INVESTIGATION OF INBREEDING RATE AND ITS INFLUENCE ON STILLBIRTH IN SOUTH AFRICAN HOLSTEIN DAIRY CATTLE

by

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DECLARATION

I, MOJAKGOMO SIDNEY MAMAKOKO, declare that this research report hereby submitted to the University of Limpopo for the degree of Master of Science in Agriculture (Animal Production) has not been submitted by me for a degree at this or any other university, this is my own work in design and execution, and that all materials contained herein has been duly acknowledged. The research was approved by the University of Limpopo Animal Research Ethics Committee (Registration no. AREC-290914-017) (Appendix A).

Signature… Date……………………

The above declaration is confirmed by.

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DEDICATION

This work is dedicated to my parents; Chego Mamakoko and Nelly Mamakoko, my siblings who were always in support of me in my journey.

Abstract

There is no study done at the population level on stillbirth in Holstein dairy cattle population of South Africa. The current study was conducted to evaluate the inbreeding rate and its influence across generations on incidences of stillbirth in South African Holstein dairy population. The dataset included about 1 million Holstein dairy cattle born between the year of 1945 to 2020. Pedigree information included animal ID, sire ID, dam ID, breeder and farm region, other information included date of birth, calf sex, dam parity and age at parturition, calving date, herd, and birth status. Generation intervals were determined using ENDOG software (version 4.8), while General Linear Model (GLM) procedure at 5% significant level was used to model incidences of stillbirth. Phenotypic trends were determined using regression procedure, and SAS was used to analyse regression of inbreeding rate effect on the incidences of stillbirth per generation. Effective population size, inbreeding coefficients and inbreeding rate were estimated using Contribution Inbreeding Coancestry (CFC) software. The results indicated that dam parity, herd, calf sex, generation, birth season and birth year had a significant effect ($P<0.05$) on the incidences of stillbirth. The multiparous, autumn (3.397±0.067), summer (3.306±0.067), and female calves (3.516±0.046) had high incidences of stillbirth than primiparous, spring (3.073±0.067), winter (3.00±0.063 and male calves (2.922±0.028). Stillbirth incidences were observed to increase with birth year and generation, while decreasing with an increase in dam parity and dam age. Incidences of stillbirth was very different across the herds ranging from a minimum of 0% and a maximum of 100%, however most of the herds had zero incidences of stillbirth (3.736±0.251). This indicated that stillbirth problem in South African Holstein dairy population is at the herd level not at the population level. Inbreds were only 0.48% of the population, with an average inbreeding coefficient ranging from 2.48% to 24.60%. Average discreet generation equivalents (DGE) approximated one for most generations, with a range of 0.226 to 1.256, this highlight that majority of the generations were complete. Animals in the 1st to the 12th generation were closely related, while from the 12th to 14th generation they were less related to each other. Inbreeding coefficients ranged from 0.0020% to 0.1099%, with inbreeding rate increasing per generation and recording the lowest value (0.0479) and the highest (0.5536) in the 13th and 2nd generations respectively.

Effective population size was observed to depend on the number of breeding males, with the 2nd generation showing lowest number of breeding males (23 sires) and the lowest effective population size (90.3), while the 13th generation had the highest number of breeding males (264 sires) and the highest effective population size (10447.7). The regression model was observed to be statistically significant ($P<0.05$) with R^2 value of 88.61%, however the evaluated factors; number of individuals, no of inbreds, no of founders, average F, average F in the inbreds, effective population size and inbreeding rate were not significant on incidences of stillbirth. Inbreeding rate in the current study had a significant effect on stillbirth incidences in the South African Holstein population. Stillbirth incidences increased with effective population size with an R square of 12% but decreased with an increase in inbreeding rate and average inbreeding coefficients in the inbreds, with R square values of 13% and 20% respectively. Inbreeding contributed only 20% towards the stillbirth incidences indicating that stillbirth in the South African Holstein population is caused by other factors other than the inbreeding. Stillbirth incidences increased with effective population size and a reduced with an increase in average inbreeding coefficients in the inbreds. However, generations increased with stillbirth incidences and effective population size with R^2 of 60% and 92% respectively, indicating that generation had 60% contribution to stillbirth incidences. Generation increased with a reduction in inbreeding rate and average inbreeding coefficients in the inbreds with R squares of 35% and 53% respectively. Stillbirth incidences in the South African Holstein population is not caused by the genetic factors such as inbreeding rate, this can be because stillbirth is a lowly heritable trait meaning it is more affected by environmental factors other than genetic factors.

Key words: Effective population size, regression, discreet generation equivalents, birth year, dam parity

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LIST OF ABBREVIATIONS

AI	Artificial Insemination
ARC	Agricultural Research Council
AR	Average relatedness
BLUP	Best Linear Unbiased Prediction
CFC	Contribution Inbreeding Coancestry
CI	Calving Interval
CR	Conception Rate
DGE	Discreet Generation Equivalents
EBVs	Estimated breeding values
F	Inbreeding coefficient
GI	Generation intervals
GLM	General Linear Model
h^2	Heritability
HPR	Holstein Profit Ranking
INTERGIS	Integrated Registration and Genetic Information System
MOP	Milk Producers Organisation
MS Excel	Microsoft Excel
Ne	Effective Population Size
Nf	Number of breeding females
Nm	Number of breeding males
PR	Pregnancy Rate
SA	South Africa

SAS	Statistical Analysis Software
SCS	somatic cell score
TMR	Total Mixed Ration
US	United States
ΔF	Inbreeding rate
σ^2	Population Variance

CHAPTER ONE

INTRODUCTION

1.1. Background and Introduction

Inbreeding occurs when individuals mated to each other have a common ancestor and is unavoidable in the commercial breeding programs in dairy cattle, it can result in loss of heterozygosity, genetic drift and decrease in genetic variation (Hinrichs and Thaller, 2011). Several schoolers reported a significant association between inbreeding and stillbirth incidences in Holstein dairy cattle, showing that an increase in inbreeding is associated with an increase in the incidences of stillbirth (Piwczyński *et al*., 2013; Mahnani *et al*., 2018; Morek-Kopeć *et al*., 2021). Stillbirth is the birth in which an animal is born dead or dies during or within 24 hours' time after parturition and after at least 260 days of gestation (Lombard *et al*., 2007; Gundelach *et al.,* 2009; Szücs *et al*., 2009; Schuenemann *et al*., 2011; Antanaitis *et al*., 2022). If the dead calf is delivered earlier than 215 days, it is categorised as an abortion (Szücs *et al*., 2009).

Stillbirth incidences follows a similar pattern to the occurrence of dystocia, with high occurrence at first parity than in later parities (Morek-Kopeć *et al*., 2021). There is a strong association between stillbirth and dystocia predominantly in heifers (Szücs *et al*., 2009) and first-time calves rate of stillbirth rages from 4 to 17% in most studies, while it ranges from 2 to 10% in later calving's (Morek-Kopeć *et al*., 2021). Dystocia is considered as the most significant factor for stillbirth, but together the stillbirth and dystocia are caused by environmental and genetical factors. Both the genotype of the calf as direct effect and that of the dam as maternal effect contribute to the risk of stillbirth and dystocia (Szücs *et al*., 2009).

Direct effect is the calf ability to be born without problems while maternal effect is the dam ability to calve without problems, other factors include birth weight, sex of calf, parity, age of dam and season of calving (Szücs *et al*., 2009; Morek-Kopeć *et al*., 2021; Ratshivhombela, 2021). Accurate animal selection to achieve genetic improvement can reduce stillbirth within the Holstein dairy cattle population, but this will depend on a sound genetic evaluation programme (Ratshivhombela, 2021). The South African dairy industry comprises various economic activities with significant differences in farming methods and the processing of dairy products, including the production and marketing of raw milk, pasteurised milk and cream, fermented milk, long-life milk and cream, yoghurt, and cheese and its by-products (SA Yearbook, 2017/18).

The selection of animals in South African dairy cattle population has been focused mostly on increasing milk yield and less focused on type improvement (National Dairy Animal Improvement Scheme, 2018). There are less publications on functional traits such as stillbirth within the South African Holstein dairy cattle population (Ratshivhombela, 2021). Development and implementation of broader breeding objectives, including all the economically important trait for South African Holstein dairy cattle such as stillbirth is important and will ensure genetic progress.

1.2. Problem statement

In all dairy cattle breeds, one of the most economically and ethically important trait is stillbirth (Steinbock *et al*., 2006). In South Africa (SA), Holstein dairy breed is the commonly used dairy breed (Banga *et al*., 2014). It is in the interest of the dairy industry, farmers and the public that stillbirth incidence is minimised (Steinbock *et al*., 2006; Antanaitis *et al*., 2022). Dairy cattle breeders are increasingly becoming interested in selection for functional traits. Currently, selection is focused on traits reducing costs (disease resistance) than those increasing profit (Eaglen *et al.,* 2012; Morek-Kopeć *et al*., 2021). Stillbirth can be classified as a trait of economic importance (Antanaitis *et al*., 2022), thus the knowledge of the cost and economic losses related to it may assist in decision-making (Mahnani *et al*., 2018).

