

University of Veterinary Medicine Budapest
Department of Obstetrics and Food Animal Medicine



Subfertility in dairy heifers in Ireland.

Ryan Gerard McElvaney

Supervisor: Dr. Rátky József DVM, PhD, DSc

Head of Department of Obstetrics and Food Animal Medicine

2023

Abstract

Subfertility in cattle can be defined as poor reproductive performance where there is difficulty getting the animal in calf, with increased breeding failures due to reduced conception rates. Unlike infertility where the animal cannot get pregnant, subfertility means the likelihood of a successful conception is reduced, and it takes longer and more inseminations to achieve conception. In cattle, decreased fertility can be temporary or permanent, and there are a multitude of possible causes of suboptimal reproductive performance. Some of the main causes of dwindling fertility levels are factors related to genetics, nutrition, infectious disease and management related issues. In this review, the aim was to focus on these main roots of subfertility in the dairy heifer population in Ireland, as well as to analyse how the implementation of good management techniques regarding these factors can eradicate such problems surrounding the breeding season. The economic impact of subfertility cannot be undermined, with increased costs occurring with higher number of inseminations, increased feeding costs, higher culling rates and loss of milk production as cows must be put in calf to provide a subsequent lactation. It highlights the importance of prudent farm management when one considers that the foundation of dairy farming is based around large scale production with small profit margins.

Table of Contents

1) Introduction	4
2) Literature Review	6
2.1) Genetics.....	6
2.1.1) Economic Breeding Index (EBI).....	6
2.1.2) Genetic merit for fertility traits	7
2.1.3) Inbreeding and crossbreeding.....	10
2.2) Nutrition	11
2.2.1) Body condition and target weights.....	11
2.2.2) Macronutrients, micronutrients, trace minerals.....	13
2.3) Disease	17
2.3.1) Viral Disease	17
2.3.1.1) Bovine Viral Diarrhoea	19
2.3.1.2) Infectious Bovine Rhinotracheitis	23
2.3.2) Bacterial Disease	25
2.3.2.1) Johne’s Disease/Paratuberculosis.....	26
2.3.2.2) Leptospirosis	28
3) Discussion.....	31
3.1) Genetic improvements.....	32
3.2) Nutritional management.....	32
3.3) Disease control	33
3.4) Use of precision technologies in breeding programme	34
4) Conclusion.....	36
5) Bibliography	38
6) Acknowledgements	44

1)Introduction

The fertility of dairy cattle has suffered greatly in the last 40 years in Ireland as well as in other countries with intense dairy farming such as the UK and New Zealand [1]. There is no doubt that the huge demand for increasing milk yields of dairy cattle has had deleterious effects on herd fertility [2, 3]. The trends have shown the inverse relationship between the two traits over the last number of decades, and the subsequent realisation of this problem has led to partly recovered fertility levels in the dairy population. However, the fertility levels of dairy cattle in Ireland and the UK are still considered suboptimal and poor reproductive performance is still considered the main cause of premature culling for dairy cows, followed by lameness and mastitis [4]. The economic impacts of subfertility are vast and multifaceted, with costs incurring through increased inseminations, more feeding until lactation, increased culling and higher replacement rates [5]. Subfertility can be observed through a variety of parameters, such as reduced conception rates, older age at first breeding and first calving at over 24 months of age. Furthermore, heifers that take longer to conceive have shown to have lower returns in lactation yield and poor fertility in later breeding seasons. It is therefore pivotal that the breeding, rearing and management of dairy heifers is done correctly to maximise the potential of that animal as well as to develop the farm financially by increasing returns and reducing costs [3].

The general improvement of heifer fertility can be based around 4 main areas: Genetics, Nutrition, Health and Reproductive management. Of course this list is not finite and there are many other possible variables to any individual farm or herd. Nevertheless, these 4 areas can be considered the most important factors of how fertility can be positively or negatively influenced [6]. The focus of this review is based on these factors to investigate the degree of importance each carries in affecting fertility and how the implementation of good practice around these elements has favourable effects on the reproductive performance of such animals. As well as that, the relationship between the aforementioned factors is reviewed in regards to how they not only affect fertility, but also affect each other.

Advancements in genomic analysis along with the establishment of the economic breeding index have made genetics an increasingly important facet of a herd's breeding program. It has allowed farmers to conveniently improve the genetic merit of their herd through the introduction of better traits from genetically superior animals.

The relationship between nutrition and fertility of heifers is well correlated. Good nutrition facilitates high growth rates allowing animals to reach puberty at an earlier age, whilst also being physically capable of carrying a calf to term. Likewise the lack or excess of feeding can be of great detriment to the fertility status of the animal [7].

The effect of infectious diseases on fertility has long been studied, from systemic diseases that hinder fertility through causing ill thrift, poor growth or immunosuppression, to diseases that are directly linked to reproductive issues. Fertility problems caused by diseases are often difficult to detect in comparison to other reproductive problems such as abortion, retained placentas or congenital deformities [8]. This review will analyse a number of agents which are considered endemic in Irish cattle herds and their true impact on fertility must be further investigated.

2)Literature Review

2.1) Genetics

In dairy cattle, genetics has become an increasingly important part of breeding programmes over the last number of decades as farmers have realised the improvements that are possible by improving the genetic merit of the herd. The huge concentration in previous decades on increasing milk yields has had detrimental effects on fertility, thus requiring action to try and fix these problems with fertility [1]. Trends have shown decreasing fertility in Irish dairy herds since the 1980s, although there has been a revival in the last decade to bring the fertility levels somewhat back up [1, 9].

2.1.1) Economic Breeding Index (EBI)

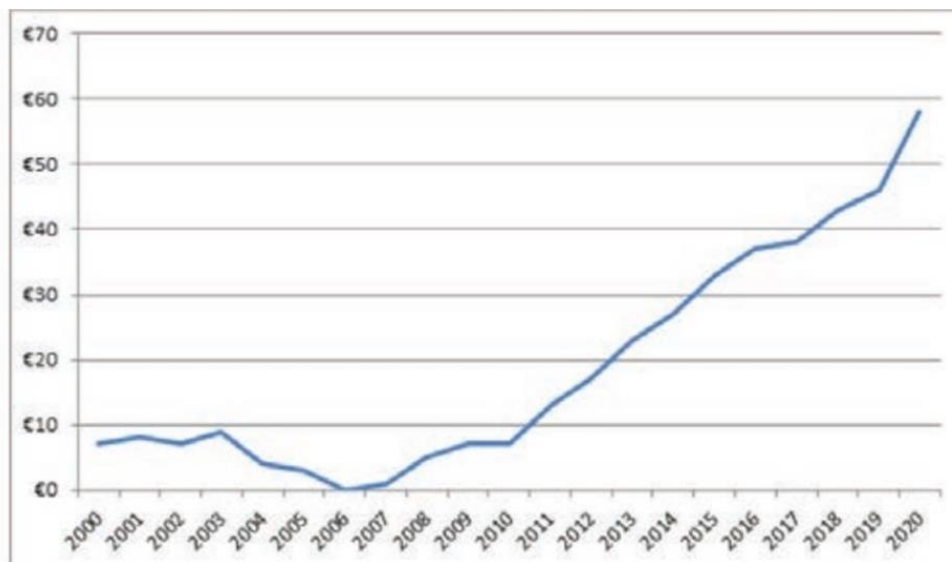
The introduction of the EBI in 2001 brought a greater awareness of the potential benefits for a farmer selecting genetically superior animals to improve the herd. The EBI previously consisted of 7 subindexes which each comprise a certain percentage towards the overall value of the animal. This has been updated to eight subindexes since 2022. The EBI was created by the ICBF (Irish Cattle Breeders Association) and they are responsible for the regular modification and updates of the index. The 8 subindexes are: Production, Fertility, Calving, Carbon, Beef, Maintenance, Management and Health. These subindexes are characterized by different traits and each carry a weighted percentage, as seen in the most recent version made by the ICBF, available on their website [Fig 1].

Figure 1: Most recent version of the EBI (2022-23).



In relation to fertility, the introduction of the EBI has increased focus on fertility which has helped to turn the tide on the trends of decreasing fertility levels. As seen in Figure 1, fertility is responsible for 25% of the valuation, second only to production (32%). Even since the introduction of the EBI, the value of fertility as a sub-index has increased in recent years, becoming a more important factor in the overall value of the animal. Figure 2 from Teagasc Oak Park demonstrates this trend [6].

Figure 2: Change in the value of the fertility sub-index of the EBI from 2001-2020 [6].



2.1.2) Genetic merit for fertility traits

The genetic ability to transfer fertility traits has long been debated and is estimated that some traits approximately have a heritability rate of 0.1[10]. However there has been a vast range of research and experiments carried out that show positive correlations between certain genetic traits with improved fertility statistics. Some potential genetically transmissible markers of fertility are SNP's (single nucleotide polymorphism), PSPB (pregnancy specific protein B) and PAG (pregnancy associated glycoprotein).

Moorepark Research farm in Co. Cork in Ireland established a program that compared 2 groups of dairy heifers, which both had come from bloodlines of high milk production but differed greatly in the fertility traits of their ancestors. These heifers have been used as a basis for multiple scientific studies by those at Moorepark farm to demonstrate the genetic relationship with fertility. The 2 groups of heifers were classified as Fert + and Fert – in relation to their reproductive potential. One study by Cummins et al. highlighted that the

Fert + group had better conception rates throughout the course of the breeding season in comparison to the Fert – heifers [11]. The same study demonstrated further parameters of reproductive performance that confirmed that the heifers with the superior traits for fertility had translated those traits into their own performance, displaying far better statistics than those from the Fert – group, showing a clear correlation between genetic merit and reproductive traits [11]. Table 1 compiles some of the data from this study by Cummins et al. and shows the results in a clear and concise matter.

Table 1: Comparison of reproductive performance in Fert + and Fert – heifers on Moorepark farm [11].

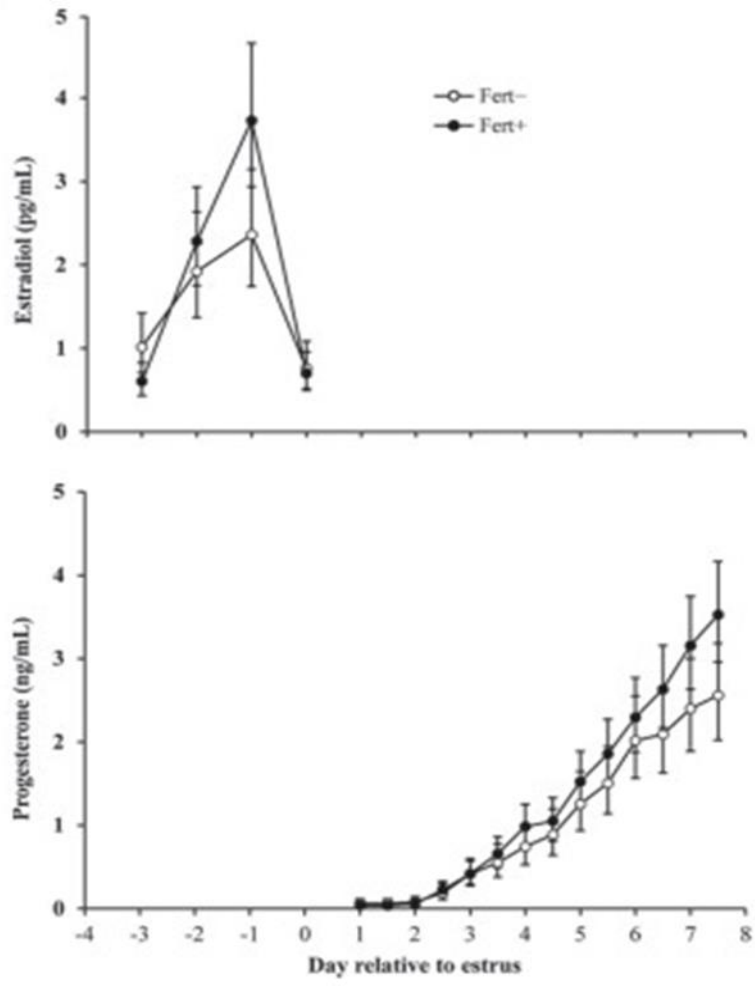
Variable	Fert '+' heifers	Fert '-' heifers
Number of heifers	41	39
No. of services per animal	1.78	2.83
21 day submission rate %	83.3	72.2
First service conception rate %	55.6	33.3
Pregnancy rate at 42 days %	72.2	41.2
Embryo mortality %	0	11.1
Overall pregnancy rate %	88.9	72.2

A follow up study by the same authors concluded that the Fert – group had poorer displays of heat and that the Fert + group had longer durations of oestrus than the Fert – group. The significance of this study shows that not only is fertility better in the Fert + group as was shown in the original study by this author, but the demonstration of being in heat is much more pronounced and consistent with the Fert + heifers [12]. This is of relevance for farmers practicing heat detection, oestrus synchronization and using artificial insemination as it is much easier to successfully impregnate animals that show behavioural signs during oestrus and have longer lasting heats.

