DIM. This contrasted with those cows which were lesion-free; ultimately cyclic cows at the point of 41 DIM amounted to 38.3% in the group with hoof lesions as opposed to 52% for the cows which were free of any lesions.

6.3 The impact of lameness on successful conception

In their study Hernandez et al., (2005) were concerned with uncovering changes to the calving-to-conception interval in cows according to varying degrees of lameness in the postpartum period. Lameness was scored based on a six-point locomotion scoring system. Their results revealed that there is a linear association between an increasing degree of lameness and time to conception. The study found that the risk of conception failure was 1.35 times higher in lame cows than in non-lame cows. In addition, those cows with higher locomotion scores were more vulnerable to conception failures as opposed to lame cows with lower locomotion scores.

The study also highlighted the importance of early detection of lameness as it was found that cows with higher locomotion scores were more likely to leave the herd during lactation versus those cows with less severe lameness. The lame cows with low scores mostly had laminitis and did not develop more severe lesions but the cows with higher scores had claw lesions including thin soles, white line disease, sole ulcers or abscesses. (Hernandez et al., (2005))

The impact of lameness on the ability of a cow to conceive remains one of the most intriguing areas during an exploration of reproductive outcomes in lame cows. An examination of different studies conducted did highlight that the First Service Conception Rate (FSCR) barely exceeds 20% for lame cows compared to their non-lame counterparts which tend to demonstrate much more pleasing First Service Conception Rates of 40-50%. (Melendez et al., (2003), Omontese et al., (2020))

Various studies such as those by Bicalho et al., (2007) and Hernandez et al., (2005) emphasise the undeniable hazard which lameness poses in terms of successful pregnancy detections in cows. The subsequent result, therefore, as emphasised by these studies, is that an extension of open days between 12 and 70 is seen in such cows also. Bicalho et al, (2007) hypothesised that cows classified as lame during the first 70 Days In Milk demonstrated more days from calving to conception and faced a greater threat of dying or being culled as opposed to non-lame cows. Cows were assigned a Visual Locomotion Score (VLS) using a 5-point scale; 1=Normal, 2=Presence of slightly asymmetric gait, 3=the cow clearly favoured one or more limbs (moderately lame), 4=Severely lame, to 5=Extremely lame (non-weight bearing). The hazard ratio of been detected pregnant was 0.85 for lame cows (VLS ≥3) versus non-lame cows meaning that lame cows were at a 15% lower risk of pregnancy than non-lame cows. In a situation where lameness was redefined as VLS≥4; then the hazard ratio of been detected pregnant was 0.76 for lame cows versus cows with a VLS <4. Overall lameness significantly decreased the hazard of pregnancy and increased the hazard of culling/death. The findings of Hernandez et al., (2005) reveal that cows classified as mildly lame were at a 29% decreased hazard for pregnancy while severely lame cows were at 58% decreased hazard of pregnancy when compared with the non-lame control cows. Indeed, Bicalho et al, (2005) summed up that it is biologically plausible that the impact of lameness on reproduction remains deeply rooted in the severity of the lameness affecting the cows.

Praxitelous et at., also conducted research work to investigate uterine and ovarian changes using ultrasound scanning techniques in 4–7-day intervals, on lame and non-lame during the

first 50 DIM. For their investigations, the researchers excluded cows with other conditions impacting on reproduction outcomes such as those cows with disorders of the uterus, cows with dystocia, cows with retained foetal membranes and cows suffering from endometritis. The study featured only multi-parous cows. The cows were scored for lameness 10 days before the expected parturition and on the day after calving. The study featured 25 sound and 22 lame cows. The findings did not reveal striking differences regarding uterine involution in the group of lame cows as opposed to the non-lame group. The diameter of the previously gravid uterine horn was measured by B-mode ultrasonography and found to be equal between the two groups on days 8, 11, 15, 23, 30 and 42-post-partum. The study found that there was a slight delay in the involution of the cervix amongst lame cows on days 15 and 42-post-partum but this trend in the measurements was not long standing. Again, this study found that lameness exerts a profound suppressive effect on the resumption of ovarian activity. Only 64% of the lame cows ovulated at least once within the first 50 DIM as opposed to 88% of the non-lame cows. In addition, persistent ovarian follicles were diagnosed in 23% of the lame cows but in just 12% of the non-lame cows. Higher rates were seen amongst the group of lame cows also for the presence of prolonged luteal phases (36% Vs. 20%) and lame cows presented more often (14%) with dominant follicles that became atretic and regressed compared to non-lame cows which did not display this pattern atall upon ultrasound examination. Normal ovarian activity was seen at 50 DIM in 76% of the healthy cows during an ultrasound which could be compared to a figure of only 45% amongst the lame cows. However, there was no difference found between the two groups for the average number of days to first ovulation. This supports findings by Morris et al., (2011); that certainly a portion of lame cows can compensate for lameness and cope with the condition to the degree that they can compete with their non-lame counterparts. Similarly, in their study Peake et al., (2011), who focused on the impact of three specific stressors on reproductive performance; namely lameness, mastitis and loss of body condition, also found that cows do have a certain degree of tolerance. They found that cows could tolerate one moderate stressor, but in instances where there were two concurrent stressors; this could prove over-whelming to the health status of the cow. They found that cows faced with say lameness and low BCS at the same time experienced a disruption of hormonal equilibrium. The consequences of such were detrimental for reproductive outcomes as they found these cows had a lowering of LH pulsation with subsequent failure of ovulation, a delay in resumption of ovarian cyclicity and possibly subsequent subfertility.