The rate of stillbirth increased with about 6% to 11% globally during the past 25 years in Holstein dairy cattle, this indicates a problem associated with enhancing milk production (Gustafsson *et al*., 2007; Gullstrand, 2017; Mahnani *et al*., 2018). Costs associated with stillbirth increase due to premature culling, veterinary services, the dam, and calf mortality (Mahnani *et al*., 2018). Ultimately, undesirable reproductive and production performance through deterioration in milk yield, longevity of cows and the increasing cost of replacement stock are envisaged to deprive enterprise revenue (Szücs *et al*., 2009; Piwczyński *et al*., 2013; Mellado *et al*., 2017; Mahnani *et al.,* 2018).

Globally in the populations of dairy cattle, the increasing rate of stillbirth is a major problem (Piwczyński *et al*., 2013). Stillbirth in Holstein dairy cattle have been reported in other countries such as Sweden with less publications in South Africa. However, Ratshivhombela (2021) reported incidences of 8.26% and 4.54% in 2014 and 2018 cows, respectively. No studies have been done on inbreeding coefficient of dairy cattle in South Africa at the population level. The Ministry of Agriculture (2007) reported that stillbirth is a much larger problem during the first calving as compared to later calving. The calving process and its postpartum phase are stressful to the cow (Antanaitis *et al*., 2022). Thus, high stillbirth incidences and dystocia are unacceptable from animals' welfare point of view (Gullstrand, 2017) and it is costly to farmers (Szücs *et al*., 2009).

1.3. Rationale

A recent study on South African Holstein by Ratshivhombela (2021) about the heritability of stillbirth incidence showed that it is lowly heritable (0.09±0.03), thus indicating a high chance of addressing the problem through mating strategies and selection decisions. Intensive selection reduces the effective population size resulting in increased inbreeding coefficient (Adamec *et al*., 2006). Inbreeding reduces the embryo survival and conception rate and result in high risk of stillbirth (Hinrichs and Thaller, 2011). Hinrichs and Thaller (2011) and Atashi *et al.* (2012) reported a significant relationship between the stillbirth and inbreeding from German Holstein cattle whereby an increase in the calf-inbreeding coefficient was associated with risk increase of stillbirth with 0.22% for every 1% rise of inbreeding coefficient.

Inbreeding has for long been linked to reduced reproductive performance of dairy cattle in general (Hinrichs and Thaller, 2011). Inbreeding effect on reproductive performance in young United States (US) Holstein cows saw a reduction in mature US Holstein cows (Adamec *et al*., 2006). Inbreeding result in high risk of stillbirth which reduces productivity and reproductive performance (Mellado *et al*., 2017) as it is correlated to rising cow culling rate due to poor reproductive performance and lower production of milk.

Globally, only few sires dominated the Holstein breeding programs due to intense selection for quick genetic progress, but there is increasing concern of the detrimental influence of utilizing few superior bulls. Selection is a vital tool to the dairy breeding programs hence it accounts for inbreeding and the relationship of animals within a population (Koenig and Simianer, 2006). There is hope of solving the stillbirth issue by looking at the inbreeding coefficients of Holstein dairy cattle, which is the largest dairy cattle breed in South Africa. The current study provides information to farmers about the effect of inbreeding on the incidences of stillbirth and identify factors contributing to stillbirth and how to overcome them.

1.3.1. Aim

The aim of the study was to outline the inbreeding rate and its influence across generations on the incidences of stillbirth in South Africa Holstein dairy cattle.

1.3.2. Objectives

The objectives of the study were to determine:

i. Incidence of stillbirth across generations in South African Holstein dairy cattle population.

ii. Inbreeding rate and the effective population size within herds of South African Holstein dairy cattle using pedigree data.

iii. Effect of inbreeding rate on the incidences of stillbirth across generations in South African Holstein dairy cattle.

1.3.3. Null Hypotheses

In this study the null hypotheses that were tested were:

i. The incidences of stillbirth across generations in South African Holstein dairy cattle are the same.

ii. There is no difference in the inbreeding rate and the effective population sizes across herds of South African Holstein dairy cattle.

iii. The inbreeding rate has no effect on the incidence of stillbirth across generations in South African Holstein dairy cattle.

CHAPTER TWO

LITERATURE REVIEW

2.1. Introduction

The aspects to be discussed under this Chapter include dairy cattle production, history and recording schemes, and breeding of Holstein dairy cattle in SA. Other aspects are general factors affecting stillbirth in dairy cattle, inbreeding in dairy cattle production, pedigree information and inbreeding rate, inbreeding coefficient, and effective population size in dairy cattle.

2.2. Dairy cattle production in South Africa

In South Africa dairy cattle production take place mostly in the Eastern and Northern Free State, North West, the KwaZulu-Natal Midlands, the Eastern and Western Cape, Gauteng and the Southern parts of Mpumalanga, this is due to the favourable weather conditions and availability of fodder (Myburgh *et al.*, 2012; Opoola, 2019). In South Africa six exotic dairy cattle breeds are recognized which include the Holstein, Jersey, Guernsey, Ayrshire, Swiss (Brown and Dairy), and Dairy Shorthorn (DAFF, 2012). Dairy cattle production is the primary sector of South African dairy industry and it represent the milk producers (MPO statistics, 2011). There was limited effect on the dairy market of South Africa due to the COVID-19 pandemic even thou it resulted in major disruption in other markets. However particular dairy products got affected with most of them having better performance under such circumstances (Coetzee, 2021). Within sub-Saharan Africa the dairy production systems of South Africa are one of the most organised aiming at fertility traits improvement and milk yield (Opoola, 2019). In January 2015 in South Africa the number of dairy farmers decreased from 1 834 to 1 053 and today this number decreased by 43% since 2015 with the largest decrease taking place in the Northern Cape (Coetzee, 2017). The Milk Producers Organisation (MOP) negotiate on behalf of the producers with the government or any other establishments and makes information about the statistical and management accessible to the produces, dairy industry, and other authorities (Kgole, 2013).

In 2018 South African milk intake was 4.64% higher than in 2017 and the demand was exceeded by the supply such that the producers’ prices dropped by 14% (MPO statistic, 2018). South African dairy cattle milk production contributes approximately 0.5 % to the world milk production (SA yearbook, 2017/18). In 2017 up to 44% were imported and 3.2% less of dairy products were exported in and out of South Africa respectively (MPO statistic, 2018). The South African dairy cattle production is in a difficult situation with about 40% of the dairy farmers leaving the dairy industry in the past 18 years (Grobler *et al*., 2008; Lassen, 2012*)*. The rising costs of inputs in dairy farming and low farm gate prices for milk paid by processors are the main causes of the decrease in the total number of dairy producers in South Africa (Theron and Mostert, 2009; Metaxas, 2016). The total milk production increases, even though the number of dairy producers is decreasing (Muller, 2017). This is mainly because of the increase in the average size of the dairy herds in South Africa.

Additionally, there is a shift in the geographic distribution of dairy farming with the coastal areas producing larger percentage of total milk, this is because of the low production costs from cultivated pastures (Gertenbach, 2007; Mkhabela and Mndeme, 2010; Metaxas, 2016). In the urban areas dairy farming is generally on total mixed ration (TMR) system and there is a need to produce fresh milk closer to the urban area with high density (Metaxas, 2016). About 2,300 dairy farmers in 2012 were producing approximately 2,6 billion litres of milk per year (Lassen, 2012).

As a result of the increased investment in the housing and milking facilities, the herd size must be double or triple so that the expansion of the enterprise be profitable (Theron and Mostert, 2009). The Agricultural Research Council (ARC) plays a major role in managing the National Dairy Animal Recording and Improvement Scheme (MPO statistics, 2011; SA yearbook, 2017/18). The main dairy cattle breeds used in the South African dairy cattle production industry are the Ayrshire, Holstein, Jersey, and Guernsey, with the Holstein covering about 60% of the South African herd (SA Yearbook, 2010/14). Meissner *et al*. (2013) reported that in South Africa there is about 1.4 million dairy cattle, and it is mostly Holstein cattle.

2.3. History of Holstein dairy cattle in SA and recoding schemes

Holstein is the most widely used dairy cattle breed in South Africa (Opoola, 2019). This is mainly due to its outstanding milk production, of all dairy breeds Holstein is the highest milk producing breed and it is reported to be the most economical producer of milk protein and fat (Dairy moos, 2016; Ratshivhombela, 2021). Van Marle-Köster and Visser (2018) reported that South African dairy industry is dominated by two cattle breeds namely the Holstein and Jersey breeds with average herd size of about 400 cows. Holstein dairy cattle were bred about 2 millennia back in the Netherlands and it is believed that two European breeds namely the Holstein from North or South America and the Friesian from Europe were responsible for the establishment of Holstein Friesian (Ratshivhombela, 2021).