Another research on the Moorepark herd of heifers examined the difference between the 2 groups in terms of important hormone concentrations, such as progesterone and oestradiol. Their findings were indicative that the heifers with superior traits for fertility had higher concentrations of oestradiol and progesterone during the days of oestrus [13]. Figure 3 is taken from this study to visually demonstrate the difference between the Fert + and Fert – groups. This would support the previous results regarding better pregnancy rates and better

displays of heat, with oestradiol important for normal oestrus and its associated behaviours, and progesterone being vital in embryo implantation and the maintenance of pregnancy.

Figure 3: Oestradiol and Progesterone levels according to day of oestrus in Fert + and Fert – heifers [13].



Genetic merit for good fertility has also been investigated in other countries with a large dairy industry. A New Zealand based research found that heifers inheriting better fertility traits reached puberty faster and had better conception rates than those of lower merit [14]. Earlier maturing heifers have been shown to have better reproductivity throughout their lifetime than late maturing heifers, and also has great financial benefits as they will calve inside 2 years of age. It also allows heifers to have one or two cycles before their first mating/insemination so that they have a higher chance of conceiving during a subsequent cycle [14]. A two-part Californian study on Holstein Friesian heifers compared them on genomic merit based on a number of parameters including heifer conception rate. They found a strong relationship between the heifers with higher genetic merit and better pregnancy rates as well as better displays of heat [15], [16]. The second part of this study

had an interesting finding in relation IGF-1 (insulin-like growth factor). Heifers with higher genetic merit for fertility had higher concentrations of IGF-1 than those of lower genetic merit [16]. It concurs with Cummins et al. who also found the same with the Fert + group [11].

2.1.3) Inbreeding and Crossbreeding

A possible factor attributable to the decline in fertility among the Irish dairy population is the narrowing of the gene pool, particularly in regards to the Holstein Friesian. Considering Ireland is a relatively small country with a huge cattle population, it is reasonable to consider there is a level of inbreeding in Irish herds. The effect of inbreeding on all aspects of animal productivity is well documented and in contrast, it is also well known of the beneficial effects of crossbreeding to achieve hybrid vigour.

Although the effects of inbreeding in Irish dairy herds are probably quite small on individual performance or a herd's performance over the course of a year, the cumulative effects of decades of inbreeding depression may be inimical over time on reproductivity, herd health and milk production in such herds [17]. A study by Mc Parland et al. has found that cattle with higher levels of inbreeding showed poorer productivity in terms of milk production, with the biggest decreases in yield associated with those with the highest rate of inbreeding. In terms of fertility, the animals with the highest degree of inbreeding showed greater calving intervals and greater age at first calving, although the differences were not wholly remarkable [18]. Nevertheless it does demonstrate how a prolonged concentration of the gene pool would decrease productivity and fertility over time.

The positive effects of crossbreeding on fertility have been examined in several papers. A study comparing the fertility statistics of purebred Holsteins to Holsteins crossed with Norwegian Red cattle showed a greater benefit to the Holstein for crossbreeding [19]. The results of this investigation can be seen in Table 2 below.

Table 2: Comparison of reproductive performances in purebred Holstein Friesian, purebred Norwegian Red and Holstein Friesian x Norwegian Red crossbreds [19].

Variable	Holstein Friesian	Norwegian Red	HF x NR
Pregnancy rate at first service %	52	60	60
Pregnancy rate after 6 weeks breeding %	62	69	77
Pregnancy rate after 13 weeks breeding %	86	90	91
Number of services	1.67	1.55	1.55

2.2) Nutrition

Nutrition is of great importance in all stages of cattle farming, and its influence on the reproductive potential of replacement heifers cannot be underestimated.

2.2.1) Body condition and Target weights

There is a clear correlation in cattle between body weight and age of puberty, meaning it is something that the farmer has control and influence over [20].

There is also a clear link between poor nutrition and poor fertility with underconditioned, deficiency-laden animals found to have poorer conception rates and poorer cyclicity [21]. Conversely, over conditioning and excess BCS is also associated with suboptimal fertility. It is therefore vital to understand that a balanced diet is important for breeding animals so that they are neither deficient or in excess of any particular nutrient, and that a proper feeding regime is established for replacement heifers [22].

It has been well reported that for cattle, the onset of puberty is highly associated with bodyweight. It means that an animal who is properly conditioned and fed a good diet can become ready for mating at an earlier age than an animal not as well-conditioned [7].

The advantage of getting a heifer to puberty at an earlier age is that it can be bred earlier thus meaning it will calve earlier. The sooner a heifer has calved and begins milking, the better it is for the farmer financially as the rearing of replacement heifers is one of the biggest expenses for the dairy farm, estimated at approximately 20-25% of his annual costs [20]. Of these costs, over half of it is related to feeding [7].

Another benefit of getting heifers in heat sooner is that it allows the farmer to breed the heifer on her 2nd or 3rd oestrus as it is well documented that there is a much higher chance of conception if they are bred on the third heat compared to their first one [14, 23].

The goal is for a heifer to be at 55% of its full adult bodyweight at the time of mating. This would roughly equate to 330kg in a Holstein Friesian who would expect to be around 600kg at adulthood. The goal is to reach this weight at 14-15 months so that the heifer calves down before 2 years of age (<24 months) [23].

It is evident that heifers that are bred and calve earlier have better fertility statistically than those who have their first calf at over 24 months of age, with heifers that calved earlier having better conception rates as well as shorter intervals between calving and conception [3].

To reach these targets, an average daily gain (ADG) of approximately 0.8kg/day is what the farmer should be aiming for to ensure his heifers are well conditioned and 13-15 months old [20]. Several studies have compared the impact of increasing ADG with reproductive parameters and with age of first mating. These have consistently shown a strong correlation between higher ADG with earlier onset of puberty and better fertility [3, 20, 23]. Table 3 is adapted from a study by Hayes et al. and demonstrates such correlation.

Table 3: Comparison of different ADG on Days Open and days to 90% chance of conception [23]. ADG = average daily gain. Days Open = time between start date of mating season and conception.

Average daily gain (kg/day)	Days Open	Days to 90% conception chance
0.4	27	103
0.5	23	87
0.6	19	73
0.7	16	61
0.8	13	51
0.9	11	43

The nutritional aspect of rearing heifers can be looked at as a biphasic program: 1) From birth to weaning and 2) from weaning to breeding. In regards to birth to weaning, the feeding of milk replacer is the key component of the diet. The normal age of weaning dairy calves in Ireland is 7-8 weeks. The goal for farmers should be to double the calf's body weight in this time frame. Considering the average calf is 40kg at birth, a target of 80kg at weaning is achievable by an ADG of 0.75kg [7].

A study by Curtis et al. compared feeding calves ad libitum with milk replacer (MR) with calves fed a restricted amount of MR. The findings showed that the ad lib fed calves reached puberty 2-3 weeks earlier than their restricted counterparts whilst also having marginally better conception rates. They also calculated the estimate financial costs of an increased age at first conception as almost £3 a day, with the benefit of ad libitum feeding equating to saving roughly £46 on each heifer [20].

After weaning, the feeding of a high quality balanced diet is vital in maximising reproductive potential of replacement heifers. It is also important to understand the nutritional demand required by the heifer for growth and development of the mammary glands, which has a direct influence on the future productivity of the animal once it begins lactating. The growth of the mammary gland is considered allometric between 3 and 10 months of age meaning

that post-weaning, a huge amount of energy taken in by the heifer goes to the development of the mammary tissue [24].

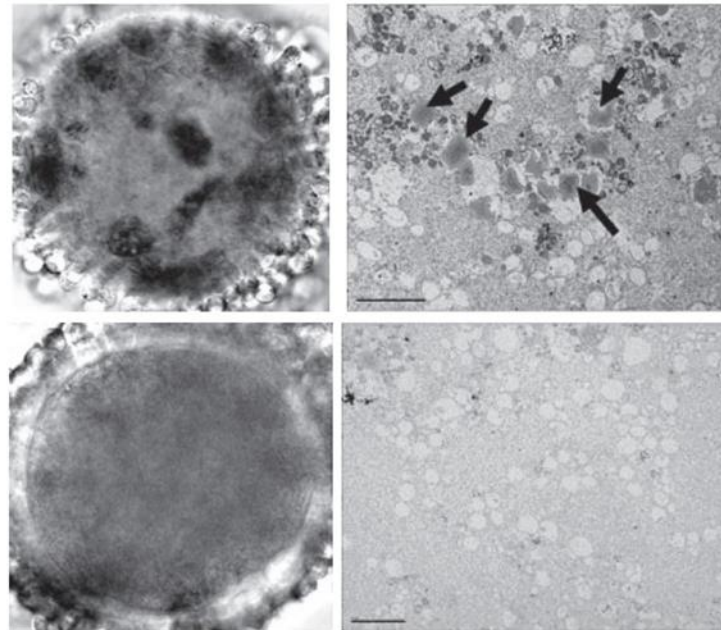
The findings of Hayes et al, seen in Table 3, demonstrate the reproductive benefits of enhanced feeding regimes, with heifers with a higher ADG taking fewer days to have a 90% chance of conceiving and also fewer days open, where days open is considered as days between the commencement of the breeding season and the animal conceiving [23].

Body condition scoring is an important and convenient way of monitoring the animals weight and level of fat cover. Dairy farmers should aim for a BCS of 2.5-3 for heifers at time of breeding. This ensures they are neither under or over conditioned. As important as conditioning and weight gain is, the effects of overfeeding and excess condition can also be detrimental to fertility and reproduction. A study by Wathes et al. found that in a herd where a number of heifers were culled due to not conceiving after 3 services, a large proportion of these heifers were significantly over conditioned and heavier than others in the herd who had successfully conceived [3].

2.2.2) Macronutrients, micronutrients, trace minerals

An interesting research finding by Awasthi et al. in relation to over conditioned heifers was the presence of lipid molecules in abundance in the cytoplasm of oocytes, believed to negatively affect the development of the oocyte and may even inhibit fertilization. Such findings occurred in heifers that were overweight and were failing to conceive on a consistent basis [25]. Figure 3 is adapted from this research and shows the presence of such lipid molecules in the oocyte cytoplasm of an over-conditioned individual. The hypothesis drawn is that these molecules interfere with the normal development of the oocyte and therefore affect its interaction with sperm during fertilisation. It should be mentioned that this study was carried out in Swedish Red cattle and they may be more prone to becoming over conditioned than the Holstein Friesian [25]. However it does not mean they cannot become overweight and this study would imply that over conditioning heifers may impair their fertility. As well as that, it is proven that over conditioned cattle at calving often have difficulty during delivery, as well as develop more post parturient problems like ketosis and fatty liver disease due to the excess mobilization of fat.

Figure 3: Upper set of images of an oocyte from an over-conditioned ‘repeat breeder’ heifer. Note the lipid droplets denoted by the arrows. Note the heterogenic appearance of the cytoplasm. In comparison, the lower set of images show a normal oocyte from a normal-weight heifer with no reproductive problems [25].



The hormone IGF-1 has been found to play a role in reproductive performance and nutrition has been demonstrated to influence this hormone. IGF -1 is regulated by GH (growth hormone) and plays an important part in myogenesis and tissue development. This includes reproductive tissue [26].

A study by Butler et al. described that IGF-1 concentrations decreased in tandem with feed restriction and increased when such restrictions were lifted [21]. Wathes’ study digressed that IGF-1 plays an important role in determining the age at which puberty occurs in heifers. It concurred with Butlers findings that a reduced feed intake aligned with lower concentrations of the hormone and resulted in an older age of puberty [3].

In terms of specific nutrients, many studies have investigated the potential effects of deficiencies or excesses of a certain nutrient on cattle fertility. This involves macronutrients like carbohydrates, protein and fats as well as various minerals and vitamins.

The effect of high protein in the diet on fertility has long been debated and reviewed. Many studies have found links between high rumen degradable protein (RDP) diets and substandard reproductivity [2, 27]. High levels of RDP and NPN (non-protein nitrogen) results in production of increased ammonia and consequently increased urea. It is widely

considered that urea has detrimental effects on fertility, affecting both the male and female gamete as well as affecting embryo development and suppressing important hormones such as progesterone [22]. High BUN (blood urea nitrogen) levels have also been reported to cause a change in the pH of the uterus [27]. Conversely, the lack of protein in the diet leading to poor growth and maturation has negative impacts on sexual development [22].

Starch is another macronutrient with conflicted findings related to fertility. The previously discussed research by Wathes et al. found that over conditioned animals to have very high insulin levels, and an association between high amounts of starch in diets and poorer oocytes has been drawn [3]. However we know the importance of carbohydrates for energy and meeting the demands of a fast growing animal.

The effect of certain lipids, namely unsaturated fatty acids, on fertility have long been investigated. The general finding is that addition of Omega 3 in the diet has been linked with better fertility, and would appear to be more beneficial than omega 6 [28]. The positive effect of healthy fats on fertility have been attributed to better embryo development, increased hormone concentrations and reduced fertilisation failure [22, 29].

In relation to minerals, there are a number of deficiencies associated with causing reproductive problems in cattle. A table summarizing the respective minerals and the impact of their deficiencies on reproduction can be seen in Table 4.

