The effect of yearling weight on fertility measures in Holstein heifers and primiparous cows

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ABSTRACT

The Canadian dairy industry consists of approximately 10,000 dairy farms with about 1.4 million head dairy cattle population, 70% of which are cows, and the rest are heifers. Cows constitute the productive segment of the herd, as they produce milk whereas the heifers form the non-productive segment. However, the heifers are equally important as they determine the future of the herd. After attaining puberty, the heifers become reproductively active and capable of replacing the dairy cows in the herd that may fail to reproduce or are culled from the herd. A producer should, therefore, select heifers with a good reproductive potential as replacements for the herd.

Data from the Québec Dairy Herd Improvement Agency (Valacta) were analysed to determine the relationship between yearling weight in Holstein heifers and various fertility measurements in heifers and primiparous cows. The fertility parameters analyzed, depending on developmental stage included: Days to first service; Number of services for conception; Days from first to successful breeding; Days open; and Calving Interval (first to second calving). Since yearling body weights were rarely reported, yearling weight in heifers was predicted from available weight data, using the approach of Cue *et al.* (2012). The study covered a period of 8 years from 2008 – 2015, and the fertility parameters were analyzed as three groups: heifers that had a successful first calving; cows that had both a first and a second calving; and cows that had a first calving but failed to conceive a second time.

Results showed that predicted yearling body weight had a significant effect on only some fertility traits of heifers; heifers that were predicted to weigh less than 300 kg at 12 months of age required a significantly fewer number of services to conception and had a significantly fewer number of days between first and successful breeding. In cows that failed to conceive the second time, the interaction between predicted weight at 12 months of age and age at first calving, had a significant effect on days to first service. Predicted body weight at 12 months did not have a significant effect on other fertility measures in those two data sets, and no significant effect on any of the fertility parameters in the data set of cows that that had both a first and a second calving. Not surprisingly, an earlier age at first calving had a significant effect on most first-lactation measures of fertility, as well as on days open and calving interval in cows that had both a first and a second calving. The data also demonstrated a considerable proportion of first calvers that failed to have a second conception. In conclusion, body weight at 12 months had no significant effect on

post-first calving fertility measures. However, a lack of actual body-weight measurements in the data should not be overlooked (animals with no recorded weight prior to first calving could not receive a predicted body weight at 12 months), and this emphasises the importance of data recording for a better understanding of the dairy industry's reproductive challenges.

RÉSUMÉ

L'industrie laitière canadienne se compose d'environ 10 000 exploitations laitières comptant environ 1,4 million de têtes de bétail laitier, dont 70 % sont des vaches et le reste des génisses. Les vaches constituent le segment productif du troupeau, car elles produisent du lait, tandis que les génisses forment le segment non productif. Cependant, les génisses sont tout aussi importantes car elles déterminent l'avenir du troupeau. Après avoir atteint la puberté, les génisses deviennent actives sur le plan de la reproduction et sont capables de remplacer les vaches laitières du troupeau qui ne parviennent pas à se reproduire ou qui sont éliminées du troupeau. Un producteur devrait donc sélectionner des génisses ayant un bon potentiel de reproduction pour remplacer les vaches du troupeau.

Les données du centre d'expertise en production laitière (Valacta) ont été analysées dans le but d'évaluer la relation entre le poids à l'âge d'un an des génisses Holstein et diverses mesures de fertilité chez les vaches primipares : Jours avant la première saillie ; Nombre de saillies pour la conception ; Jours entre la première et la réussite de la reproduction ; Jours ouverts ; et Intervalle entre vêlages (premier et deuxième vêlage). Les poids à 12 mois étant rarement rapportés, le poids à l'âge d'un an des génisses a été prédit à partir des données de poids disponibles, en utilisant l'approche de Cue *et al.* (2012). L'étude a couvert une période de 8 ans, de 2008 à 2015, et les paramètres de fertilité ont été analysés en trois groupes : les génisses qui ont eu un premier vêlage réussi ; les vaches qui ont eu à la fois un premier et un deuxième vêlage ; et les vaches qui ont eu un premier vêlage mais n'ont pas réussi à concevoir une deuxième fois.

Les résultats ont montré que le poids prédit à l'âge de 12 mois n'avait un effet significatif que sur certains traits de fertilité dans les données sur les génisses ; les génisses dont le poids prédit était inférieur à 300 kg à l'âge de 12 mois ont eu besoin d'un nombre significativement moins élevé de services avant la conception et ont eu un nombre significativement moins élevé de jours entre le premier et le succès de la reproduction. Chez les vaches qui n'ont pas réussi à concevoir une deuxième fois, l'interaction entre le poids prédit à 12 mois et l'âge au premier vêlage a eu un effet significatif sur le nombre de jours avant la première saillie. Le poids corporel prédit à 12 mois n'a pas eu d'effet significatif sur les autres mesures de fertilité dans ces deux ensembles de données, et aucun effet significatif sur aucun des paramètres de fertilité dans l'ensemble de données des vaches qui ont eu à la fois un premier et un deuxième vêlage. Il n'est pas surprenant

qu'un âge plus précoce au premier vêlage ait un effet significatif sur la plupart des mesures de fertilité de la première lactation, ainsi que sur les jours d'ouverture et l'intervalle entre vêlages chez les vaches qui ont eu un premier et un deuxième vêlage. Les données ont également démontré qu'une proportion considérable de vaches ayant vêlé pour la première fois n'ont pas réussi à avoir une deuxième conception. En conclusion, le poids corporel à 12 mois n'avait pas d'effet significatif sur les mesures de fertilité après le premier vêlage. Cependant, il ne faut pas négliger le manque de mesures réelles du poids corporel dans les données (les animaux dont le poids n'a pas été enregistré avant le premier vêlage ne pouvaient pas recevoir un poids prédit à 12 mois), et cela souligne l'importance de l'enregistrement des données pour une meilleure compréhension des défis de l'industrie laitière en matière de reproduction.

1. GENERAL INTRODUCTION

Humans subsist on the products of agriculture for their existence. These products include grains, vegetables, milk, meat, etc. Not only food the agricultural products are also a source of fiber, fuel, and energy. This makes the agriculture sector a substantial research area over the years. A very crucial element of the agricultural sector is livestock farming. The animals that are raised for the purpose of consumption or for obtaining some other values (nutritional and/or economical) are called livestock.

The dairy industry constitutes a fundamental part of the Canadian Agricultural sector. It primarily deals with the rearing of cows to obtain milk and its by-products. Just like most mammals, humans rely on milk in their initial stages of life and cow's milk provides all the nutrients (like calcium, magnesium, selenium, riboflavin, vitamin B12 and vitamin B5) along with the macro-nutrients essential to support life (Ellen Muehlhoff, 2013). Not only during the initial stages of life, but milk and its by-products such as butter, cheese, yogurt are also considered to be a vital part of the diet in all stages of life. Humans and cows produce milk with the same purpose of feeding their young ones. In comparison to a human baby, a young calf matures at a faster rate. As a consequence, its nutritional requirements are higher (Walker, 1990). In order to meet these demands, the cow's milk provides more nourishment as compared to human milk (Muehlhoff *et al.*, 2013). It provides three times more protein, four times the calcium, and other minerals (excluding iron) as compared to human milk (Patton, 2017; Wiley, 2012). In addition to its dietary values, cow's milk is also consumed because of its good taste and no distinct flavor as compared to the milk obtained from other animals like goat, horse, sheep, etc. (Rainer Haas, 2019). Besides milk, a dairy cow also produces calf as well as beef that makes it a principal animal in the dairy industry. Moreover, the cow is quite economical as it converts the waste products of agricultural farming such as roughages into nutritious products like protein-rich beef and milk in large quantities for a significant period of time.

Globally, 83% of the milk is produced by cattle, followed by buffalo (14%), goat (2%), sheep (1%), and camel (0.3%) (Muehlhoff *et al.*, 2013). In cattle, there are two species that are conventionally linked with milk production: *Bos indicus* (Zebu cattle) and *Bos taurus* (Hump-less cattle). The most common dairy cattle breed worldwide that is associated with milk production is

“Holstein” (Labatut & Tesnière, 2018). It is considered to be a pioneer breed for the dairy industry following numerous genetic selections in the 20th century (Labatut & Tesnière, 2018).

The consumption of dairy products is highly variable around the world as a result of which, the demand of the products also differs. The consumption of milk per capita per year (in kg) is illustrated in **Figure 1.1** (FAOSTAT, 2007)

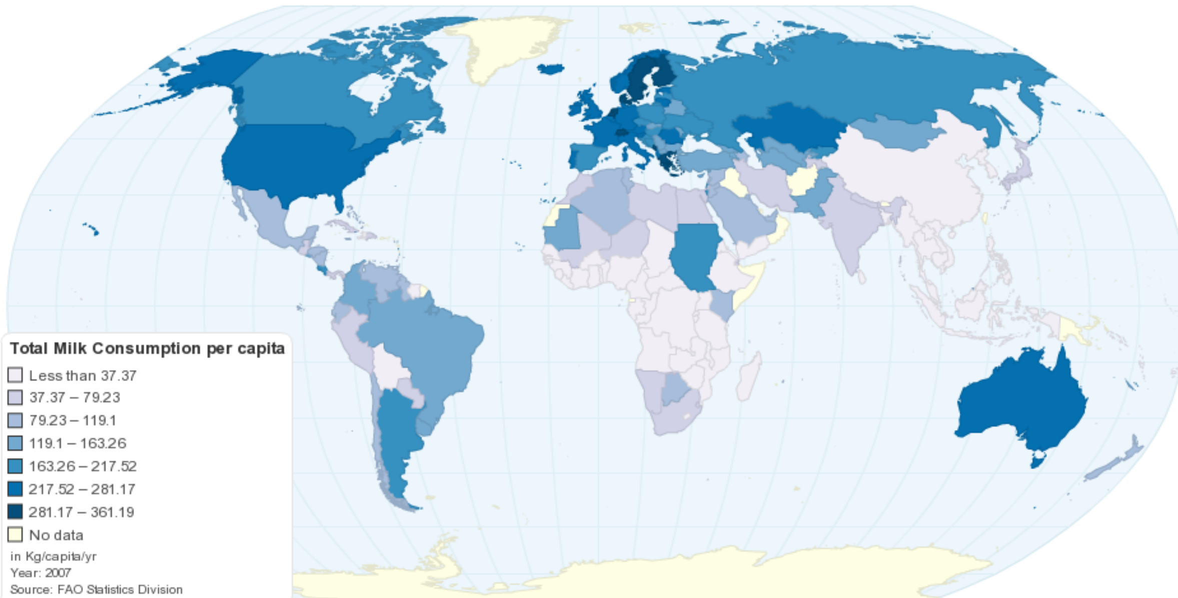


Figure 1.1: Worldwide total milk consumption (in kg) per capita in 2007

To meet the demands of dairy-based on consumption different countries have adopted pertinent strategies to ensure a safe and steady dairy supply with stable prices. In Canada, the Dairy industry runs under the supply management or the quota system. In simple words, supply management refers to matching the production of milk to the demand of Canadian consumers. For the consumers, it refers to a constant supply of quality milk and other dairy products at a stable and reasonable price. It avoids surplus production by helping the dairy farmers produce what is required ensuring a fair price for their product. In other countries where there is a lack of demand-predictability and/or a supply management system, farmers often face market fluctuations as a result of over-production. In this way, the supply management system helps Canadian dairy farmers to remain in business by not relying on government subsidies to cover their cost of production and support their livelihood.

In order to fulfill the needs of Canadian consumers, the Canadian dairy industry constitutes 10,371 farms with 1.401 million head population of dairy cattle, 70% of which are dairy cows, and the rest are dairy heifers (*Canadian Dairy Information Centre*, 2021). Cows form the productive component of the herd as these are the milk-producers whereas on the other hand heifers are considered to be non-productive since they are young and have not given birth yet, hence do not produce milk. So as to remain productive a cow has to be capable of producing milk. This can only happen when a cow is both 'productively' and 'reproductively' active. It also plays an important role in determining the profitability of a dairy farm (Arbel *et al.*, 2001). This is the reason why the dairy farmers aim at having a calf per cow per year, which corresponds to having a calving interval (interval between consecutive calvings of a cow) of 365 days. Achieving this not only benefits the farmer but also increases the herd-lifetime of a cow by reducing the number of cows that get culled as a result of reproductive failure. Moreover, it increases the number of peak-lactations in a cow's lifetime ultimately contributing to more farm-income and producing heifers at the same time. Although these heifers do not form the productive component of a herd, they are equally important for a dairy farmer as they determine the future of the dairy herd. The heifers act as a replacement of the dairy cows that may fail to reproduce or are culled or leave the herd for any possible reason. These heifers, after attaining sexual maturity (puberty) are bred and become productive members of the herd after they start producing milk following their first calving.

Once a cow gives birth (fresh cow) and starts producing milk, its body undergoes a major transition in energy. The particular reason for this is that the cow tends to mobilize its body reserves to support milk synthesis to feed its young one during the early stages of lactation when the milk production is at its peak (Wathes *et al*., 2007). This results in a lag between the energy requirements of a cow and the energy it obtains from feed intake (Berglund & Danell, 1987). As a consequence of this phase, a cow ends up in a state of negative energy balance where it begins to lose its body weight. Besides this, it also makes the cow susceptible to several metabolic disorders such as ketosis as well as various other health issues including reproductive failures (Berglund & Danell, 1987) due to loss in the ability to conceive. Constant milk production can only be possible if the cow is reproductively efficient so that it gives birth and the lactation cycle is renewed (Lucy, 2001). The loss of body condition score (BCS)[1] of a cow in the early stages of its lactation is shown in **Figure 1.2** (Pryce *et al*., 2001). As the Figure depicts, this loss of body condition is usually short-

[1] The BCS (on a scale of 1-5) is a subjective method used for evaluating the animal based on its outer appearance that is linked to its body-fat reserved and is therefore influenced by its energy balance (Anitha *et al*., 2011).

term as the cow regains its body weight after the stages of peak milk production pass, and the energy required for milk production is balanced with the energy obtained from the diet.

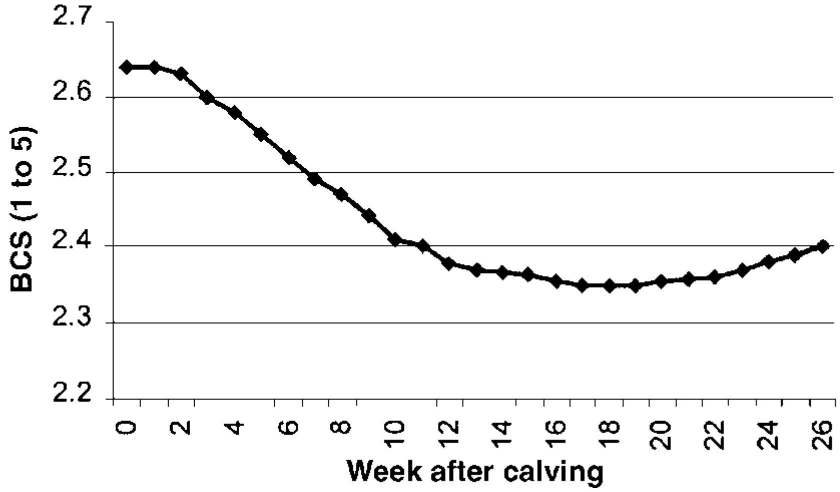


Figure 1.2: BCS against week lactation (Pryce et al., 2001)

The state of negative energy balance is directly associated with milk-yield (**Figure 1.3**). The cows that lose excessive body weight during the early stages of lactation often suffer from fertility issues following calving. Therefore, the selection of high milk-producing cows is often accompanied by a decline in fertility (Lucy, 2001; Pryce *et al.*, 1997). The cows that are in their first lactation tend to eat less and lie in a lower energy balance state. Because of this, their body is not able to meet the energy requirements to support milk production as well as growth. Though there can be other management factors, growth, and/or physiological factors, that influence the reproductive efficiency of a dairy cow. (Lucy, 2001). These factors collectively have a negative impact on the profitability of a farmer since the unproductive cows are removed from the herd/culled. Furthermore, reproductive failure is the foremost involuntary culling reason in Canada as of 2019 (*Canadian Dairy Information Centre*, 2021). All these factors contribute to a major challenge for not only the dairy farmers but also the Canadian dairy industry.

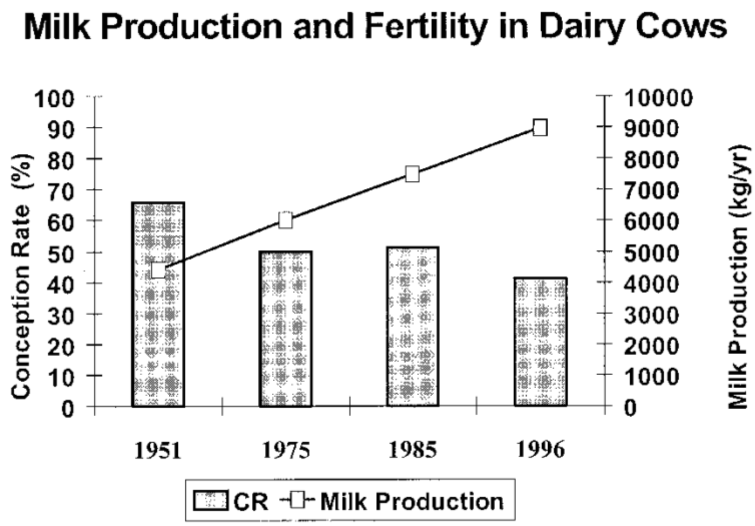


Figure 1.3: Relationship between annual milk production and fertility of Holstein dairy cows in New York (Butler, 2000)

2. LITERATURE REVIEW

2.1 The dairy industry

The Canadian dairy sector is a robust industry in Canada. As of 2019 reports, it ranks at second place after the red meat industry based on net farm receipts i.e., $6.99 billion, producing 92.26 million hl of milk (*Canadian Dairy Information Centre*, 2021). Almost 98% of the dairy farms in Canada are family-owned and operated, with each farm milking an average of 73 cows (*Holstein Canada*, 2021). Out of nearly 10,095 dairy farms present in Canada, approximately 50% of them are present in Québec (**Figure 2.1**), contributing about 36% to the total farm cash receipts making it the highest milk-producing province countrywide. The dairy sector in Québec ranks first place in the agricultural sector producing 38% of the total milk in the country (*Canadian Dairy Information Centre*, 2021).

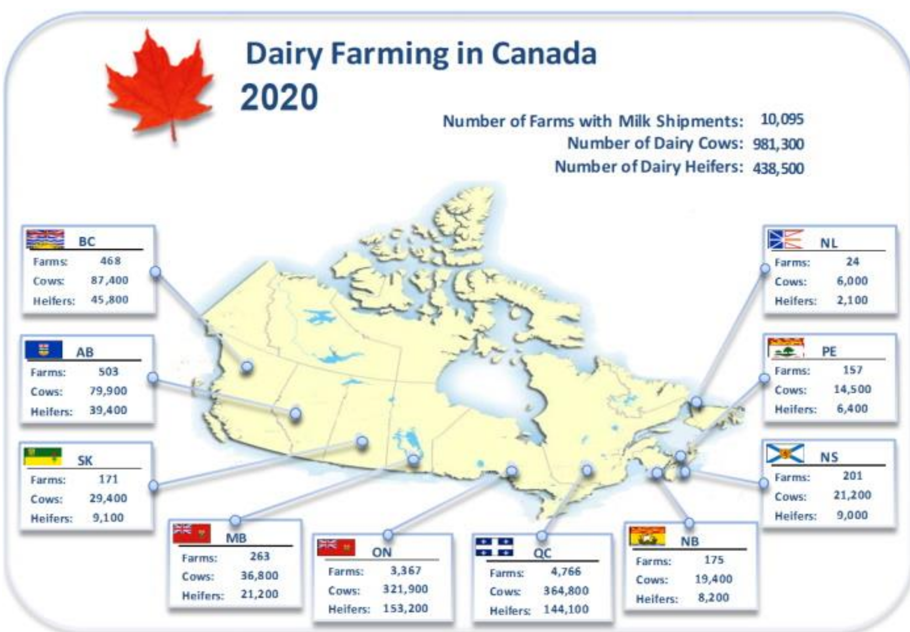


Figure 2.1: Dairy farms in Canada, 2020 (*Canadian Dairy Information Centre*, 2021)

2.1.1 Breeds

There are seven dairy breeds that are found in Canada namely Holstein, Jersey, Ayrshire, Brown Swiss, Milking Shorthorn, Guernsey and Canadienne as shown in **Figure 2.2**. Out of all these breeds, 93% of them are Holstein, followed by Jersey (4%) and Ayrshire (2%). The Holstein breed is native to the Netherlands. The first Holstein-Friesian cow brought to North America was imported from Holland to the United States in 1881. The Holstein breed gained popularity during the years of depression when the feed-conversion economics were critical because of its high milk production and feed conversion rate (*Holstein Canada*, 2021). One Holstein cow produces 10,909 kg of milk on average, which makes it the highest milk-producing dairy breed in Canada. Its milk composition comprises approximately 3.98% milk fat and 3.27% milk protein components. Although the average milk production per cow of the Jersey breed is very low as compared to Holstein (7,035 kg), its milk fat percentage and milk protein percentage is the highest of all dairy breeds i.e., 5.10% and 3.85% respectively (*Canadian Dairy Information Centre*, 2021). Jerseys are also recognized for their good temperament and are easy to handle. The third most popular breed is Ayrshire. It is well-adapted to the Canadian climate and is regarded as Québec's second-leading breed. Apart from the most common breeds, the Canadienne is known for its hardiness, adaptability to cold climate and is well-liked for cheese production. It is regarded as the first cattle breed that was developed in the North American continent. The Brown Swiss is known for its strong makeup, climate adaptability, disease resistance and longevity. Because of the high protein content found in its milk, it is also in demand for cheese production (*Producteurs de Lait du Québec*, 2021).