South African Stud Book registered the breed for the first time in 1906 and the Holstein-Friesland Cattle Breeders Society of South Africa was established six years later (1912) (Dairy moos, 2016; Ratshivhombela, 2021). In South African milk recording, the largest participating breed is the Holstein dairy breed, accounting to 57% to the total of dairy cows, Jersey (38%), Ayrshire (4%) and Guernsey (4%) breed followed respectively. During the test year 2004 in Milk Recording more commercially registered Holsteins participated than registered Guernsey cows (Mostert, 2007).

In the national milk recoding scheme over the past decade participation has reduced with 24% among commercial producers, with the movement to automatic milk systems and recording particularly in larger herds (Van Marle-Köster and Visser, 2018). On average per 305-day lactation Holstein cows produce 7 441 kg of milk as compared to other dairy breeds such as Ayrshire, Guernsey and Jersey producing 6 072 kg, 5 570 kg, and 5 187 kg respectively (Ratshivhombela, 2021).

Breeders and producers tend to disregard traits like calving performance/stillbirth focusing on the high milk productivity of the Holstein dairy cattle. Estimated breeding values (EBVs) of Holstein cattle have been routinely produced under the National Genetic Evaluation Programme for five milk production traits, 17 linear-type traits, somatic cell score (SCS) and calving interval (Banga *et al*., 2014). Holstein dairy cattle are well adapted to all systems of management and utilization but are equally suitable for grazing and the heifers can be bred at 1 year 3 months weighing about 362.87 kg (Ratshivhombela, 2021). Figure 2.1. bellow shows the Holstein cattle breed cows.

Figure 2.1: Holstein cattle breed (Farmer's weekly, 2018).

2.4. Breeding of Holstein dairy cattle in SA

Genetics contribute a lot to increasing milk production, but there are other factors that take part such as good nutrition, sound management, and other environmental conditions (Kgari, 2020). Breeding of cattle generally includes measures that are organized and zootechnical aimed at improvement of economic and useful qualities of animals (Banga *et al*., 2014; Kharina *et al*., 2021). The main objective for breeding is to rear animals with high production, improve animals' productivity and create new breeds (Kharina *et al*., 2021), but in dairy cattle the primary goal of breeding is to improve milk production profitability as a result a breeding objective should cover all traits that are economically relevant in the dairy production (Banga *et al*., 2014; Kidane *et al*., 2019). Breeding objectives in the past for dairy cattle solely focused on production traits, however in recent years they comprise a wider range of traits of economic importance (Banga *et al*., 2014).

Since mid-1950s there was massive expansion in dairy breeding due to semen freezing and development of artificial insemination industry. Dairy cattle breeding is currently a highly specialised science that breeders can use it advantageously to improve the herds' profit (Mostert, 2007). Breeding in dairy cattle focuses on increasing the milk yield, protein, and fat content in milk; preserving fertility and productive longevity and developing high adaptive properties of suitability for modern industrial technologies (Kharina *et al*., 2021). Breeding programme that are perfectly designed and correctly implemented in the commercial enterprises results in increased rate of cost-effective genetic improvement. Recently, there has been a pressing need to apply advanced knowledge to developing more comprehensive dairy cattle breeding objectives in South Africa (Banga *et al*., 2014).

2.4.1. Selection criteria of dairy cattle

Selection in the Holstein dairy cattle of South African population mainly focused on increased yields of solids and on improved type of animals to a lesser extent as indicated by the genetic trends (Banga *et al*., 2014). In Holstein dairy cattle, selection focuses mainly on the production traits, and this led a remarkable increase in milk yield, but it causes a reduction in non-yield functional traits (Morek-Kopeć *et al*., 2021). Several features such as product quality, productivity level, milk production rate, diseases resistance and udder shape are used in selecting animals. As a result, animals are highly suitable in the modern high-tech condition (Kharina *et al*., 2021). Furthermore, several non-production traits play a vital role in limiting losses due to illness and mortality and maximizing the longevity (Szücs *et al*., 2009).

Selection of animals having best breeding values is used to achieve genetic improvement with a population of animal, hence it is vital to estimate breeding values accurately (Mostert, 2007). In the past 2 decades a large increase have been realized in the genetic merit for yield traits and a significant genetic difference in some linear type traits. However, there is no clear indication if these genetic trends are all towards the desirable direction or what the overall value of this amounts to (Banga *et al*., 2014). Initially in South Africa dairy sires were genetically evaluated based on progeny groups utilizing data from National Livestock Improvement Scheme and this resulted to breeding value estimation using contemporary methods (Mostert, 2007).

First time in 1987 the breeding values of South African dairy animals were received from Best Linear Unbiased Prediction (BLUP) and for breeding value estimation the Sire Model was used. Animal Model was used from 1992 for single trait analyses but in 1999 there were developments of multiple trait analyses which used genetic correlation between traits (Mostert, 2007). In South Africa the breeding value index have been used in defining the breeding objective for Holstein cattle, which resulted from a consent approach that is lacking economic and scientific basis and might be misleading (Banga *et al*., 2014).

South Africa in 2004 was listed as one of the countries incorporating only production traits and conformation traits in their selection index, but later Holstein Profit Ranking (HPR) index system was adopted. This system uses five traits including milk protein, fat, volume, calving interval and somatic cell count by combining their breeding values (Kgari, 2020).

2.4.2. Mating strategies in dairy cattle

For dairy cattle breeding, artificial insemination (AI) and selective bull mating/ natural breeding are reflected as strong methods and they are used to improve productivity and realize fast genetic gains (Mwanga *et al*., 2019). Despite popularity of AI, a lot of dairy farmers use natural breeding, there is several reasons including the perception that it is easier to manage and less expensive than AI. Farmers normally complain about rising cost of production but manly they underestimate and ignore the cost of keeping natural service bulls on their farms (Milk South Africa, 2014). AI is a highly specialised and technical process, and the procedure should only be performed by a trained person. It is recommended that one should attend AI course before trying it otherwise consult veterinarian or an expert who has experience in performing AI procedures (Milk SA, 2014). AI has been introduced about 60 years ago and since then its diffusion and usage was rapid worldwide due to its potential (Mwanga *et al*., 2019).

South African dairy industry relies on semen which are imported from superior bulls available across the globe as there is a significant decline in the local dairy bull industry (Van Marle-Köster and Visser, 2018). Semen from superior bulls is packed in straws and stored in liquid nitrogen at a temperature of -196 °C and it is very important to handle semen with care and according to the instructions. Only good quality, fertile semen of proven sires is to be used for any AI procedure (Milk SA, 2014).

South Africa dairy industry currently uses AI as the main mating method in the commercial sector. AI is the most widely used mating method in dairy farming and its well adopted by developed countries and on a commercial level by developing countries (Mwanga *et al*., 2019). AI is an economical way for a farmer to genetically improve their dairy herd, as it allows for the semen of top proven sires to be used to inseminate several cows annually. Estimated breeding values will help to determine what kind of calf you can expect from a specific bull, and therefore it will influence the choice of semen to be used for AI purposes. By selecting the sire, the dairy farmer can improve the production of his herd based on milk volume, butterfat, and protein percentages, as well as the characteristics of his animals, such as better udders, legs, and hooves (Milk SA, 2014).

Some other advantages of AI include: reduction of spreading venereal diseases as there is no direct contact between bulls and cows; increases efficiency of bull usage; accidents during mating are avoided; the best genetic material of proven sires can be used to improve a dairy herd; the occurrence of dystocia (difficult birth) is reduced; dry cow management can be better controlled, due to accurate drying off and calving dates, it is cheaper to buy semen than to keep a bull and the safety of farm personnel is ensured (Milk SA, 2014; Mwanga *et al*., 2019). At the individual herd level, AI has allowed producers to use multiple sires in their herds, which has potential to increase the within-herd diversity compared with natural mating (Baes *et al*., 2019).

2.5. Factors affecting stillbirths in dairy cattle

2.5.1. Animal factors

Animal factors affecting stillbirth in dairy cattle include age of the dam at calving, sex of the calf, parity, calf size/birth weight and dam size, and pelvic conformation (Morek-Kopeć *et al*., 2021).

2.5.1.1. Dam age at calving

Age at calving has a significant effect on stillbirth (Szücs *et al.*, 2009; Adrian and Barragan, 2015). The age at which primiparous calve influences the risk of stillbirth and this is mediated via pelvic size at calving, but this effect is small within industry norms for age at first calving (Mee *et al*., 2014). Bluel (2011) reported that stillbirth incidences increase as the age at first calving decreases. The incidence of stillbirth increases as the primiparous calve at a younger age, with less incidences in multiparous (Mee *et al*., 2014). The risk of stillbirth is greatest in primipara calving at

24 months of age or less (Szücs *et al.*, 2009; Bleul, 2011; Mee, 2013). The increase in stillbirth incidence observed in young heifers can be linked with the high risk of dystocia because of small pelvic size (Mee *et al.*, 2014).