Phosphorous deficiency has been proven to affect reproductive capability, with a lack of this mineral associated with the likes of lower conception rates, abnormal oestrus cyclicity, delayed puberty and increased occurrence of ovarian cysts [30, 31]. One study showed that a group of cattle fed a diet with insufficient phosphorus required 3.7 inseminations to conceive, yet once the diet was changed and enough phosphorus was given, the conception rate improved to 1.3 services to successfully conceive [22].

Copper is another mineral with a very important role in reproduction. Copper deficiency is common in Irish soils, often due to high molybdenum levels which binds up the copper. The deficiency of this mineral has been demonstrated to reduce conception rates, delay onset of puberty, suppress oestrus and increase the incidence of early embryonic death [31, 32]. It is therefore a vital mineral in breeding cattle.

Zinc plays a substantial role in sexual development and its inadequacy is associated with underdeveloped gonads in both males and females [30]. Another role of zinc is that it

increases the concentration of beta carotene which is a beneficial compound in relation to conception and embryo survival [22].

Iodine deficiency is linked with oestrus abnormalities and fertilisation issues, whilst excess iodine has been related to abortions and negatively affecting immune systems [30]. It plays a role in regulating the ovary due to its role in increasing the release of gonadotropin from the pituitary gland [32]. Cobalt and Manganese are also important and deficiencies of these minerals can lead to anoestrus, poor conception rates and the delay of puberty [31].

Table 4: List of important minerals in cattle fertility, with the recommended dietary concentration and the main effects of their deficiency/excess on the reproductive system [30, 32].

Mineral	Recommended amount in diet	Effects on reproduction
Phosphorous	0.45 -0.5 % of DM intake	Anoestrus Delayed puberty onset Lowered conception rates Poor gonad development
Copper	12-15 ppm	Lowered conception rate Suppressed oestrus Delayed puberty Reduced libido
Zinc	56-67 ppm	Cystic ovaries Abnormal oestrus Delayed puberty Poor testicular development Testicular atrophy
Iodine	0.56 -0.67 ppm	Anoestrus Infertility Poor conception rates
Iodine (excess)		Abortions Congenital deformations Immunosuppression
Cobalt	0.11 ppm	Reduced ovarian activity Reduced conception rate Delayed puberty
Manganese	15-19 ppm	Hidden or delayed oestrus Reduced conception rates Calves with tendon contraction
Selenium	0.3 ppm	Weak calves Retained placenta White muscle disease

2.3) Disease

One of the major factors involved in herds with unsatisfactory reproductive performance is the presence of infectious diseases. Disease can affect reproductive parameters both directly and indirectly and can present in various ways such as lowering fertility levels, causing embryonic death/abortion or the birth of calves with congenital disorders.

In relation to fertility of heifers the most important parameters include conception rate (CR), age at first insemination, age at first conception and first service conception rate. Other parameters relevant for cows but not for heifers such as Calving Interval or Days Open may be used in this report to demonstrate fertility issues even though they are not a variable in heifers.

The focus of this review in relation to disease is based on the most prevalent viral and bacterial agents in Ireland that are linked to subfertility.

2.3.1) Viral Disease

Viral diseases play a major role in all livestock industries, with the dairy industry being no different in that regard. With relation to fertility the main 2 viral agents involved in reproductive failure in Irish dairy cattle are Bovine Viral Diarrhoea (BVD) and Infectious Bovine Rhinotracheitis (IBR). Both diseases are notifiable by law in the Republic of Ireland. Both agents can be described as complex in their manifestation and can present in various forms, with the 2 diseases often showing similar signs but in other cases are very distinguishable [33]. They both spread in various routes, and can present in subclinical forms making them difficult to detect as the cause of poor performance. Both viruses are of high importance in affecting herd health and impacting the industry economically [33].

BVD and IBR play an important role in reproductive failure in cattle. Both diseases have been shown to affect fertility and also cause problems in gestating cattle such as embryonic death and abortion. It is widely understood that the timing of infection can determine the outcome – with infection prior to breeding associated with conception difficulties, whilst infection during gestation can lead to loss of calf. Viral diseases also play a part in causing immunosuppression which further leads to the colonization of bacterial agents that can affect the reproductive parameters [8].

Currently Ireland has had a BVD eradication programme for PI (persistently infected) animals in place since 2013 [34]. This involves testing tissue samples taken from ear tags of

newborn calves. Upon detection of a BVD + calf, the herd is restricted and the calf is culled and its dam must also be blood tested [35]. Results on Animal Health Ireland's open database showed that in 2013 there was a prevalence rate for PI of 0.66% in newborn calves which resulted in the cull of 13,877 calves. The most recent results in 2023 show a prevalence rate of 0.02% and the cull of 530 calves. Along with a decrease in the number of PI BVD positive calves, there is a reduction in the number of herds with PI BVD positive animals – down from 11.27% to 0.35% in the 10 years. Figures 4 and 5 adapted from Animal Health Ireland (AHI) visually demonstrate this progress.

These are encouraging statistics in the fight to eradicate the disease. However it must be mentioned that a significant challenge exists in the border regions due to Northern Ireland's (NI) prevalence rate being much higher – 2022 statistics released from the AHWNI (Animal Health and Welfare of Northern Ireland) show a herd level positivity rate of 3.76% in NI compared to approximately 0.4% in the Republic of Ireland. Northern Ireland's higher statistics is partly attributed to the fact they did not begin their eradication programme until 2016. The higher prevalence of the disease on the northern side of the border poses a huge risk to the eradication of the disease in farms on the southern side of the border, particularly in counties Donegal, Monaghan and Louth [Fig 6].

In relation to IBR no eradication programme has been initiated [34], however a recent scheme carried out by the NBWS (National Beef Welfare Scheme) involving blood testing for IBR to examine the prevalence of the disease is a sign that IBR is beginning to gain more attention with speculation that an IBR eradication programme may be introduced once BVD has been officially eliminated. Prior investigations into the prevalence of IBR in Irish herds suggest the virus is widespread in the country with seroprevalence rates at herd level at approximately 75-80% [34, 36, 37]. Another more recent study displayed individual seroprevalence levels at nearly 22% [38]. Considering that the statistics are quite high and one source reporting that in 2009 only around 2% of herds were believed to be vaccinating against IBR [36], it is reasonable to say that it is an endemic in Irish herds and eradication may be unfeasible at this stage. A higher percentage of farms, particularly dairy, are currently vaccinating against IBR in the last few years, with reports of an average of 18-20% of herds vaccinating in Ireland today [39]. Data shows that NI vaccination levels are even higher, in the region of 30% of dairy farmers vaccinating against the herpes virus [40]. The incidence of co-infection with BVD and IBR has been initially but not majorly investigated [41].

2.3.1.1) Bovine Viral Diarrhoea

Bovine Viral Diarrhoea Virus (BVDV) is an agent caused by Pestiviruses A & B, more recently known as BVDV-1 & 2 making it closely related to Border Disease of sheep (Pestivirus D) and Classical Swine Fever of pigs (Pestivirus C), all of which belong to the greater family of Flaviviridae [42]. BVDV-1 & 2 are a worldwide distributed disease that are endemic in many countries where cattle are widely farmed [43]. Cattle are the main hosts but other ruminants can be less frequently affected [44].

The economic and epidemiological importance of the disease is well documented with the virus associated with decreased production in both milk and beef sectors, poor reproductive performance, health problems and increased culling [43, 45, 46].

BVD infection can have a plethora of presentations and manifestations, varying from asymptomatic or subclinical infections to a lethal and acute mucosal disease [41]. The vast range of disease forms is due to the difference in virus strains along with the timing of infection [47].

Animals can be infected with non-cytopathic (nCp) and less frequently, cytopathic strains. The cytopathic (Cp) form is associated with abortion, congenital deformations and the deadly mucosal disease (MD) which arises from a mutation of the non-cytopathic strain internally in a PI animal leading to them being superinfected with CP virus which their immune system tolerates [44]. In utero infection with nCp strain between 40 and 120 days leads to persistently infected calves due to the infection occurring prior to the development of the calf's immune system [48]. Therefore it becomes immunotolerant to the virus and will remain a lifelong carrier and shedder, posing a major risk to the rest of the herd [49]. These PI animals play the most important epidemiological role in the herd, spreading the virus to naïve members of the herd [46].

In relation to reproduction, BVD has been shown to cause embryonic death, foetal abortion, in utero infection leading to immunotolerant or seropositive calves and congenital malformations and it also has shown to effect fertility leading to conception issues [44]. Just as timing of infection determines the outcome of the foetus, multiple studies have discussed the importance of timing of infection in relation to fertility, with animals infected in and around the time of insemination showing reduced conception rates [8, 41, 50]. A study by McGowan et al. implied that animals infected with BVD at the time of insemination had a lower CR than animals that were seropositive for BVD from older infections [51]. Another

research by Fray et al. links active infection at the time of oestrus with up to 50% reduction in CR [52].

The virus has been demonstrated to cause reproductive failure due to various mechanisms including direct damage to the ovary. Kale et al. discussed how the virus' high affinity for replicating cells was a clear indication that the ovary is a place of significant susceptibility to the virus [53]. Experimental tests carried out on the ovaries of 3 persistently infected heifers found the virus present in significant titres in ovarian tissue, and immunofluorescence tests on the same ovaries found the virus distributed throughout the tissue, with the NS3 antigen of the virus detected not only in the ovarian tissue but also found in over 18% of oocytes [52]. The direct damaging effect on the ovary has been highlighted by cases of ovarian inflammation in heifers and cows infected with BVD [41], and the collection of degenerative embryos from infected uterine horns [54]. Along with the physical damage or inflammation of either the ovary (ovaritis) or oocytes (oophoritis), the virus appears to inhibit ovarian function by affecting the normal cyclicality and hormonal activity of the ovary. Fray et al. investigated the effect of the virus on oestradiol levels, with results showing a significant drop in oestradiol after intranasal infection with a nCp virus strain. How exactly it affects steroidogenesis is not fully clear but it is obvious that a markedly reduced oestradiol concentration would have negative effects on oestrus and ovulation, and subsequently lead to reduced conception rates [52].

It must be noted that many of these findings occur in the case of experimental infection of the virus into naive heifers/cows, and that they may not be a complete reflection on natural infection with the virus, or in animals with some level of immunity. However the compelling findings of several papers are consistent to show that the infection with the virus around the time of breeding has a detrimental effect on conception rates [8, 41, 50, 51].

The ovary is not the only part of the reproductive tract affected, with the virus showing to replicate in the uterine endometrium [8]. Archbald et al's study described that the presence of the virus replicating in the uterine horns had a harmful effect on the implantation of embryos [54]. Another example suggesting the negative influence of the virus in the uterine environment is how seronegative individuals inseminated with BVD infected semen had poorer conception rates than similar members of the same group who had been challenged with the virus intranasally as well as animals inseminated with the infected semen but were already exposed to the virus i.e. seropositive [47]. This information would highlight the

importance of using BVD free semen, from BVD free bulls or using AI as there is data to show the damaging effect of infected semen on fertility levels [8].

Wathes et al. described that the virus had inhibitory effects on local immune system mechanisms in the uterus which would normally react in the presence of bacterial LPS (lipopolysaccharide), suggestive that the virus has immunosuppressive effects on the endometrium, potentially leading to endometritis and the colonisation of harmful bacterial species that would normally be kept under control [8].

Statistically, the negative effects of BVD on fertility have been measured using parameters like conception rate (CR), age at first conception and number of services to first conception. These parameters have been used as the basis of multiple studies into the effect of BVD on reproductive performance. An Austrian study showed that dairy cattle from control herds had a 1.1 times higher CR at first service than cattle with BVD infection. The same study compared first service conception rate (FSCR) in herds during BVD infection with herds after BVD had been eradicated with the FSCR being 0.68 times lower when BVD was present in the herd [55]. Another study showed an increased duration (32 days) to first conception in heifers with high levels of BVD virus at 10 months old than those uninfected or with lower infection titres [50]. Likewise Kale et al. found an increased age at first insemination in BVD positive heifers than for BVD negative heifers. This statistic was consistent with both seropositive and persistently infected heifers [53].

Figures 4-6 are AHI maps of the island of Ireland demonstrating the number of herds with PI animals detected. Figures 4 and 5 show the success of the eradication programme in the Republic of Ireland from 2013 to 2023. Figure 6 shows how the eradication programme in Northern Ireland is not yet up to the same level, posing a major risk to counties on the southern side of the border.