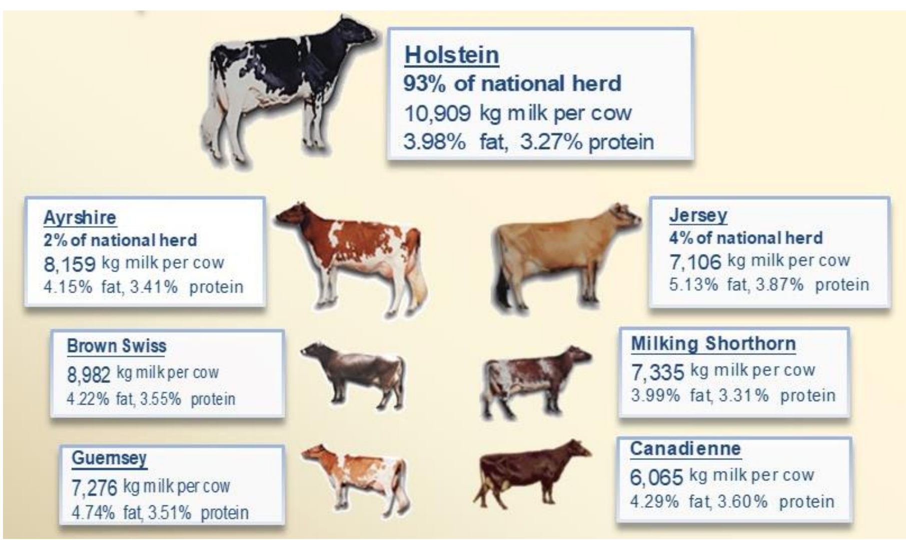


Figure 2.2: Dairy breeds in Canada, 2019 (*Canadian Dairy Information Centre*, 2021)

2.1.2 Types of dairy barns

Dairy farms are widely spread throughout Canada with a significant concentration present in Québec and Ontario (81%), followed by Western (13%) and Atlantic provinces (6%). Canada witnesses intense winters for a significant time around the year as it mostly lies in the temperate zone. The cows cannot survive such a harsh climate unless they are kept inside the shelters. These shelters ensure that the animals are safeguarded from cold during the winters along with ensuring proper care, comfort, food, and water supply, etc. In this type of arrangement, the cows can be put on pasture feed when it is available during the summer days and inside the shelter during the colder times of the year or can be kept confined throughout the year (Haskell *et al.*, 2006).

There are two types of housing systems available for dairy cows in Canada i.e., tie stall and free stall housing systems or barns. In a tie stall housing system, the cows are confined to a stanchion with a neck chain in their own individual stalls. The cows are fed and monitored in their individual stalls where they can also easily lunge and rest or groom. Each stall has a trainer that trains the cow to urinate and defecate in the gutters attached to its stall so as to reduce the bacterial load inside the stall's bedding along with minimizing labour. Whereas, in a free-stall housing system the cows are not confined to one place and hence are provided more space where they can move around freely. They are also provided a bedded area where they can rest when required. The choice of the barn in which the cows will spend most of their lifetime is a very crucial decision for a dairy producer as it would determine the well-being, productivity, health, milk production, and reproductive capability of a cow. The decision of a producer is influenced by various factors like the climatic conditions of the place, the construction cost, labour availability, investments, etc. (Bewley *et al.*, 2017). Both tie stall and free stall barns have several pros and cons. In a tie stall barn, the producer can individually monitor and feed each cow which helps to increase productivity. However, one of its disadvantages is that the cow is confined to a place that reduces its exercise and comfort, but the main advantage of its design is that it is good for research purposes. On the other hand, a free stall barn provides more comfort to the cows however, it does not allow the farmer to individually monitor or look after a cow. The tie stall housing systems are more common in Canada (almost 73%). Also, Nearly 91% of the dairy barns in Québec are tie stall as shown in **Figure 2.3**. (*Canadian Dairy Information Centre*, 2021).

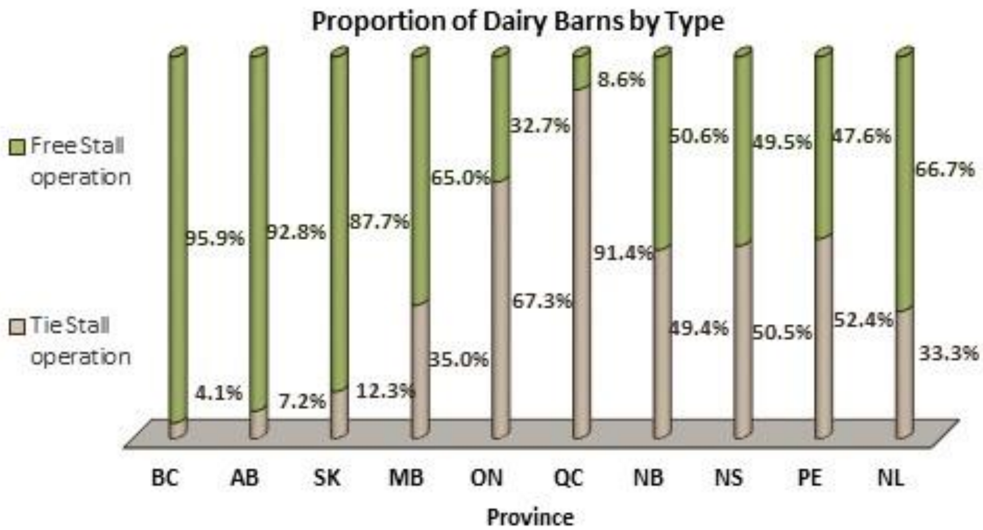


Figure 2.3: Types of dairy barns in different provinces across Canada (*Canadian Dairy Information Centre*, 2021)

2.1.3 Challenges for the Canadian dairy industry

The main objective for a dairy industry is that the cows stay productive in the dairy herd for the longest time possible; because of which the dairy cows have been selected for an increased milk production over the years. However, this does not happen as the producer desires, as high milk production is often accompanied by a decrease in the fertility of a dairy cow (Butler, 2000). There are mainly two culling reasons based on which the cows are removed from the herd. These are either voluntary reasons that are entirely influenced by the producer's wish and/or involuntary reasons that result against the will of the producer. There are various reasons that compel the producer to involuntarily cull the cows. And for every cow that is removed from the herd or is culled, the producer reports one or more reasons associated with it. Some of these reasons are low milk production, injured cows, feet and leg problems, mastitis, displaced abomasum, sickness, bad temperament, and many others. The culling rate in the dairy herds of Canada has increased from being 26.16% in 2017 to 32.70% in 2019. Out of 621,509 cows that were enrolled on a milk-recording program in Canada, there were above 203,000 cows that were culled in the year 2019 based on various reasons **(Table 2.1)**. Out of all these, reproductive failure is the foremost cause for involuntary culling of dairy cows in Canadian dairy herds i.e., almost 16% of all the culling reasons in 2019 (*Canadian Dairy Information Centre*, 2021).

Table 2.1: Culling rate in Canadian dairy herds in 2019 (*Canadian Dairy Information Centre*, 2021).

COWS	**2019**	
Number of herds enrolled on a milk recording program	6,787	
Number of cows enrolled on a milk recording program	621,509	
CULLING REASONS	**NUMBER OF COWS**	**PERCENT**
Reproductive	39,993	15.9%
Mastitis	23,832	9.5%
Feet and leg problems	16,151	6.4%
Low milk production	18,872	7.5%
Sickness	10,521	4.2%
Injury to udder/teats	10,063	4.0%
Injury/accident	8,321	3.3%
Old age	5,006	2.0%
Difficult calving	1,367	0.5%
Bad temperament	1,902	0.8%
Conformation	1,646	0.7%
Displaced abomasum	816	0.3%
Milk fever	1,005	0.4%
Slow milker	1,254	0.5%
Arthritis	700	0.3%
Pneumonia	913	0.4%
Staph aureus	458	0.2%
Leukosis	457	0.2%
Low fat	82	0.0%
Paratuberculosis	25	0.0%
Low protein	23	0.0%
Unknown	59,819	23.7%
Total culling reasons	**203,226**	**80.6%**
Culling rate	**32.70%**	

There are only 2% of animals that have ‘old age’ as a reason for leaving the herd. This implies that almost 98% of the animals leave way before showing their full potential in the herd. In Québec, fertility is seen to be decreasing continually since 1990. The rate of a dairy cow conceiving at first artificial insemination has gone down from 44% to 39% whereas the same for conceiving at second artificial insemination has decreased from 47% to 41% which has resulted in a decline of fertility by approximately 5% in the past 10 years (Bouchard & Du Trembly, 2003). The cost of inseminating a dairy cow is about 35 CAD per insemination. With each failure in conception, the farmer is compelled to bear the extra cost of inseminating the cow multiple times, leading to economic losses. Besides, the cost of a 24 months-old replacement heifer is around 1300 CAD (Dekkers *et al*., 1998). A recent study from Canada reported that a technician performs one artificial insemination at the cost of 17.86 CAD per cow. It also revealed that the total cost of fixed-time artificial insemination of the cows adds up to 139.76 CAD which includes the cost of a controlled intravaginal drug-releasing device (CIDR®), needle, syringe, semen, labour, management, etc. Furthermore, it was also reported that the cost of replacing a cow is 1,400 CAD (Lardner *et al.*, 2020). All these issues lead to an increase in the calving interval of a dairy cow as each time a cow fails to conceive, the producer is bound to wait for at least 18-24 days to re-inseminate the cow on the return of its estrous cycle. Each day increase of the calving interval as an outcome of failure in conceiving is estimated to be 4.7 CAD per cow (Plaizier *et al*., 1997). This indicates that reproductive efficiency directly affects the profitability of a farm and is also a major challenge for the dairy industry in Québec as well as the rest of the country.

2.2 Growth and physiological changes

Growth is one of the sundry characteristics of a living being. It can be considered as an overall increase in the size and structure of an organism. Heinrichs and Hargrove (1987) defined growth as: ‘maturation of the reproductive system, as well as an increase in body size and weight, is affected by many factors such as genetics, nutrition, and management’. It has a direct impact on the fertility of the cow, as the rate of growth defines when a cow reaches puberty and becomes reproductively and eventually productively active. Productivity is also related to age and body weight that triggers ovulation (Lawrence *et al*., 2012). A young calf’s growth rate is faster than that of a human baby; along with that is a chain of physiological events that occur concurrently. A human baby takes 20 weeks to double its birth weight whereas, a heifer takes only 10 weeks for the same (Walker, 1990). Swanson (1967) described growth as the most important feature to consider in a dairy heifer as it not only affects the fertility and/or lactation of a cow but also determines its value if it fails to reproduce or produce milk. For instance, if the former happens the cow would indubitably be used for beef and the body weight is an important factor that is taken into consideration in that case. Emphasising the milk-production traits, a cow must have an appropriate body size and weight for supporting the milk production as the body size would also determine the size of its udder. The growth of a dairy cow commencing right from its birth and is of utmost importance that governs its value in the herd and the farm. The chain of events constituting the growth process will be now elaborated.

2.2.1 The birth to weaning period

A bovine fetus grows till 6 months of age, after which very little development occurs before birth (Lawrence *et al*., 2012). At birth, a Holstein calf weighs somewhere between 34-52 kg (Beal *et al*., 1978). A bovine placenta is structured in such a way that the intra-uterine transfer of the serum immunoglobulins from mother to fetus does not occur, because of which the calf is born without any immunity of its own. This makes it highly susceptible to diseases and increases mortality rates. Therefore, it is very essential to provide the protective immunoglobulins passively during the neonatal period for optimal calf health and protection from diseases. It is provided to the calf by feeding the dam's colostrum that is not only rich in immunoglobulins but also provides the young calf with supplementary ingredients like cytokines, hormones, antimicrobial, and essential growth factors. Its quality is thus superior as compared with whole milk as depicted in **Table 2.2**. The colostrum levels in Holstein cows are about 68.5 g/L that is lower than that of beef breeds which is 100 g/L on an average. The colostrum with a specific gravity of less than 1.05 is considered to be poor in immunoglobulin concentration and hence immunity transfer. In addition to the quality of colostrum, the transfer of desired immunoglobulins also depends on the volume and concentration of the colostrum ingested, the time when it was ingested plus the ability of the calf to absorb it from the small intestine into its blood (Lorenz *et al*., 2011).

Table 2.2: Composition of colostrum, transition milk and whole milk of a Holstein dam. (Godden, 2008)

	Colostrum	Transition milk (milking postpartum)		Milk
Parameter	1	2	3	6
Specific gravity	1.056	1.040	1.035	1.032
Total solids (%)	23.9	17.9	14.1	12.9
Fat (%)	6.7	5.4	3.9	4.0
Total protein (%)	14.0	8.4	5.1	3.1
Casein (%)	4.8	4.3	3.8	2.5
Albumin (%)	6.0	4.2	2.4	0.5
Immunoglobulins (%)	6.0	4.2	2.4	0.09
IgG (g/100 mL)	3.2	2.5	1.5	0.06
Lactose (%)	2.7	3.9	4.4	5.0
IGF-I (μg/L)	341	242	144	15
Insulin (μg/L)	65.9	34.8	15.8	1.1
Ash (%)	1.11	0.95	0.87	0.74
Calcium (%)	0.26	0.15	0.15	0.13
Magnesium (%)	0.04	0.01	0.01	0.01
Zinc (mg/100 mL)	1.22	—	0.62	0.3
Manganese (mg/100 mL)	0.02	—	0.01	0.004
Iron (mg/100 g)	0.20	—	—	0.05
Cobalt (μg/100 g)	0.5	—	—	0.10
Vitamin A (μg/100 mL)	295	190	113	34
Vitamin E (μg/g fat)	84	76	56	15
Riboflavin (μg/mL)	4.83	2.71	1.85	1.47
Vitamin B_{12} (μg/100 mL)	4.9	—	2.5	0.6
Folic acid (μg/100 mL)	0.8	—	0.2	0.2
Choline (mg/mL)	0.7	0.34	0.23	0.13

A newborn calf can absorb all the vital immunoglobulins present in the maternal colostrum through its small intestine. However, its ability to absorb starts declining rapidly after 4-6 hours following birth and completely stops after 24 hours of age. This cessation is called gut closure and is not influenced by the quantity of colostrum fed to the calf till that time. Furthermore, the concentration of the mother's colostrum is highest in the first milking because the immunoglobulin transfer to the mammary gland halts after calving. Therefore, it is always beneficial to feed the calf earliest after birth to ensure substantial levels of immunoglobulin absorption **(Figures 2.4 & 2.5)**. The IgG_1 is the predominant colostral immunoglobulin present in maternal as well as calf's serum after it is fed (Besser & Gay, 1994). The quality of colostrum depends on various factors like the age of the dam, the volume of the colostrum produced (higher quantity is often correlated with poor quality), nutrients fed to the dam, the season of calving, etc. (Godden, 2008). If a cow is incapable of producing the required quality and/or quantity of colostrum or due to any other reason (like bad mothering behaviour of the dam, failure of the newborn to rise and suckle or poor conformation of the dam's teats) the colostrum is unavailable, the calf can be fed commercially available colostrum replacement products through feeding tubes.

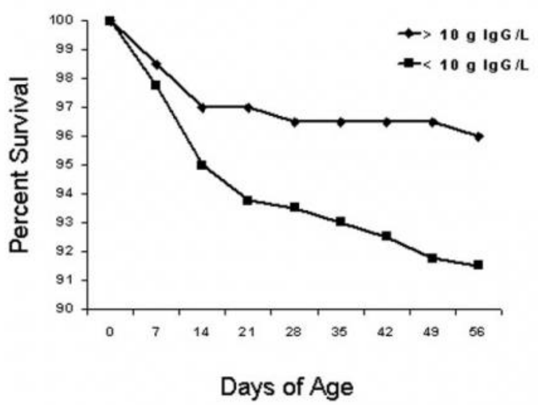


Figure 2.4: Effect of passive immunity transfer on newborn survival

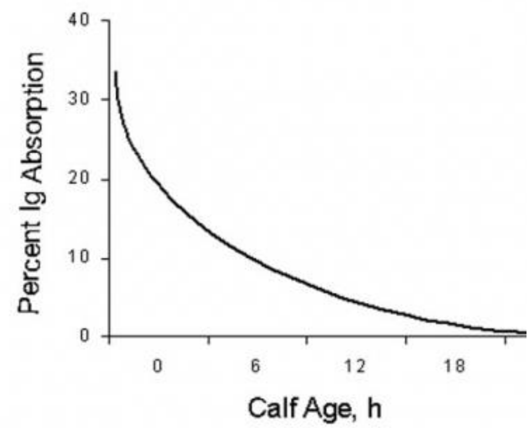


Figure 2.5: Effect of age (in hours) on immunoglobulin absorption (in percent)

Source: Gould (2012)

2.2.2 Physiological aspects of the birth-to-weaning period

The digestive tract of a mature cow comprises four stomach compartments namely rumen, abomasum, omasum, and the reticulum. A mature bovine stomach is dominated by rumen that accounts for 80% of the total capacity. However, the digestive system of a neonatal calf is very distinct from that of a mature cow **(Table 2)**. A calf is monogastric by birth, which implies that only one of its four stomach compartments is physiologically active. This compartment is the abomasum (true stomach) which occupies 60% of the total volume of the stomach of a calf whereas the rumen covers only 25% of the total capacity (Govil *et al.*, 2017; Guzman *et al.*, 2016). During the monogastric phase, the colostrum/milk bypasses the rumen and directly enters the abomasum through the oesophageal groove (or rumoreticuler groove) where the enzymatic digestion takes place. It is a unique feature of the digestive system of calves that helps the liquid feed to directly flow into the only active and fully functional compartment of their stomach i.e., abomasum. The digestion of fats, proteins, and carbohydrates also totally depends on the digestive enzymes that are produced by the small intestine and the abomasum of the monogastric calves (Diao *et al.*, 2019).

Table 2.3: Relative size of bovine stomach compartments from birth to maturity. (Source: Penn State: http://extension.psu.edu/animals/dairy/nutrition/calves)

	Percent of Total stomach capacity	Percent of Total stomach capacity	Percent of Total stomach capacity	Percent of Total stomach capacity
Age	***Rumen***	***Reticulum***	***Omasum***	***Abomasum***
Newborn	25	5	10	60
3 to 4 months	65	5	10	20
Mature	80	5	7 to 8	7 to 8

Traditionally, a calf is fed approximately 10% of its body weight each day for the least weight gain and body maintenance requirements (Jasper & Weary, 2002). Albright and Arave (1997) affirmed that a calf consumed greater amount of milk (about 20% of body weight) when left with the dam as it would suckle 7 to 10 times per day in its natural habitat, resulting in more rapid weight gain hence better growth. Consequently, the growth rate of these calves as measured by the average daily weight gain reaches up to 1 kg per day (Flower & Weary, 2001). In addition to this, Jasper and Weary (2002) have also found in their study that the cause of nutritional diarrhea in milk-fed calves is not excessive liquids in their diet but poor management and/or poor quality of the liquid fed to them.

2.3 Pre-ruminant state and rumen development

The calf remains in the liquid-diet phase until 3 weeks of age, following which it starts ingesting solid food (hay, starter meal, or hay grass) that triggers the development of rumen in terms of its weight and tissue thickness (Guzman *et al*., 2016). The introduction to solid feed increases the production of fibriolytic bacteria in the rumen that produces volatile fatty acids stimulating the development of rumen papillae. These papillae help the absorption of the volatile fatty acids into the bloodstream and act as a source of energy for the growth and development of other organs of the calf (Khan *et al*., 2011). Lesmeister *et al*. (2004) considered the length of rumen papillae to be the best indicator of rumen development in the calves, followed by its thickness along with the ruminal epithelium thickness. Rumen aids in the digestion of solid feed along with providing protection, allowing transportation, absorption, and metabolism of short-chain fatty acids however, for its development exposure to solid feed is required because of which the rumen papillae develop making the rumen capable of absorbing more nutrients from the diet consequently accelerating the growth (Diao *et al*., 2019). In addition to that, the solid feed is less expensive as compared with the liquid feed. Therefore, a dairy farmer desires an early development of rumen of a monogastric calf and to reduce its nutritional dependence on liquid feed/milk. Although some new findings by Eckert and coworkers (2015) have also proven that calves that are weaned at 6-8 weeks of age or later with a higher plane of nutrition have higher rates of starter feed consumption than the early-weaned ones. Additionally, these late-weaned calves also had an improved gastrointestinal development with fewer signs of distress caused by weaning in their behaviour. Regardless of the feeding system, Khan *et al.* (2007) claimed that calves should be provided with water and concentrates constantly to increase the rate of digestion in the rumen as the consumption of concentrates allows ruminal epithelium development, essential for the digestion of solid feeds. Also, Davis *et al.* (1998) suggested that calves may be weaned once the daily dry-matter consumption of concentrates reaches and is consistent at 1kg. To be able to reach this DMI, it is recommended to gradually introduce concentrates, while decreasing the intake of a liquid diet over a short time.

After the first 3 weeks, the calves gradually start consuming the starter concentrated diet. This increases their growth rate as the amount of nutrition increases in the diet (Marshall & Smith, 1970; Woodward, 1923). Since this intake is negligible at the start, the calves are only able to attain 20-30% of their standard growth (Appleby *et al*., 2001). Various studies have proven that the dairy calves that are fed a raised plane of nutrition during their pre-weaning phase show a higher growth rate with respect to their body weight gain, body frame measurements, and development of organs without negatively impacting their health (Geiger *et al*., 2016).