6.4 Laminitis

The study by Hernandez et al., (2005) comparing the calving-to-conception interval in dairy cows with different degrees of lameness during the pre-breeding postpartum period found that most cows classified as lame in the study had Laminitis. This article explains that while it is known that hoof lesions such as White Line Disease and Sole Ulcers can cause clinical lameness; lesions associated with Laminitis such as red or yellow discoloration of the sole and white line; do not cause lameness. The writers suggest that possible explanations for the association between Laminitis and lameness during the first 8 weeks postpartum may be related to cows in this study being in the initial phase of Laminitis. This initial phase of Laminitis is very painful. The lame cows with Laminitis which were examined had thin soles and reacted to the hoof testers suggesting that these cows were in the acute phase of Laminitis and hence demonstrated signs of severe pain and lameness. This initial, acute phase of Laminitis is associated with Acidosis. Rumen acidosis is responsible for the decrease in systemic pH which activates vasoactive processes that increase the digital blood flow. Vasodilation and vasoconstriction are intensive due to the release of Endotoxins and Histamine. The vessel walls become damaged and exude serum which is followed by classic signs such as oedema and ultimately there are detrimental processes underway as there is a risk of thrombosis and internal haemorrhages in the solar corium. There may also be expansion of the corium inside the hoof wall which causes significant pain. Similarly to previous studies, Hernandez et al.. (2005) also found that cows in the postpartum period were more susceptible to experiencing such acidosis given that they are transitioned from a lower to higher energy diet to achieve optimal milk production. These transition diets can signify a metabolic insult that can begin a cascade of negative influences on fertility outcomes.

Hernandez et al., (2005) described an increased prevalence of Laminitis amongst primiparous cows which were classified as lame more often than multiparous cows in the first 8 weeks postpartum. The researchers highlight that primiparous cows may be at an increased risk of developing Laminitis because they are exposed to significant changes in husbandry practices during this period. Primiparous cows experience significant stress as they must adapt to being introduced to mature groups around the time of calving as well as facing demands posed by adapting to changes in housing conditions and bedding which are all new challenges for primiparous cows. Ultimately Hernandez et al., (2005) found that cows classified as lame during the pre-breeding postpartum period had a longer calving to conception interval by 36 days compared to non-lame cows.

The study by Hernandez et al., (2005) also found that the delayed resumption of ovarian cycling could be attributed to ketosis. Lame cows are more likely to face the threat of ketosis as Dry Matter Intake (DMI) is suppressed in these cows. Subsequently these cows can demonstrate a NEB and there is a platform for the development of ketone bodies. The formation of such ketone bodies can delay the onset of ovarian activity. The article highlights that lame cows with delayed ovarian cycling during the pre-breeding postpartum period can be expected to have longer calving to conception intervals. This emphasises the importance of early treatment and resolution of lameness cases as there are huge economic losses associated with increased days open (not pregnant) for dairy cows. (Hernandez et al., (2005))

6.5 The consequences of pain and discomfort

Uncovering some possible explanations for the effects of lameness on production and reproduction; a study by Logrono et al., (2021) referred to the widely accepted fact that lameness is associated with a sustained painful experience for cows. As a result of such pain cows demonstrate altered feeding patterns as they eat less often, having fewer daily meals and in addition to this it was also found that lameness causes the cows to have overall shorter feeding periods (Bach et al., 2007). Logrono et al., (2021) also referred to findings that lame cows have lower rumination bouts (Walker et al., 2008). This reduction in Dry Matter Intake (DMI) over a sustained period will lead to a Negative Energy Balance (NEB).