Fig 4: 2013 PI animal herds in Republic of Ireland

Fig 5: 2023 PI animal herds in Republic of Ireland

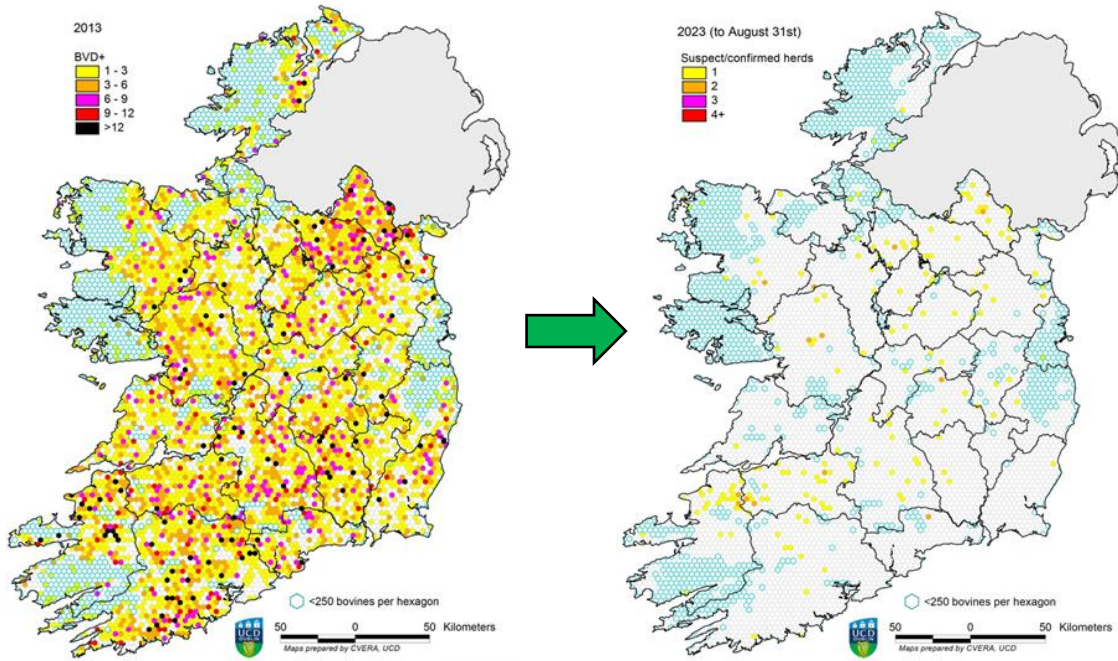
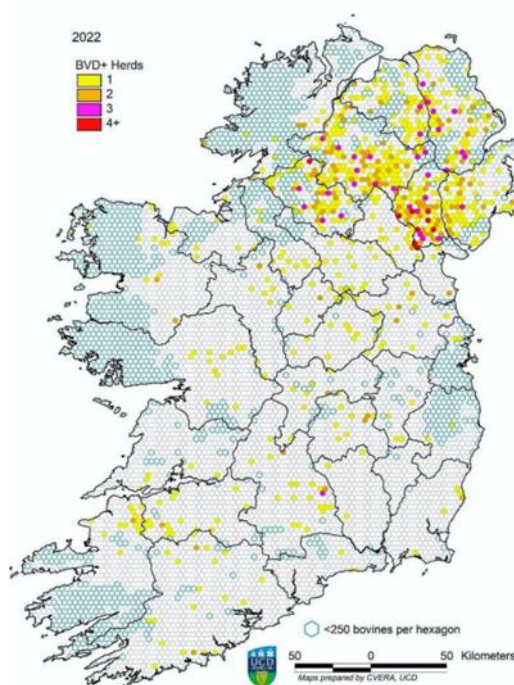


Fig 6: Herds with PI animals across island of Ireland in 2022.



2.3.1.2) Infectious Bovine Rhinotracheitis (IBR)

IBR is another important viral disease affecting cattle worldwide. It is caused by Bovine Herpes Virus, with the most significant serotype being Bovine herpes virus type 1 (BHV - 1), although there is studies to show that BHV-4 is also capable of affecting the reproductive performance of cattle [8]. BHV-5 is associated with encephalitis of young animals under 6 months of age. BHV-1 has been found to manifest in many different forms, with its most common form being a febrile upper respiratory disease characterized by inflammation of the nares, larynx and trachea with a profuse nasal discharge that progresses from serous to purulent and necrotic, with the appearance of a classical red nose [56]. However it also occurs in the form of keratoconjunctivitis, abortion, enteritis, reproductive failure and a genital disease characterized by pustular lesions, swelling and discharge of the vulva and vagina in females – IPV, and the penis and prepuce of males – IBP [57, 58].

BHV is of great economic and epidemiological importance with the disease having drastic effects on the productivity and performance of cattle herds with drop in milk production, weight loss, increased culling and reproductive problems all a result of the disease [59]. The negative effects on milk production are of serious concern for dairy farmers, with Irish research showing reductions in milk yield as well as fat and protein content of herds exposed to BHV-1 [39]. Such findings are consistent with those of Sayers et al. who also found a reduction in yields as well as milk protein and fats in BHV-1 positive herds in comparison with herds free of the disease [37].

Similar to BVD, the severity of the disease varies from asymptomatic or subclinical to acute clinical disease with an array of presentations. The severity varies on virulence, immune status of host, previous exposure and presence of a secondary bacterial infection [60]. Like any herpes virus, it leads to lifelong latency with the virus residing in the nervous system, with the potential of reactivation at a time of stress. Such animals are carriers and shedders of the virus and pose a serious epidemiological threat in the spread of disease [38, 61]. The disease is spread in a number of different routes, through direct contact, respiratory and ocular discharge, to venereal infection either by natural or artificial breeding and is known to be highly contagious [61]. IBR has become highly endemic in Ireland with bulk tank antibody results showing approximately 80% prevalence in dairy herds [34, 36, 37].

It is worth mentioning that the occurrence of the severe clinical forms of the disease are less common in Ireland due to the widespread seropositivity meaning that most herds have some level of immunity , along with the increased practice of vaccinating against the disease.

In regard to fertility there has been sufficient research done to show that the virus has a negative effect on reproductive performance. Similar to BVD, insemination with IBR infected semen appears to have a deleterious effect on reproductive performance [41]. The virus has been demonstrated to interfere with reproduction by causing shortened oestrus cycles with Graham et al. reporting that in a study where 8 seronegative heifers were inseminated with IBR infected semen, 6 of them showed reduced oestrus cycles of 11-15 days [36]. These same animals showed necrotizing endometritis in biopsy samples taken. This finding of necrotizing endometritis has been alluded to in several other studies along with necrotizing oophoritis [8, 41, 59].

Graham et al. also related to a subsequent study where in a group of 10 seronegative heifers inseminated with infected semen, only 2 of them had a successful conception on the first service, with the group taking an average of 4.5 services until conception, in contrast to a control group that had an average of 1.7 inseminations to conceive, and 9 of the 10 conceived successfully [36]. Another study reported in their review that in a herd of dairy cattle with IBR, 69% of them had reproductive problems in comparison to 30% in cattle without IBR. Ring et al. also found a strong correlation with positive antibody results for IBR with suboptimal fertility [61]. The virus' influence on reproduction has been shown through its effects on conception rates, number of services to conception and in case of cows- days open and calving interval [59, 62]. Although IBR abortions more commonly occur in the second half of pregnancy, there are findings to show early embryonic and foetal death in the first trimester [56, 60].

Considering IBR infection often paves the way for secondary bacterial infection in respiratory disease, it is reasonable to assume that infection of the reproductive tract increases the potential for bacterial species such as *Trueperella pyogenes* and *E. Coli* to cause further inflammation of the endometrium [8].

The culmination of these effects on the reproductive system leads to the use of the term 'repeat breeder', where animals continue to return to oestrus despite inseminations. IBR has been demonstrated to be a factor in such animals, shown by shortened oestrus cycles,

reduced conception rates, increased number of inseminations to conceive and as a result; a longer time until first conception and subsequently older age at first calving than desired.

2.3.2) Bacterial Disease

Bacterial infections can also play an important role in fertility of cattle, although bacterial infections are much more common in cows than maiden heifers and are of less significance in this review as the concentration is more so on heifers.

Uterine infection can occur due to possible contamination around calving time, especially with cows that experienced difficult calving, having required manual assistance or experiencing post parturient issues such as vaginal or uterine prolapse. Retained foetal membranes are another source of potential metritis/endometritis in the recently calved dam [63, 64].

However poor hygiene practices, poor sterility during insemination and the presence of viral infections causing immunosuppression can increase the risk of bacterial infection in the reproductive tract, with the potential of having detrimental effects on fertility in heifers also.

The normal uterus and vagina is not sterile of course and many bacterial species associated with infections are also normal residents in the uterine microflora, such as Enterobacteriaceae spp, Trueperella pyogenes, Ureaplasma spp, Streptococcus spp and Bacteroides spp. However in normal cases they are not harmful and kept under control by the uterus' own defence system [65].

Aside from bacterial infection of the uterus and reproductive tract, other bacterial diseases causing systemic infection can also have serious impacts on fertility. Two of such diseases in Ireland that are of economic and clinical importance are Johne's / Paratuberculosis and Leptospirosis.

It is worth including that *Campylobacter fetus* ssp. *venerealis*, commonly known as Vibriosis is another bacterial infection that causes reduced fertility and is transmitted during mating with bulls being long term carriers of the disease [66]. However the disease is not currently found in Ireland and will not be discussed in depth in this report as a result.

2.3.2.1) Johne's Disease/Paratuberculosis

Johne's disease, also known as Paratuberculosis is a worldwide distributed bacterial infection that causes a chronic enteritis caused by the species *Mycobacterium avium* subspecies paratuberculosis (MAP) [67].

The disease primarily affects cattle but has the ability to infect a wide range of hosts including other ruminants, pigs and various wild animal species. It may also be of zoonotic importance, with a connection between the bacterium and Crohn's disease in humans yet to be fully investigated [68]. MAP is considered a disease of great economic importance to the dairy industry, with the infection associated with causing a reduction in milk production and milk quality, along with increased culling rates and suboptimal reproductivity [69, 70].

Paratuberculosis is considered widespread in European countries with intensive dairy farming, with Ireland and the UK being no exception. Irish reports show herd level positivity at around 30% [71], which is considerably lower than the estimated average of 50% in Europe [69]. The disease seems to be much more of an endemic issue in the UK with findings of greater than 70% of dairy herds having at least one animal with positive ELISA results [72].

The disease is contracted by ingestion, through feed or water contaminated with infected faeces or through milk of seropositive cows [73, 74]. Neonates are the most vulnerable to becoming carriers of the disease, and there is evidence to show that intrauterine infection is also possible [73]. It is widely known that calves infected in the first 6 months of life are those individuals that will display typical chronic signs later in life, whereas animals that become infected at an older age are usually able to fight off the infection [68, 72].

Johne's is characterized by a slowly progressing disease course, with infected animals usually asymptomatic or subclinical until they are greater than 2 years of age [74]. When clinical signs do develop they typically manifest as a chronic wasting syndrome coupled with persistent, profuse diarrhoea, often with a classical jet-like appearance [75]. A granulomatous enteritis causes protein losing enteropathy, often highlighted by a submandibular oedema and an emaciated appearance [70].

The subclinical phase of the disease is believed to be of significant epidemiological relevance as the animals are capable of shedding the bacterium in high titres despite showing little to no signs of the disease [76]. This phase can be also considered extremely important in sub productivity in terms of milk and fertility, leading to premature culling and increased

replacement costs [74]. The lack of an effective therapy for the disease further stresses the importance of control and prevention of infection [72, 77].

Studies investigating the relationship between MAP and reproductive performance have returned varied results [78]. A study by Oszvari et al. found that cows that had positive ELISA antibody tests had lower FSCR than comrades who had negative ELISA results. From cows in their 1st, 2nd and 3rd parities, the average FSCR for positive cows was 12.9% compared to disease free counterparts that had an average of 24.9% [69]. In another research by Reynolds et al. on UK herds, a correlation was drawn between a recent positive antibody test and a reduced rate of conception [79]. Meanwhile a Canadian study found that seropositive heifers in their first lactation required on average 49 days more to conceive than those who were seronegative [76]. A commonly suggested hypothesis behind poor fertility in Johne-positive animals is that the effects of a severe enteritis on energy balance and the ability to absorb important nutrients, may lead to untoward effects on conception rates and an increase in number of services required to successfully conceive [76, 78, 79, 80].

Other studies would suggest Johne's has no detrimental effect on fertility, some even finding improved fertility statistics in MAP-positive animals in comparison to individuals who were negative for the infection [72, 73, 78].

It would appear that the severity of fertility depletion in animals with Johne's disease is dependent on the physical state of the animal and the stage in the disease's course, with poor fertility occurring as a secondary effect from malnourishment and ill health [72]. Further research is needed to fully understand the influence MAP has on the breeding capabilities of dairy cattle. It is nonetheless a major economic burden to the dairy industry, as potential subfertility along with reduced milk production, poorer milk quality as well as increased culling rates and cost of replacements have a great financial impact on dairy farming in an industry that is already based on marginal profits [81].

2.3.2.2) Leptospirosis

Leptospirosis is a worldwide disease caused by a form of spirochaetes and they are classified into many different serovars with approximately 300 reported, which are assigned to serogroups of certain species [82].