2.4 The Pre-pubertal period

A calf goes through extensive changes, both morphologically and physiologically before it completely shifts to solid feed (Vi *et al*., 2004). Its stomach transitions from being a pseudo-monogastric one which only one functional compartment (i.e., abomasum) to having a fully developed rumen and becoming a functionally ruminant animal. The dairy heifers must grow to reach an optimum body size and weight so that they become capable of reproducing themselves. To attain this goal, the heifers have to utilize their feed to the fullest during their pre-pubertal phase that is from weaning till puberty. This makes this period equally important as it determines the growth and eventually the age at which the heifers reach puberty (Lawrence *et al*., 2012). Also, this is the phase during which the mammary glands undergo an accelerated growth (Sinha & Tucker, 1969). The growth phases of the mammary gland and the endocrine changes during the pre-pubertal stage will now be addressed.

2.4.1 Endocrine changes prior to puberty

The animals undergo numerous steady and/or abrupt changes in their endocrine mechanisms before or as puberty occurs. These changes may begin right before they reach puberty whereas, some may begin prior to their birth. Day *et al*. (1984) stated that in heifers, the frequency of LH pulses intensifies prior to puberty. In an immature animal, the concentrations of LH in the plasma are maintained at minimal levels by the negative feedback mechanism of the estradiol on the hypothalamo-pituitary axis. Conversely, in a mature animal, the concentration of estradiol receptors decreases that allows follicular development to take place. The mature follicles stimulate the production of estrogen inducing uterine growth and development. Estradiol induces the release of gonadotropin as the follicle reaches the pre-ovulatory stage, followed by ovulation (Duggan, 1993).

2.5 The mammary gland growth and development

Mammary glands are an important part of the reproductive system of a mammal. The mammary gland development is a complex process that consists of various organised events and interactions between various types of cells in the body. Calves are born with a rudimentary mammary gland. This underdeveloped mammary gland comprises a teat, a primary duct, and several secondary ducts. The primary duct serves as a passage for the milk to go into the teat cistern (Sheffield, 1988). The growth of which commences during the pre-pubertal phase. At around 3 months of age, the growth of the mammary gland of a heifer becomes positively allometric to the body weight which implies that the parenchymal tissues start growing at a considerably higher rate as compared with the body weight of the calf. This allometric growth continuous till the heifer is about 6 months old followed by a higher plane of growth later at around 9 months of age. The mammary gland development continues even after the onset of puberty (Sinha & Tucker, 1969). Followed by allometric mammary growth, the growth rate reduces and becomes isometric again **(Figure 2.6)**.

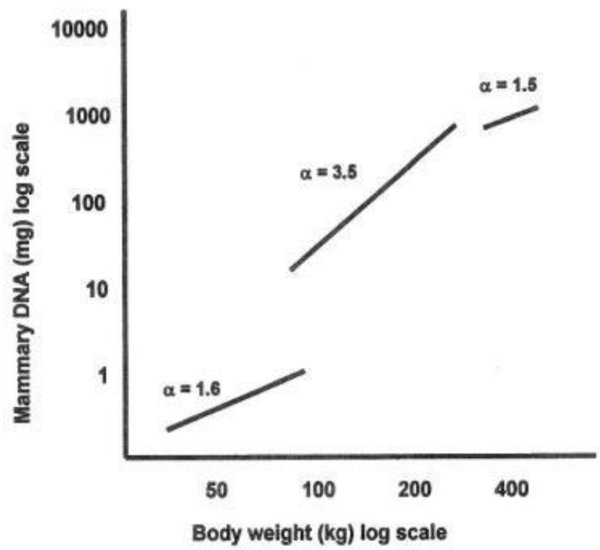


Figure 2.6: Relationship between mammary DNA (mg) and body weight (kg) in Holstein heifers (Sinha & Tucker, 1969)

Once a heifer becomes pregnant the isometric growth ceases and very negligible development takes place. A significant mammary growth takes place near the end of heifer's pregnancy period. An exponential growth of the udder is also observed during the gestation period (Sejrsen, 1978). Cowie and Tindal (1971) reported that very less cell division takes place near the end of pregnancy however a significant number of secretions are accumulated in the udder resulting in a significant increase in the parenchymal tissues and the size of the udder.

2.6 The pubertal period

Robinson and Shelton (1977) broadly defined puberty as a process by which the heifers become capable of reproducing themselves. To be more precise, attainment of puberty refers to the time at which the heifer shows a rise in its progesterone levels, which is followed by a normal luteal phase (Moran *et al.*, 1989). Moran and co-workers (1989) also stated that: 'In heifers, first ovulation is triggered when the hypothalamo-pituitary axis loses its sensitivity to the negative feedback effect of oestradiol-17β, allowing an LH surge to occur.' Normally in heifers, puberty occurs at 12 to 14 months of age. The time or age at which a heifer attains puberty is very critical, as it determines its lifetime productivity. The heifers that are younger when they reach puberty are bred sooner and produce more calves in their productive lifetime, hence are more beneficial for the farm.

While the sexual differentiation commences prior to the calf's birth, the development of its reproductive organs lasts even after the heifer reaches puberty (Atkins *et al*., 2013). The following sections will categorize some of these physiological changes like the uterine growth, ovarian development, and follicular growth, that take place in a heifer as puberty is initiated.

2.6.1 Uterine growth

Honaramooz *et al*. (2004) reported that the diameter of the uterus rapidly increases from 9 mm to 14 mm when the heifer is around 2 to 10 weeks old. It gradually continues to grow till 24 months of age at which it becomes about 16 mm thick. The progression becomes steady after this stage. It recommences as the heifer turns nearly 32 weeks old and grows 21 mm thick at approximately 60 weeks of age. This is called a biphasic growth pattern. Consequently, both length and weight of the uterus continue to increase from the birth of a heifer where it is 7.7 cm long and weighs 6 g, till the attainment of puberty where it becomes 24.3 cm long and 150 g respectively. Along with length and weight, there is also a rapid increase in its RNA and protein content after the heifers turn 6 months old however the DNA content remains the same (Desjardins & Hafs, 1969). These changes continue till the heifers turn 6 months old following which it stabilizes, marking the completion of uterine development in heifers (Bartol *et al*., 1995).

2.6.2 Ovarian development

Desjardins and Hafs (1969) also stated a similar pattern of ovarian growth in heifers. An elevation in the growth of ovaries is observed starting from 5 months until 8 months of age. The growth recommences in a similar fashion to the uterine growth at 12 months of age, but at a slower pace. Like the biphasic growth of the uterus, Honaramooz *et al*. (2004) also reported a similar growth pattern in ovaries where the length and diameter increase starting from 2 to 14 weeks of age, pauses for a while, and then restarts as the heifers grow older. An increase in the follicle count ($\geq$3mm) is observed on the ovaries, that is followed by negligible change in the number of antral follicles. Furthermore, as the heifers mature, an increase in the size of follicles and number of antral follicles is also seen (Desjardins & Hafs, 1969). The quality of nutrition given to the dam during its gestation period also has an impact on the number of antral follicles present in the heifer. An adequate number of antral follicles (>25) is associated with high pregnancy rates in heifers. Therefore, the antral follicle count is positively correlated with fertility in heifers (Cushman *et al*., 2009). Because of this reason the replacement heifers or the embryo donor cows can be selected based on their antral follicular count (Atkins *et al.*, 2013).

2.6.3 Follicular waves

The development of follicles is controlled by the feedback mechanism of the gonadotrophin-releasing hormone (GnRH), luteinising hormone (LH), follicle-stimulating hormone (FSH), oestrogens, progestins, androgens, and numerous inhibin-related proteins that are secreted by the ovaries (Webb *et al.*, 1994; Webb *et al.*, 1992). As a result of this, the follicles continue to grow in discrete waves that tend to last for a period of 7 to 10 days. In an oestrus cycle of 21 days, there are around two to four follicular waves. Each follicular wave recruits a cohort of five to seven antral follicles of about 5 mm diameter, one of which becomes large regressing the growth of other follicles. A new follicular wave is instigated by the loss of the dominance of the large follicle. If the development of the dominant follicle concurs with the luteal regression phase, it rapidly matures and ovulates. If fertilization takes place, the embryo resides in the reproductive tract of the cow until implantation takes place at around the 19th day of the pregnancy (Wathes & Wooding, 1980). **Figure 2.7** below by Atkins *et al.* (2013) illustrates the stated process in detail.

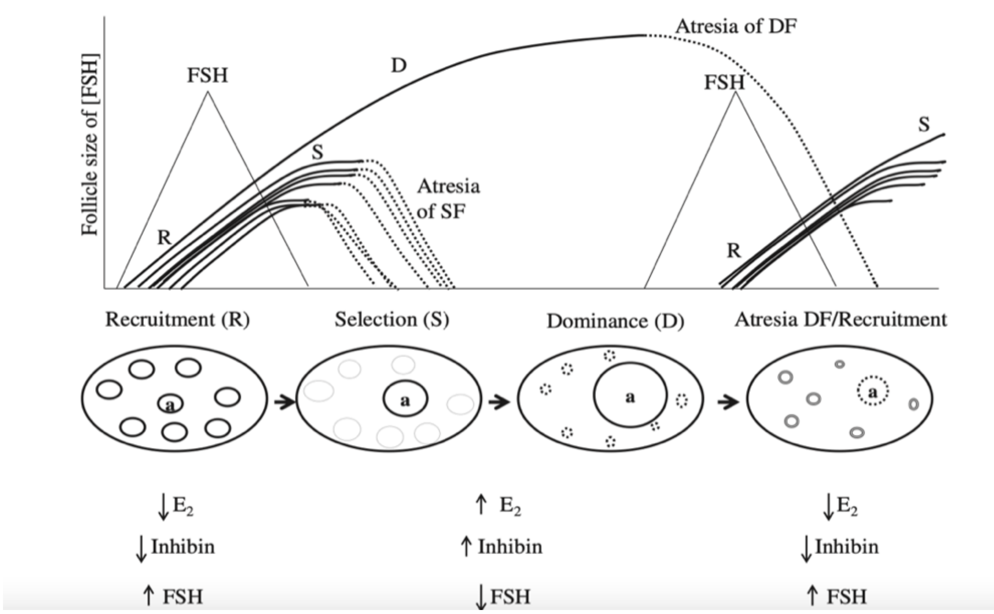


Figure 2.7: Diagram of stages of follicle growth during the stages of a follicular wave (Atkins *et al.*, 2013). E_2 = estradiol, FSH = follicle-stimulating hormone, SF = subordinate follicles.

2.7 Factors affecting puberty

The age at which a heifer reaches puberty is of great significance in the dairy industry as it directly influences its breeding efficiency. Interactions have been observed between the age at puberty and mammary development, fertility as well as growth rates (Hawk *et al*., 1954). It is subject to various genetic and non-genetic factors. The non-genetic factors are equally important as they help to improve the expression of the genetic potential of the heifers. Some of these non-genetic factors will now be highlighted.

2.7.1 Breed

Studies by Wiltbank *et al*. (1966), Gregory *et al*. (1979), Baker *et al*. (1988), Ferrell (1982), and Laster *et al*. (1976) state that the breed of the cattle highly influence the age and body weight at which it reaches puberty. Ferrell (1982) found that the heifers that were larger in size reached puberty earlier than those of smaller size. Also, Laster *et al*. (1976) claimed that heterosis and maternal effects determine the age at which heifers reach puberty however, these factors do not influence the age of the heifer when it attains puberty. Furthermore, Baker *et al*. (1988) stated that cattle breeds that are selected based on their high milk yields reach puberty earlier and at a lighter body weight than the breeds that are selected for beef production respectively. Since genetic makeup of the breed is a significant factor that can be useful to foretell the age at which the heifer would attain puberty, Short and Bellows (1971) proposed to select a breed that has younger age at puberty and cross-breed it with another breed to obtain the desired age at puberty in heifers.

2.7.2 Nutrition

As the growth rate significantly effects age at the attainment of puberty, studies show that higher levels of feed promote better growth resulting in early attainment of puberty in heifers along with higher body weight at puberty (Schillo *et al.*, 1992; Short & Bellows, 1971). Moreover, it is reported that an inadequate level of nutrition delays the occurrence of puberty in Holstein heifers whereas, a proficient one hastens the process. Therefore, the heifers are fed to accomplish substantial daily gains from their diet, either pre- or post-weaning (Akins, 2016). They also weigh heavier at puberty than those that are fed a comparatively lower plane of nutrition. Wiltbank *et al*. (1969) illustrated an interaction between the intake of nutrients and cattle breed for the age and weight at puberty. It is also seen that the cross-bred heifers are superior to the straight-bred ones as they reach puberty at a younger age at a comparatively lower feed of nutrition. Yelich *et al*. (1991) reported greater body weight, body fat and body condition score (BCS) at puberty in the heifers that were fed at an elevated plane of nutrition. Studies by Moseley *et al*. (1982), McCartor *et al*. (1979), and Bushmich *et al*. (1980) claimed that Monensin (an antibiotic used in animal feed) is effective in hastening puberty as it provides additional energy to the heifers to support their growth and development in the early stages of their life. Similarly, McCartor *et al*. (1979) also reported the use of rumen fermentation products (acetate, butyrate, propionate) in the feed to have a similar effect on the age at the onset of puberty in heifers.

2.7.3 Season

Kinder *et al*. (1987) indicated that season influences the age at which puberty in heifers is initiated. Grass *et al*. (1982) explained that the heifers that were born in the spring season attained puberty earlier than the heifers that were born in the fall season. A possible reason behind this can be that the calves born in fall have no choice but to go through the winter season before they reach puberty which was not true for the calves born in the spring season. This can also be attributed to the photoperiod factor. This was verified by the studies of Petitclerc *et al.* (1983), Ringuet *et al.* (1994), and Rius *et al.* (2005), which showed that supplementary lighting increased the rate of weight gain in addition to increasing the feed efficiency of heifers. Hansen *et al*. (1982) supported this by suggesting that the reason behind this is the alterations in the positive feedback mechanism of estradiol that enhances the ovarian growth as well.

2.7.4 Body weight

The body weight of the heifers and/or the body condition score is one of the many factors that affect the age at which puberty commences. The nutrition provided, environment, and/or the management practices determine the body weight or body condition score of the heifers. The body weight can be regarded as an effective parameter to predict the time at which the heifers would reach puberty also attaining the target body weight. Chebel *et al.* (2007) found that Holstein heifers attain puberty when they reach approximately 30-40% (i.e., 240-320 kg) of their mature body weight. Yet merely acquiring a certain body weight does not always instigate puberty in heifers therefore it should not be considered as the only crucial factor. However, it is the accelerated rate of growth by consumption of greater amounts of energy through the diet that influences the age at puberty (Ferrell, 1982). Furthermore, Siebert and Field (1975) asserted that the commencement of the estrous cycles in heifers is very closely related to body fat. They predicted 18.8 kg to 21.8 kg body fat content of the heifers when they reached puberty in accordance with their body weight and total estimation of body water content. A study done by Frisch *et al*. (1977) exhibited the same, as alterations in the fatness of the body also modify the rate of metabolism, impacting puberty initiation.

2.7.5 Environment

The social environment around the heifers, such as the exposure to bulls, relocating the environment, and/or various other biostimulators may also help fasten the attainment of puberty. These factors may also contribute in synchronizing estrus in various other species thus increasing the importance of the role of the environment in determining the age at puberty (Duggan, 1993). Roberson *et al*. (1991) found that the growth rate interacts with the presence of bulls around the heifers hence decreasing their age at puberty, although the duration of exposure to the bulls did not have a much effect on the age or body weight of the heifers.

2.8 The reproductive cycle and conception

Cows undergo estrous cycles throughout their productive life. This cycle recurs approximately every 21 days that repeatedly gives them chances to become pregnant, produce a young one and start their lactation cycle. The estrous cycle can be divided into two phases **(Figure 2.8)** i.e., the follicular phase and the luteal phase (Donadeu *et al*., 2012). Various hormones are responsible for the regulation of the cycle and are regulated by the hypothalamic-pituitary-gonadal axis comprising of the hypothalamus, pituitary, and ovary. The corpus luteum is responsible for the production of progesterone hormone whereas the ovulatory follicle triggers the release of estrogen hormone that is responsible for the signs of heat like mounting behaviour, red swollen vulva, chin resting, etc. (Esslemont *et al*., 1980).

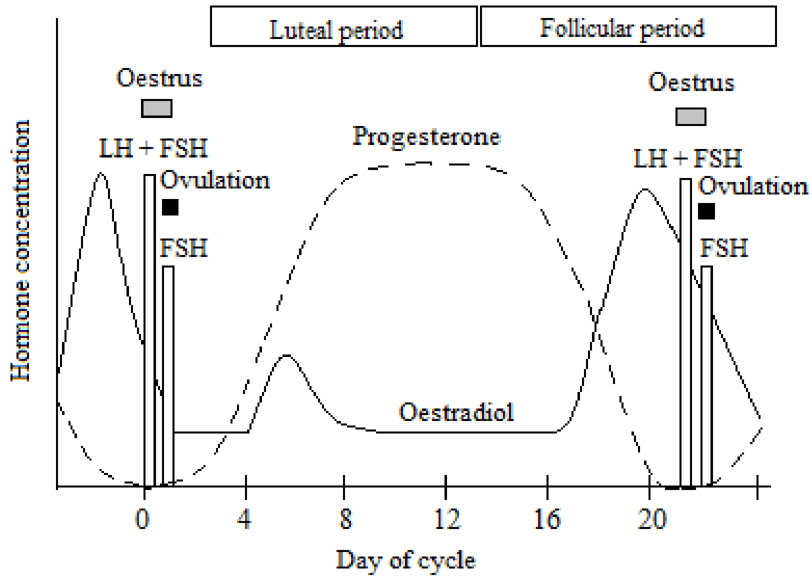


Figure 2.8: Hormone concentrations during the oestrous cycle of a cow (Mukasa-Mugerwa, 1989). LH = Luteinising hormone, FSH = Follicle stimulating hormone.

The cow becomes ready to be inseminated when its estrogen level rises i.e., it starts showing the signs of heat/oestrus. This is a very short time span of only a few hours ranging 14.1 ± 4.5 (Kerbrat & Disenhaus, 2004). If the cow conceives during this time, its progesterone level stays high as the corpus luteum is retained (Zaied *et al*., 1979). However, not all cows have a regular estrous cycle. Some of them show delayed ovulation resumption (Crowe & Mullen, 2013), whereas some of them have an extended luteal phase (Shrestha *et al*., 2004). This results into reproductive problems because of which the producer is not able to maintain a 365-days calving interval time. Other than these factors it can also be possible that some of the cows are not inseminated at a proper time, the quality of insemination can be compromised, and/or the cow may be suffering from health disorders related to the reproductive tract. Apart from this, the chief hormone that is required for pregnancy is cattle is progesterone. The progesterone hormone levels rise within the first seven to ten days following insemination (GE *et al*., 1999), but the levels of progesterone in the blood decrease as a result of low body weight and/or poor diet (Beal *et al*., 1978), which implies that growth and body weight are important factors for an efficient reproductive performance in a dairy cow.

2.9 Factors affecting growth

The primary objective of a dairy producer is to maintain an efficient growth rate so that puberty is initiated at the right time and the age at first calving of the heifer is reduced with the purpose of increasing the cow's lifetime productivity and profitability. The optimum calving age at first calving as reported by Heinrichs (1993), Pirlo *et al*. (2000), and Ettema and Santos (2004) is considered to be 23 months to 24.5 months with a target body weight of 515 kg to 600 kg (Keown & Everett, 1986; Moore *et al*., 1991). To achieve this goal, the producers have been altering the growth of heifers. The performance of a heifer is predominantly determined by its genetic potential. The genetic factors include disease resistance, feed conversion efficiency, and unusual growth rate. There are various non-genetic factors such as proper management, optimum nutrition, disease control, environment, etc. that allow the maximum expression of this genetic potential (Alemneh & Getabalew, 2019) of the heifers. Donovan *et al*. (1998) have shown that the passive transfer of colostral immunoglobulins from the dam to the calf affect the calf-height and body weight by directly impacting its health. Some of the non-genetic factors that influence the growth of the heifers will now be elucidated.

2.9.1 Environment

Bornstein (1989) described infancy as the most crucial period of growth and development of mammals emphasising on the role of the environment. Typically, in a dairy industry, the calf is immediately separated from the dam, right after its birth. The calf is then kept and raised in individual pens till it is weaned. This social isolation of the calf during the infancy stage is often seen to have many side effects which include abnormality in its behaviour and/or growth-related issues. In addition to these, the calves that are raised in isolation exhibit inadequate social skills, a difficult time handling unfavourable situations, along with lack of cognitive skills which can also be seen later in life (Costa et al., 2016). House *et al*. (1988) also associated individual housing systems with mortality and morbidity in young calves. Although there are problems associated with group housing too such as cross-suckling, disease incidence, belligerence, and competition among each other. However, significant studies have exhibited strong evidence of positive effects of grouped housing with better health and growth of calves (Costa *et al*., 2016). A study on housing systems done by Costa *et al*. (2016) has proved that group housing improves development by increasing the body weight gains of the calves particularly due to an elevated dry matter intake.

Moreover, studies have shown that the calves that are kept in contact with older animals or the dam itself, start consuming solid feed earlier and in greater quantities (Key & MacIver, 1985; Nolte *et al*., 1990). Correspondingly, these calves have been reported to show greater body weight gains post-weaning as a result of enhanced concentrate intake as compared with the calves raised in individual pens (Curtis *et al*., 2018; Warnick *et al*., 1977).

2.9.2 Season and temperature

The cows in temperate climates like Canada are highly susceptible to cold stress. A prolonged exposure to even slight cold conditions can lead to unfavourable changes in dairy cows. To maintain the thermal body temperature, the ruminants may undergo physiological changes that results in changes in their appetite, basal metabolic strength, along with variations in their digestive processes (Young, 1983). Collier *et al*. (2006) showed that the seasons have an impact on the growth, performance, reproduction as well as lactation of a dairy cow as it significantly effects the average daily gain of the heifers (Place *et al*., 1998). Some studies by Rhoads *et al*. (2009) and Cook *et al*. (2007) have shown that the temperature has a significant effect on the feed efficiency of dairy cows that ultimately effects their growth. The Holstein breed is more tolerant to cold temperatures as it is a bulky breed (Hammami *et al*., 2015).