As a consequence of the pain associated with lameness; cows are also forced to demonstrate altered behaviour patterns as they favour sitting for longer periods. The cows deploy a less intensive expression of oestrus and they are less available to be mounted given that they stand for shorter periods. (Walker et al., (2008))

6.6 Negative Energy Balance (NEB)

The Negative Energy Balance mentioned above contributes not only to a lower milk yield but also has a devastating impact on reproductive performance (Logrono et al., 2021). Ovarian activity is impaired (Lucy et al., 1992) and there is a delayed return to cyclicity after parturition (Garbarino et al., 2004).

Similarly to Garbarino et al., (2004), an article by Butler, W., (2000) revealed that cows that maintain an optimum body condition score and have a good nutrient intake are expected to display shorter intervals to the first postpartum oestrus. NEB diminishes gonadotropin

pulsatility and ovarian steroid synthesis. Both these effects of NEB can reduce the cow's expression of oestrous. (Butler, W., (2000))

Logrono et al., (2021) highlighted further detrimental effects of a lowered DMI and NEB on critical physiological processes of cattle reproduction. Oocyte competence is reduced (Roth, Z., 2018), the uterine environment is disturbed (Ribeiro et al., 2016) and embryo survival and development are impaired (Soto et al., 2003).

These studies provide ample evidence that all stages of the reproductive cycle demand for the cows every nutritional need to be addressed. Taking account of such research grants an understanding as to why lame cows with compromised nutritional status due to lameness are subsequently found to perform less triumphantly in terms of achieving reproductive targets.

6.7 Inflammation in the CNS

Herzberg et al., (2020) aimed to conduct a study specifically to determine the profile of cytokines in the spinal cord dorsal horn of dairy cows with painful chronic inflammatory lameness. Herzberg et al., (2020) hypothesized that chronic inflammatory lameness serves to promote persistent nociceptive input which could subsequently induce changes to the various cytokines being synthesized in the spinal cord. The study involved seven adult dairy cows with chronic lameness and seven adult dairy cows with no lameness. A mobility scoring system was used to evaluate lameness in all the cows. Immediately after euthanasia the spinal cord was removed from the cows and 20cm lumbar segments were obtained. Dorsal horn removal and processing followed and this allowed for quantification, using a multiplex array, of various cytokines including TNF-α, Interleukin-1-α, Interleukin-13, Chemokine-10 (CKCL-10), Chemokine-9 (CXCL-9), Interferon-Alpha, Interferon-Gamma, Interleukin-21, Interleukin-36ra and Macrophage Inflammatory Protein-1-Beta (MIP-1-β).

Herzberg et al., (2020) found that lame cows had higher concentrations of TNF-α, IL-1-α, IL-13, CXCL-10, CXCL-9, IFN-α and IFN-γ in their dorsal horn compared to non-lame cows. IL-21 concentration was decreased. There was no difference in the concentrations found of IL-36ra and MIP-1-β between lame cows and non-lame cows. It is well documented that peripheral and central nervous system cytokines enhance pathological pain sensation and contribute to central sensitization during chronic pain. Spinal cord cytokines contribute hugely to the maintenance of chronic pain. As has been discussed previously, this chronic pain which can be present in a bovine lameness case; will cause altered behaviour and feeding patterns which lead

to an overall compromised health status of the cow and can induce production shortcomings and reproductive failures.

In addition, the article by Herzberg et al., (2020) emphasizes that pro-inflammatory cytokines such as those mentioned; can induce resting microglia and astrocytes into a reactive state. These cytokines facilitate neuron to glia and glia to glia communication, maintaining a state of neuro-inflammation. Of course, it is necessary to consider the specific impact of such pro-inflammatory cytokines as TNF- α and Interleukins. Trevisi et al., (2016) affirmed that pro-inflammatory cytokines, not only having a role in chronic pain, individually also exert profound neuroendocrine and metabolic effects and act in the brain to reduce feed intake. In their study; cows with a higher concentration of pro-inflammatory cytokines before calving, showed a lower DMI, had lower milk yield and were more likely to develop mastitis. This proves fundamental for reproductive outcomes as has been discussed, whereby a low DMI and NEB are often predictors of sub-optimal fertility.