The disease has a vast range of hosts with certain strains considered to have maintenance or reservoir hosts, whilst others cause incidental infections in species of animals that are not considered reservoirs [83]. Humans are also susceptible to infection making it of great significance in the veterinary profession [84]. In relation to cattle, the hardjo serovar is of most clinical importance and cattle are recognised as the maintenance host for this serovar [85]. Bovine leptospirosis is associated with great economic loss caused by loss in milk production, poor conception rates, embryonic death, stillbirth and abortion [84, 86].

In Irish cattle herds, the two *L. hardjo* strains of greatest significance are 1) *L. interrogans* serovar hardjo and 2) *L. borgpetersenii* serovar hardjo [87]. These are considered to be 2 of the most important *Leptospira*'s in cattle populations not only in Ireland but across the world [88]. It is considered an endemic disease in Irish herds with multiple studies reporting average herd seroprevalence rates of 80-90% [87, 89]. Data would strongly suggest that a vast percentage of the dairy population in Ireland have been in contact with *L. hardjo* [90].

Incidental infections of cattle are most commonly caused by other serovars of *L. interrogans* such as Pomona and Icterohaemorrhagiae [88]. These infections are usually associated with other animals such as pigs or rodents, with the disease often transmitted through contact with urine of the reservoir species [83]. Such infections are often causative of acute and severe leptospirosis, as they are often naïve to those particular strains [88]. These sort of acute infections are considered rare in cattle populations in Europe [84]. In comparison, infection with hardjo usually involves a more subclinical or chronic infection residing in the herd [85]. This chronic form of disease is highly associated with poor reproductive performance and it has been demonstrated in a number of studies that hardjo serovars are heavily linked with reproductive failure, having drastic effects on the productivity and profitability of cattle farming [86, 87, 91].

Although Leptospirosis has long been defined as a causative factor of bovine abortion, it appears the chronic effects the disease can have on causing poor herd fertility may be of even greater importance, as such effects are much more subtle and difficult to detect [88]. Increased embryonic loss, reduced conception rates, and repeated oestrus are all less obvious

consequences and indicators of leptospirosis infection compared to the likes of abortions and retained foetal membranes [82]. In fact abortions involving *L. hardjo* strains are considered sporadic in comparison to reports of abortion storms caused by acute infections with strains such as *L. pomona* and *L. grippotyphosa* [92, 93], with infection with the former more associated with long term depression on herd fertility [94]. The significance of this inconspicuous infection with *L. hardjo* is that it can be easily overlooked and more difficult to detect due to the lack of ‘classic’ leptospirosis symptoms [82, 95].

The data of multiple researches indicates a strong correlation between *L. hardjo* infections and fertility issues [83, 84, 95]. When compared with seronegative cattle, those seropositive with *L. hardjo* had lower conception rates, increased number of services required to conceive and in case of cows – prolonged calving intervals [89, 91, 96]. In Table 5, adapted data taken from a study carried out by Dhaliwal et al. statistically demonstrates the decimating effect of increasing leptospiral MAT (microagglutination test) titres on reproduction [91].

Table 5: Comparison of the conception rates in cows with varying titres of *L. hardjo* versus seronegative individuals [91]. FSCR=first service conception rate.

Variable	Seropositive animals and their MAT titre			Seronegative
	MAT > 1:10	MAT > 1:30	MAT >1:100	
FSCR %	50.53	48.83	41.66	51.83
Overall CR %	55.76	53.93	50.86	59.33

Another common fact related to leptospirosis is how the kidneys are a primary site of replication and persistence for the disease. However it has been proven to colonize the reproductive tract of cattle [86]. Its persistence in the uterine environment would appear significant in its effect on reproductive parameters [83, 92]. One hypothesis to the negative influence the disease has on reproduction is that it causes inflammatory reactions in the uterus which have an inhibiting effect on implantation [88], however a contradictory finding by Molinari et al. who found that in an experiment conducted on bovine endometrial cells that the uterus did not respond when challenged with *L. borgpetersenii* serovar *hardjo*, yet the same cells reacted when exposed to other pathogens [86]. It is reasonable however to hypothesize that due to the fact that it may not elicit an immune response from the

endometrium, it is able to persist in the uterine environment and chronically affect the reproductive cycle.

A positive note related to the practice of vaccination against *L. hardjo* shows a benefit on fertility after vaccination in herds that had high levels of infection [94, 97]. Despite herd level seroprevalence rates in Ireland being extremely high (~90%), it is encouraging to report one recent study that found an average of 47% of herds were vaccinating against leptospirosis [89].

3) Discussion

Subfertility is a complex issue with dairy herds, as the causative factor can be variety of things, and these can occur coincidingly with each other. Unlike some reproductive problems such as dystocia, abortion and retained foetal membranes, the diagnosis of a fertility issue is not as simple and obvious. As it is not always a unifactorial problem, it means there is usually not a one-step solution to improve herd fertility. The combination of good genetics, adequate nutrition, disease control and an effective breeding program is essential for farmers to optimize their herds fertility. Veterinarians have an increasingly important role in consulting with farmers about managing fertility levels, through advice on improving the herd's bloodline, working on feeding regimes, improving herd health, implementation of disease control measures and construction of a breeding program that involves accurate heat detection and successful service. A study by Mee et al. gathered data from a questionnaire of veterinary practices from 3 countries with a large dairy customer base. It asked vets their opinions of the causes behind declining fertility in dairy herds. Table 6 compiles the results of this questionnaire [98]. An observation from the results was the volume of Irish vets that believed the fall in fertility was due to demand for increased milk production and insufficient nutrition, as well as too much emphasis of genetic selection on production. Another interesting take was the difference in opinions between Irish and Portuguese vets, with almost half of the latter saying there was not a decline in fertility on their farms, which one could possibly attribute to the difference in dairy farming intensity in the 2 countries.

Table 6: Questionnaire on veterinary opinions for reduced fertility in dairy herds [98]. Answers displayed as a percentage of practitioners who selected the option.

	Ireland	Netherlands	Portugal	Total
Number of participating veterinary practitioners	47	44	31	122
Has dairy fertility rates fallen in your practice?				
Yes	80.9	88.6	51.6	76.2
No	0	2.3	48.4	13.1
Not sure	19.1	9.1	0	10.7
What do you think is the reason?				
Inadequate management	19.1	25	16.1	20.5
Increased milk yields	23.4	15.9	16.1	18.9
Inadequate nutrition	21.2	9.1	29	18.9
Genetic focus based on milk production	21.2	9.1	16.1	15.6
Increase in herd size	6.4	18.2	0	9
Increased disease prevalence	6.4	4.5	3.2	4.9
What are the main reproductive problems you encounter on dairy farms?				
Poor oestrus detection	55.3	90.9	77.4	73.8
Puerperal problems	80.9	59.1	48.4	64.8
Repeat breeders	59.6	54.5	87.1	64.8
Low conception rates	66	38.6	67.7	56.5

3.1) Genetic improvements

There has been extensive and widespread studies to support that genetics play an important role in reproductive potential and performance. The introduction of the EBI and its development in recent years to increase the overall weight that fertility carries in the valuation of the animal has shown the progress made in Irish cattle breeding, with the realisation that this constant effort for breeding to increase milk production year on year was sending fertility levels in the opposite direction. The graph in Figure 2 has shown how the fertility sub index value has increased by approximately €5 a year since 2007. It is no coincidence that it correlates with improving fertility statistics in Irish herds in this same time period [6, 9]. The benefit of the EBI for farmers is that it allows them to select superior animals to improve the aspect of their herd they feel requires the most attention. So for a farmer who feels his herd's fertility is suffering, he can select to use semen from a bull with high EBI values for fertility, or can buy in replacement heifers bred from stock with high EBI values. For example an animal with an EBI value of €250 is proposed to return a profit of €250.

The use of artificial insemination with semen from top EBI sires has become a widely practiced and financially viable tool for dairy farmers to improve herd performance and profitability [6]. The EBI has become the basis for AI companies in how they promote their bulls and cows as superior individuals to produce better progeny for farmers.

Genotyping is another service available to dairy farmers to measure the genetic merit of their herd for specific traits such as fertility. It involves the analysis of single nucleotide polymorphisms from the DNA of the herd, taken using hair samples. It can help determine the predicted performance of the animal as well as its offspring as these genes will be passed on to the calf [99].

3.2) Nutritional management

The importance of good nutrition in the rearing of replacement heifers has been clearly demonstrated in a plethora of studies across multiple countries and cattle herds. The correlation between body weight and onset of puberty is also undeniably strong.

There is also a reciprocal relationship between nutrition and genetics as one will only get so far without the other. Well-bred animals may have the potential to be high yielders with good

fertility, however if they are not adequately fed during the maturation process then the potential will never translate into physical results. Likewise, an optimum diet will help animals reach target weights and puberty but there will be a ceiling to their potential based on the genetic merit of the animals. Therefore the importance of the intertwining relationship cannot be ignored [28].

Target weights and body condition scoring are the two main tools for farmers to achieve optimum conditioning at breeding so that heifers reach puberty at earlier ages and have better conception rates. Although the use of target weights can be loosely interpreted depending on breeds and industry, the knowledge of your adult herds average body weight allows the farmer to calculate an approximate weight for heifers at breeding. This is roughly estimated to be 55-60% of adult bodyweight at mating.

The role of multiple minerals in reproduction is covered in many reports and the impact of their deficiencies is often drastic on fertility levels. It is vital for farmers to understand the importance of feeding a balanced diet that supports growth and development and also contains sufficient amounts of those trace minerals [7]. Seeking advice from their veterinarian on formulating a diet for rearing heifers is one way in which the farmer can take action to ensure his feeding regime does not have gaping holes in the form of mineral deficiencies. Soil testing is another way for farmers to check if his farmland is naturally lacking or deprived of a particular nutrient. Irish soils are commonly low in copper due to a high level of molybdenum, with these two minerals having an antagonistic relationship.

3.3) Disease control

Infectious diseases can wreak havoc in a multitude of ways in herd health and productivity, and their effect on fertility and other reproductive parameters is no exception. Again, similar to other issues, those reproductive risks caused by disease can be greatly reduced and even prevented by good farm management.

It is no secret that good hygiene and strict biosecurity can minimize the risk of infectious diseases arriving in an individual's farm. The use of disinfection, proper fencing and control of what and who enters the farm is paramount in prevention of disease breakout on a holding. Dairy farmers that rear their own replacements also greatly reduce the risk by not buying in potential disease carriers into their herd. Those who do buy in animals should operate a quarantine regime to test new individuals for harmful agents [100].

Disease screening for important diseases can be a voluntary or mandatory practice. The state controlled eradication programme of PI animals for BVD in Ireland is one example of such. Great developments in diagnostic techniques has allowed for the increased offering of sampling procedures by veterinary practitioners to their farmers. Results of such tests can allow the veterinarian consult with the farmer on possible treatment or vaccination protocols to implement. Vaccination against the likes of BVD, IBR and Leptospirosis have proven to be of great benefit for fertility rates [97, 101, 102].

3.4) Use of precision technologies in breeding programme

Heat detection and oestrus synchronization are technological advances to help farmers improve the success rate of breeding. The benefit of using such methods is seen by increasing conception rates, shortening the breeding season which subsequently shortens the calving period, with the goal of many large dairy farms to calve all gestating animals in a 6 week period. There is also the matter of the cost of artificial insemination, with farmers wanting to make sure they inseminate successfully to avoid increased costs and elongated breeding seasons [10].

Heat detection is a popular and effective way of improving conception rates and maximising efficiency around artificial insemination. Traditional heat detection was based on visual observation of cows in standing heat, however studies show the incidence of fervid heat expression to be less common in dairy cattle nowadays. It is believed that the huge concentration on milk yield has led to the reduction in observable heat demonstration [103].

The general practice is that cows should be inseminated after oestrus based on the AM-PM rule whereby depending on what time they display heat, they should be inseminated 12 hours from then [10]. Some tools commonly used for heat detection include pedometers that pick up on increased movement indicating the animal is in oestrus, accelerometers that monitor head and neck movement, rumination time and heat patches [103, 104].

Oestrus synchronization is another commonly practiced method of improving the success of a condensed breeding season. There are a number of different methods involved in oestrus synchronization, using various hormonal interventions to get the herd's oestrus cycles in tandem. These programs such as the "OvSynch" protocol involve a series of injections and implants over the course of a set number of days, after which artificial insemination can be performed with great success rates [105].

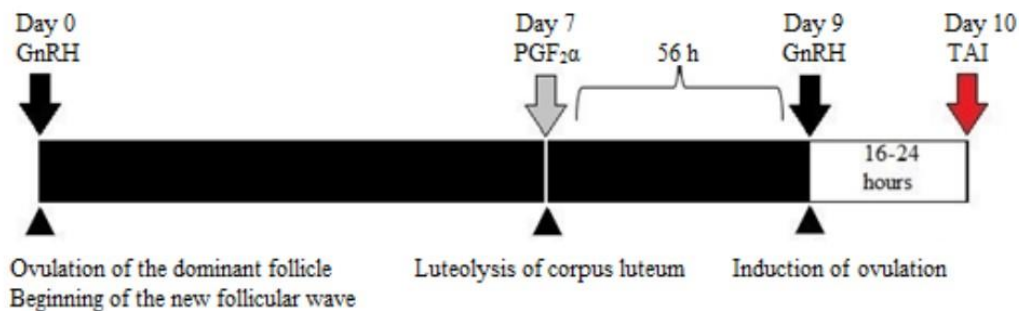
The basis of the OvSynch protocol is as follows:

- Day 1: GnRH (Gonadotropin regulating hormone) injection to initiate the ovulation of a dominant follicle in the ovary
- Day 7: Injection with prostaglandins (PGF 2a) to induce luteolysis of the corpus luteum. This facilitates the further development of the dominant follicle by reducing the progesterone levels.
- Day 9: Second GnRH injection to bring about ovulation of the follicle in approximately 16 – 24hours [106].