2.9.3 Nutrition

The nutritional status of a dairy heifer is the most obvious factor that determines its growth and development. Although Alemneh and Getabalew (2019) explain that if the feed intake exceeds the amount of energy required for optimum tissue growth, it contributes to excess fat deposition which has unfavourable effects on the growth and performance of the heifers. Therefore, heifer nutritional programs target high feed-efficiency along with minimizing the risks of over-conditioning the heifers. The rates of weight gain in pre-pubertal heifers should be based on the desired age at first calving and a proper nutritional program should be designed according to that. Over-conditioning of the heifers can also lead to dystocia and different metabolic diseases such as ketosis, milk fever, etc. (Akins, 2016).

2.9.4 Disease

A study by Donovan *et al*. (1998) has shown that the health status of dairy heifers has a significant effect on their growth rates, especially during the first 6 months of their life. The incidence of diseases like diarrhea, respiratory diseases, septicemia, etc. can substantially reduce the growth rate of the heifers with respect to their height and body weight. The heifers that suffered from septicemia and/or pneumonia grew around 13 to 15 days slower than the healthy calves to reach a particular body weight whereas, diarrhea had a comparatively less effect on their growth rate. The mentioned study has also shown a difference in the pelvic height growth of the heifers from their birth to 6 months of age.

2.10 Fertility

For a very long time, dairy cows have been selected for high milk yield which has been directly linked to a decline in fertility of the dairy cows globally. Good fertility is defined as the ability of a cow to conceive at the desired time. The cows that have the highest milk yield have been observed to have maximum incidences of infertility (Lucy, 2001). It may give rise to abnormal oestrous cycles, low conception rates, delay in the resumption of oestrous cycle post-calving (Royal *et al*., 2002). The transition phase (3 weeks before and after calving) is very critical for a dairy cow as its endocrine status suddenly changes to support milk production. There is a rapid increase in nutrient demand to meet the energy requirements for the development of the fetus as well as milk secretion. On the other hand, the cow's dry matter intake during this phase is not sufficient and the mobilization of body reserves takes place causing a state of negative energy balance. The tissue mobilization at the commencement of lactation is almost unavoidable, but the extensive secretion and consumption of lipid stores is problematic (Butler & Smith, 1989). It totally depends on the dry matter intake of the cow that is influenced by environmental conditions, body condition of the cow, feeding management, diet composition, and/or metabolic disorders. Though it's not only the high productivity that negatively impacts the fertility of the cows, it is a combination of several management practices (for instance inefficient heat detection and improper timing of insemination) and/or physiological factors that vary from place to place (Lucy, 2001).

A cow does not become fertile immediately after calving. Post-calving, the uterus of a cow undergoes uterine involution, in which there is an abrupt decrease in the weight and size of the uterus as it transitions from a pregnant to its normal/non-pregnant state. This is the physical involution that takes about 30-40 days to complete (Roche, 2006). It involves physical shrinkage of the uterus, necrosis of uterine caruncles, and endometrium regeneration (Gier & Marion, 1968). Following this process, the endometrium of the cow still may or may not be able to support the pregnancy until 60 days post-calving. The chief factors that hinder this process are retained placenta, metabolic disorders, and/or dystocia (Gier & Marion, 1968; Morrow, 1966). Other factors such as uterine infections that interfere with uterine health may also delay the occurrence of first ovulation post-calving decreasing the conception rates, resulting in involuntary culling of the cows due to infertility. Following the involution, the regeneration of the endometrium takes place, followed by the return of the oestrus cycle (Sheldon, 2004). **Figure 2.9** illustrates the sequence of events that take place in a dairy cow's reproductive system in detail (Garnsworthy *et al.*, 2008).

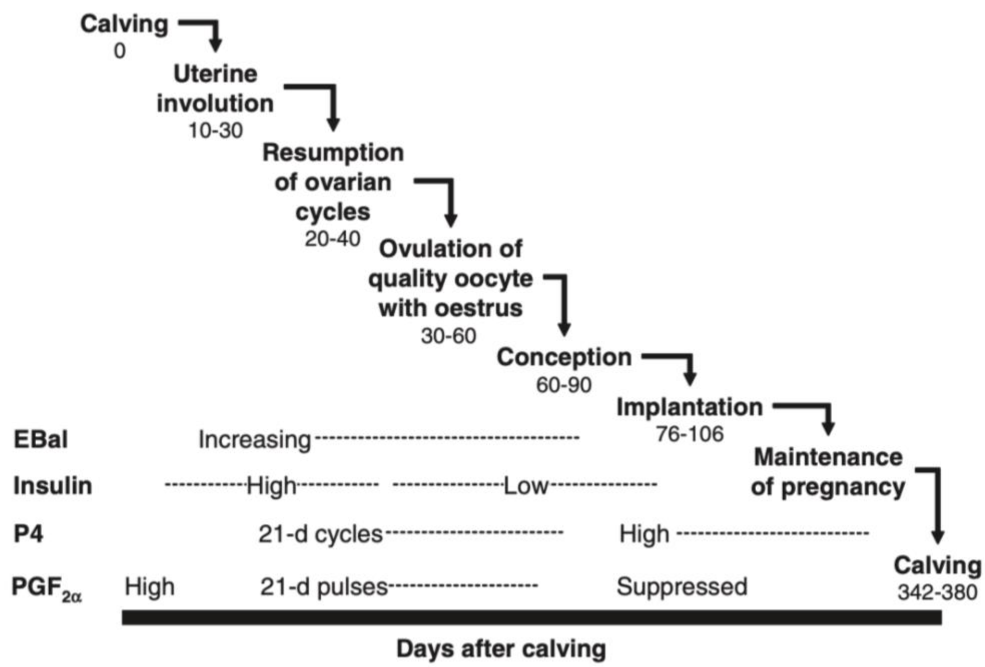


Figure 2.9: Sequence of reproductive events in the dairy cow (Garnsworthy *et al.*, 2008)

Energy deficiency also interferes with the resumption of the bovine oestrous cycle post-partum. Literature documents that early commencement of ovulatory cycles to be directly associated with improvement in the following conception rates. The negative energy causes impaired Luteinizing hormone secretion, preventing ovulation (Formigoni & Trevisi, 2003). Furthermore, Garnsworthy and Webb (2002) reported a decrease in glucose and insulin levels in a postpartum dairy cow. This reduces the ovarian response to the gonadotropins, which prevents follicle growth, negatively affecting ovulation.

The fertility status of a dairy cow has a direct impact on its reproductive performance and is a multi-factorial aspect. Studies by Filteau *et al.* (2003) and Fricke (2002) show a significant decrease in the fertility of the cows as compared to heifers. Also, Lucy (2001) reported an association of increased milk yield with infertility over the years. Furthermore, Webb *et al.* (1999) claimed that the phase of negative energy balance in postpartum dairy cows is determined by the nutrition provided to the cow, which in turn is linked with fertility. Another important factor specified by Eicker *et al.* (1996) that affects bovine fertility is disease incidence. Gröhn and Rajala-Schultz (2000) and Loeffler *et al.* (1999) also supported this by stating that the incidence of diseases such as mastitis, lameness, dystocia, retained placenta, etc. have a more profound effect on the reproductive performance than a dairy cow's milk production. Along with these factors, a strong relationship between the growth and fertility of dairy cows is also observed (Titterton & Weaver, 2001). Besides the mentioned factors that affect a dairy cow fertility, the contribution of environment (that accounts for variation) and genetics cannot be neglected although its heritability estimates is observed to be extremely low (Foote, 1970; Maijala, 1964).

2.11 Growth and fertility

In addition to all the factors mentioned above, growth is one of the leading factors that influences the onset of puberty as well as the fertility of the dairy cattle. Literature has documented the use of body weight as a representative of growth as it has been considered a good measure of the body size of an animal (Blackmore *et al.*, 1958; Miller & McGilliard, 1959; Taylor, 1955). An animal's body weight can be influenced by various factors that are previously discussed in section 2.9. Several studies have used the body condition score as a proxy for the growth of dairy cows, confirming an association between the former and reproductive efficiency (Markusfeld *et al.*, 1997; Titterton & Weaver, 2001). While some studies (Buckley *et al.*, 2003; Gillund *et al.*, 2001; Waltner *et al.*, 1993) have reported otherwise i.e., lack of any relationship between body condition score and the reproductive performance of the cow. There can be multiple possible reasons responsible for these discrepancies such as the kind of population that was analyzed, the stage at which the body condition score was observed or the measurement frequency, genetic makeup of animals, distinct management practices, parameters considered, variations amongst seasons, and/or regions, etc. There are comparatively fewer studies that have been done using the body weight as a measure of growth and investigate the effect of the former on the reproductive success (Roche *et al.*, 2007). Anyhow, Buckley and colleagues (2003) did report a significant effect of body weight of the cows (measured at the commencement of breeding season) on the pregnancy rate at first service signifying it to be a considerable element to determine a dairy cow's reproductive potential.

Correspondingly, to evaluate the reproductive performance or fertility of a dairy cow, many researchers have proposed numerous indicators of fertility. For instance, Pryce *et al*. (2001) used calving interval, days to first service, etc. to evaluate the reproductive performance of Holstein cows in relation to their body condition score at week 1 and 10 of first lactation; the change between the BCS at weeks 1 and 10; and the average of week 1 and 10. Calving interval can be defined as the span of time between successive parturitions. This implies that only those cows that have two or more lactations will have a calving interval. Williams in 1919, defined 12 months to be an ideal length of calving interval, assuming the heifer calves at the age of two years for the first time. In addition to the reproductive status of a cow, the calving interval can also be determined by gestation length of the cow and the farm management practices like voluntary waiting period. The voluntary waiting period is the period between calving and the next insemination of the cow. Although a minimal 45 to 60 days waiting period is recommended to allow complete uterine involution and resumption of ovarian activity to take place post-calving (Fetrow *et al.*, 2007); the producer might intentionally extend or shorten this waiting period as per his will. It is therefore called a 'voluntary' waiting period. Nevertheless, to achieve the ideal calving interval, the cow should be successfully bred at 80 days postpartum (i.e., 80 days to successful service) assuming a gestation period of around 285 days.

Similarly, Buschner *et al*. (1950) made use of the number of services per conception to study the fertility of the cows. If a cow does not conceive when bred, the producer will be compelled to wait for its next oestrus cycle to successfully breed it, which would return after approximately 21 days. The ability of the cow to successfully conceive when bred is also determined by management practices, fertility of the sire and not solely on its reproductive potential. For example, the inefficiency of the breeder to detect heat and/or inseminate the cow at the appropriate time may lead to conception failures. This would negatively impact the profitability of the farm as the producer will have to bear the insemination expenses multiple times, alongside increasing the calving interval. In addition to using the number of services per conception, Johansson (1961) also used the number of days between first and successful service in his study to examine dairy cattle fertility. Each failure in conception increases the number of days between first and successful service. Likewise, Everett *et al*. (1966) emphasised that the days open is an important determinant of fertility as well. The days open are affected by the days to first service and the number of services per conception as they are directly proportional. Days open can be defined as the number of days between calving and the next conception of the cow.

An increase in any of the above-mentioned fertility indicators (calving interval, days open, number of services to conception, number of days between first and successful service, and days to first service) are considered expensive as they would cause a reduction the productivity per unit time (Louca & Legates, 1968). Studies have indicated that the primiparous cows encounter maximum difficulties in the recovery of their ovarian cyclicity postpartum during their first lactation as they go through a greater negative energy balance phase as compared with the multiparous cows. This can be influenced by the fact that the cows in their first lactation have not fully grown; since their body is still growing, the metabolic and endocrine responses during this phase are likely to be compromised as the nutrients obtained from the diet are apportioned towards their growth (Senatore *et al*., 1996; Taylor *et al*., 2003).

2.12 Objectives

Considering all the challenges present in the dairy industry, the aim of this study was to determine the effect body weight of Holstein heifers observed at 12 months of age as a representation of growth during the rearing period on their reproductive performance as heifers and primiparous cows, using the database of the Québec Dairy Herd Improvement Agency (Valacta).

The present study aimed at the following:

- To determine the effect of yearling weight on the fertility measures in Québec Holstein dairy heifers
- To determine the effect of yearling weight on the fertility measures in Québec Holstein primiparous dairy cows

This study considered different regions across Québec province. The heifer's body weight at 12 months of age was analyzed as it represents the growth of heifers at the attainment of puberty; followed by the evaluation of their fertility with respect to their growth. Fertility was reviewed using such indicators as calving interval, days open, number of services to conception, number of days between first and successful service, and days to first service. Each indicator of fertility was analyzed for heifers and for cows that had their first lactation to investigate its relationship with the growth of the heifers along with other influencing factors.

Based on previous studies, it was hypothesised that the yearling body weight of heifers would have a significant effect on the fertility of the heifers as well as primiparous cows. It was also postulated that the heifers exhibit better reproductive performance than the primiparous cows.

3. Materials and Methods

3.1 Data preparation

For this study, the data were obtained from Valacta, a dairy production center of expertise that provided its milk recording data that was collected over a period of 16 years from January 2000 to December 2015. The data provided were in the form of separate SAS datasets, which were: lactation records, breeding records, and body weight records of the Holstein cows. In addition to these, two more files were also obtained from Valacta in the form of Microsoft Excel spreadsheets describing variables and codes used in the SAS datasets.

To begin with, the lactation records file was examined using the SAS® software (SAS Institute, 2018). There were two files corresponding to the lactation records of the cows. The first file contained the Lactation number 1 records of the cows following their first calving whereas the second file contained the Lactation number 2 records of the cows that had first lactation and a second calving. There were 127,847 records with 43,544 animals that belonged to 2,081 herds in the former file and 85,163 records of 29,314 animals that belonged to 1,708 herds in the latter. There were multiple observations per animal because of the multiple body weight measurements recorded at different ages and dates. The first lactation records comprised of all the records for the first lactation of the cows and the second lactation records corresponded to the cows from the first lactation dataset that had a second calving. These lactation files contained important information regarding the lactation of the cows like the start and end date of each lactation, the start reason for each lactation, the end date of the first lactation along with the animal identification number, herd identification number, and date of birth of each cow. To further examine the lactation records, only one record per animal was retained as the body weight information was not important at this point.

3.2 Data editing and merges

3.2.1 Lactation records file edits

The first step was to remove all the animals born before January 1st, 2008, because of the unavailability of reliable individual body weight records for these animals. This resulted in the deletion of 26,539 animals from the first lactation file and 17,841 from the second one. The former was left with 17,005 animals and the latter with 11,473. These datasets were then merged using the common variables that are animal and herd identification numbers. The resulting dataset had information on both lactations of the cows. However, not all cows had a second calving as they did not conceive throughout their first lactation, their second lactation records were missing. These missing cows were still retained in the data (their first lactation records) with the aim of determining the possible reason behind their inability to conceive during the first lactation. Therefore, the merged data consisted of 17,005 animals, out of which 11,473 cows had records for two lactations and 5,532 of them had records for only their first lactation. These cows had different lactation start reasons (**Appendix 1)**; for instance, some animals start their lactation because of hormone induction, some of them did that because of an abortion whereas some of them started a lactation as a result of calving or giving birth to a calf. For the study, only those animals were retained in the data that had 'Birth' as the reason behind the commencement of their lactation (**Appendix 2)**. The thought behind this was simply that the commencement of a lactation due to any reason other than calving does not provide an understanding of the reproductive status of a cow. So, to accomplish this, the animals that had 'hormone induction' or 'abortion' as their lactation start reason in both lactation files were removed (i.e., 242 animals). This implies that the cows that had two lactations and both the lactations started as a result of a calving were retained; there were 11,231 such cows. A similar edit was done on the other group of cows that had a missing second lactation record, which resulted in the loss of 57 cows leaving 5,475 of them for the study (**Figure 3.1**).

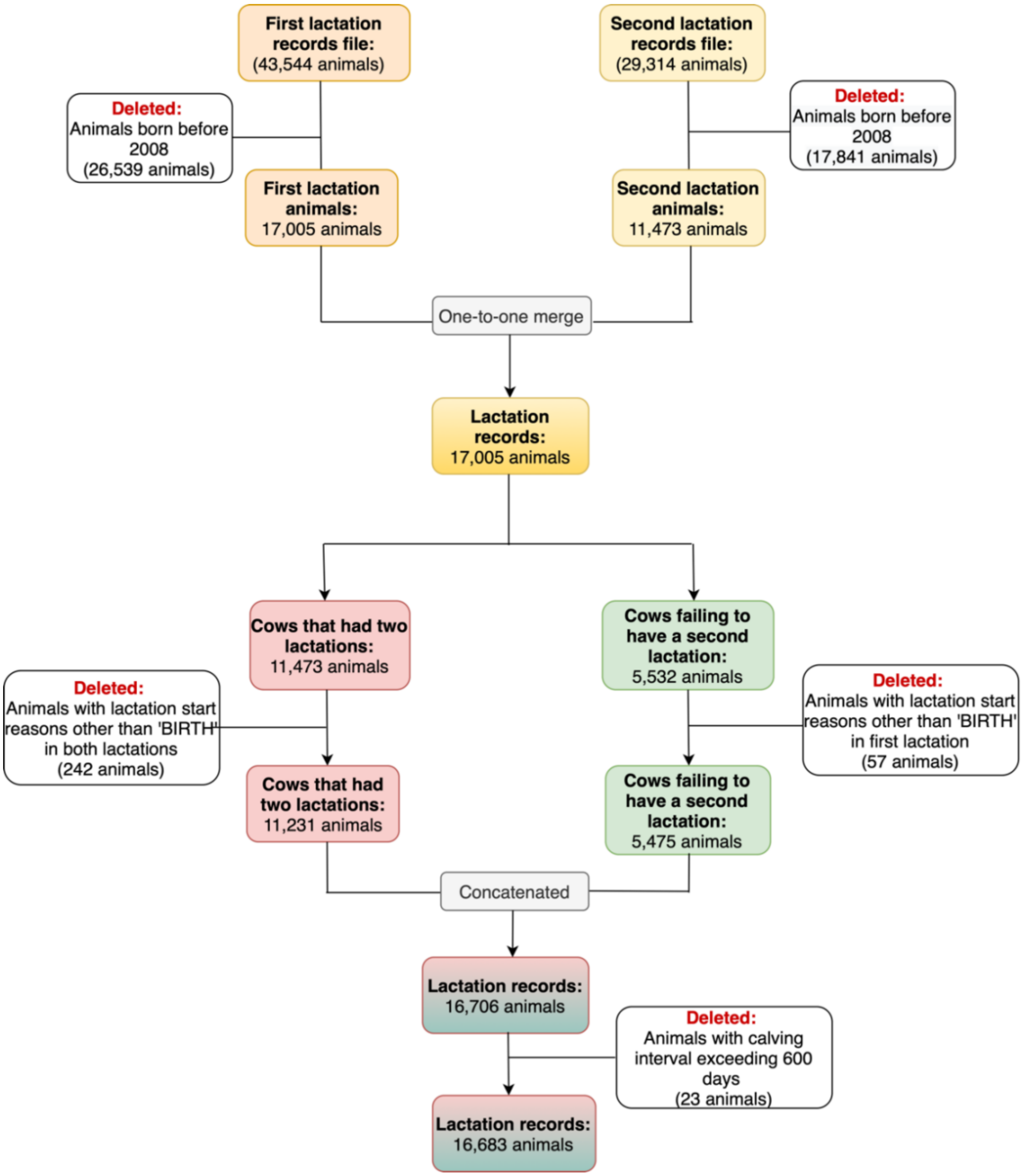


Figure 3.1: Lactation file data edits

Following this, a new variable called 'calving interval' was created by calculating the number of days between reported calvings (i.e., the difference between second lactation start-date and the first lactation start-date). It reflects the time period in which a cow reproduces again following a calving. As a result, only 11,231 cows (67.2%) had a calving interval since they had two calvings and 5,475 of them did not. Following this edit, the cows that had a calving interval exceeding 600 days were not included in the data which further removed 23 cows from the data, resulting in 16,683 heifers and cows obtained from the lactation files (**Appendix 3)**.

3.2.2 Breeding records file edits

There were 16,683 cows and heifers present in the cleaned lactation records dataset. Thereafter the breeding records of heifers and cows were obtained (8,754,432 records). These breeding records provided the breeding information of each animal such as the number of services required to conceive before, during, and after the first lactation plus information regarding the service type of an animal whether it was inseminated by an A.I. technician or the producer or was naturally bred, had an embryo transfer or pasture/paddock (the undetermined method present in the dataset) along with some missing values as shown in **Appendix 4**. Since the natural breeding would not provide the exact number of breedings required to impregnate the cow and/or the precise date of the successful conception, it was removed from the data. Similarly, an embryo transfer method reflected that the animal might have a compromised reproductive system and hence were removed from the data too. Along with these was an unknown service type that was not properly defined in the descriptions file i.e., pasture/paddock method, and was eliminated too. In a nutshell, the first step was to remove all the cow and heifer breeding records that were not inseminated by a technician or producer (8,578,144 breeding records retained).

These cleaned breeding records were then merged with the previously cleaned lactation records using the common variables that are animal and herd identification numbers. There were 149,955 breeding records for 16,683 cows. For 11,208 cows that successfully conceived during their first lactation and had a second calving, all the breeding records during the first lactation were retained to study their first lactation fertility. However, this resulted in the loss of 211 animals as their breeding records during the first lactation were missing/not recorded. The same procedure was then repeated on the 5,475 cows that failed to have a second calving due to a conception failure during their first lactation. This was done to study the number of attempted artificial inseminations for this group of animals. In this case, 182 animals had a missing breeding record and were therefore lost. Following this step, the heifer records were considered to study heifer fertility. To accomplish this, all the inseminations that were done before the first calving date were retained. Out of 16,683 heifers 1,772 of them had missing breeding records. The total number, therefore, came down to 10,881 (with 24,287 breeding records) for the group of cows that conceived during their first lactation and had a second calving; 5,293 (with 34,954 breeding records) for the group of cows that did not conceive during their first lactation and 14,911 (with 25,799 breeding records) for the group of heifers as illustrated in **Figure 3.2**.