2.5.1.2. Dam size and calf size

The size of the dam and calf relative to each other is also a contributing factor to stillbirth, with dam experiencing dystocia if they have a low weight relative to its calf (Hossein-Zadeh, 2013). Dystocia is defined as difficulties experienced by a female animal during parturition/ calving and dams experiencing dystocia have high stillbirth rate than those that are not experiencing dystocia (Szücs *et al.*, 2009). Dams having lower body size relative to their calf size are the most likely to experience difficulties during calving than those with high body size relative to their calf. Male calves normally have high birth weight than the female calves' hence male calves have high rate of stillbirth than the female calves (Morek-Kopeć *et al*., 2021). Excess body condition prior to calving mostly in heifers is associated with difficult calving hence high chances of stillbirth incidences (Mee, 2013).

2.5.1.3. Sex of the calf

The incidence of stillbirth in dairy cattle has been widely reported to be influenced by the sex of the calf (Atashi *et al*., 2011; Hossein-Zadeh, 2013; Mee *et al*., 2014; Gullstrand, 2017; Morek-Kopeć *et al*., 2021; Ratshivhombela, 2021). Calf sex is the main source of variability particularly on birth weight, with male calves having higher birth weight than female calves (Morek-Kopeć *et al*., 2021). Berry *et al*. (2007) and Rahbar *et al*. (2016) reported an association between high incidence of stillbirth and heavier calves. Male calves have shown to be associated with high incidences of stillbirth than female calves, this may be due to heavier birth weights and the different conformation of male calves which result in higher rates of dystocia and stillbirth (Hickey *et al*., 2007; Fiedlerova *et al*., 2008; Mee *et al*., 2014; Gullstrand, 2017).

Szücs *et al*. (2009) reported 69.73% and 30.27% incidences of stillbirth in male and female calves in United State respectively. Al-Samarai (2012) reported 5.51%, 5.42% and 7.54% incidence of stillbirth for female calves and 12.48%, 14.15% and 12.76% for male calves from cows in parity 1, 2 and 3, respectively. Giving birth to a male calf is associated with 1.4 times chances of stillbirth than giving birth to a female calf

(Gullstrand, 2017). Ratshivhombela (2021) reported that male calves are associated with high incidences of stillbirth than female calves.

2.5.1.4. Pelvic conformation

Internal pelvic area measurement is one of the most useful tools for reducing the rate of stillbirth, as cow with small pelvises appears to have high chances of contributing to high rate of stillbirth (Morek-Kopeć *et al*., 2021). Some studies (Bicalho *et al*., 2007; Atashi *et al*., 2011; Hossein-Zadeh, 2013) on dairy cattle focused on relationship between the external measured pelvic parameters and both the calving ease and calf survival and reported a relationship between externally measured length of the pelvis and perinatal mortality. Cow with small external measured length of the pelvis have high perinatal mortality and stillbirth compared to those with longer pelvis measured length (Hossein-Zadeh, 2013). The disproportion between the calf size and the pelvis size of the dam result in calving difficulties hence high chances of stillbirth (Bicalho *et al*., 2007; Atashi *et al*., 2011).

2.5.1.5. Dam parity

A significant influence on the stillbirth by dam parity was reported by several scholars (Fiedlerova *et al*., 2008; Szücs *et al*., 2009; Atashi, 2011; Gullstrand, 2017). The stillbirth incidences in primiparous cows differs significantly from that in cows in all other parities, with high incidences in the first parity as compared to later parities (Fiedlerova *et al*., 2008; Atashi, 2011; Ratshivhombela, 2021). This might be because of the disproportion between the size of the calf and the pelvic area, which causes a difficult calving and increases stillbirth parturition incidence (Bicalho *et al*., 2007; Atashi, 2011). Primiparous cows are 2.50 time and 2.35 time more likely to require birth assistance and to produce stillborn respectively, than calves from multiparous cows (Olson *et al.,* 2009).

Atashi (2011) reported the stillbirth incidences to be 7.97%, 4.61%, 4.00% and 4.93%, for first, second, third and fourth parity, respectively. In the past five years the incidence of stillbirth in Canadian first calving Holstein dairy cattle increased from 10 to 12% while increased from 5 to 6 % for cows calving for the second time or more. In the Norwegian study of calving difficulties and stillbirths in Norwegian Red cattle found the incidence of stillbirths to be 3% for first calving and 1.5% for second and later calving (Szücs *et al*., 2009).

2.5.2. Environmental factors

Environmental factors affecting stillbirth include herd, year and season of calving, disease, and nutrition (Szücs *et al*., 2009; Al-Samarai, 2012; Ratshivhombela, 2021).

2.5.2.1. Herd

Stillbirth incidences are influenced significantly by the herd (Bicalho *et al*., 2007; Vallée *et al*., 2013; Kayano *et al*., 2016; Ratshivhombela, 2021). Bicalho *et al*. (2008) reported a large variation in the stillbirth incidences among herds, with as low as 4.1% and as high as 14.3% incidences of stillbirth. Environmental conditions and the management practices which the herds are raised in can be contributing factors to this variation (Bicalho *et al*., 2008; Vallée *et al*., 2013). Bleul (2011) and Kayano *et al*. (2016) reported that the larger the herd becomes, the lower the overall level of attention to management details and this result in high incidences of stillbirth and dead calves during the first 24 hours. There is a difference the proportionality of monitored and none monitored calving events between herds as not all calving's are monitored (Gullstrand, 2017; Ratshivhombela, 2021). The way of recording these events in relation to their true values and the way in which recordings are handled may also vary (Vallée *et al*., 2013; Gullstrand, 2017).

2.5.2.2. Year and season of calving

In dairy cows a significant influence by the year and season of calving on stillbirth have been reported by several researchers (Hossein-Zadeh *et al*., 2008; Atashi, 2010; Atashi *et al*., 2011; Al-Samarai, 2012; Kayano *et al*., 2016; Ratshivhombela, 2021). However, Atashi (2011) reported a significant effect by the year of calving with no significant effect by the season of calving. Fiedlerova *et al*. (2008) reported easier calving in autumn and difficult calving in spring, hence high incidences of stillbirth in spring as compared to the spring autumn. The highest incidences of stillbirth were observed in winter calvers with lowest incidences in summer calvers (Del Río *et al*., 2007; Fiedlerova *et al*., 2008; Hossein-Zadeh *et al*., 2008; Al-Samarai, 2012). Calving in the colder months is associated with high incidences of stillbirth compared to calving during the warm months (Del Río *et al*., 2007; Kayano *et al*., 2016). In contrary Atashi *et al*. (2011) reported highest incidences of stillbirth when calving occurred during the summer seasons.

2.5.2.3. Nutrition

Animals that are provided with enough and balanced nutrition are at less risk of experiencing stillbirth as compared to those animals that do not get enough and balanced nutrition. However, overfed animals are normally fat and that is associated with difficulties during calving which result in high incidence of stillbirth (Atashi *et al*., 2011; Hossein-Zadeh, 2013). Underfeeding dams during gestation produces significantly lighter calves which translate to weak calves and subsequently increase the risk for stillbirth (Micke *et al*., 2010). The deficiencies of micro-nutrients such as iodine, selenium, copper, and zinc have been associated with high stillbirth rates, hence it is imported to provide the animals with enough nutrients (Mee, 2013).

2.5.3. Genetic factors

Genetic factors affecting stillbirth include Twinning, gestation length and heritabillities (Szücs *et al*., 2009; Hossein-Zadeh, 2013; Gullstrand, 2017).

2.5.3.1. Twinning

In dairy herds there is high incidences of twin birth which is 3 to 5% on average and it is strongly affected by age and parity of the dam (Hossein-Zadeh, 2013). Olson *et al.* (2009) reported that twins normally have low body weight and 7.80 times chances of being stillbirth than single birth. Increase occurrence of twinning rises the potential of getting more progeny from genetically superior female animals by giving them the allowance to contribute a larger role in selection program. However, twinning is associated with number of unfavourable effects such as increased culling rate, lower potential calf survival (Lombard *et al*., 2007) and poorer cow reproductive performance and stillbirth (Bicalho *et al*., 2007; Olson *et al.,* 2009; Kayano *et al*., 2016). Risk of stillbirth increases, and the herd profitability decreases as the occurrence of twinning increases and twinning has more incidences of stillbirth as compared to single birth (Lombard *et al*., 2007; Atashi *et al*., 2011; Hossein-Zadeh, 2013). Atashi *et al*. (2011)

reported 8.28%, 7.12% and 5.93% of the incidences of stillbirth in male twins, mixed sex twins and female twins respectively.