Figure 7 shows an example of such protocol using GnRH and prostaglandins to synchronize the oestrus cycles. It is important to note that after injection with PGF2a leading to a fall in progesterone due to luteolysis, the animal will come into heat and ovulate. The use of the second GnRH injection is to induce ovulation during a specified window so that the chances of successful AI are increased.

The use of progesterone releasing intravaginal devices (PRID) in conjunction with the OvSynch protocol is also common practice. The device is placed into the vagina on day 1 along with the initial GnRH injection and removed 24hrs after the prostaglandin injection. The use of progesterone (P4) is believed to help in anoestrus or non-cycling animals as well as aiding cows lacking a corpus luteum. The removal of the PRID further causes a drop in P4 and subsequent onset of oestrus [106].

Fig 7: OvSynch protocol. TAI = timed artificial insemination [106].



4) Conclusion

From the various academic sources analysed in this report, it can be concluded that the fertility of dairy heifers can be compromised and affected by a multitude of factors, and that sufficient data has shown that to be true. Fertility is negatively influenced by poor genetics, inadequate nutrition and infection with diseases such as BVD, IBR, Johne's and Leptospirosis. The findings of the studies reviewed in this paper not only show how these factors have detrimental effects on reproductivity, but also show that the opposite of these factors (better genetics, good nutrition and elimination/prevention of harmful infectious agents) are contributory to improving herd fertility. The economic impact of this cannot be underestimated and it is evident that one of the main causes of extra costs on dairy farms is due to fertility issues, combined with the cost of increased culling rates of animals that cannot be put in calf. As previously mentioned in this report, fertility problems on dairy farms are usually not due to a single factor. The relationship between the various factors are intimately intertwined. For example, despite a heifer having a high EBI and being on a high plane of nutrition, if she is congenitally infected with BVD she may display serious fertility problems. Furthermore the chronic effects of such infection will hinder growth and development meaning regardless of nutrition quality, the likelihood this individual can reach puberty at an early age is unlikely as there average daily gain will be reduced, with the knock on effect being increased feeding costs during the rearing period to reach puberty.

The data has shown that the industry's drive to incessantly increase milk yields has inversely led to the decline in fertility performance of the dairy cow in Ireland. The effect of such demand can be seen by comparing dairy to beef where fertility problems are not as widely reported. A realisation of this issue in recent times has led to the partial recovery of breeding success, with the introduction of the EBI helping shift some of the focus from production to fertility and other important traits. It is paramount for farmers to understand that without high fertility, the production is irrelevant as a cow won't produce milk unless she can produce a calf first.

It is worthwhile to mention that a vast majority of fertility problems that occur in dairy herds are occurring in cows rather than heifers. There are even more factors at play for cows when one considers the physical changes around calving, dystocia, lameness, post parturient diseases and metabolic conditions. For that reason, this review was constructed with the concentration based mainly on heifers as there were less factors involved allowing thorough review of the most important issues. Another consideration for focussing on heifers was that

good management practices in relation to the aforementioned factors in the first year of life will lead on throughout the animal's lifespan. For example it is known that an over conditioned animal with too much fat cover will mobilise her reserves much more readily than an animal who is in correct condition. Hence nutrition and management of rearing is paramount not only in getting a heifer to conceive early and calve inside 24 months before producing a great lactation period, but also to ensure this animal is capable of doing the same thing in following mating, calving and milking periods.

The model heifer is one that is a well-bred animal with good genes for production and fertility, that reaches puberty at approximately 13-14 months old at the correct bodyweight, with good health and free from harmful diseases through good biosecurity and/or vaccination programs and will almost certainly become a high value animal with good reproductive abilities. The beauty of this is that it not only is a great concept but that it is actually achievable for a farmer to produce a replacement heifer of such potential. Understanding the multivariable relationship between genetics, nutrition and health with bovine fertility along with efficient management is vital in ensuring that the future of the dairy industry is safe. As veterinarians who are interested in working with bovines, it is our role to work alongside farmers to advise, consult and assist them in developing good management practices to maximise the potential of their herd in regards to production, fertility, health and longevity.

5) Bibliography

1. Mee, J.F. Temporal trends in reproductive performance in Irish dairy herds and associated risk factors. *Ir Vet J* **57**, 158 (2004). <https://doi.org/10.1186/2046-0481-57-3-158>
2. Chagas, L.M., Bass, J.J., Blache, D., Burke, C.R., Kay, J.K., Lindsay, D.R., Lucy, M.C., Martin, G.B., Meier, S., Rhodes, F.M., Roche, J.R., Thatcher, W.W. and Webb, R. (2007). Invited Review: New Perspectives on the Roles of Nutrition and Metabolic Priorities in the Subfertility of High-Producing Dairy Cows I. *Journal of Dairy Science*, [online] **90**(9), pp.4022–4032. <https://doi.org/10.3168/jds.2006-852>.
3. Wathes, D.C., Pollott, G.E., Johnson, K.F., Richardson, H. and Cooke, J.S. (2014). Heifer fertility and carry over consequences for life time production in dairy and beef cattle. *animal*, [online] **8**(s1), pp.91–104. doi:<https://doi.org/10.1017/s1751731114000755>.
4. Macrae, A. and Esslemont, R., 2015. The prevalence and cost of important endemic diseases and fertility in dairy herds in the UK. *Bovine Medicine*, pp.323-337.
5. Ahmet Keskin, Yasemin Öner, Gülnaz YILMAZBAŞ-MECİTOĞLU, Barış Güner, Ebru KARAKAYA, Cengiz Elmacı and Ahmet GÜMEN (2015). Fertil ve Subfertil Siyah Alaca Düveler Arasında CYP19, ER α , PGR Allel Frekanslarının Dağılımı. *Kafkas Üniversitesi Veteriner Fakültesi Dergisi*, **21**(6). doi:<https://doi.org/10.9775/kvfd.2015.13827>.
6. Ramsbottom, G. (2020). *Trends in genetic merit for dairy cow fertility*. [online] www.veterinaryirelandjournal.com. Available at: <https://www.veterinaryirelandjournal.com/large-animal/184-trends-in-genetic-merit-for-dairy-cow-fertility> Teagasc Oak Park.
7. Akins, M.S. (2016). Dairy Heifer Development and Nutrition Management. *Veterinary Clinics of North America: Food Animal Practice*, **32**(2), pp.303–317. doi:<https://doi.org/10.1016/j.cvfa.2016.01.004>.
8. Wathes, D.C., Oguejiofor, C.F., Thomas, C. and Cheng, Z. (2020). Importance of Viral Disease in Dairy Cow Fertility. *Engineering*, **6**(1), pp.26–33. doi:<https://doi.org/10.1016/j.eng.2019.07.020>.
9. Cummins, S. and Butler, S. (2011). *High And Low Fertility In Dairy Cows*. [online] www.thedairysite.com. Available at: <https://www.thedairysite.com/articles/2694/high-and-low-fertility-in-dairy-cows>.
10. Crowe, M.A., Hostens, M. & Opsomer, G. Reproductive management in dairy cows - the future. *Ir Vet J* **71**, 1 (2018). <https://doi.org/10.1186/s13620-017-0112-y>
11. Cummins, S.B., Lonergan, P., Evans, A.C.O., Berry, D.P., Evans, R.D. and Butler, S.T. (2012). Genetic merit for fertility traits in Holstein cows: I. Production characteristics and reproductive efficiency in a pasture-based system. *Journal of Dairy Science*, **95**(3), pp.1310–1322. doi:<https://doi.org/10.3168/jds.2011-4742>.
12. Cummins, S.B., Lonergan, P., Evans, A.C.O. and Butler, S.T. (2012b). Genetic merit for fertility traits in Holstein cows: II. Ovarian follicular and corpus luteum dynamics, reproductive hormones, and estrus behavior. *Journal of Dairy Science*, **95**(7), pp.3698–3710. doi:<https://doi.org/10.3168/jds.2011-4976>.
13. Moore, S.G., Scully, S., Browne, J.A., Fair, T. and Butler, S.T. (2014). Genetic merit for fertility traits in Holstein cows: V. Factors affecting circulating progesterone concentrations. *Journal of Dairy Science*, **97**(9), pp.5543–5557. doi:<https://doi.org/10.3168/jds.2014-8133>.
14. Meier, S., McNaughton, L.R., Handcock, R., Amer, P.R., Beatson, P.R., Bryant, J.R., Dodds, K.G., Spelman, R., Roche, J.R. and Burke, C.R. (2021). Heifers with positive genetic merit for fertility traits reach puberty earlier and have a greater pregnancy rate than heifers with negative genetic merit for fertility traits. *Journal of Dairy Science*, [online] **104**(3), pp.3707–3721. doi:<https://doi.org/10.3168/jds.2020-19155>.
15. Veronese, A., Marques, O., William, R., Anna Luiza Belli, R.S. Bisinotto, Bilby, T.R., Peñagaricano, F. and R.C. Chebel (2019). Genomic merit for reproductive traits. I: Estrous characteristics and fertility in Holstein heifers. *Journal of Dairy Science*, **102**(7), pp.6624–6638. doi:<https://doi.org/10.3168/jds.2018-15205>.
16. Veronese, A., Marques, O., Peñagaricano, F., Bisinotto, R.S., Pohler, K.G., Bilby, T.R. and Chebel, R.C. (2019a). Genomic merit for reproductive traits. II: Physiological responses of Holstein heifers. *Journal of Dairy Science*, **102**(7), pp.6639–6648. doi:<https://doi.org/10.3168/jds.2018-15245>.
17. Evans, R.J., Dillon, P., Buckley, F., Berry, D.P., Wallace, M.B., Véronique Ducrocq and Garrick, D.J. (2006). Trends in milk production, calving rate and survival of cows in 14 Irish dairy herds as a result of the introgression of Holstein-Friesian genes. *Animal Science*, **82**(4), pp.423–433. doi:<https://doi.org/10.1079/asc200660>.
18. Parland, S.Mc., Kearney, J.F., Rath, M. and Berry, D.P. (2007). Inbreeding Effects on Milk Production, Calving Performance, Fertility, and Conformation in Irish Holstein-Friesians. *Journal of Dairy Science*, **90**(9), pp.4411–4419. doi:<https://doi.org/10.3168/jds.2007-0227>.
19. Begley, N., 2008. An Evaluation of Norwegian Red and Norwegian RedxHostein Friesian Dairy Cows Under Irish Production Conditions (Doctoral dissertation, University College Dublin).