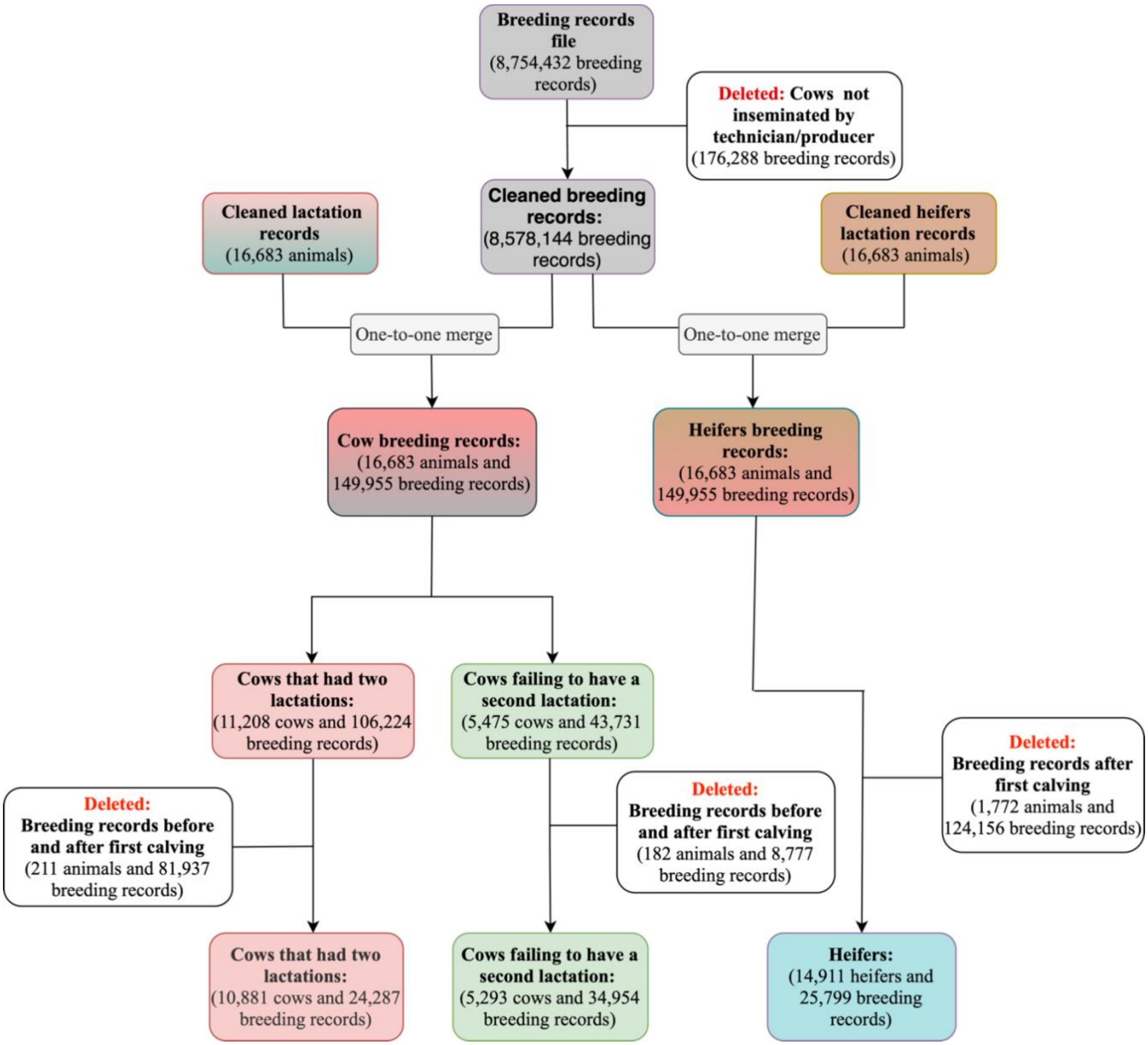


Figure 3.2: Breeding file data edits

It is common among many producers to implement multiple-insemination practices following heat detection to ensure that the cow was not inseminated too early or too late. This helps the producer to make sure that the timing of insemination is correct, and it synchronizes with the ovulation timing. For the study, the cows that underwent this practice would have exhibited a greater number of inseminations attempted to ensure pregnancy as compared to the others that did not. Therefore, for all the animals only the first service record was kept for the ones that had multiple inseminations done within 72 hours (i.e., less than 3 days). This led to the loss of 1,532 breeding records from the groups of cows that had two calvings; loss of 2,130 breeding records from the group of cows that failed to have a second calving and 1,262 breeding records from the heifer group as shown in **table 3.1**

Table 3.1: Breeding records retained: the first breeding record of the animals bred multiple times within 3 days

Editing criteria	**Group**	**Number and breeding records of animals:** Before editing	After editing	**Number of records removed**
Retaining the 1st breeding record of the animals bred multiple times within 3 days	Cows that had two calvings	10,881 cows and 24,287 breeding records	10,881 cows and 22,755 breeding records	1,532
	Cows that failed to have a second calving	5,293 cows and 34,954 breeding records	5,293 cows and 32,824 breeding records	2,130
	Heifers	14,911 heifers and 25,799 breeding records	14,911 heifers and 24,537 breeding records	1,262

Subsequently, the gestation length variable was created by calculating the number of days between the last/successful breeding date and the following calving date. Therefore, it could only be obtained for the group of cows that had two lactations and the heifers, as for the group of cows that failed to have a second calving did not have any successful breeding date. After the examination, the values of gestation length (in days) were found to be as low as 28 days to as high as 434 days which indicated some errors since all the cows had ‘Birth’ as a reason behind the start of their lactation. This indicated that there some false entries in the data. To filter out these records, the limits of 265-295 days were imposed on the gestation length variable. All the animals that had gestation lengths less than 265 days or greater than 295 days were dropped from the data. This led to the deletion of 453 cows that had two calvings and 755 heifers as shown in **Table 3.2**

Table 3.2: Breeding records retained: Animals with gestation length lying between 265 days to 295 days

Editing criteria	Group	Number of animals: Before editing	After editing	Animals removed
Gestation length	Cows that had two calvings	10,881	10,428	453
	Heifers	14,911	14,156	755

3.2.3 Body weight records file edits

A total of 2,283,550 body weight records were obtained that had to be merged with the cleaned breeding records of the animals. Only one observation per animal was retained in the cleaned breeding records with all the required breeding information. There were 15,721 cows present in the data (the cows that had two lactations i.e., 10,428 plus the cows that failed to have a second lactation i.e., 5,293) along with 14,156 heifers. At first, the 15,721 cow records were taken and merged with the body weight records using the common variables i.e., animal and herd identification number. For the study the heifer body weight was required, therefore all the body weights recorded after the first calving date were removed from the data. This led to the removal of 50,877 body weight records along with 5 animals since these animals did not have any body weight records prior to their first calving. Furthermore, there were 16 regions present in the data, that corresponded to different regions in Québec to which the animals belonged. There were some animal records that had missing region information and some of them were not very informative as there were less than ten animal records present in them. Therefore, three such regions were removed from the data. This led to the omission of 45 cows that had 158 body weight records, leaving only 13 regions for the study as tabulated in **Appendix 5**. On the resulting 15,671 cows, the limits of 21-32 months were applied on the age at first calving variable to remove all the cows that first calved at the age of less than 21 or greater than 32 months.

Following this step, the age at which the body weight was recorded was plotted against the body weight of the animals using PROC GPLOT to visualize if there were any outliers present. As depicted in **Figure 3.3 (a)**, these outliers represented the unusual heifer body weights.

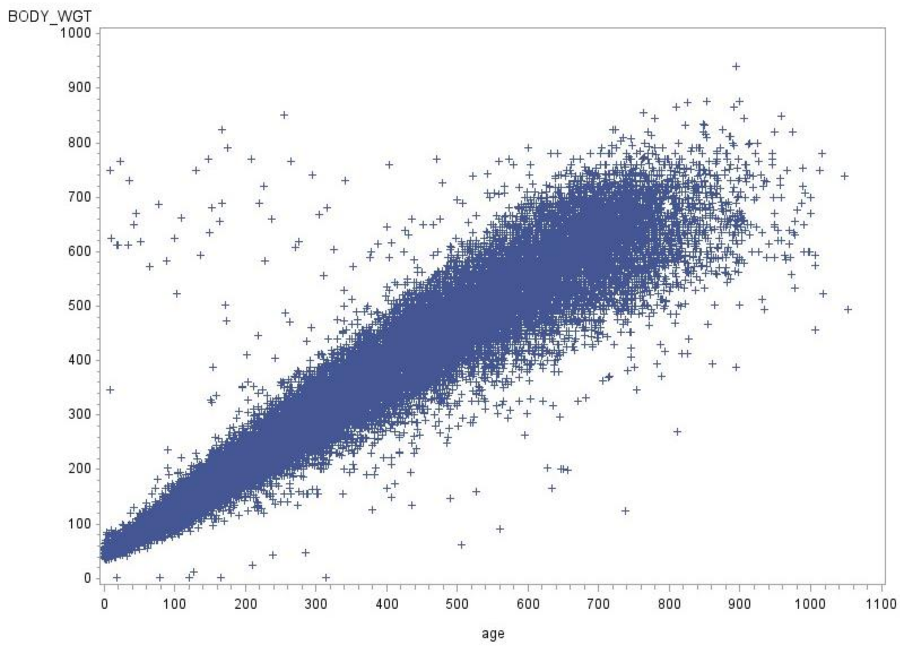


Figure 3.3: (a) Body weight measurements (in kg) against age (in days, at which body weight was measured) of all the cows

Therefore, all the animals that had anomalous body weight records lying outside the 1-99% quantile range, for thew corresponding age were removed from the data. This resulted in the deduction of 174 cows that corresponded to 243 body weight records (**Figure 3.3 (b)**).

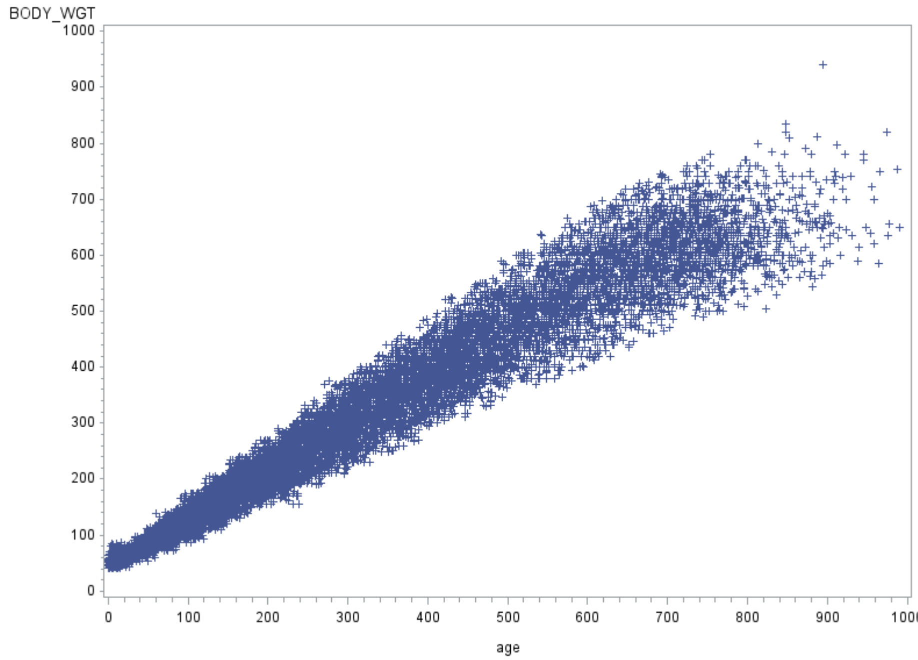


Figure 3.3: (b) Cleaned body weight measurements (in kg) against age (in days, at which body weight was measured) of all the cows after removing the outliers

The body weights present in the data had four codes associated with them that represented different methods using which the weights were measured. These codes denoted 'tape measurement', 'scale measurement', 'visual measurement', and 'method not recorded' methods of body weight measurement. In addition to these four methods, there were some animals that had valid body weight records, but their weight measurement method was not entered. These can be considered to be the same as the 'method not recorded' method of measurement as tabulated in **Appendix 6**. For the study, all the body weight measurements were retained as more than 85 percent of the animals did not have their body weight measurement method recorded while having an acceptable body weight record before their first calving date. The resulting 15,282 animals having 34,450 body weight records consisted of 10,230 cows that had two lactations and 5,052 cows that failed to have a second lactation.

Likewise, with the group of heifers, the same edits were done. At first, all the regions which had less than ten heifers present or heifer records with missing region information were removed, eliminating 51 heifers from the data. Among the remaining 14,105 heifers, 82 records were discovered in which the heifers had an irrational age at first breeding (i.e., less than 12 months) and were therefore removed, retaining the heifers that were at least 12 months old or above when they were first bred. Subsequently, for the resulting 14,023 heifers, the limits of 21-32 months were imposed on the age at first calving variables leaving 13,871 heifers behind for the study. A subset of this data was then created, which contained only one breeding record per heifer with the required breeding information (i.e., number of services to first conception and service type). These clean heifer breeding records were then merged (one-to-one merge) with the body weight records using the common variables i.e., animal and herd identification number. After retaining all the body weight records measured before the first calving date of the heifers, the unusual records were visualized using the PROC GPLOT (**Figure 3.4 (a)**) where the age at which the body weight was recorded was plotted against the body weight of heifers.

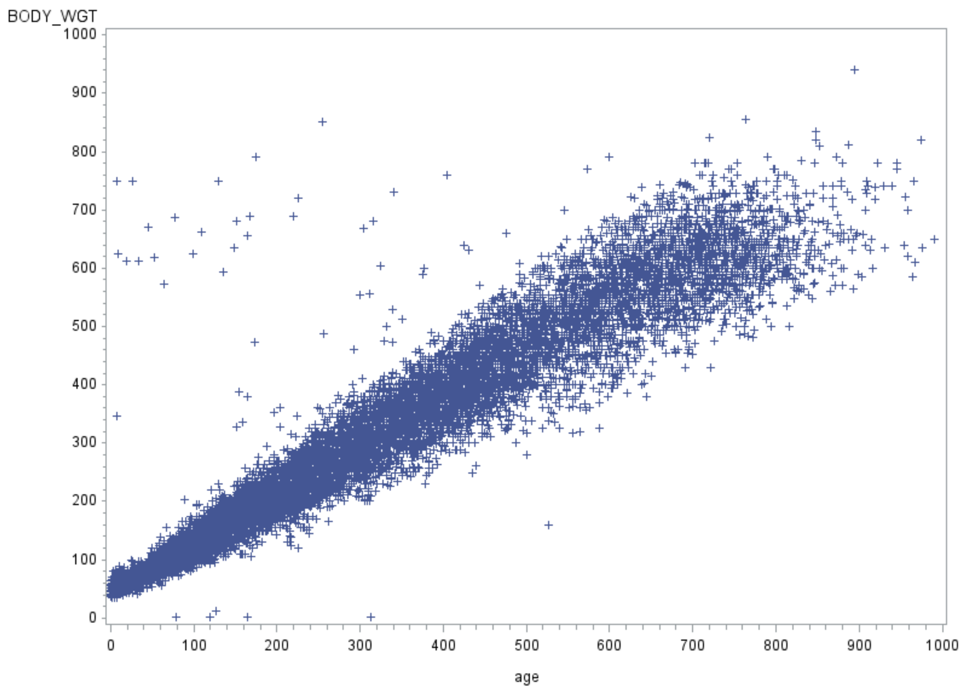


Figure 3.4: (a) Body weight measurements (in kg) against age (in days, at which body weight was measured) of the heifers

All the heifer body weight records lying beyond the 1-99% quantile range, of the corresponding age were removed from the data in the same as the anomalous cow body weight records were. This omitted 130 heifers from the data that had 367 body weight records, leaving 13,741 heifers with 31,844 body weight records left behind (**Figure 3.4 (b)**).

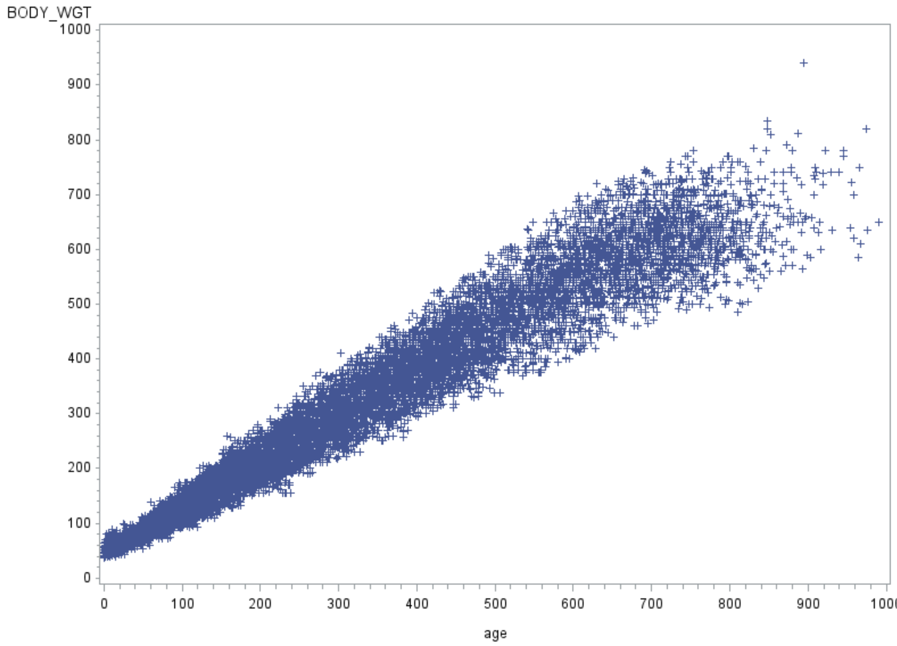
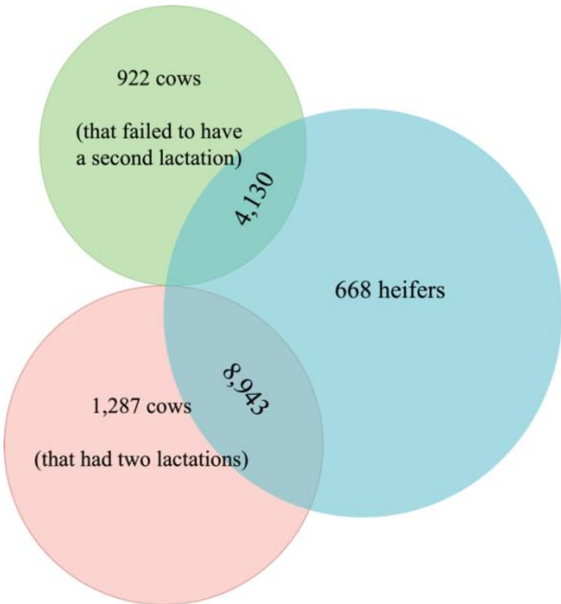


Figure 3.4: (b) Cleaned body weight measurements (in kg) against age (in days, at which body weight was measured) of the heifers after removing the outliers

Thereafter, the three datasets i.e., the cows that had two lactations (10,230), the heifers (13,741), and the cows that failed to have a second lactation (5,052) were merged using the common variables (animal and herd identification number) with all the required lactation, breeding, and body weight information so that their body weight at 12 months of age could be predicted using the growth curve equation from the prediction model by Cue *et al*. (2012). Body weight at 12 months of age was considered for the study because at or around this age the cows attain puberty and become reproductively active. The merged dataset consisted of 15,950 animals in total that had 36,806 body weight records as there were some animals in common among the three groups as elucidated in **Figure 3.5**.



- Cows that had two lactations:
  1,287 + 8,943 = 10,230
- Heifers:
  688 + 4,130 + 8,493 = 13,741
- Cows that failed to have a second calving:
  922 + 4,130 = 5,052
- Total number of animals:
  922 + 4,130 + 668 + 8,943 + 1,287 = 15,950

Figure 3.5: Number of animals in each group after predicting body weight at 12 months of age using weight prediction model by Cue *et al.* (2012)

As the Venn diagram above depicts, there are 8,493 animals common among the group of heifers and the group of cows that had two lactations; plus 4,130 animals are common among the group of heifers and the group of cows that failed to have a second lactation.

There were 15,950 individual animals in total, out of which some of them were common among two groups (i.e., appeared in more than one group). Out of these 15,950 animals, 48 of them did not give a sensible growth rate using the weight prediction equation and were therefore dropped from the data. This led to the removal of 20 cows that had two lactations, 44 heifers, and 18 cows that failed to have a second calving. As a result of this, 15,902 animals were left for the study; out of which 10,210 were the cows that had two lactations, 13,697 were heifers, and 5,034 of them failed in having a second lactation. Each animal's body weight was predicted at 12 months of age (see **Table 3.3**).

Table 3.3: Body weights retained: animals with rational body weights at 12 months of age

	Before BW12 prediction	After BW12 prediction	No. of animals lost
No. of cows that had two lactations	10,230	10,210	20
No. of heifers	13,741	13,697	44
No. of cows that failed to have a second lactation	5,052	5,034	18
Total number of animals	15,950	15,902	48

Thereafter, a variable called 'year-season' was created that had the information of the year, month, and date on when the animal was last inseminated (i.e., its last service attempted that led to a second calving in case of the cows that had two lactations, or led to the first calving in case of heifers and the failed last service that was attempted during the first lactation of the cows that failed to result into a second calving). In addition to this, another variable called 'season' was created that corresponded to the four seasons present in the data. These seasons were fall, spring, summer, and winter that also corresponded to a particular season when the animal was last inseminated (whether or not it resulted in a conception).