2.5.3.2. Gestation length

Gestation length is the time from conception to parturition and average duration of the gestation period is 282 days. Some cattle breeds such as Jerseys, Holsteins and Ayrshires have shorter gestation period than the average (279 days), while other cattle breeds such as Guernsey and Brown Swiss have longer gestation period (283-288 days) (USDA, 2007). Gestation length has a significant effect on the rate of stillbirth, the more the gestation length deviated from the mean, the more likely the calf is to be stillborn, especially if the gestation is shorter than normal (Szücs *et al*., 2009). Gestation length of 15 to 12days below the mean is one of the vital rick factors for stillbirth. (Hossein-Zadeh, 2013). The gestation period of about 120 to 260 days in dairy cows is associated with high incidences of stillbirth (Mee, 2020). Longer than average gestation length is associated with high incidences of stillbirth (Bleul, 2011; Piwczyn´ski *et al*., 2013).

2.5.3.3. Heritability – direct and maternal effects

All the genetic contributions to the phenotypic variance in a population are reflected by heritability including the maternal and direct effects. Heritability (h^2) is expressed on a scale of zero to one and it affect accuracy of selection and rate of genetic progress hence it is important in selection (Gullstrand, 2017). Steinbock *et al*. (2003) reported the heritability of stillbirth for direct and maternal effect as 4% and 3% respectively on a visible scale using linear model and 12% direct effect and 8% maternal effect when using the threshold model. There is low and little difference in heritability of stillbirth between the first and second parities, but heritability is slightly high in the first than in the second parity (Steinbock *et al*., 2003; Szücs *et al*., 2009).

The heritability for direct effect is higher than those for maternal effect. Heritability estimates for stillbirth is low when single traits model is used to analyse than when bivariate models are used to analyse two traits (Steinbock *et al*., 2006). Szücs *et al*. (2009) reported heritability for direct sire effect as 0.05-0.19 which is higher than that of maternal grand sire effect (0.04-0.06). Both the direct and maternal genetic effects have an influence on stillbirth. A large variation in the rate of stillbirth among bulls in

Holstein population were observed with sires and maternal grandsires ranging from 2 to 25% mainly shown in a large group of progenies (Steinbock *et al*., 2003).

2.6. Inbreeding and dairy cattle production

Inbreeding is defined as the likelihood that two alleles at any given locus are identical by descent and occurs when related individuals are mated to each other (Mc Parland *et al*., 2007). Within a population inbreeding reduces genetic variability and performance mainly in traits associated with individual fitness such as fertility, and in a large population inbreeding impact might be neglected (Maiwashe *et al*., 2006). Reis Filho *et al*. (2015) reported that an increase in inbreeding reduces productivity and reproductive performance. Inbreeding is generally known to have a negative influence on the milk production, fertility, and survival (Sewalem *et al*., 2006; Szücs *et al*., 2009; Reis Filho *et al*., 2015), its effect on milk production in Holstein dairy cattle rages from -29.6 to -19.7kg (Mc Parland *et al*., 2007).

An increase in inbreeding by 1% is associated with lengthening of the calving interval by up to 0.31 days, increased incidences of dystocia and stillbirth in Holstein dairy cattle (Adamec *et al*., 2006). Stillbirth and dystocia can lead to dam and calf mortality, premature culling, and indirect costs due to additional veterinary services, treatment, and labour (Olson *et al.,* 2009). This also reduces the milk yield and the protein and fat content of the milk (Ratshivhombela, 2021). Gorelik *et al*. (2020) reported a slight milk yield increase in inbreed dairy animals which is however associated with the decrease in milk quality indicators, exactly the mass fraction of fat and mass fraction of protein.

On contrary, Bezdicek's *et al*. (2008) and Nazokkarmaher (2016) reported that inbreeding led to decreased milk production; however, increases fat and protein content of milk. Nazokkarmaher (2016) reported that inbreeding in a long-term it negatively affects the milk production of dairy cattle population, and it also has an adverse effect on the health of the offspring. Inbreeding influence offspring survival and produce reduced survival rates and again is associated with low birth weights and mature weights. Sewalem *et al*. (2006) study on three Canadian dairy cattle the Jerseys, Holsteins and Ayrshires indicted negative effect of inbreeding on lifespan of the animal. There is positive relationship between increases in inbreeding and incidence of genetic disorders within and between populations (Nazokkarmaher, 2016). Mc Parland *et al*. (2007) reported that the effect of inbreeding on protein yield and milk fat concentration differ across parities and that on protein yield was greater in multiparous animals.

2.7. Inbreeding rate, inbreeding coefficient, average relatedness, and effective population size in dairy cattle production

2.7.1. Inbreeding rate

Inbreeding rate (ΔF) expresses the increase in average inbreeding level in a population from one generation to the next and due to the fact that a rise in inbreeding is non-linear, the rate of inbreeding is expressed relative to how much the population is away from full inbreeding (Kor and van der Waaij, 2015). Phenotypic performances such as body weight and milk yield of livestock animals decline with increasing inbreeding rate (Makanjuola *et al*., 2020a). An increase in the inbreeding rate by one percent have a significant negative effect on the reproductive traits such as fertility, survival, and calving interval (Mc Parland *et al*., 2007) and production traits such as the milk, fat, and protein yield (Makanjuola *et al*., 2020a).

Inbreeding rates exhibit negative correlation with fertility in a population and when inbreeding rate increases in small populations fertility is reduced in the next generation. The study by González-Recio *et al*. (2007) on inbred Spanish cattle, reported reduction in fertility by 3% in most inbred animals with the rate of pregnancy also being diminished. In addition, Figure 2.2. below confirm that increased inbreeding rate triggers a decline in reproductive fitness by González-Recio *et al*. (2007).

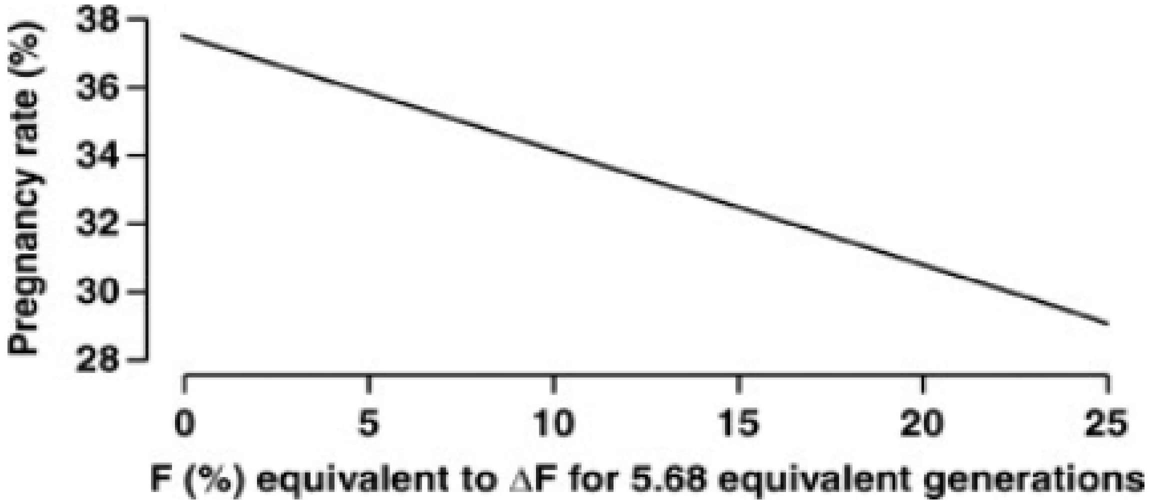

Figure 2. 2: Regression line for inbreeding rate (ΔF) on pregnancy rate as a fertility trait (González-Recio *et al*., 2007)

2.7.2. Inbreeding coefficient and Average relatedness (AR)

Inbreeding coefficient (Fx) indicates the chance that an animal receives the same allele from both parents because they are related. The inbreeding level in a particular animal is expressed as inbreeding coefficient. It takes values between 0 which means zero percent inbred (not inbreed) and 1 which means one hundred percent inbreed (fully inbred) (Kor and Van der Waaij, 2015). Increase in the inbreeding coefficient causes losses in production of milk, fat, and protein (Reis Filho *et al*., 2015) and reproductive traits in dairy cattle (Mc Parland *et al*., 2007). Poor reproductive performances result in substantial economic losses due to prolonged calving intervals, increased insemination and veterinary costs, higher culling rates and excessively late age at first calving which can result in reduced lifetime milk yield and increased replacement costs (Wondossen *et al*., 2018).

Anciently inbreeding coefficient (F) was computed by means of relationships based on pedigrees and the accuracy of F is very dependent on the quality and depth of the pedigree information (Letko *et al*., 2020). Average relatedness (AR) is normally used to estimate the effective population size which is a key parameter in genetic conservation (Mandal *et al*., 2021). Average relatedness can be used to manage genetic diversity in a population (Mokhtari *et al*., 2015) and is inversely related to the genetic diversity, also serves as an indicator of the long-term inbreeding rate (Mandal *et al*., 2020). Random selection and mating of available progeny in closed population result in AR estimating average inbreeding coefficient in the next generation (Mokhtari *et al*., 2015; Mandal *et al*., 2021). Introduction of few outside breeding males disrupt estimated relationship between F and AR, because introduction of small number of breeding males has a small impact on AR but superior use of the introduced males and their progeny have a vital negative impact on F (Mandal *et al*., 2021).