20. Curtis, G., McGregor Argo, C., Jones, D. and Grove-White, D. (2018). The impact of early life nutrition and housing on growth and reproduction in dairy cattle. *PLOS ONE*, 13(2), p.e0191687. doi:<https://doi.org/10.1371/journal.pone.0191687>.
21. Butler, S.T. (2014). Nutritional management to optimize fertility of dairy cows in pasture-based systems. *animal*, 8(s1), pp.15–26. doi:<https://doi.org/10.1017/s1751731114000834>.
22. Bindari, Y.R., Shrestha, S., Shrestha, N. and Gaire, T.N., 2013. Effects of nutrition on reproduction-A review. *Advances in Applied Science Research*, 4(1), pp.421-429.
23. Hayes, C.J., McAloon, C.G., Carty, C.I., Ryan, E.G., Mee, J.F. and O’Grady, L. (2019). The effect of growth rate on reproductive outcomes in replacement dairy heifers in seasonally calving, pasture-based systems. *Journal of Dairy Science*, 102(6), pp.5599–5611. doi:<https://doi.org/10.3168/jds.2018-16079>.
24. Lacasse, P., Block, E., Guilbault, L.A. and Petitclerc, D., 1993. Effect of plane of nutrition of dairy heifers before and during gestation on milk production, reproduction, and health. *Journal of dairy science*, 76(11), pp.3420-3427.
25. Awasthi, H., Saravia, F., Rodríguez-Martínez, H. and Båge, R., 2010. Do cytoplasmic lipid droplets accumulate in immature oocytes from over-conditioned repeat breeder dairy heifers?. *Reproduction in domestic animals*, 45(5), pp.e194-e198.
26. Taylor, V.J., Beever, D.E., Bryant, M.J. and Wathes, D.C., 2004. First lactation ovarian function in dairy heifers in relation to prepubertal metabolic profiles. *Journal of Endocrinology*, 180(1), pp.63-76.
27. D. O’Callaghan, Lozano, J.M., Fahey, J.L., Gath, V., S. Snijders and Boland, M.P. (2001). Relationships between nutrition and fertility in dairy cattle. *British Society of Animal Science*, 26(1), pp.147–159. doi:<https://doi.org/10.1017/s0263967x00033656>.
28. Friggens, N.C., Disenhaus, C. and Petit, H.V. (2010). Nutritional sub-fertility in the dairy cow: towards improved reproductive management through a better biological understanding. *animal*, 4(7), pp.1197–1213. doi:<https://doi.org/10.1017/s1751731109991601>.
29. Bisinotto, R.S., Greco, L.F., Ribeiro, E.S., Martinez, N., Lima, F.S., Staples, C.R., Thatcher, W.W. and Santos, J.E.P., 2018. Influences of nutrition and metabolism on fertility of dairy cows. *Animal Reproduction (AR)*, 9(3), pp.260-272.
30. Pugh, D.G., Elmore, R.G. and Hembree, T.R., 1985. A review of the relationship between mineral nutrition and reproduction in cattle. *The Bovine Practitioner*, pp.10-13.
31. Velladurai, C., Selvaraju, M. and Napolean, R.E., 2016. Effects of macro and micro minerals on reproduction in dairy cattle a review. *International Journal of Scientific Research in Science and Technology*, 1, pp.68-74.
32. Ahuja, A. and Parmar, D. (2017). Role of Minerals in Reproductive Health of Dairy Cattle: A Review. *International Journal of Livestock Research*, 7(10), p.1. doi:<https://doi.org/10.5455/ijlr.20170806042724>.
33. Shope Jr, R.E., 1970. Bovine virus diarrhea: infectious bovine rhinotracheitis complex. *Journal of dairy science*, 53, pp.619-621.
34. Sayers, R.G., Byrne, N., O’Doherty, E. and Arkins, S. (2015). Prevalence of exposure to bovine viral diarrhoea virus (BVDV) and bovine herpesvirus-1 (BoHV-1) in Irish dairy herds. *Research in Veterinary Science*, 100(2015), pp.21–30. doi:<https://doi.org/10.1016/j.rvsc.2015.02.011>.
35. Martínez-Ibeas, A.M., Power, C., McClure, J. et al. Prevalence of BoHV-1 seropositive and BVD virus positive bulls on Irish dairy farms and associations between bull purchase and herd status. *Ir Vet J* 68, 28 (2015). <https://doi.org/10.1186/s13620-015-0059-9>
36. Graham, D.A. (2013). Bovine herpes virus-1 (BoHV-1) in cattle—a review with emphasis on reproductive impacts and the emergence of infection in Ireland and the United Kingdom. *Irish Veterinary Journal*, 66(1). doi:<https://doi.org/10.1186/2046-0481-66-15>.
37. Sayers, R.G. (2017). Associations between exposure to bovine herpesvirus 1 (BoHV-1) and milk production, reproductive performance, and mortality in Irish dairy herds. *Journal of Dairy Science*, 100(2), pp.1340–1352. doi:<https://doi.org/10.3168/jds.2016-11113>.
38. Barrett, D., Lane, E.A., Jose Maria Lozano, O’Keeffe, K. and Byrne, A. (2023). Bovine Herpes Virus Type 1 (BoHV-1) seroprevalence, risk factor and Bovine Viral Diarrhoea (BVD) co-infection analysis from Ireland. *Research Square (Research Square)*. doi:<https://doi.org/10.21203/rs.3.rs-3099921/v1>.
39. Hanrahan, K., Shalloo, L., Crossan, P., Donnellan, T., Sayers, R., Parr, M., Kenny, D.A., Barrett, D. and Lynch, R., 2020. Analysis of the economics of BoHV-1 infection in Ireland. A report produced by Teagasc and published by Animal Health Ireland.

40. Cowley, D.J.B., Graham, D.A., Guelbenzu, M., Doherty, M.L. and More, S.J. (2014). Aspects of bovine herpesvirus 1 and bovine viral diarrhoea virus herd-level seroprevalence and vaccination in dairy and beef herds in Northern Ireland. *Irish Veterinary Journal*, 67(1). doi:<https://doi.org/10.1186/2046-0481-67-18>.
41. Biuk-Rudan, N., Cvetnić, S., Madic, J. and Rudan, D., 1999. Prevalence of antibodies to IBR and BVD viruses in dairy cows with reproductive disorders. *Theriogenology*, 51(5), pp.875-881.
42. Guelbenzu-Gonzalo, M.P., Cooper, L., Brown, C. et al. Genetic diversity of ruminant Pestivirus strains collected in Northern Ireland between 1999 and 2011 and the role of live ruminant imports. *Ir Vet J* 69, 7 (2015). <https://doi.org/10.1186/s13620-016-0066-5>
43. Thomann, B., Tschopp, A., Ioannis Magouras, Meylan, M., Gertraud Schüpbach-Regula and Häsler, B. (2017). Economic evaluation of the eradication program for bovine viral diarrhoea in the Swiss dairy sector. *Preventive Veterinary Medicine*, 145(2017), pp.1–6. doi:<https://doi.org/10.1016/j.prevetmed.2017.05.020>.
44. Grünberg, W. (2021). Bovine Viral Diarrhoea and Mucosal Disease Complex - Generalized Conditions. [online] Merck Veterinary Manual. Available at: <https://www.msdsvetmanual.com/generalized-conditions/bovine-viral-diarrhoea/bovine-viral-diarrhoea-and-mucosal-disease-complex>.
45. Yue, X., van der Voort, M., Steeneveld, W., van Schaik, G., Vernooij, J.C.M., van Duijn, L. and Hogeveen, H. (2021). The effect of new bovine viral diarrhoea virus introduction on somatic cell count, calving interval, culling, and calf mortality of dairy herds in the Dutch bovine viral diarrhoea virus-free program. *Journal of Dairy Science*, 104(9), pp.10217–10231. doi:<https://doi.org/10.3168/jds.2021-20216>.
46. Stott, A.W., Humphry, R.W., Gunn, G.J., Higgins, I., Hennessy, T., O’Flaherty, J. and Graham, D.A. (2012). Predicted costs and benefits of eradicating BVDV from Ireland. *Irish Veterinary Journal*, 65(1). doi:<https://doi.org/10.1186/2046-0481-65-12>.
47. Moennig, V. and Liess, B. (1995). Pathogenesis of Intrauterine Infections With Bovine Viral Diarrhoea Virus. *Veterinary Clinics of North America: Food Animal Practice*, 11(3), pp.477–487. doi:[https://doi.org/10.1016/s0749-0720\(15\)30462-x](https://doi.org/10.1016/s0749-0720(15)30462-x).
48. Morarie-Kane, S.E., Смирнова, Н.П., Hansen, T., Mediger, J., Braun, L.J. and Chase, C. (2018). Fetal Hepatic Response to Bovine Viral Diarrhoea Virus Infection in Utero. *Pathogens*, [online] 7(2), pp.54–54. doi:<https://doi.org/10.3390/pathogens7020054>.
49. Barrett, D.J., More, S.J., Graham, D.A., O’Flaherty, J., Doherty, M.L. and Gunn, H.M. (2011). Considerations on BVD eradication for the Irish livestock industry. *Irish Veterinary Journal*, [online] 64(12), p.12. doi:<https://doi.org/10.1186/2046-0481-64-12>.
50. Muñoz-Zanzi, C.A., Thurmond, M.C. and Hietala, S.K. (2004). Effect of bovine viral diarrhoea virus infection on fertility of dairy heifers. *Theriogenology*, 61(6), pp.1085–1099. doi:<https://doi.org/10.1016/j.theriogenology.2003.06.003>.
51. Kale, M., Ata, A., Sibel Yavru, O. Yapkiç, Oya Bulut and Mehmet Şükrü Gülay (2006). The effect of infection with bovine viral diarrhoea virus on the fertility of cows and heifers. *Acta Veterinaria-beograd*, 56(5-6), pp.467–477. doi:<https://doi.org/10.2298/avb0606467k>.
52. Fray, M. (2000). Bovine viral diarrhoea virus: its effects on ovarian function in the cow. *Veterinary Microbiology*, 77(1-2), pp.185–194. doi:[https://doi.org/10.1016/s0378-1135\(00\)00275-3](https://doi.org/10.1016/s0378-1135(00)00275-3).
53. Kale, M., Sibel Yavru, Ata, A., Mesih Kocamüftüoğlu, Orhan YAPICI and Sibel HASIRCIOGLU (2011). Bovine Viral Diarrhoea Virus (BVDV) Infection in Relation to Fertility in Heifers. *Journal of Veterinary Medical Science*, 73(3), pp.331–336. doi:<https://doi.org/10.1292/jvms.10-0254>.
54. Archbald, L.F., Fulton, R.W., Seger, C.L., Al-Bagdadi, F. and Godke, R.A. (1979). Effect of the bovine viral diarrhoea (BVD) virus on preimplantation bovine embryos: A preliminary study. *Theriogenology*, 11(1), pp.81–89. doi:[https://doi.org/10.1016/s0093-691x\(79\)80020-5](https://doi.org/10.1016/s0093-691x(79)80020-5).
55. Johann Burgstaller, W. Obritzhauser, Kuchling, S., Kopacka, I., Beate Pinior and Köfer, J. (2016). The effect of bovine viral diarrhoea virus on fertility in dairy cows: two case-control studies in the province of Styria, Austria. *PubMed*, 129(3-4), pp.103–10.
56. Campbell, J. (2022). Viral Infections Associated with Bovine Respiratory Disease Complex in Cattle - Respiratory System. [online] MSD Veterinary Manual. Available at: <https://www.msdsvetmanual.com/respiratory-system/bovine-respiratory-disease-complex/viral-infections-associated-with-bovine-respiratory-disease-complex-in-cattle>.
57. Ayahan, A., Kale, M., Yavru, S., Bulut, O. and Buyukyoruk, U. (2006). The effect of subclinical bovine herpesvirus 1 infection on fertility of cows and heifers. *Acta veterinaria*, [online] 56(2-3), pp.267–273. doi:<https://doi.org/10.2298/avb0603267a>.
58. Mitchell, D. and Greig, A.S. (1967). The incidence and significance of bovine herpesvirus (infectious bovine rhinotracheitis) antibodies in the sera of aborting cattle. *PubMed*, 31(9), pp.234–8.