Then, the herd-year-seasons with only one cow or heifer record in them were removed as they were uninformative due to possible overfitting of model in their case. Likewise, based on the same thought the year-seasons with only one herd record were also removed. This further brought down the number of animals present in each group to 7,890 animals in the group of cows that had two lactations, 11,154 heifers, and 2,779 animals in the group of cows that failed to have a second lactation respectively. Since the cows that failed to have a second lactation did not have any second lactation records, this indicates that these cows failed to conceive during their first lactation. The information on whether these cows conceived and then underwent an abortion (during their first lactation) or had a fetal mortality was not provided. The flowchart below (**Figure 3.6**) explains the described progression in detail.

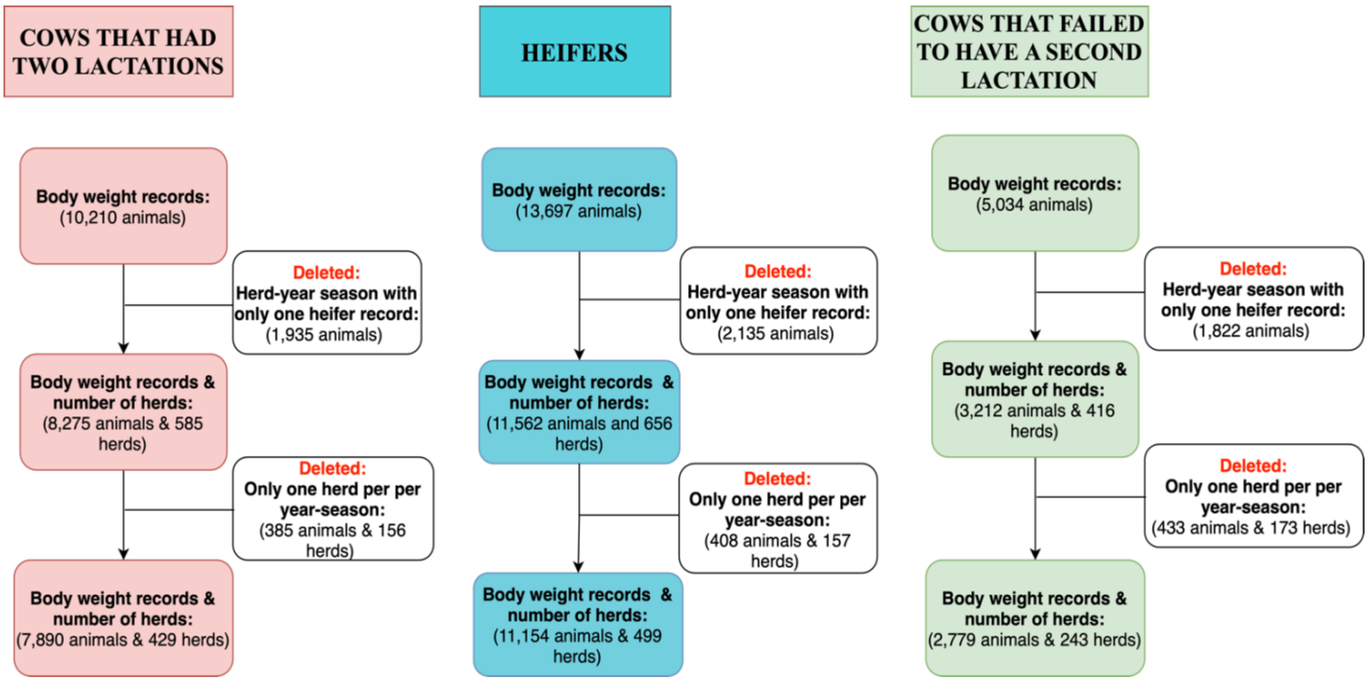
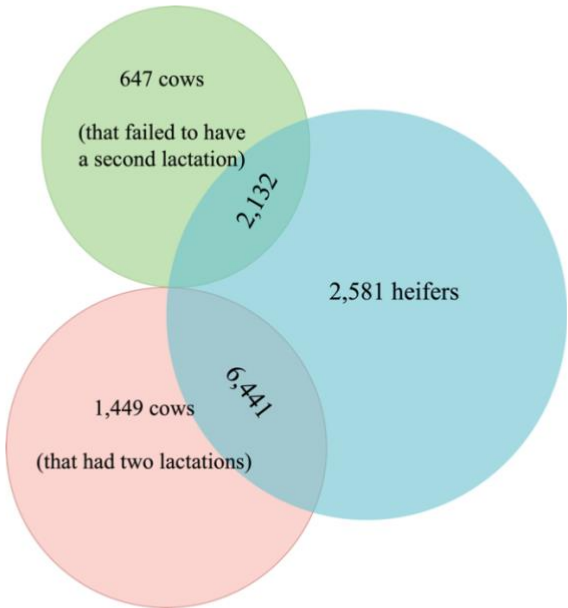


Figure 3.6: Number of animals available for analyses

Following all the edits, there were total 13,250 individual animals available for the study, which also included some animals common among the three groups as explicated in the **Figure 3.7** below.



- Cows that had two lactations:
 1,449 + 6,441 = 7,890
- Heifers:
 2,581 + 2,132 + 6,441 = 11,154
- Cows that failed to have a second calving:
 647 + 2,132 = 2,779
- Total number of animals:
 647 + 2,132 + 6,441 + 2,581 + 1,449 = 13,250

Figure 3.7: Number of animals in each group available for analyses

3.3 Statistical analysis and model construction

A statistical model was designed to analyze the fertility parameters of the cows and heifers using the SAS® software (SAS Institute, 2018). It contained both random and fixed effect parameters, therefore the PROC MIXED procedure was used for its analyses. The effect of sire was not included in the model since the information on the sire whose sperm was used to artificially inseminate the animals was not available. The following model was run multiple times using different dependent variables to determine the fertility status of the cows and heifers present in the data:

$$\mathbf{Y_{ijkmnp} = \mu + Body\ Weight_i + Region_j + Herd_{jk} + Age\ at\ first\ calving_m + Season_n + Year\text{-}season_{np} + e_{ijkmnp}}$$

- Y_{ijkmnp}: The dependent variable
    - (For cows that had two lactations: **calving interval** or **days open** or **number of services to second conception** or **days to first service** or **days between first and successful service**; For heifers: **number of services to first conception** or **days**

between first and successful service; For cows that failed to have a second lactation: **number of services attempted for second conception** or **days to first service**).

- μ: The overall mean.

- Body weight$_i$: The fixed effect of the ith predicted body weight (in kg) category at 12 months of age.

  i = 1, 2,……12

  - The12 levels of the body weight variable refers to the 12 non-overlapping categories that had a range of 10 kg each for the body weight of the animals at 12 months of age. These body weights were predicted by Cue's model (Cue *et al.*, 2012) at 12 months of age. (**Table 3.4**).

Table 3.4: Body weight categories

BWG12 category	Body weight (in kg)	BWG12 category	Body weight (in kg)
1	< 300	7	≥ 350 < 360
2	≥ 300 < 310	8	≥ 360 < 370
3	≥ 310 < 320	9	≥ 370 < 380
4	≥ 320 < 330	10	≥ 380 < 390
5	≥ 330 < 340	11	≥ 390 < 400
6	≥ 340 < 350	12	≥ 400

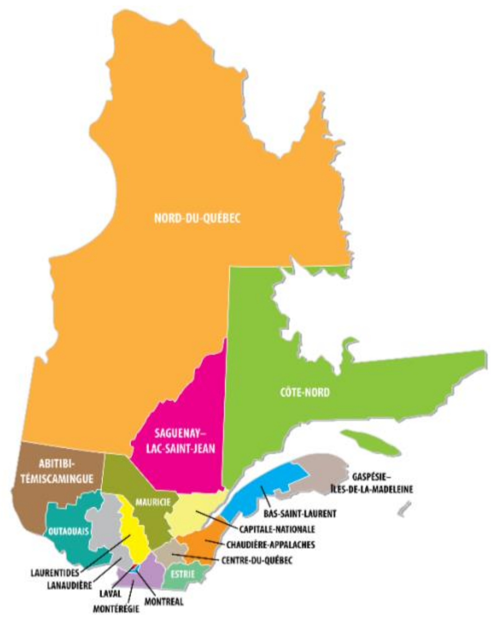
- Region$_j$: The fixed effect of the jth region category.

  j = 1,2,……13

  - There were 13 of Québec's 17 regions that contained sufficient data for analyses as shown in **Table 3.5** and the corresponding map below.

Table 3.5: Regions in Québec that contained sufficient data for analyses

Obs.	Regions
1	Bas-Saint-Laurent
2	Saguenay-Lac-Saint-Jean
3	QuébecCapitale-Nationale
4	Mauricie
5	Estrie
6	Outaouais
7	Abitibi-Témiscamingue
8	Chaudière-Appalaches
9	Lanaudière
10	Laurentides
11	Montérégie-Est
12	Montérégie-Ouest
13	Centre-du-Québec



- Herd (Region)$_{jk}$: The random effect of the herd nested within region.

$$\text{Herd (Region)}_{jk} \sim N\left(0, \sigma^2_{\text{Herd (Region)}}\right)$$

  - There were 429 herds present in the group of animals that had two lactations, 499 in the group of heifers, and 243 in the group of animals that failed to have a second calving.

- Age at first calving$_m$: The fixed effect of the mth age (in months) at first calving category.

$$m = 1, 2, \ldots 12$$

  - The12 levels of the Age at first calving variable refers to the 12 non-overlapping categories that had a range of 1 months each (Starting from 21 months to 32 months) for the Age at first calving of the animals as illustrated in **Table 3.6**

Table 3.6: Age at first calving categories

Age FC category	Age (in months)	Age FC category	Age (in months)
1	$\geq 21 < 22$	7	$\geq 27 < 28$
2	$\geq 22 < 23$	8	$\geq 28 < 29$
3	$\geq 23 < 24$	9	$\geq 29 < 30$
4	$\geq 24 < 45$	10	$\geq 30 < 31$
5	$\geq 25 <26$	11	$\geq 31 < 32$
6	$\geq 26 < 27$	12	$\geq 32 < 33$

- Season_n: The fixed effect of the n^{th} season.

  $n = 1, 2, 3, 4$

  - The 4 levels of the Season variable represent the 4 seasons present in the data. These seasons represent when the animals were last inseminated as elucidated in **Table 3.7**

Table 3.7: Seasons

SEASONS	SEASON START & END DATE
Winter	21 Dec – 19 Mar
Spring	20 Mar – 20 Jun
Summer	21 Jun – 22 Sep
Fall	23 Sep – 20 Dec

- Year-season $(\text{Season})_{np}$: The fixed effect of year-season (when the cows were last inseminated) nested within season.

  $p = 1, 2,\ldots.22$

- e_{ijkmnp}: The random residual associated with the experimental unit (i.e., cow/heifer).

$$e_{ijkmnp} \sim N(0, \sigma^2_e)$$

Also, a fixed effect interaction between the yearling body weight category and age at first calving category was considered in the model (wherever significant) to fathom if the body weight at 12 months of age and the age at first calving collectively influenced the dependent variable.

The dependent variables differed from group to group for the analyses of fertility parameters as demonstrated in **Figure 3.8**. For the group of cows that had two lactations, five dependent variables were created and analyzed. These variables were Days to first service i.e., the number of days to the first service post-partum (during the first lactation), Number of services (artificial inseminations) for second conception i.e., the number of services (artificial inseminations) done during the first lactation of the cows that led to a second pregnancy, Days from first to successful breeding i.e., the number of days between the first breeding post-partum and the last breeding that impregnated the cow during the first lactation, Days open i.e., the number of days from first calving to the next conception of the cow. For the group of heifers two dependent variables were created and analyzed. These were Number of services for first conception and Number of days from first to successful breeding of the heifers. Similarly, for the group of cows that failed to have a second calving, two variables were analyzed. These were Days to first service (during the first lactation) and the Number of services attempted for the second conception.

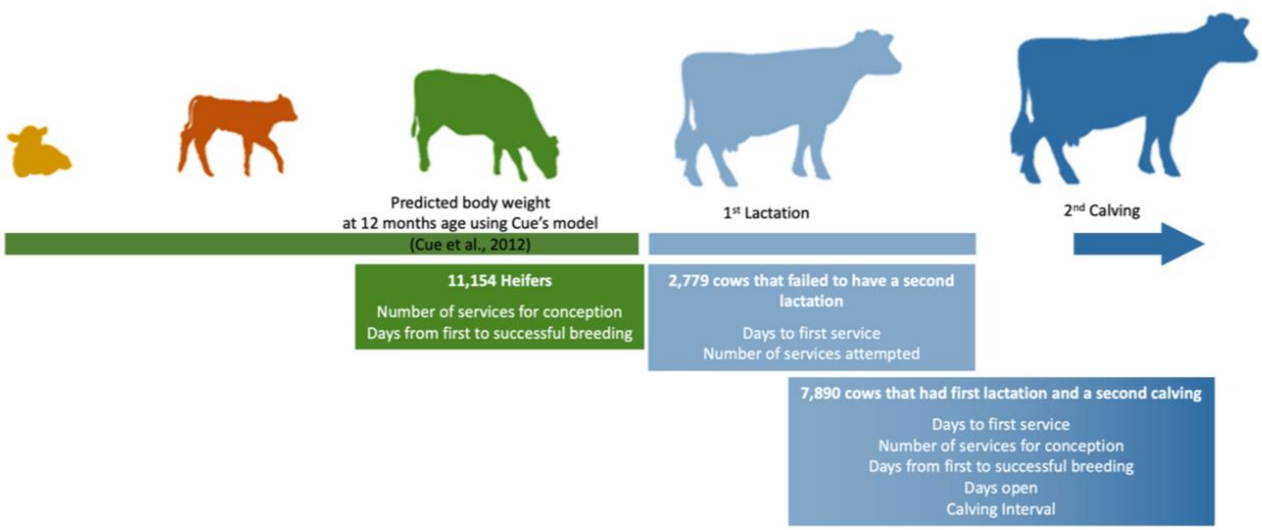


Figure 3.8: Number of animals and fertility parameters analyzed in each group

The Days to first service variable (number of days from first calving to the subsequent insemination during first lactation) was same for the group of cows that had two lactations and the group of cows that failed to have a second calving. An attempt was made to merge the two groups of cows and analyse the common variable (Days to first service after first calving) with a greater number of animals in it. However, due to large memory requirements of the model, it required an exorbitant amount of time (approximately over a month) to execute and hence was aborted. Thus, the said variable was analyzed separately in both groups of cows.

4. Results

4.1 Descriptive statistics

The **Tables 4.1, 4.2** and **4.3** below present the descriptive statistics of the analyzed dependent variables as well as the independent variables used in the study. A five percent probability level was selected for determining the statistical significance ($P \leq 0.05$) of the independent effects on the fertility parameters based on one of the statements of R.A. Fisher that says that an unusual sampling occurrence can be represented by one in twenty chances (Moore & McCabe, 1993).

Table 4.1: Descriptive statistics for the group of heifers

Variable	Mean	SD	Min	Max
Days from first to successful breeding	22.98 days	40.81	0 days	286 days
No. of services for first conception	1.60 services	0.97	1 service	9 services
Predicted yearling body weight (kg)	357.41 kg	26.81	274.04 kg	439.14 kg
Age at first calving	25 months	2.07	21.03 months	32.99 months

Table 4.2: Descriptive statistics for the group of cows that had first lactation and a second calving

Variable	Mean	SD	Min	Max
Days to first service	74.61 days	20.86	11 days	269 days
No. of services for second conception	2.06 services	1.31	1 service	12 services
Days from first to successful breeding	37.81 days	48.02	0 days	250 days
Days open	112.42 days	51.23	25 days	319 days

Calving interval	392.08 days	51.41	298 days	598 days
Predicted yearling body weight (kg)	356.01 kg	26.27	279.87 kg	439.14 kg
Age at first calving	25.34 months	2.02	21.09 months	32.99 months

Table 4.3: Descriptive statistics for the group of cows that had failed to have a second calving

Variable	Mean	SD	Min	Max
Days to first service	73.91 days	29.99	8 days	526 days
No. of services attempted for second conception	6.58 services	3.58	1 service	25 services
Predicted yearling body weight (kg)	362.02 kg	26.45	282.29 kg	432.96 kg
Age at first calving	25 months	2.07	21.09 months	32.99 months

From the tables above **(Tables 4.2 and 4.3)**, it can be observed that some primiparous cows have been re-bred by the producer unusually early (post-partum) although it is highly unlikely that the cows show the signs of estrus at this stage e.g., 11 days or 8 days post-partum. Even though it seems to be unreal (as, in Québec, a voluntary waiting period of 60 days in milk is practiced (Bonneville-Hébert *et al.*, 2011)), it was considered in the study, since the Days to first service of 269 days or 526 days seemed to be equally irrational. It also gave rise to irrational values for the Days open variable for the group of cows that had two calvings **(**see **Table 4.2)**.

As hypothesised, the above-mentioned tables suggest a decline in the fertility of the first-lactation cows as compared to the heifers as the heifers require a fewer number of services on an average to conceive than the group of cows that conceived during the first lactation. Similarly, the heifers were also seen to have a smaller average of the number of days between their first and successful breeding. The data above represent the fertility measurements of the animals recorded over a period of 8 years (from 2008-2015). In **Table 4.3**, the number of services were attempted for second conception of the primiparous cows but, were not successful which implies that none of the cows in this group conceived (i.e., 2,779 cows) and never had a second calving/lactation.

The **Appendices 7-18** show the distribution of animals present in each of the body weight categories (at 12 months of age), age at first calving categories (21-32 months old), regions, and seasons for the three groups of animals being studied.

4.2 Random effect parameters

The only random effect present in the model was herd identification number. After running the model, with the PROC MIXED procedure of the SAS® software, the variance parameter estimates were obtained. The random effect was then tested for statistical significance using the BIC (Bayesian information criterion) information. The BIC criterion is a method of selection of the best model based on the BIC values obtained from the F-tests. The model with the lowest BIC value is preferred. To accomplish this, the desired model (for which the significance of the random variable is to be tested) was run twice; once with the random effect (i.e., herd identification number) and then without it, keeping the rest of the model the same. Then the BIC values from both the models were obtained in the fit statistics table and the model that had a lower BIC value was chosen. A difference of greater than 8 between the two models (BIC value with herd random effect – BIC value without herd random effect) shows a very substantial evidence of the herd variability. **Table 4.4** shows the BIC values obtained with and without the herd random effect in all the models, along with the covariance parameter estimates of the herd variability among different models with the same dependent variable. The residual variances for several traits (or σ^2_e) used in the models is also mentioned.

Table 4.4: Covariance parameter estimates for the herd random effect and residual variances in the models

Dependent variable	σ^2_{Herd}	σ^2_e	BIC values with herd effect	BIC values without herd effect	Difference
	COWS THAT HAD TWO LACTATIONS				
No. of services for second conception	0.04676	1.6536	26653.5	26708.9	55.4
No. of days from first to successful service	61.0399	2200.59	83045.9	83085.1	39.2
Days to first service	79.6105	330.16	69153.6	69547.9	394.3
Days open	109.84	2424.17	83926.5	84000.2	73.7
Calving interval	106.17	2446.27	83981.2	84051.5	70.3
	HEIFERS				
No. of services for first conception	0.1581	0.5037	25941.2	26659.9	718.7
No. of days from first to successful service	322.59	629.45	105630.6	106619.7	989.1
	COWS THAT FAILED TO HAVE A SECOND LACTATION				
No. of services attempted for second conception	1.8594	10.3138	14612.9	14696.6	83.7
Days to first service	88.3057	581.36	25158.4	25191.3	32.9

In this study, all the models that included the herd random effect showed lower BIC values with a difference exceeding 8 as compared with the BIC values obtained from the corresponding model that was run without the herd random effect. Thus, the herd variability for all the dependent variables was considered to be statistically significant for all groups of animals.

Also, from the covariance parameter estimates it can be inferred that for the dependent variable - Number of services required to conceive, a greater herd variability was observed in the group of heifers as compared to the group of cows that had two lactations. A similar pattern can also be seen for the dependent variable - Number of days between first and successful breeding. Whereas, for the dependent variable - Days to first service, a greater herd variability was observed in the group of cows that had failed to have a second lactation than the group of cows that had two lactations.

4.3 Fixed effect parameters

The objectives of this study (as explained in section 2.12) were to determine the effects of growth on the fertility of dairy heifers and first-lactation cows. In addition to these, the fertility status of the cows that failed to conceive during their first lactation was also studied. Body weight at 12 months of age by weight prediction model by Cue *et al*. (2012) has been used as a proxy of the growth of the animals. The statistical model, therefore, consisted of the fixed effect of body weight (12 months old), along with the fixed effects of region, age at first calving, season, and year-season (nested within seasons). The body weight and age at first calving variables were grouped into 12 distinct and non-overlapping categories as explained in section 3.2. The season and year-season represent the season when the animal was last inseminated and the latter represents the year, month, and date when the animal was last inseminated (i.e., the last service in the records which impregnated the heifer or cow or failed to impregnate the cow in its first lactation).

In addition to the said fixed effects, a fixed effect interaction of body weight and age at first calving was also tested for significance against each fertility parameter in the models using the F-tests. Wherever it was significant (based on at $P \leq 0.05$), the main effects of yearling body weight and age at first calving were dropped and only the interaction was retained in the model. A total of nine fertility traits were used for the analyses of the three groups of animals; five for the group of cows that had two lactations, two for the group of heifers, and two for the group of cows that failed to have a second lactation.