Trevisi et al., (2016) explain that TNF-α is involved in systemic inflammatory responses and its presence means that the normal balance is compromised throughout the physiological systems of the animal. IL-1-β is associated with acute phase reactions after calving. Trevisi et al., (2016) outline that cows with higher Il-1-β concentrations during late pregnancy, tend to exhibit a worse health status during lactation. In this way, these cows have a lower milk yield and are more susceptible to mastitis. Trevisi et al., (2016) also discussed IL-6, which they revealed; may contribute to oxidative stress that is in turn damaging for fertility outcomes and overall productivity. Hence, Trevisi et al, (2016) highlight the importance of monitoring Pro-Inflammatory Cytokines during late pregnancy in order to establish if there may be an increased risk to the health status of a cow in the transition period if these cytokines are found to be off-balance.

6.8 Lameness, Infertility and ovarian cysts

The study of Melendez et al., (2003) was conducted on a large commercial dairy farm in North Central Florida. The objective was to establish the association between ovarian cysts, lameness and infertility in lactating dairy cows. Cows on this farm were bred using both heat detection and timed insemination protocols. As part of the reproductive management a postpartum examination was performed on every cow between 30 and 50 days after calving. This was to enable the veterinarian to diagnose and record diseases such as ovarian cysts, uterine infections

and pyometra. Pregnancy diagnosis was by way of a palpation per rectum 42-49 days after artificial insemination.

This was an observational study which compared the lame cases group and the control group. The cows considered as lame cases were those diagnosed within the first 30 days postpartum as having an abnormal and painful gait attributable to claw origin. 90% of the lesions in the lame cows were related to white line disease, sole ulcer, sole abscesses and double sole. These are all horn growth abnormalities which are associated with sub-clinical laminitis. The paper revealed that lameness is most likely to occur in the first 60 days postpartum which can be explained by the vast changes which this period brings in terms of the environment as the housing and floor surfaces are changed as well as alterations to feeding management and nutrition.

The study found that correcting for calving season, parity and milk yield; the control group of cows were 4.22 times more likely to conceive at first service than lame cows. The group of cows experiencing lameness within the first 30 days after calving were 2.63 times more likely to develop ovarian cysts before the first breeding than the control group. It is noteworthy that this study did not find an increased calving to first service interval in the group of lame cows. Although this contrasts with findings of other studies; an explanation may be that the voluntary waiting period for the cows in this study was higher than in many other instances at 95 days. This offered the lame cows a higher amount of time in which to resume ovarian activity.

The findings of the study revealed that there was a multi-collinearity relationship between lameness and ovarian cysts. A higher incidence of ovarian cysts, a lower likelihood of pregnancy and lower fertility than the control group was found amongst the cows that became lame in the first 30 days postpartum.

It was not possible to conclude a cause-effect relationship given that this was an observational study but the paper did proceed to reveal the researchers hypotheses regarding their findings.

The first hypothesis related to the fact that 90% of the claw injuries for the lame cows in this study were laminitis-related lesions. It is well-known that laminitis may develop due to a release of Histamine or Endotoxin by animals suffering from rumen acidosis. The authors acknowledged that cows just like those in this study are very vulnerable to rumen acidosis as the transition period before and after calving coincides with huge metabolic shifts and pronounced adaptations to the nutritional input. The rumen must adapt to the newly lactating

diet which is higher in highly fermentable non-structural carbohydrates and lower in fibre. In their study Melendez et al., (2003) also referred to broader research findings which have demonstrated that the low-energy prepartum diet and the high-energy postpartum diet are statistically associated with sole haemorrhages and low quality horn (Donavan et al., 1998). Melendez et al., (2003) proposed a scenario where this interpretation is assumed correct; that hoof lesions are associated with sub-clinical rumen acidosis and so therefore it could be said that "endotoxins released by the lysis of gram-negative bacteria in the rumen could play a very important role in both the pathophysiology of laminitis-related disorders and the alteration of the follicular phase and formation of ovarian cysts".

The study cited the research of Battaglia et al., (1999) which could offer great insight on the effect of these endotoxins on follicular patterns. Battaglia et al., (1999) had found that endotoxins can act at a neuroendocrine level, potently suppressing the pulsatile Luteinizing Hormone release after the Progesterone levels have decreased. Endotoxins were also found to impede the LH surge system. It was also found that Endotoxins may exert suppressive effects at the ovarian level with this being entirely detrimental for the fertility outcomes as follicular development is not encouraged and there is inhibition by the endotoxins to the Estradiol secretion in response to gonadotropic stimulation.