Rokouei *et al*. (2010) reported that dams with F of 0-12.5% and 12.5-25% had 1 and 3% greater incidence of dystocia, respectively, over non-inbred dams. Reis Filho *et al*. (2015) in their study on dairy Gyr cattle observed a linear decrease in milk yield due to the increase of inbreeding coefficient (F). Figure 2.3. below confirm that increased inbreeding coefficient is associated with decline in Days in Milk (DIM).

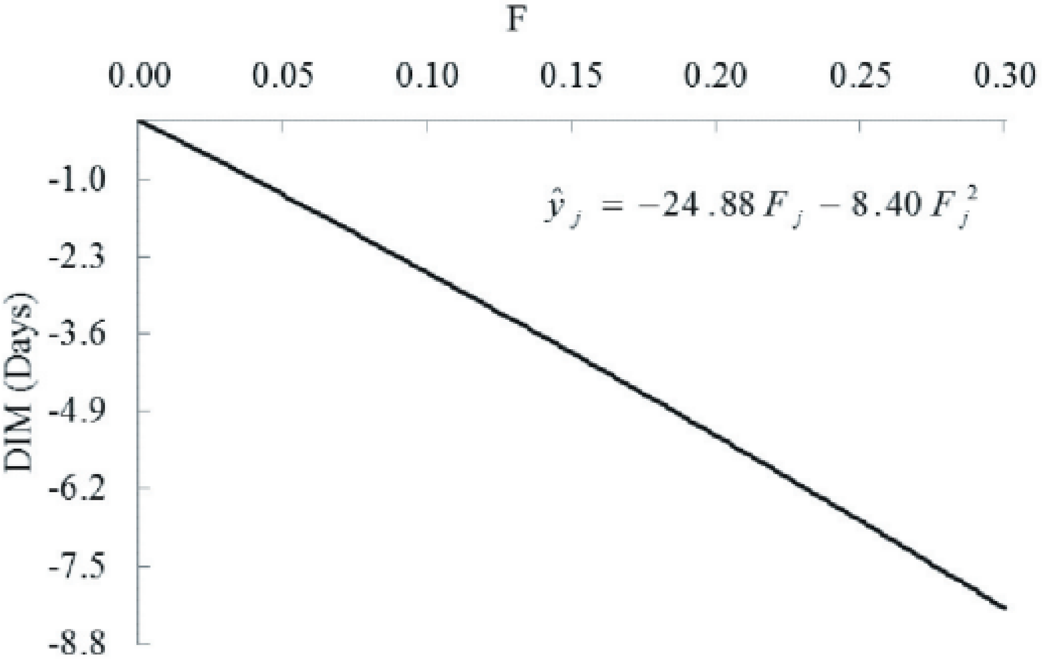

Figure 2.3: Effect of inbreeding coefficient (F) on days in milk (DIM) in dairy Gyr cattle (Reis Filho *et al*., 2015)

2.7.3. Effective Population Size

Effective population size (Ne) can be defined as the number of individuals that effectively participates in producing the next generation (Sbordoni *et al*., 2012) and is sensitive to changes in census population size over time (Kliman *et al*., 2008). Effective population size is a key parameter in conservation genetics and normally can be estimated from the increase in average relativeness (Gutiérrez *et al*. 2003). Ne it depends largely on the number of breeding males and females contributing an offspring to the next generation within a population (Maiwashe *et al*., 2006; Makanjuola *et al*., 2020b). Small Ne can result in inbreeding depression, which is the noticeable decline in the phenotypic mean performance of economically important traits within a population (Makanjuola *et al*., 2020a). Lower Ne is associated with less genetic variability within the population (Maiwashe *et al*., 2006).

Stachowicz *et al*. (2011) estimated an Ne for Holsteins to be 155 and that it is not expected to change significantly if generation interval remains the same. A large population size does not imply free from problems typical for small, endangered populations. For instance, world Holstein population suffers from inbreeding depression and significant loss of genetic diversity (Maiwashe *et al.,* 2006). High Ne is associated with reduced inbreeding rate and lower incidences of stillbirth in dairy cattle population (Stachowicz *et al*., 2011). Kliman *et al*. (2008) in their study of genetic drift and effective population size they reported that *Ne* is dependent on the number of founders (N_f). Figure 2.4. below shows the relationship between the *Ne* and N_f in a population of 1,000 mating individuals by Kliman *et al*. (2008).

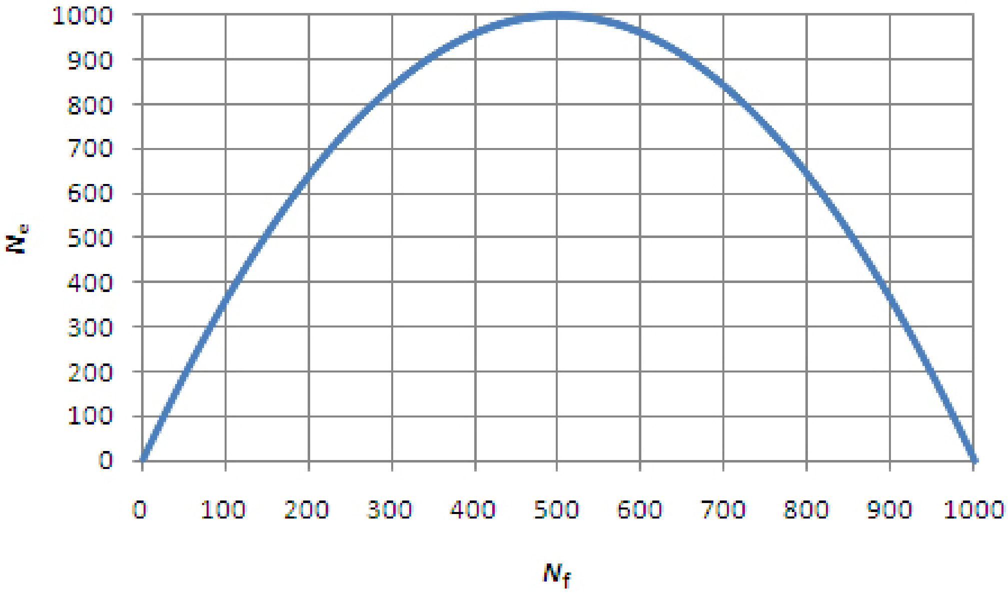

Figure 2.4: The relationship between Ne and N_f in a population of 1000 mating individuals (Kliman *et al*., 2008)

2.8. Conclusion

This Chapter highlighted that there is limited publications or research done on stillbirth and inbreeding in South African Holstein dairy cattle. Holstein dairy cattle breed dominates the dairy industry across the world and in South Africa experiences stillbirth which is currently one of the major reproductive problems in the dairy industry. The present study seeks to evaluate the inbreeding rate and its influence across generations on the incidences of stillbirth in Holstein dairy cattle of South Africa. This study will also provide recommendations on the inbreeding rates levels that are acceptable. This will help to improve the reproductive performance of the dairy cows in terms of their calving rate and reduce costs associated with stillbirth. There is a need to look into the inbreeding incidences across generations of the South African Holstein dairy population.

CHAPTER THREE

Incidence of stillbirth across generations in South African Holstein dairy cattle

Abstract

Stillbirth incidences is a major contributor to the productivity of the dairy industry, as its increases reduces the profitability of the dairy enterprise. The aim of the study was to determine the stillbirth incidences and phenotypic trends across generations of South African Holstein dairy cattle. Herd, birth season and year, dam parity, calf sex, and generation effect on the incidences of stillbirth were determined. Data set of 1 million cows born between 1945 to 2020 were extracted from INTERGIS database of registered Holstein dairy cattle in South Africa, cows with incomplete records and herds with 10 or less cows were removed. Therefore, a total of 308 157 cows from 1 769 herds were used. ENDOG Software (Ver 4.8) (Gutiérrez and Goyache, 2005) was used to determine generation intervals. General Linear Model (GLM) procedure of SAS was used to model incidences of stillbirth at 5% significance level. Regression procedure in SAS (2021) was used for analyses of the phenotypic trends. Herd and birth season significantly ($P<0.05$) affected the incidence of stillbirth. There was a great variation in the incidences of stillbirth across the herds ranging from 0% to 100%. With most of the herds having zero incidences of stillbirth (3.736±0.251). Autumn (3.397±0.067) and summer (3.306±0.067) had the highest incidences of stillbirth while spring (3.073±0.067) and winter (3.00±0.063) had the lowest. Dam parity, calf sex, generation, and birth year had a significant effect ($P<0.05$) on the incidences of stillbirth, with multiparous and female calves (3.516±0.046) having high incidences of stillbirth than primiparous and male calves (2.922±0.028). Incidences of stillbirth were found to be positively and significantly correlated ($P<0.05$) to birth year (r= 0.251) and generation (r= 0.245), but negatively and significantly correlated to dam parity (r = -0.009) and dam age (r= -0.024) ($P<0.05$). The observed great variation in the incidences of stillbirth across herds in small scale farming warrant a need for further investigation of the inbreeding rate and the effective population size within the herds of South African Holstein dairy population.