59. Ata, A., Kocamuftuoglu, M., Hasircioglu, S., Kale, M. and MS, G., 2012. Investigation of Relationship Between Bovine Herpesvirus-1 (BHV-1) Infection and Fertility in Repeat Breeding Dairy Cows in Family-Type Small Dairy Farmsı. *Kafkas Üniversitesi Veteriner Fakültesi Dergisi*, 18(4).
60. Muylkens, B., Thiry, J., Kirten, P., Schynts, F. and Thiry, E. (2007). Bovine herpesvirus 1 infection and infectious bovine rhinotracheitis. *Veterinary Research*, 38(2), pp.181–209. doi:<https://doi.org/10.1051/vetres:2006059>.
61. Ring, S.C., Graham, D.A., Sayers, R.G., Byrne, N., Kelleher, M.M., Doherty, M.L. and Berry, D.P. (2018). Genetic variability in the humoral immune response to bovine herpesvirus-1 infection in dairy cattle and genetic correlations with performance traits. *Journal of Dairy Science*, 101(7), pp.6190–6204. doi:<https://doi.org/10.3168/jds.2018-14481>.
62. Wedajo, M.T., Alemayehu, L., Tefera, Y., Hagos, A. and Abadi, A.R. (2021). Seroprevalence of infectious bovine rhinotracheitis and brucellosis and their effect on reproductive performance of dairy cattle. *Journal of Veterinary Medicine and Animal Health*, 13(2), pp.106–113. doi:<https://doi.org/10.5897/jvmah2020.0889>.
63. Piersanti, R.L., Zimpel, R., Molinari, P.C.C., Dickson, M.J., Ma, Z., Jeong, K.C., Santos, J.E.P., Sheldon, I.M. and Bromfield, J.J. (2019). A model of clinical endometritis in Holstein heifers using pathogenic *Escherichia coli* and *Trueperella pyogenes*. *Journal of Dairy Science*, 102(3), pp.2686–2697. doi:<https://doi.org/10.3168/jds.2018-15595>.
64. De Kruif, A., 1978. Factors influencing the fertility of a cattle population. *Reproduction*, 54(2), pp.507–518.
65. Mounir Adnane and Aspinas Chapwanya (2022). A Review of the Diversity of the Genital Tract Microbiome and Implications for Fertility of Cattle. *Animals*, 12(4), pp.460–460. doi:<https://doi.org/10.3390/ani12040460>.
66. Peter, D. (2020). Bovine Genital Campylobacteriosis - Reproductive System. [online] MSD Veterinary Manual. Available at: <https://www.msdsvetmanual.com/reproductive-system/bovine-genital-campylobacteriosis/bovine-genital-campylobacteriosis?query=campylobacter>
67. Pritchard, T., Mrode, R., Coffey, M., Bond, K. and Wall, E. (2017). The genetics of antibody response to paratuberculosis in dairy cattle. *Journal of Dairy Science*, 100(7), pp.5541–5549. doi:<https://doi.org/10.3168/jds.2016-12300>.
68. Richardson, E., More, S. Direct and indirect effects of Johne's disease on farm and animal productivity in an Irish dairy herd. *Ir Vet J* 62, 526 (2009). <https://doi.org/10.1186/2046-0481-62-8-526>
69. Ozsvári, L., Harnos, A., Lang, Z., Monostori, A., Strain, S. and Fodor, I. (2020). The Impact of Paratuberculosis on Milk Production, Fertility, and Culling in Large Commercial Hungarian Dairy Herds. *Frontiers in Veterinary Science*, 7(2020). doi:<https://doi.org/10.3389/fvets.2020.565324>.
70. Sergeant, E.S.G., McAloon, C.G., Tratalos, J.A., Citer, L.R., Graham, D.A. and More, S.J. (2019). Evaluation of national surveillance methods for detection of Irish dairy herds infected with *Mycobacterium avium* ssp. *paratuberculosis*. *Journal of Dairy Science*, 102(3), pp.2525–2538. doi:<https://doi.org/10.3168/jds.2018-15696>.
71. McAloon, C.G., Doherty, M.L., Whyte, P., O'Grady, L., More, S.J., Messam, L.L.McV., Good, M., Mullowney, P., Strain, S. and Green, M.J. (2016). Bayesian estimation of prevalence of paratuberculosis in dairy herds enrolled in a voluntary Johne's Disease Control Programme in Ireland. *Preventive Veterinary Medicine*, 128(2016), pp.95–100. doi:<https://doi.org/10.1016/j.prevetmed.2016.04.014>.
72. Pritchard, T.C., Coffey, M.P., Bond, K.S., Hutchings, M.R. and Wall, E. (2017b). Phenotypic effects of subclinical paratuberculosis (Johne's disease) in dairy cattle. *Journal of Dairy Science*, 100(1), pp.679–690. doi:<https://doi.org/10.3168/jds.2016-11323>.
73. Garcia, A.B. and Shalloo, L. (2015). Invited review: The economic impact and control of paratuberculosis in cattle. *Journal of Dairy Science*, 98(8), pp.5019–5039. doi:<https://doi.org/10.3168/jds.2014-9241>.
74. Tiwari, A., VanLeeuwen, J.A., McKenna, S.L., Keefe, G.P. and Barkema, H.W., 2006. Johne's disease in Canada: Part I: Clinical symptoms, pathophysiology, diagnosis, and prevalence in dairy herds. *The Canadian Veterinary Journal*, 47(9), p.874.
75. Donat, K., Soschinka, A., Erhardt, G. and Brandt, H.R. (2014). Paratuberculosis: decrease in milk production of German Holstein dairy cows shedding *Mycobacterium avium* ssp. *paratuberculosis* depends on within-herd prevalence. *Animal*, 8(5), pp.852–858. doi:<https://doi.org/10.1017/s1751731114000305>.
76. McKenna, S.L., Keefe, G.P., Tiwari, A., VanLeeuwen, J. and Barkema, H.W., 2006. Johne's disease in Canada part II: disease impacts, risk factors, and control programs for dairy producers. *The Canadian veterinary journal*, 47(11), p.1089.

77. Stott, A.W., Jones, G.M., Humphry, R.W. and Gunn, G.J. (2005). Financial incentive to control paratuberculosis (Johne's disease) on dairy farms in the United Kingdom. *Veterinary Record*, 156(26), pp.825–831. doi:<https://doi.org/10.1136/vr.156.26.825>.
78. Marcé, C., Beaudéau, F., Bareille, N., Seegers, H. and Fourichon, C. (2009). Higher non-return rate associated with *Mycobacterium avium* subspecies paratuberculosis infection at early stage in Holstein dairy cows. *Theriogenology*, 71(5), pp.807–816. doi:<https://doi.org/10.1016/j.theriogenology.2008.10.017>.
79. Reynolds, J.A., Bradley, A.J., Sherwin, G., Remnant, J. and Hudson, C. (2023). Associations between Johne's disease and fertility in UK dairy herds. *Veterinary Journal*, 298-299(2023), pp.106015–106015. doi:<https://doi.org/10.1016/j.tvjl.2023.106015>.
80. Smith, R.L., Strawderman, R.L., Schukken, Y.H., Wells, S.J., Pradhan, A.K., Espejo, L.A., Whitlock, R.H., Van Kessel, J.S., Smith, J.M., Wolfgang, D.R. and Gröhn, Y.T. (2010). Effect of Johne's disease status on reproduction and culling in dairy cattle. *Journal of Dairy Science*, 93(8), pp.3513–3524. doi:<https://doi.org/10.3168/jds.2009-2742>.
81. Hoogendam, K., Richardson, E. & Mee, J. Paratuberculosis sero-status and milk production, SCC and calving interval in Irish dairy herds. *Ir Vet J* 62 (Suppl 4), 265 (2009). <https://doi.org/10.1186/2046-0481-62-4-265>
82. Lilenbaum, W. (2022). Leptospirosis in Ruminants - Generalized Conditions. [online] Merck Veterinary Manual. Available at: <https://www.msdvetmanual.com/generalized-conditions/leptospirosis/leptospirosis-in-ruminants>.
83. Lilenbaum, W. and Martins, G. (2014). Leptospirosis in Cattle: A Challenging Scenario for the Understanding of the Epidemiology. *Transboundary and Emerging Diseases*, 61(2014), pp.63–68. doi:<https://doi.org/10.1111/tbed.12233>.
84. Sohm, C., Steiner, J., Jöbstl, J., Wittek, T., Firth, C.L., Steinparzer, R. and Amélie Desvars-Larrive (2023). A systematic review on leptospirosis in cattle: a European perspective. *bioRxiv* (Cold Spring Harbor Laboratory). doi:<https://doi.org/10.1101/2023.03.07.531463>.
85. Pandian, S.J. (2015). Seroprevalence of *Brucella abortus* and *Leptospira hardjo* in cattle. [online] Doi.org. Available at: <https://doi.org/10.14202%2Fvetworld.2015.217-220> [Accessed 27 Oct. 2023].
86. Molinari, P.C.C., Nally, J.E. and Bromfield, J.J. (2021). Bovine endometrial cells do not mount an inflammatory response to *Leptospira*. *Reproduction and Fertility*, 2(3), pp.187–198. doi:<https://doi.org/10.1530/raf-21-0012>.
87. Ryan, E.G., Leonard, N., O'Grady, L. et al. Seroprevalence of *Leptospira Hardjo* in the Irish suckler cattle population. *Ir Vet J* 65, 8 (2012). <https://doi.org/10.1186/2046-0481-65-8>
88. Loureiro, A.P. and Lilenbaum, W. (2020). Genital bovine leptospirosis: A new look for an old disease. *Theriogenology*, 141(2020), pp.41–47. doi:<https://doi.org/10.1016/j.theriogenology.2019.09.011>.
89. Barrett, D., Parr, M., Fagan, J., Johnson, A., Tratalos, J., Lively, F., Diskin, M. and Kenny, D. (2018). Prevalence of Bovine Viral Diarrhoea Virus (BVDV), Bovine Herpes Virus 1 (BHV 1), Leptospirosis and Neosporosis, and associated risk factors in 161 Irish beef herds. *BMC Veterinary Research*, 14(1). doi:<https://doi.org/10.1186/s12917-017-1324-9>.
90. Leonard, N., Mee, J.F., Snijders, S. and Mackie, D. (2004). Prevalence of antibodies to leptospira interrogans serovar hardjo in bulk tank milk from unvaccinated Irish dairy herds. *Irish Veterinary Journal*, 57(4), p.226. doi:<https://doi.org/10.1186/2046-0481-57-4-226>.
91. Dhaliwal, G.S., Murray, R.D., Dobson, H., Montgomery, J. and Ellis, W.A. (1996). Reduced conception rates in dairy cattle associated with serological evidence of *Leptospira interrogans* serovar hardjo infection. *Veterinary Record*, 139(5), pp.110–114. doi:<https://doi.org/10.1136/vr.139.5.110>.
92. Grooms, D.L. (2006). Reproductive losses caused by bovine viral diarrhoea virus and leptospirosis. *Theriogenology*, 66(3), pp.624–628. doi:<https://doi.org/10.1016/j.theriogenology.2006.04.016>.
93. Orjuela, A.G., Parra-Arango, J.L. and Sarmiento-Rubiano, L.A. (2022). Bovine leptospirosis: effects on reproduction and an approach to research in Colombia. *Tropical Animal Health and Production*, 54(5). doi:<https://doi.org/10.1007/s11250-022-03235-2>.
94. Ellis, W.A., 1994. Leptospirosis as a cause of reproductive failure. *Veterinary Clinics of North America: Food Animal Practice*, 10(3), pp.463-478.
95. Mori, M., Bakinahe, R., Vannoorenberghe, P., Maris, J., De Jong, E., Tignon, M., Marin, M., Desqueper, D., Fretin, D. and Behaeghel, I. (2017). Reproductive Disorders and Leptospirosis: A Case Study in a Mixed-Species Farm (Cattle and Swine). *Veterinary Sciences*, [online] 4(4), p.64. doi:<https://doi.org/10.3390/vetsci4040064>.

96. Guitian, J., Thurmond, M.C. and Hietala, S.K., 1999. Infertility and abortion among first-lactation dairy cows seropositive or seronegative for *Leptospira interrogans* serovar hardjo. *Journal of the American Veterinary Medical Association*, 215(4), pp.515-518.
97. Dhaliwal, G.S., Murray, R.D., Dobson, H., Montgomery, J. and Ellis, W.A., 1996. Effect of vaccination against *Leptospira interrogans* serovar hardjo on milk production and fertility in dairy cattle. *The Veterinary Record*, 138(14), pp.334-335.
98. Mee, J. Veterinary dairy herd fertility service provision in seasonal and non-seasonal dairy industries - a comparison. *Ir Vet J* 63, 230 (2010). <https://doi.org/10.1186/2046-0481-63-4-230>
99. Pryce, J.E., Hayes, B.J. and Goddard, M.E., 2012, May. Genotyping dairy females can improve the reliability of genomic selection for young bulls and heifers and provide farmers with new management tools. In *Proceedings of the 38th international committee for animal recording meeting* (Vol. 28, p. 28).
100. McCarthy, M.C., O'Grady, L., McAloon, C.G. and Mee, J.F. (2021). A survey of biosecurity and health management practices on Irish dairy farms engaged in contract-rearing. *Journal of Dairy Science*, 104(12). doi:<https://doi.org/10.3168/jds.2021-20500>.
101. Souto, L.A.S., Maturana Filho, M., Lemes, K.M., Torres, F.D. and Madureira, E.H., 2015. 160 strategic vaccination against bovine viral diarrhoea (bvd), infectious bovine rhinotracheitis (ibr) and leptospirosis improves pregnancy rate in ftai protocols in elore beef cows. *Reproduction, Fertility and Development*, 27(1), pp.171-171.
102. Perry, G.A., Geary, T.W., Walker, J.A., Rich, J.J., Northrop, E.J., Perkins, S.D., Mogck, C.L., Van Emon, M.L., Zezeski, A.L. and Daly, R.F., 2018. Influence of vaccination with a combined chemically altered/inactivated BHV-1/BVD vaccine or a modified-live BHV-1/BVD vaccine on reproductive performance in beef cows and heifers. *The Bovine Practitioner*, pp.53-58.
103. Das, S., Arsha Shaji, Nain, D., Shubham Singha, Karunakaran, M. and Rubina Kumari Baithalu (2023). Precision technologies for the management of reproduction in dairy cows. *Tropical Animal Health and Production*, 55(5). doi:<https://doi.org/10.1007/s11250-023-03704-2>.
104. Saint-Dizier, M. and Chastant-Maillard, S. (2012). Towards an Automated Detection of Oestrus in Dairy Cattle. *Reproduction in Domestic Animals*, 47(6), pp.1056–1061. doi:<https://doi.org/10.1111/j.1439-0531.2011.01971.x>.
105. Ryan, D.P., Snijders, S., Yaakub, H. and O'Farrell, K.J. (1995). An evaluation of estrus synchronization programs in reproductive management of dairy herds. *Journal of Animal Science*, 73(12), p.3687. doi:<https://doi.org/10.2527/1995.73123687x>.
106. Nowicki, A., Barański, W., Baryczka, A. and Janowski, T. (2017). OvSynch protocol and its modifications in the reproduction management of dairy cattle herds – an update. *Journal of Veterinary Research*, [online] 61(3), pp.329–336. doi:<https://doi.org/10.1515/jvetres-2017-0043>.

6) Acknowledgements

I would firstly like to thank my supervisor, Professor Rátky for his patience, help and guidance throughout the duration of compiling this thesis.

I would also like to thank all the teaching staff I have had the pleasure of learning from during the 5 and a half years of education I received. The knowledge I have gained in the process was of great benefit to me in this study, as the topic consists of various departments of veterinary medicine.

Finally, I would like to thank my parents for the opportunity and support they have given me over the last 5 years to help me achieve becoming a veterinarian.