The second hypothesis discussed by Melendez et al., (2003) in their study centred on the phenomenon of pain experienced by lame cows. Lameness represents a significant "stressor" and is associated with a disturbance of homeostasis and the animal is forced into a catabolic state. Stress and pain are followed by increased blood levels of Catecholamines, Glucocorticoids and stress-induced Progesterone from the adrenal glands. The study pointed to earlier research which had found that increased levels of Adrenocorticotrophic hormone or Cortisol or Progesterone have been related to disturbances to the release of GnRH and/or LH as well as altering normal follicular activity. All this in turn led to the development of persistent ovarian follicles. (Nanda et al., (1990))

An article by Thatcher & Santos, (2003) explored factors associated with the pre-ovulatory period that reduce fertility and hence shed light on the concept of persistent ovarian follicles. The writers explain that when a persistent follicle ovulates, the oocyte is at a later stage of maturation. The oocyte can still be fertilised but early embryonic death will occur. The article revealed that reduced fertility can be anticipated if there is a period of follicular dominance of longer than 8 days. (Thatcher & Santos, 2003)

In their study, Melendez et al., (2003) found a 25% incidence of persistent ovarian follicles in lame cows, compared to 11% in healthy cows. Melendez et al, (2003) declared that they expect to see that the calving to first service interval is extended by 4-17 days in lame compared to healthy cows with variations seen according to the severity of the lesions.

The third hypothesis by Melendez et al., (2003) was related to the Negative Energy Balance (NEB) of lame cows. Lame animals spend more time lying down and less time eating, ruminating and interacting with other animals. These lame cows lose weight as a direct consequence of their abnormal behaviour patterns stimulated by pain and discomfort. It is well documented that NEB and weight loss do not represent a positive force on follicular development. In addition, Insulin Growth Factor-1 and Luteinizing Hormone must work in harmony to promote follicular growth. However, pulses of Luteinizing Hormone and plasma concentrations of Insulin Growth Factor-1 have both been found lacking in cows losing weight as opposed to healthy cows. Finally, Melendez et al. (2003) cross referenced the finding by Beam et al., (1999); that lame cows experiencing pronounced weight loss or a NEB can be expected to require more time for the dominant follicle to reach a larger size so that sufficient Estradiol levels are achieved which are needed to guarantee a successful ovulation.

For all these reasons outlined, Melendez et al., (2003) could reasonably conclude that the inhibitory effect on ovarian follicles is less apparent in non-lame cows than in lame cows with weight loss and a NEB.

7 Summary

There are different modes of action of lameness which culminate to guarantee that lameness reduces the capacity for a dairy cow to fulfil her capacity to produce healthy off-spring. Lameness causes the behaviour of dairy cows to change as pain and discomfort dents the desire of cows to feed for the usual periods. Cows are more inclined to have shorter feeding periods as they favour lying down to relieve suffering. This reduced intake has a knock-on effect on the nutritional status as cows are vulnerable to experience a negative energy balance. This negative energy balance has a detrimental influence on calving to conception interval and embryo viability.

Lameness causes reduced mobility as cows are seen to not socialise in the usual manner as they are less inclined to move amidst their counterparts. As a result, these cows are less social and less available to be mounted during oestrous. This is a major concern for farmers as the lame cows are less triumphant in respect to successful conception as compared to the non-lame cows in the herd.

Lameness represents a resounding source of stress for dairy cows. Stress causes increased levels of Glucocorticoids such as Cortisol. This in turn leads to detrimental hormonal imbalances as progesterone levels are reduced and luteal function is compromised.

Lameness accelerates profound inflammatory processes which are responsible for damaging effects on different stages of the reproductive process. In addition, the immune system is activated in response to inflammation and so more of the reserves of the dairy cow are taken up with fighting the inflammation. The dairy cow experiences a depletion in energy reserves and is less capable to achieve reproductive targets. Not only is there in increase in Haptoglobin levels and inflammatory cytokines as the inflammation can also predominate in the Central nervous system. Again this diminishes the normal balance of hormones seen in healthy cows.

Ultimately lameness prevention must be a paramount consideration for dairy farmers. Proper hygiene practices and lameness prevention strategies must be implemented. Farmers should be prompt in ensuring that lameness cases are identified and properly treated in a timely manner to prevent escalation of inflammatory processes. Farmers will reap the rewards of successful lameness management as the welfare of the herd will be to a higher standard and such cows are better placed to achieve reproductive targets and produce healthy offspring.

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