Key words: Birth season, birth year, calf sex, dam parity, herd

3.1. Introduction

Stillbirth and dystocia affect cows calving which is a very stressful period to the cow, and currently on dairy farms stillbirth and dystocia are the major economic problems (Kebede *et al*., 2017; Antanaitis *et al*., 2022). Stillbirth can lead to direct losses such as dam mortality, calf mortality, premature culling, and indirect costs due to additional veterinary services, treatment, and labour (Adamec *et al*., 2006; Olson *et al.,* 2009; Piwczyn´ski *et al*., 2013; Sasaki *et al*., 2014; Mahnani *et al*., 2018). Trends of stillbirth and dystocia are rising internationally, this has been partly attributed to the introduction of Holstein genes (Adamec *et al*., 2006). There are little publications on calving problems such as stillbirth and dystocia in South Africa. However, a recent study by Ratshivhombela (2021) reported 8.26% in 2014 and 4.54% in 2018 cows' incidences of stillbirth. Because the heritability is low, improvement of stillbirth incidences thorough selection can be a long process. Therefore, environmental factors determining changeability of stillbirth should be taken into consideration (Piwczyn´ski *et al*., 2013).

Stillbirth increase is due to multifactorial causes, and it is currently different because it is reported to be less related to high birth weight and to dystocia than it used to be (Berglund *et al*., 2003). Inbreeding contributes to stillbirth incidences whereby at first parity there was 0.20 to 0.25% increase in stillbirth incidences per 1% increase in inbreeding, but the incidence percentage declined with parities (Adamec *et al*., 2006). Piwczyn´ski *et al.* (2013) reported that increase in the incidences of stillbirth in the Holstein dairy cattle is very detrimental to the profitability of the dairy enterprises and dairy industry. Limited studies have been done on the incidences of stillbirth in South African Holstein dairy cattle. The objective of the study was to determine the stillbirth incidences and phenotypic trends across generations of South African Holstein dairy cattle.

3.2. Material and methods

3.2.1. Study site

The study was conducted using secondary data records from all South African provinces as this is a population study. South Africa occupies the most southern tip of Africa with its long coastline stretching more than 3 000 km from the desert border with Namibia to Mozambique. South Africa has numerous vegetation such as trees, shrubs, and grass, with the common grass species being Guinea grass, sweet thorn, finger grass, ubiquitous cosmopolitan weed, weeping lovegrass, and kikuyu grass. It is a semi-arid region receiving rainfall of 464mm on average and experiencing a minimum temperature of about 8^0C in winter and a maximum of about 32^0C (Alexander, 2018).

3.2.2. Study animals and Management

The study was conducted using the Holstein breed of dairy cattle which are the greatest milk-producing dairy breed in the world with cows weighing about 680kg and standing 147cm tall at the withers on average. Secondary data of Holstein dairy cattle that was used in this study were collected from animals in small scale farming which were managed conventionally. The animal husbandry practices included putting cattle on a total mixed ration (TMR) containing 51% and 49% of forage and concentrates respectively (Schären *et al*., 2016), and semi-continuous feed access (Herdt, 2014).

Vaccinating cattle against brucellosis, bovine viral diarrhoea and leptospirosis and bovine respiratory syncytial virus were mandatory (Erika *et al*., 2014; Mellado *et al*., 2017). Animals were housed in holdings that have shelter and allow ease handling, feeding, breeding, milking, and health activities (Hristov *et al*., 2008). Replacement heifers were selected based on breeding values on productivity potential and pure breeding using good quality semen sources at international markets.

3.2.3. Study design and data collection

A longitudinal observational study design was used, and secondary data were extracted from the INTERGIS database of registered South African Holstein dairy cattle. The data set included 1 million Holstein dairy cattle born from 1945 to 2020. Purposive sampling was done with a criterion of including cows with complete records and herds with more than 10 animals. Then a total of 308 157 cows from 1 769 herds were used.

The pieces of information that were gathered over different generations included; pedigree data (animal ID, sire ID, dam ID, date of birth), calf sex, dam factors (parity, age at parturition, calving date), herd/farm, and birth status. Stillbirth was recorded as 0 for alive and 1 for dead. The birth season and birth year effect were derived from the date of calving of the dams.

3.2.4. Statistical analyses

Preliminary data editing (adding birth seasons and generations) was done using Microsoft (MS) Excel (2020) while advanced data editing (pruning the herds, removing animals with incomplete data) was done using SAS Version 9.4 (SAS, 2021). Then generation interval and incidences of stillbirth were determined as outlined below.

3.2.4.1. Generation interval

Generation intervals (GI) were computed across the four paths of selection, sire to sire (L_{ss}), sire to dam (L_{sd}), dam to sire (L_{ds}) and dam to dam (L_{dd}), as the average age of parents when their offspring were born (Menezes *et al.*, 2015). The average generation interval was computed as follows:

$$GI = \frac{Lss + Lsd + Lds + Ldd}{4}$$

The above calculation was carried out using ENDOG Software (Version 4.8). (Gutiérrez and Goyache, 2005).

3.2.4.2. Incidences of stillbirth

Incidences of stillbirth across generations was modelled using fixed factors (herd, birth year, birth season, generation, calf sex and dam parity) and random factors (dam effects and dam age). The General Linear Model (GLM) procedure of SAS (SAS, 2021) was used to fit the following model in the matrix notation at 5% significance level:

$$y = Xb + Za + e$$

Where:

y = vector of observations for stillbirth (0 = alive, 1 = dead)

b = vector of fixed effects (herd, birth year, birth season, generations, calf sex and dam parity)
a = vector of random effects (dam effect and dam age)
X = incidence matrix relating observations to fixed effects
Z = incidence matrix relating observations to random dam effects
e = vector of random residual effects

3.2.4.3. Phenotypic trends

The phenotypic trends were determined from the mean values based on the calculated generations. The following model was used:

$$Yij = \mu + b1X1 + eij$$

Where:

Y = mean incidences of stillbirth per generation, dam parity and birth year,

μ = overall constant

b1 = regression co-efficient of the fixed effects (generation, dam parity and birth year),

X1 = fixed effect of j^{th} generation of the cow, and

eij= the residual effect, N (0; $I\sigma^2$)

Regression procedure in SAS (2021) was used for analyses.

3.3. Results

3.3.1. Determination of generation intervals

Table 3.1. below indicate the generation intervals determined and used in this study, together with the birth year period and the total count of animals in each generation. Total of 14 generations were determined and had different birth year periods. The results showed that the number of animals increased from the first generation until 9th generation and then declined until the 14th generation.

Table 3. 1. Generation intervals with birth year periods and total count of animals per generation

Birth year period	Count of animals	Generation
1949 – 1959	837	1
1960 – 1965	4044	2
1966 – 1971	10291	3
1972 – 1976	28469	4
1977 – 1981	67613	5
1982 – 1985	121926	6
1986 – 1989	172822	7
1990 – 1993	196100	8
1994 – 1997	223347	9
1998 – 2001	103973	10
2002 – 2005	22495	11
2006 – 2009	56315	12
2010 – 2013	31090	13
2014 – 2020	9253	14

3.3.2. Incidences of stillbirth across the herds in South African Holstein dairy cattle.

Figure 3.1. below shows the incidences of stillbirth across the herds in South African Holstein dairy cattle. Incidences of stillbirth were very variable across herds with minimum of zero and maximum of 100, but most (70%) of the herds had zero incidence as demonstrated by the boxplot on Figure 3.1. The incidences of stillbirth were positively skewed (4.84) with an average mean of 3.736%, standard deviation of 10.542, range of 100 and a variance of 111.134.