4.3.1 Fixed effects: Cows that had first lactation and a second calving

The five fertility parameters that were analyzed for the group of cows that conceived during their first lactation and had a second calving have been described in **Table 4.5**. This table presents all the type III tests of the fixed effects analyzed along with their significance (at $P \leq 0.05$ in **green/bold**) or non-significance (at $P \leq 0.05$ in Black/no bold). The effect that was not included in the model, based on its significance is marked in red as '**x**'.

The five fertility parameters that were analyzed are Number of services to second conception, Days between first and successful breeding, Days to first service, Days open, and Calving interval. **Table 4.5** below shows all the type III tests obtained from running all the five models. Each of the five models were run twice; firstly, with the interaction of body weight (12 months) and age at first calving fixed effects and their significance was checked. For all the fertility parameters the said interaction was found to be non-significant and was hence dropped from the model. Then, a second corresponding model was executed which included only the main fixed effects of body weight (12 months) and age at first calving separately, keeping the rest of the model unchanged.

Table 4.5: Cows that had two lactations: P-values obtained from type III tests of Fixed effects

Fixed effects	Number of services to second conception	Days between first and successful breeding	Days to first service	Days open	Calving interval
BW12*AGE AT 1ST CALVING	**x**	**x**	**x**	**x**	**x**
PREDICTED YEARLING BODY WEIGHT (kg)	0.2147	0.4251	0.8142	0.3121	0.2624
AGE AT 1st CALVING (months)	0.4144	0.1106	**0.0065**	**0.0117**	**0.0032**
YEAR-SEASON OF THE LAST SERVICE	**<.0001**	**<.0001**	0.4528	**<.0001**	**<.0001**
SEASON OF THE LAST SERVICE	0.2346	0.1051	0.4825	**0.0216**	**0.0116**
REGION	0.098	**0.0071**	**0.0044**	**0.0005**	**0.0005**

As illustrated in Type III tests in the table above, the yearling body weight and the age at first calving interaction does not seem to have a significant effect on any of the dependent variables tested for the group of cows that had two lactations. In addition to that, the fixed effect of body weight of cows at the age of 12 months also does not appear to have a significant influence on any of the dependent variables, which means that the yearling body weight of cows did not affect their first-lactation fertility based on the five parameters that were tested. This finding was not in agreement with the postulation. On the other hand, the fixed effect of age at first calving variable was found to be significant for three of the five fertility parameters that are, Days to first service, Days open, and Calving interval of the cows being non-significant for the Number of services and the Days between first and successful breeding of the cows done during their first lactation. Besides, the fixed effect of the year-season variable was statistically significant for four of the five dependent traits that are Number of services to second conception, Days between first and successful breeding, days open, and Calving interval being non-significant for the Days to first service parameter. Furthermore, the fixed effect of the season variable was found to be statistically significant for the Days open and Calving interval parameters. Lastly, the fixed effect of the region variable was statistically significant for all the fertility parameters tested except the Number of services to second conception.

4.3.2 Fixed effects: Heifers

Identical tests were performed for the group of heifers to examine their fertility before first calving. Therefore, all the records (lactation and breeding records) prior to their first calving were utilized for this study. Instead of five, only two fertility traits were available and examined for this group that are, Number of services to first conception and Days between first and successful breeding of the heifers. Similar to the previous model, a fixed effect interaction of yearling Body weight and the Age at first calving was included in both of the models. However, it was then dropped as it turned out to be statistically non-significant (at $P \leq 0.05$), thus the main effects of yearling Body weight and the Age at first calving were retained in both models as shown in **Table 4.6** below.

Table 4.6: Heifers: P-values obtained from type III tests of Fixed effects

Fixed effects	Number of services to first conception	Days between first and successful breeding
BW12*AGE AT 1ST CALVING	x	x
PREDICTED YEARLING BODY WEIGHT (kg)	<.0001	<.0001
AGE AT 1st CALVING (months)	<.0001	<.0001
YEAR-SEASON OF THE LAST SERVICE	<.0001	<.0001
SEASON OF THE LAST SERVICE	0.5051	0.0006
REGION	0.0015	0.0003

As stated in the hypothesis, the Type III tests (**Table 4.6**) obtained from the analyses show that main effect of the yearling body weight of the heifers did have a significant effect on the Number of services required for their first conception as well as on the Days between their first and successful breeding. Similarly, the fixed effect of Age at first calving also influences the Number of services the heifers required for their first conception as well as the Days between their first and successful breeding respectively. The fixed effect of Year-season (when the last/successful breeding was done) also had a significant effect on the fertility of the heifers, based on the Number of services to their first conception and the Days between their first and successful conception. Conversely, the fixed effect of season did not seem to have a significant effect on the Number of services the heifers required for their first pregnancy however, it did influence the Days between their first and successful breeding. Lastly, the fixed effect of region also had a significant effect on both fertility parameters tested for the group of heifers.

4.3.3 Fixed effects: Cows that failed to have a second lactation

For the group of cows that failed to have a second lactation, two fertility parameters (Number of services attempted for a second conception and Days to first service) were analyzed to look into the reasons behind their poor fertility. Similar to other models, each model was run twice; first with the fixed effect interaction of yearling Body weight and Age at first calving to check its significance. As shown in **Table 4.7**, the interaction was found to be statistically significant for the Days to first service dependent variable. As a result, the main effects of yearling Body weight and Age at first calving were removed from the model and only the interaction effect was retained to analyze the Days to first service (during the first lactation) of the cows that failed to have a second lactation. However, since the interaction was not statistically significant for the other model that analyzed the Number of services attempted for a second conception, it was dropped from the model and the main effects were retained. The main effects of yearling Body weight and Age at first calving were also non-significant for both fertility parameters. On the contrary, the fixed effect of year-season (of the last insemination, that was not successful) was statistically significant for both fertility parameters i.e., Number of services attempted for a second conception and Days to first service. The fixed effect of season, on the other hand, was only significant for one of the two fertility parameters analyzed i.e., the Days to first service. The fixed effect of the region variable was found non-significant for both fertility parameters analyzed.

Table 4.7: Cows that failed to have a second lactation: P-values obtained from type III tests of Fixed effects

Fixed effects	Number of services attempted for a second conception	Days to first service
BW12*AGE AT 1ST CALVING	**x**	**<.0001**
PREDICTED YEARLING BODY WEIGHT (kg)	0.8146	**x**
AGE AT 1st CALVING (months)	0.3132	**x**
YEAR-SEASON OF THE LAST SERVICE	**<.0001**	**0.0476**
SEASON OF THE LAST SERVICE	0.2781	**0.0288**
REGION	0.2319	0.4598

4.4 Fixed-effect estimates and the differences in the Least square means

Concurrently, the estimates of all fixed effects (or the least square means) along with the differences in their least square means were observed using the PROC MIXED procedure of SAS® software. The PDIFF option in the LSMEANS statement was used to generate the pair-wise differences in the least square means of all the fixed effects. The outputs obtained were then captured in a new dataset using the ODS (Output Delivery System) function. The least square means of the yearling body weight and their significant differences for all the dependent variables in each group will now be presented.

4.4.1 Fixed-effect estimates and differences in the least square means: Cows that had two lactations

Predicted yearling Body weight: As described in previously, the model consisted of the fixed effect of yearling body weight categories was examined against the dependent variables for the group of cows that had two lactations. The yearling body weights were divided into 12 distinct categories of 10 kg range each, starting from animals weighing less than 300 kg weight in category 1, 300-309 kg in category 2, 310-319 kg in category 3, and so on till category 12 which contained the animals weighing equal to and above 400 kg. The estimates and least square means of yearling body weight were not examined for this group as this variable was not significant for any of the dependent variables examined for fertility. It can thus be inferred that the body weights of the cow at 12 months of age does not impact their first-lactation fertility.

Age at first calving effect: The age at first calving effect was significant for Calving interval, Days open, and Days to first service dependent variables. Although there were no significant differences among the age at first calving categories, the cows falling in the category 1 (the cows that first calved when they were less than 22 months old) had a better fertility status as compared to the cows that calved older in terms of their Calving interval, Days open, and Days to first service. **Appendix 19 - 21** display the least square means estimates of the Age at first calving variable for Calving interval, Days open, and Days to first service dependent variables.

Region effect: The fixed effect of the region was found to be significant for the group of cows that had two lactations however, there were no significant differences found among the different regions. The cows belonging to the Estrie region had the shortest Calving intervals and Days to first service, whereas the Québec Capitale-Nationale region had the cows with the shortest Days open and the least number of days between their first and successful breeding during first breeding (see **Appendix 22-25)**.

Year-season and Season effect: The fixed effect of year-season (when the cow was last inseminated) was significant for the Calving interval, days open, Days between first and last breeding, and Number of services to the second conception. When the LSMEANS estimates were plotted against the Year-seasons in a chronological order, no trend could be determined as the data was scattered. Whereas the effect of seasons was only significant for the Calving interval, and Days open dependent variables. The cows that were artificially inseminated (last insemination during their first lactation) in the fall season had the smallest calving interval and Days open estimates. In addition, the fall and spring seasons were found to be significantly different from each other (based on $P \leq 0.05$) for the Calving interval variable. The cows that were inseminated in the fall season (last insemination during their first lactation), had a shorter calving interval as compared to the spring inseminated cows (see **Appendix 26)**.

4.4.2 Fixed-effect estimates and differences in the least square means: Heifers

Predicted yearling Body weight: There were two fertility parameters analyzed for the group of heifers. The body weight at 12 months of age was significant for both dependent traits tested for the fertility of heifers. The heifers falling in the Body weight category 1 (heifers that weighed less than 300 kg when they were 12 months old) had a better fertility as compared to the heavier heifers (**Appendix 27 and 28**) based on their Number of services required for first conception and the Days between first and successful breeding. In addition to that, there were some statistically significant differences among the different body weight categories as illustrated in **Appendix 29 & 30**.

Age at first calving effect: The age at first calving variable was significant for the Number of services to first conception and Number of days between the first and successful breeding of the heifers, as it is obvious that because the heifers that required fewer number of services had fewer number of days between their first and last insemination and hence calved early.

Region effect: The fixed effect of region was also found to be significant for the Number of services to first conception and Number of days between the first and successful breeding of the heifers, however, there were no significant differences found among various regions. The region estimates obtained from the analyses depicted that the heifers belonging to the Estrie region required a fewer number of services to their first conception and had least number of days between their first and successful breeding to their first pregnancy (**Appendix 31 & 32)**.

Year-season and Season effect: The year-season (when the cow was last inseminated) effect was statistically significant for both fertility parameters tested for the group of heifers although no chronological trend was observed in the plots of LSMEANS against the year-seasons. Whereas the Season (when the cow was last inseminated) effect was only significant for one of the two fertility traits i.e., Days between first and successful breeding. The heifers that were last serviced during the Fall season had the least number of Days between first and successful breeding. (**Appendix 33**). No significant differences among the seasons were found.

4.4.3 Fixed-effect estimates and differences in the least square means: Cows that failed to have a second lactation

Predicted yearling Body weight and Age at first calving interaction: There were two fertility parameters analyzed for the group of cows that failed to have a second lactation that are Number of services attempted during first lactation and Days to first service. The main effects of yearling body weight and age at first calving were not significant for either of the dependent variables. Whereas the yearling Body weight and Age at first calving interaction was found to be statistically significant for the Days to first service dependent variable with no significant differences among the different categories. From the estimates it can be deduced that the cows that belonged to the body weight category 1 (less than 300 kg at 12 months of age) resumed their cyclicity (following first calving) earlier than the heavier cows.

Year-season and Season effect: The year-season (when the cow was last inseminated during its first lactation) effect was statistically significant for both fertility parameters (Number of services attempted during first lactation and Days to first service) tested for the group of cows that failed to have a second lactation whereas the Season (when the cow was last inseminated during its first lactation) effect was only significant for one of the two fertility traits i.e., Days to first service following first lactation. However, no trend could be determined from the LSMEANS estimates and year-seasons plots as the data was scattered.

Region: Since the fixed effect of the region was not statistically significant for any of the dependent variables tested for the group of cows that failed to conceive during their second lactation, the region estimates were not examined for either of the fertility traits.

5. DISCUSSION

Although the fertility of heifers and cows can be influenced by a number of factors such as the age of the sire when the semen was collected, the dilution of semen before freezing, the artificial insemination technician, etc. (Jamrozik *et al*., 2005), the present study has only considered some of the fertility-influencing factors into account. These factors are Body weight of the heifer at 12 months of age, age at first calving, region, year and season (when the animal was last inseminated) effect.

Overall, it was found that the heifers required fewer number of services than the primiparous cows, reflecting a better reproductive performance than the lactating cows. This finding was in agreement with that of Badinga *et al.* (1985), Ron *et al.* (1984), and Orr *et al.* (1993). Furthermore, it was supported by the findings of Hansen and Areéchiga (1999), Wolfenson *et al.* (2000), and Thatcher and Collier (1986), which state that the lactating dairy cows are more susceptible to high temperatures than heifers, due to increase in the production of metabolic heat (leading to hyperthermia) associated with milk synthesis and feed intake. The heat stress also aggravates the effects of negative energy balance in primiparous cows (Shehab-El-Deen *et al.*, 2010). They also show increased incidence of anestrus and silent ovulation (De Rensis & Scaramuzzi, 2003). Therefore, the primiparous cows exhibit a compromised fertility as compared with heifers. The literature also supports this finding as Jansen *et al*. (1987) state that the fertility can change as the heifers age.

5.1 Heifers:

In the present study, it was found that the predicted yearling body weight has a significant effect on both fertility traits analyzed for the heifers. The fixed effects estimates showed that the heifers falling in the lighter body weight categories had better reproductive potential than the ones that lied in the heavier ones. This finding is in agreement with a recent study by Handcock *et al*. (2020) which stated that the heifers that lied towards the heavier body weight range used in the study experienced a decline in their reproductive performance compared with the heifers with lighter or mid-ranged body weight; their body weight was measured right before their breeding. Therefore, Handcock and colleagues (2020) concluded that a hike in the pre-breeding body weight of the heifers might not always be advantageous with respect to their fertility (measured as the ability to stay longer in the herd). In addition, Lucy (2001) also reported a negative relationship between accelerated growth (pre-pubertal growth, influenced by high average daily gain) and reproductive performance of the heifers. Correspondingly, Leaver's study (1977) also concluded that the heifers that did not conceive to the first artificial insemination were the ones that were heavier or were gaining body weight at a faster rate at the pre-pubertal stage. On the other hand, Donovan *et al*. (2003) found that the fertility of heifers with respect to their first service was not associated with any measure of body size or body condition.

Similarly, both the fertility traits used to study the heifer fertility were also influenced by the effect of age at first calving of the heifers. It is obvious that the heifers that conceived early had a better reproductive performance, due to which they calved early. Research by Wathes *et al*. (2008) found similar results where they reported that the heifers that calved early had the best reproductive performance. Also, these heifers stayed in the herd longer because of their good reproductive performance as cows as well. Van Amburgh *et al*., (1998) also reported in their study that the fertility of heifers was positively influenced by the age at first calving. Krpálková *et al*. (2014) also associated the age at first calving of the heifers with their body condition score.

The heifer fertility was also found to be influenced by the year and the season in which the heifer was inseminated. However, the number of services to first conception of the heifers was not found to be influenced by the separate season effect. This disagrees with the findings of Stevenson *et al*. (1984) that indicated that the services per conception were affected by seasons and agrees with those of Chebel *et al.* (2007), that state otherwise. Other findings (Al-Katanani *et al*., 1999; Cavestany *et al*., 1985) implied that cooler temperatures at the time of breeding showed better conception rates in heifers. This can be because of unobserved estrous during the summer season (Al-Katanani *et al*., 1999) caused by the heat stress. Various studies have shown that the heat stress during summers increases the length of estrus cycle and decreases the expression of estrus in heifers (Abilay *et al.*, 1975; Gangwar *et al.*, 1965; Madan & Johnson, 1973). A significant effect of seasons was also found on the Days to first service variable of heifers. This agrees with other findings of Al-Katanani *et al*. (1999) and De Rensis and Scaramuzzi (2003), which suggest that the heat stress of the summer season increases the number of days between first and successful breeding in the heifers. Wilson *et al.* (1998) also found that heat stress in heifers, inhibits the development of follicle during their preovulatory period. As a result, the estradiol concentrations in serum are reduced and late luteal phase progesterone decline is also delayed along with a greater number of follicular waves per estrus cycle.

5.2 Primiparous cows:

In the present study, it was found that the predicted yearling body weight of the heifers has no effect on any of the fertility traits analyzed for the primiparous cows that conceived during their first lactation. This finding is consistent with that of Macdonald *et al*. (2005) who affirmed that the pre- and post-puberty body weight of heifers did not affect their first-lactation reproductive performance. Whereas, another study by Wathes *et al*. (2014) reported that high (pre-breeding) growth rates can be detrimental to the fertility of cows during their first lactation. As the dry matter intake of the (post-calving) over conditioned cows is reduced, they tend to have greater fat mobilization and more severe and extended negative energy balance phase. They also take longer to increase their dry matter intake post calving (Roche *et al.*, 2009). In addition, Pryce *et al*. (2001) stated that cows with a low body condition score as heifers lose more body weight in their early lactation which compromises their first-lactation fertility.

The age at first calving variable was found to influence the days to first service, days open, and calving interval of these primiparous cows that conceived during their first lactation. The cows that calved early for the first time exhibited better reproductive performance than the late calvers. This trend is mirrored in a study done by Eastham *et al*. (2018) and Wathes *et al*. (2008), which showed that the heifers that calved early had a shorter subsequent calving interval than the late calvers as these heifers had better fertility as cows. On the contrary, Hare *et al*. (2006) reported a negative effect of age at first calving on the calving interval (between first and second calving) of the cows. Another study by Ettema and Santos (2004) also showed no effect of age at first calving on the subsequent calving interval of the cows, whereas (Krpálková *et al.,* 2014) found that the heifers that calved at less than 23 months of age had a better reproductive performance overall in their subsequent lactation. A similar study by Cooke *et al*. (2013) showed that the age at first calving exhibited a significant effect on Days to first service during the first lactation of the cows. On the other hand, Abeni *et al*., (2000) and Van Amburgh *et al*., (1998) outlined similar results, stating that earlier calving results in reduced reproductive performance of the cows overall. Simerl *et al*. (1992) reported that the first calving age did not affect the conception rate of the cows in their first lactation. In accord with the aforementioned findings, the present analysis showed that the age at first calving variable did not seem to have an effect on the Number of services to second conception and the Days between first and successful breedings of the cows during their first lactation.

The present study also showed that the season in which the cow was inseminated affected her Days open and Calving interval fertility traits. These results were in agreement with Doren *et al*. (1986) that also indicated that environmental variations influence Days open and Calving interval of cows. Hayes *et al*. (1992) also found similar results in their study. In addition to the season, Thaller (1998) also reported a significant effect of the month of insemination of the cows on their fertility, which was in accord with the findings from the current study. This can possibly be influenced by the exposure to specific environmental conditions during the month of insemination, such as heat stress that may impair the fertility of the dairy cows by negatively affecting estrus detection rates, thus decreasing the proportion of inseminated cows that maintain pregnancy along with affecting fertilization and embryo survival (Wolfenson *et al.*, 2000). An exposure to high temperatures may lead to more energy expenditures as the cows attempt to get rid of the excess heat (Fox & Tylutki, 1998). To be precise, an exposure to temperatures exceeding 27.5 °C result in lower pregnancy rates (Orr *et al.*, 1993). Similarly, cold stress can also have detrimental effects on the fertility of the dairy cows as it greatly increases the body maintenance requirements (Chebel *et al.*, 2007; Fox & Tylutki, 1998).

The effect of regions was also found to be significant for the Days to successful service, Days to first service, Days open and Calving interval variables tested for the first-lactation fertility of the cows. Likewise, Garcia-Peniche *et al*. (2005) analyzed seven different regions within the United States and found the effect of regions on the calving interval to be statistically significant. Variable management practices/decisions, environmental factors, semen used for the artificial insemination, and genetic makeup of the cows across different regions can be a possible reason behind the obtained results.

There were some cows present in the data that failed to conceive during their first lactation due to poor fertility. It can be seen from **Table 4.7** that their poor fertility is influenced by the year and season when they were last inseminated which is in agreement with other literature (Hayes *et al.*, 1992; Thaller, 1998). Their Days to their first service following first calving is also influenced by yearling body weight and age at first calving interaction.

6. CONCLUSION

The objective of this study was to determine if the growth of heifers during the rearing period affects their reproductive performance before and after their first calving. To study this, the yearling body weight of heifers was used to represent their growth at the attainment of puberty. The heifer body weights of all the animals measured at different ages were present in the data. Since the body weights at 12 months for all the animals were rarely reported, the heifer body weight at 12 months of age was predicted using Cue's body weight prediction model (Cue *et al.*, 2012). The fertility measures in Québec Holstein dairy heifers and primiparous cows were studied to investigate the effect of yearling body weight on the fertility measures. The data for this study were obtained from Valacta and a total of 13,250 animals were used for the analyses, which included 11,154 heifers, 7,890 cows that had two lactations, and 2,779 cows that failed to have a second calving with some animals among these three groups. Different fertility traits were examined to attain the objective of the study (Calving interval, Days open, Days between first and successful service, number of services, and Days to first service).

The findings from the present study supported the following conclusions:

- The yearling body weight of heifers only had a significant effect on the fertility of heifers by affecting the number of services they required for their first conception and the number of days between their first and successful breeding.
- The yearling body weight did not appear to have an effect on any of the fertility traits of the primiparous cows reviewed during their first lactation.
- The yearling body weight seemed to affect one of the two fertility traits examined for the group of cows that failed to have a second lactation. However, their yearling body weight effect interacted with the age at first calving effect. It implies that the age at first calving and yearling body weight together influenced the days to first service variable of the cows that failed to have a second lactation.

Overall, the findings of this study are in agreement with recent literature which states that the yearling body weight of the heifers (representing the attainment of puberty) affects the reproductive performance of heifers however, it does not affect the fertility of primiparous cows during their subsequent lactation. The study has also revealed that the fertility of heifers is better than that of primiparous cows as they require fewer number of services to first conception and also had fewer days between their first and successful service, which agrees with most of the recent findings. The disagreements between the present findings and others can be influenced by various factors, for instance, the genetic makeup of animals, distinct herd management practices, parameters considered for the analysis, variations amongst seasons, and/or regions present in the study, age at which the body weight was measured for the study, etc.

In addition to all the findings, it is also very important to note that a significant number of animals were lost from the analyses due to a lack of sufficient or reliable body weight and/or breeding records that were required for the study. There were 11,154 heifers analyzed in the study, but first lactation records of these heifers that went on to have a second lactation in the future were only present for 6,441 of them. This throws a light on the importance of consistent data recording practices by the producers in the dairy farms. This would not only help in a better understanding of the reproductive status of the animals but also help in providing other valuable information (like financial records, production records) to keep the farm running smoothly.

7. References

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APPENDICES

Appendix 1: Lactation start reasons

	LACTATION START REASON			
Lactat-ion no.	**Birth**	**Abortion**	**Hormone induction**	**Total animals**
1	16,844 (99.05%)	145 (0.85%)	16 (0.09%)	17,005
2	11,331 (98.76%)	139 (1.21%)	3 (0.02%)	11,473

Appendix 2: Animals that had 'Birth' as their lactation start reasons in both lactations

Lactation 1 start reason	Lactation 2 start reason	Number of animals
Birth	-	5,475
Birth	Birth	11,231
Birth	Abortion	135
Birth	Hormone induction	3
Abortion	Birth	89
Abortion	Abortion	4
Abortion	Hormone induction	0
Hormone induction	Birth	11
Hormone induction	Abortion	0
Hormone induction	Hormone induction	11

Appendix 3: Calving interval

Obs.	Calving interval	Number of animals
1.	Less than 300 days	06
2.	300 – 600 days	11,202
3.	No calving interval	5,475
	TOTAL	16,683

Appendix 4: Types of services

Obs.	Service type	Number of animals	Percent
1.	Technician	7693381	87.88
2.	Herd	884657	10.11
3.	Natural	68031	0.78
4.	Embryo transfer	105969	1.21
5.	Pasture/paddock	909	0.01
6.	In vitro	1379	0.02
Animals with missing service types = 106			

Appendix 5: Regions

Obs.	REGIONS AVAILABLE
1.	Bas-Saint-Laurent
2.	Saguenay-Lac-Saint-Jean
3.	QuébecCapitale-Nationale
4.	Mauricie
5.	Estrie
6.	Outaouais
7.	Abitibi-Témiscamingue
8.	Chaudière-Appalaches
9.	Lanaudière
10.	Laurentides
11.	Montérégie-Est
12.	Centre-du-Québec
13.	Montérégie-Ouest
	REGIONS REMOVED
14.	Montréal
15.	Côte-Nord
16.	Nord-du-Québec

Appendix 6: Body weight measurement methods

Obs.	Measurement method	No. of records	Percent
1.	Tape measurement	227,429	9.95
2.	Scale measurement	7,463	0.32
3.	Visual measurement	43,451	1.90
4.	Method not recorded	1,984,697	86.91
5.	Method with missing values	20,510	0.89

Appendix 7: Cows that had two lactations: Distribution of animals in Body weight categories

BW 12	ANIMALS	PERCENT
< 300	102	1.29
≥ 300 < 310	223	2.83
≥ 310 < 320	394	4.99
≥ 320 < 330	603	7.64
≥ 330 < 340	853	10.81
≥ 340 < 350	1077	13.65
≥ 350 < 360	1169	14.82
≥ 360 < 370	1111	14.08
≥ 370 < 380	885	11.22
≥ 380 < 390	669	8.48
≥ 390 < 400	383	4.85
< 300	421	5.34
TOTAL	7890	

Appendix 8: Cows that had two lactations: Distribution of animals in Age at first calving categories

AGE FC	ANIMALS	PERCENT
≥ 21 < 22	75	0.95
≥ 22 < 23	525	6.65
≥ 23 < 24	1492	18.91
≥ 24 < 45	1927	24.42
≥ 25 <26	1497	18.97
≥ 26 < 27	936	11.86
≥ 27 < 28	577	7.31
≥ 28 < 29	358	4.54
≥ 29 < 30	202	2.56
≥ 30 < 31	154	1.95
≥ 31 < 32	85	1.08
12	62	0.79
TOTAL	7890	

Appendix 9: Cows that had two lactations: Distribution of animals in the Regions

Obs.	REGION NAME	ANIMALS	PERECNT
1.	Bas-Saint-Laurent	881	11.17
2.	Saguenay-Lac-Saint-Jean	423	5.36
3.	QuébecCapitale-Nationale	412	5.22
4.	Mauricie	441	5.59
5.	Estrie	1455	18.44
6.	Outaouais	62	0.79
7.	Abitibi-Témiscamingue	179	2.27
8.	Chaudière-Appalaches	1395	17.68
9.	Lanaudière	133	1.69
10.	Laurentides	144	1.83
11.	Montérégie-Est	953	12.08
12.	Centre-du-Québec	791	10.03
13.	Montérégie-Ouest	621	7.87
	TOTAL	7890	

Appendix 10: Cows that had two lactations: Distribution of animals in the Seasons

Season	Animals	Percent
Fall	2343	29.70
Spring	1566	19.85
Summer	1905	24.14
Winter	2076	26.31
TOTAL	7890	

Appendix 11: Heifers: Distribution of animals in the Body weight (at 12 mo) categories

BW 12	ANIMALS	PERCENT
< 300	150	1.34
≥ 300 < 310	302	2.71

≥ 310 < 320	542	4.86
≥ 320 < 330	799	7.16
≥ 330 < 340	1119	10.03
≥ 340 < 350	1441	12.92
≥ 350 < 360	1659	14.87
≥ 360 < 370	1562	14.00
≥ 370 < 380	1310	11.74
≥ 380 < 390	956	8.57
≥ 390 < 400	622	5.58
< 300	692	6.20
TOTAL	11154	

Appendix 12: Heifers: Distribution of animals in Age at first calving categories

AGE FC	ANIMALS	PERCENT
≥ 21 < 22	119	1.07
≥ 22 < 23	759	6.80
≥ 23 < 24	2198	19.71
≥ 24 < 45	2610	23.40
≥ 25 <26	2059	18.46
≥ 26 < 27	1323	11.86
≥ 27 < 28	814	7.30
≥ 28 < 29	510	4.57
≥ 29 < 30	297	2.66
≥ 30 < 31	221	1.98
≥ 31 < 32	143	1.28
≥ 32 < 33	101	0.91
TOTAL	11154	

Appendix 13: Heifers: Distribution of animals in the Regions

Obs.	REGION NAME	ANIMALS	PERECNT
1.	Bas-Saint-Laurent	1270	11.39
2.	Saguenay-Lac-Saint-Jean	646	5.79
3.	QuébecCapitale-Nationale	537	4.81
4.	Mauricie	675	6.05
5.	Estrie	2061	18.48
6.	Outaouais	80	0.72
7.	Abitibi-Témiscamingue	303	2.72
8.	Chaudière-Appalaches	1839	16.49
9.	Lanaudière	199	1.78
10.	Laurentides	221	1.98
11.	Montérégie-Est	1311	11.75
12.	Centre-du-Québec	1155	10.36
13.	Montérégie-Ouest	857	7.68
	TOTAL	11154	

Appendix 14: Heifers: Distribution of animals in the Seasons

Season	Animals	Percent
Fall	3209	28.77
Spring	2554	22.90
Summer	2570	23.04
Winter	2821	25.29
TOTAL	11154	

Appendix 15: Cows that failed to have a second lactation: Distribution of animals in the Body weight (at 12 mo) categories

BW 12	ANIMALS	PERCENT
< 300	16	0.58
≥ 300 < 310	56	2.02
≥ 310 < 320	97	3.49
≥ 320 < 330	145	5.22
≥ 330 < 340	269	9.68
≥ 340 < 350	338	12.16
≥ 350 < 360	404	14.54
≥ 360 < 370	399	14.36
≥ 370 < 380	344	12.38
≥ 380 < 390	273	9.82
≥ 390 < 400	203	7.30
< 300	235	8.46

TOTAL	2779	

Appendix 16: Cows that failed to have a second lactation: Distribution of animals in the Age at first calving categories

AGE FC	ANIMALS	PERCENT
≥ 21 < 22	67	2.41
≥ 22 < 23	304	10.94
≥ 23 < 24	646	23.25
≥ 24 < 45	590	21.23
≥ 25 <26	460	16.55
≥ 26 < 27	276	9.93
≥ 27 < 28	173	6.23
≥ 28 < 29	104	3.74
≥ 29 < 30	67	2.41
≥ 30 < 31	47	1.69
≥ 31 < 32	27	0.97
≥ 32 < 33	18	0.65
TOTAL	2779	

Appendix 17: Cows that failed to have a second lactation: Distribution of animals in the Regions

Obs.	REGION NAME	ANIMALS	PERECNT
1.	Bas-Saint-Laurent	295	10.62
2.	Saguenay-Lac-Saint-Jean	182	6.55

3.	QuébecCapitale-Nationale	126	4.53
4.	Mauricie	202	7.27
5.	Estrie	585	21.05
6.	Outaouais	4	0.14
7.	Abitibi-Témiscamingue	78	2.81
8.	Chaudière-Appalaches	393	14.14
9.	Lanaudière	44	1.58
10.	Laurentides	66	2.37
11.	Montérégie-Est	366	13.17
12.	Centre-du-Québec	290	10.44
13.	Montérégie-Ouest	148	5.33
	TOTAL	2779	

Appendix 18: Cows that failed to have a second lactation: Distribution of animals in the Seasons

Season	Animals	Percent
Fall	797	28.68
Spring	609	21.91
Summer	622	22.38
Winter	751	27.02
TOTAL	2779	

Appendix 19: Cows that had two lactations: LS means estimates of Age at 1st calving variable tested against Calving interval

Obs.	Age at 1st calving	Estimate
1	≥ 21 < 22	389.31
2	≥ 22 < 23	392.47
3	≥ 23 < 24	392.86
4	≥ 24 < 45	394.30
5	≥ 25 <26	397.12
6	≥ 26 < 27	397.74
7	≥ 27 < 28	397.24
8	≥ 28 < 29	401.83
9	≥ 29 < 30	394.38
10	≥ 30 < 31	407.32
11	≥ 31 < 32	400.41
12	≥ 32 < 33	383.07

Appendix 20: Cows that had two lactations: LS means estimates of Age at 1st calving variable tested against Days open

Obs.	Age at 1st calving	Estimate
1	≥ 21 < 22	110.01
2	≥ 22 < 23	113.84
3	≥ 23 < 24	113.73
4	≥ 24 < 45	115.00
5	≥ 25 <26	117.57
6	≥ 26 < 27	118.18
7	≥ 27 < 28	117.46
8	≥ 28 < 29	121.44
9	≥ 29 < 30	114.00

10	≥ 30 < 31	127.20
11	≥ 31 < 32	120.20
12	≥ 32 < 33	102.94

Appendix 21: Cows that had two lactations: LS means estimates of Age at 1st calving variable tested against Days to first service

Obs.	Age at first calving	Estimate
1	≥ 21 < 22	71.7876
2	≥ 22 < 23	75.9277
3	≥ 23 < 24	76.2510
4	≥ 24 < 45	76.7137
5	≥ 25 <26	77.5940
6	≥ 26 < 27	78.2907
7	≥ 27 < 28	79.2160
8	≥ 28 < 29	78.2233
9	≥ 29 < 30	77.6443
10	≥ 30 < 31	78.2052
11	≥ 31 < 32	80.2931
12	≥ 32 < 33	72.1766

Appendix 22: Cows that had two lactations: LS means estimates of Region variable tested against Calving interval

Obs.	Region	Estimate
1	Bas-Saint-Laurent	393.81
2	Saguenay-Lac-Saint-Jean	396.83
3	QuébecCapitale-Nationale	387.73
4	Mauricie	403.07
5	Estrie	387.42
6	Outaouais	417.90
7	Abitibi-Témiscamingue	398.01
8	Chaudière-Appalaches	391.05
9	Lanaudière	405.18
10	Laurentides	393.57
11	Montérégie-Est	386.13
12	Centre-du-Québec	390.06
13	Montérégie-Ouest	392.93

Appendix 23: Cows that had two lactations: LS means estimates of Region variable tested against Days to first service

Obs.	Region	Estimate
1	Bas-Saint-Laurent	78.0671
2	Saguenay-Lac-Saint-Jean	75.5050
3	QuébecCapitale-Nationale	76.4875
4	Mauricie	75.3519
5	Estrie	71.9676
6	Outaouais	90.7075
7	Abitibi-Témiscamingue	74.5349
8	Chaudière-Appalaches	74.9215
9	Lanaudière	86.3078
10	Laurentides	73.2630

11	Montérégie-Est	73.4557
12	Centre-du-Québec	72.4198
13	Montérégie-Ouest	76.1945

Appendix 24: Cows that had two lactations: LS means estimates of Region variable tested against Days open

Obs.	Region	Estimate
1	Bas-Saint-Laurent	113.66
2	Saguenay-Lac-Saint-Jean	116.57
3	QuébecCapitale-Nationale	107.59
4	Mauricie	124.49
5	Estrie	108.11
6	Outaouais	138.83
7	Abitibi-Témiscamingue	117.77
8	Chaudière-Appalaches	111.07
9	Lanaudière	124.76
10	Laurentides	114.07
11	Montérégie-Est	106.26
12	Centre-du-Québec	110.70
13	Montérégie-Ouest	113.67

Appendix 25: Cows that had two lactations: LS means estimates of Region variable tested against Days between first and successful service

Obs.	Region	Estimate
1	Bas-Saint-Laurent	35.9028
2	Saguenay-Lac-Saint-Jean	41.5247
3	QuébecCapitale-Nationale	31.9039
4	Mauricie	48.1876
5	Estrie	36.1614
6	Outaouais	47.8892
7	Abitibi-Témiscamingue	43.8296
8	Chaudière-Appalaches	36.3149
9	Lanaudière	40.4554
10	Laurentides	39.8939
11	Montérégie-Est	33.1103
12	Centre-du-Québec	38.1067
13	Montérégie-Ouest	37.5800

Appendix 26: Cows that had two lactations: LS means estimates of Season variable tested against Calving interval

Obs.	Season	Estimate
1	Fall	387.88
2	Spring	402.50
3	Summer	395.90
4	Winter	396.39

Appendix 27: Heifers: LS means estimates of BW12 variable tested against Days between first and successful service

Obs.	BW (12 mo)	Estimates
1	< 300	42.9367
2	≥ 300 < 310	43.8970
3	≥ 310 < 320	48.1686
4	≥ 320 < 330	47.7887
5	≥ 330 < 340	51.5904
6	≥ 340 < 350	53.3590
7	≥ 350 < 360	55.2820
8	≥ 360 < 370	57.9693
9	≥ 370 < 380	58.9101
10	≥ 380 < 390	60.2185
11	≥ 390 < 400	62.2245
12	≥ 400	61.7929
TOTAL	2779	

Appendix 28: Heifers: LS means estimates of BW12 variable tested against Number of services to first conception

Obs.	BW 12 (kg)	Estimates
1	< 300	1.8770
2	≥ 300 < 310	1.8844
3	≥ 310 < 320	1.9682
4	≥ 320 < 330	1.9455
5	≥ 330 < 340	2.0425
6	≥ 340 < 350	2.0670
7	≥ 350 < 360	2.1495
8	≥ 360 < 370	2.2033
9	≥ 370 < 380	2.2155
10	≥ 380 < 390	2.2864
11	≥ 390 < 400	2.3087
12	≥ 400	0.65
TOTAL	2779	

Appendix 29: Heifers: Significant differences between BW12 categories tested against Days between first and successful service

BW 12 (kg)	BW 12 (kg)	Difference
< 300	≥ 350 < 360	-12.3453
< 300	≥ 360 < 370	-15.0326
< 300	≥ 370 < 380	-15.9733
< 300	≥ 380 < 390	-17.2818
< 300	≥ 390 < 400	-19.2878
< 300	>400	-18.8562
≥ 300 < 310	≥ 340 < 350	-9.4619
≥ 300 < 310	≥ 350 < 360	-11.3850
≥ 300 < 310	≥ 360 < 370	-14.0722
≥ 300 < 310	≥ 370 < 380	-15.0130
≥ 300 < 310	≥ 380 < 390	-16.3215
≥ 300 < 310	≥ 390 < 400	-18.3274
≥ 300 < 310	≥ 400	-17.8958
≥ 310 < 320	≥ 350 < 360	-7.1134
≥ 310 < 320	≥ 360 < 370	-9.8007
≥ 310 < 320	≥ 370 < 380	-10.7415
≥ 310 < 320	≥ 380 < 390	-12.0499
≥ 310 < 320	≥ 390 < 400	-14.0559
≥ 310 < 320	≥ 400	-13.6243

≥ 320 < 330	≥ 340 < 350	-5.5703
≥ 320 < 330	≥ 350 < 360	-7.4934
≥ 320 < 330	≥ 360 < 370	-10.1806
≥ 320 < 330	≥ 370 < 380	-11.1214
≥ 320 < 330	≥ 380 < 390	-12.4299
≥ 320 < 330	≥ 390 < 400	-14.4358
≥ 320 < 330	≥ 400	-14.0042
≥ 330 < 340	≥ 360 < 370	-6.3789
≥ 330 < 340	≥ 370 < 380	-7.3197
≥ 330 < 340	≥ 380 < 390	-8.6281
≥ 330 < 340	≥ 390 < 400	-10.6341
≥ 330 < 340	≥ 400	-10.2025
≥ 340 < 350	≥ 360 < 370	-4.6103
≥ 340 < 350	≥ 370 < 380	-5.5511
≥ 340 < 350	≥ 380 < 390	-6.8595
≥ 340 < 350	≥ 390 < 400	-8.8655
≥ 340 < 350	≥ 400	-8.4339
≥ 350 < 360	≥ 390 < 400	-6.9425
≥ 350 < 360	≥ 400	-6.5109

Appendix 30: Heifers: Significant differences between BW12 categories tested against Number of services to first conception

BW 12 (kg)	BW 12 (kg)	Difference
< 300	≥ 360 < 370	-0.3263
< 300	≥ 370 < 380	-0.3385
< 300	≥ 380 < 390	-0.4094
< 300	≥ 390 < 400	-0.4317
< 300	≥ 400	-0.4266
≥ 300 < 310	≥ 350 < 360	-0.2652
≥ 300 < 310	≥ 360 < 370	-0.3189
≥ 300 < 310	≥ 370 < 380	-0.3312
≥ 300 < 310	≥ 380 < 390	-0.4020
≥ 300 < 310	≥ 390 < 400	-0.4244
≥ 300 < 310	≥ 400	-0.4192
≥ 310 < 320	≥ 350 < 360	-0.1814
≥ 310 < 320	≥ 360 < 370	-0.2351
≥ 310 < 320	≥ 370 < 380	-0.2473
≥ 310 < 320	≥ 380 < 390	-0.3182
≥ 310 < 320	≥ 390 < 400	-0.3406
≥ 310 < 320	≥ 400	-0.3354
≥ 320 < 330	≥ 350 < 360	-0.2040
≥ 320 < 330	≥ 360 < 370	-0.2577
≥ 320 < 330	≥ 370 < 380	-0.2700
≥ 320 < 330	≥ 380 < 390	-0.3408
≥ 320 < 330	≥ 390 < 400	-0.3632
≥ 320 < 330	≥ 400	-0.3580
≥ 330 < 340	≥ 360 < 370	-0.1607
≥ 330 < 340	≥ 370 < 380	-0.1730
≥ 330 < 340	≥ 380 < 390	-0.2438
≥ 330 < 340	≥ 390 < 400	-0.2662
≥ 330 < 340	≥ 400	-0.2610
≥ 340 < 350	≥ 360 < 370	-0.1362
≥ 340 < 350	≥ 370 < 380	-0.1485
≥ 340 < 350	≥ 380 < 390	-0.2193
≥ 340 < 350	≥ 390 < 400	-0.2417
≥ 340 < 350	≥ 400	-0.2365

Appendix 31: Heifers: LS means estimates of Region variable tested against Days between first and successful service

Obs.	Region	Estimate
1	Bas-Saint-Laurent	47.4576
2	Saguenay-Lac-Saint-Jean	56.3150
3	QuébecCapitale-Nationale	51.4567
4	Mauricie	62.7572
5	Estrie	41.3897
6	Outaouais	58.3733
7	Abitibi-Témiscamingue	60.3162
8	Chaudière-Appalaches	49.7866
9	Lanaudière	62.1737
10	Laurentides	51.2000
11	Montérégie-Est	53.1689
12	Centre-du-Québec	50.9863
13	Montérégie-Ouest	52.4345

Appendix 32: Heifers: LS means estimates of Region variable tested against Number of services to first conception

Obs.	Region	Estimate
1	Bas-Saint-Laurent	1.9494
2	Saguenay-Lac-Saint-Jean	2.2113
3	QuébecCapitale-Nationale	2.0725
4	Mauricie	2.2996
5	Estrie	1.8539
6	Outaouais	2.0792
7	Abitibi-Témiscamingue	2.1775
8	Chaudière-Appalaches	2.0507
9	Lanaudière	2.2855
10	Laurentides	2.1177
11	Montérégie-Est	2.0932
12	Centre-du-Québec	2.0736
13	Montérégie-Ouest	2.0918

Appendix 33: Heifers: LS means estimates of Season variable tested against Days between first and successful service

Obs.	Season	Estimate
1	Fall	51.8242
2	Spring	56.1233
3	Summer	53.1117
4	Winter	53.6534