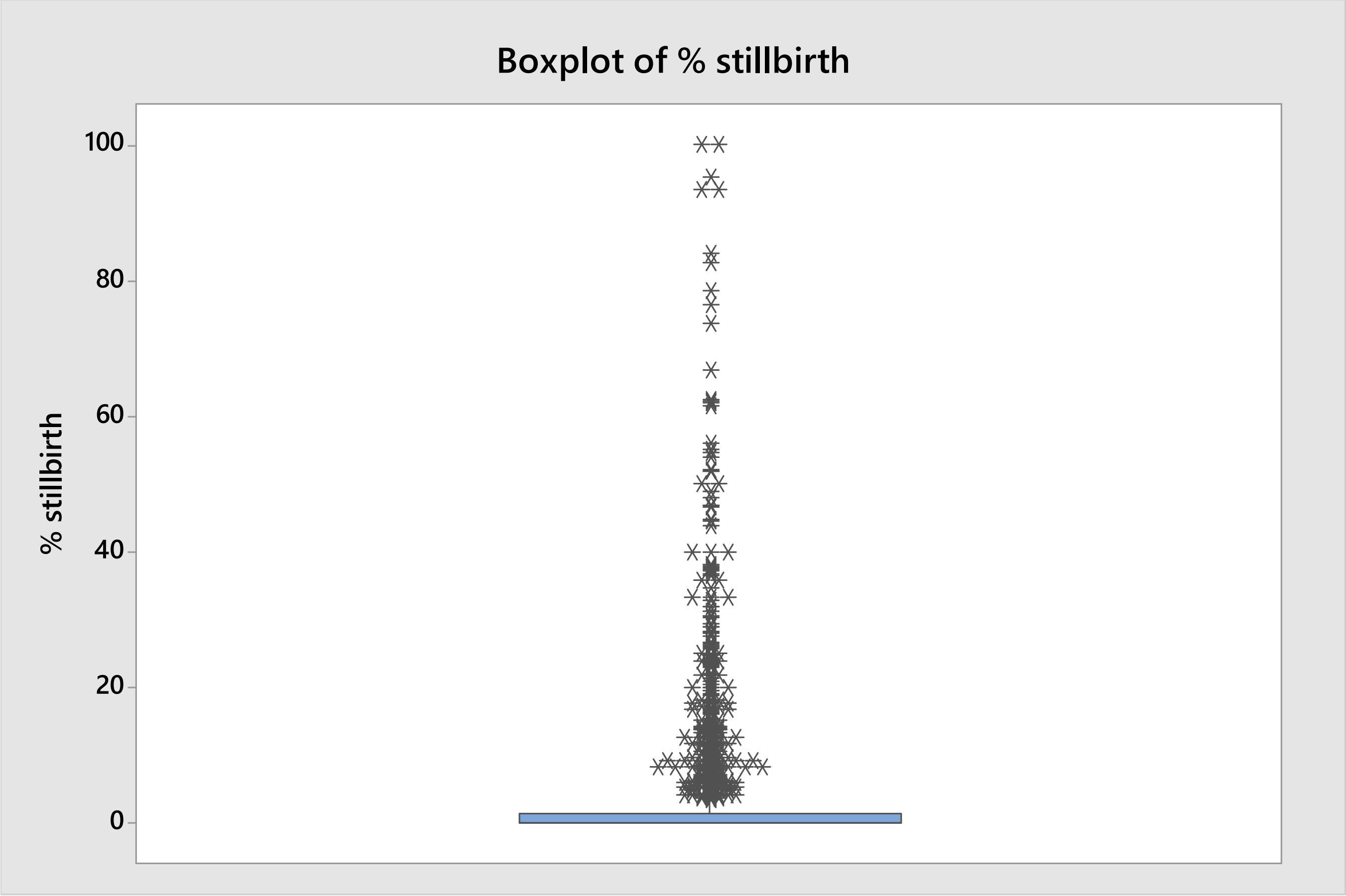

Figure 3. 1. Incidences of stillbirth across the herds in South African Holstein dairy cattle

3.3.3. Effect of birth seasons on the incidences of stillbirth across the generations in South African Holstein dairy cattle.

Effect of birth season on the incidences of stillbirth across generations in South African Holstein dairy cattle is presented in Table 3.2. Birth season had a significant effect (*P<0.05*) on the incidences of stillbirth, with summer (3.306±0.067) and autumn (3.397±0.067) performing significantly higher than the winter (3.100±0.063) and spring (3.073±0.067) seasons.

Table 3. 2. Effect of birth seasons on incidences of stillbirth across generations in South African Holstein dairy cattle

Birth seasons	Mean percentage
Summer	3.306 ± 0.067^a
Autumn	3.397 ± 0.067^a
Winter	3.100 ± 0.063^b
Spring	3.073 ± 0.067^b

3.3.4. Effect of birth year on the incidences of stillbirth across the generations in South African Holstein dairy cattle.

The effect of birth year on incidences of stillbirth across generations in South African Holstein dairy cattle is represented in Figure 3.2. Stillbirth incidences observed were different within birth years, it was below 5% between 1985 to 2004, with a constant of 0% from 1994 to 2001, start increasing in 2005 until it reached a maximum of 30.4% in 2007, then it started to decline until 2011 where it then stayed between 10% and 12%. The incidences of stillbirth become high starting from 2003, hence animals from 2003 are having high incidences of stillbirth.

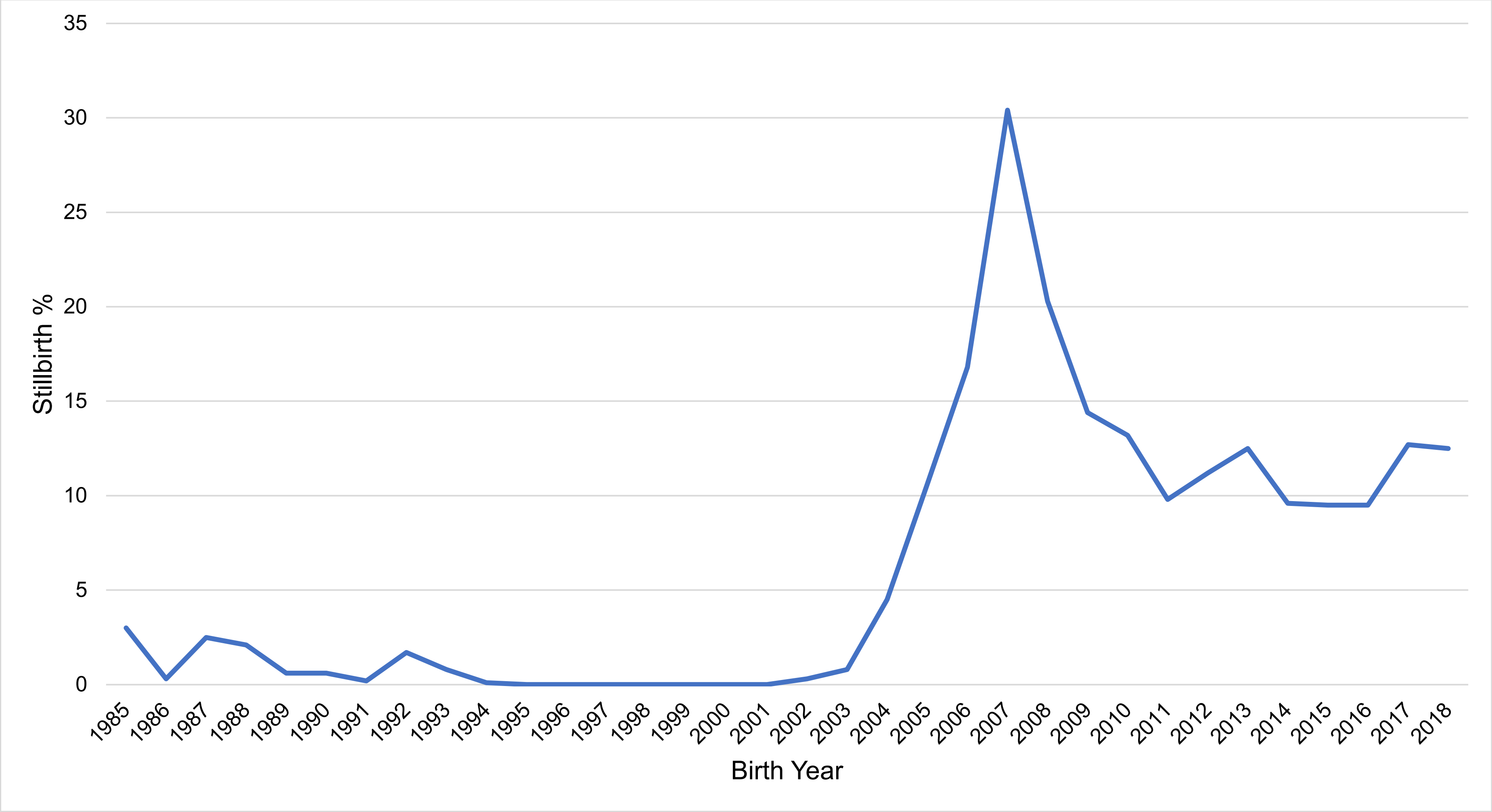

Figure 3. 2. Effect of year on incidences of stillbirth across generations in South African Holstein dairy cattle

3.3.5. Effect of generation on the incidences of stillbirth across the generations in South African Holstein dairy cattle

Figure 3.3. below shows the effect of generation on incidences of stillbirth across generations in South African Holstein Dairy cattle. Generation had a significant effect (*$P<0.05$*) on the incidences of stillbirth, with the 14th and 11th generations having the highest effect followed by the 12th and 13th generations with the second highest and 10th generation having the third highest. The other generations were close to zero percent incidences of stillbirth. The stillbirth observation declined from 3% in the 5th generation until it reached 0% in the 8th and 9th generations, then start increasing until reaching 16.9% in the 11th generation and declined again until the 13th generation then increased to 12.9% in the 14th generation. After the 9th generation the stillbirth incidences became very high.

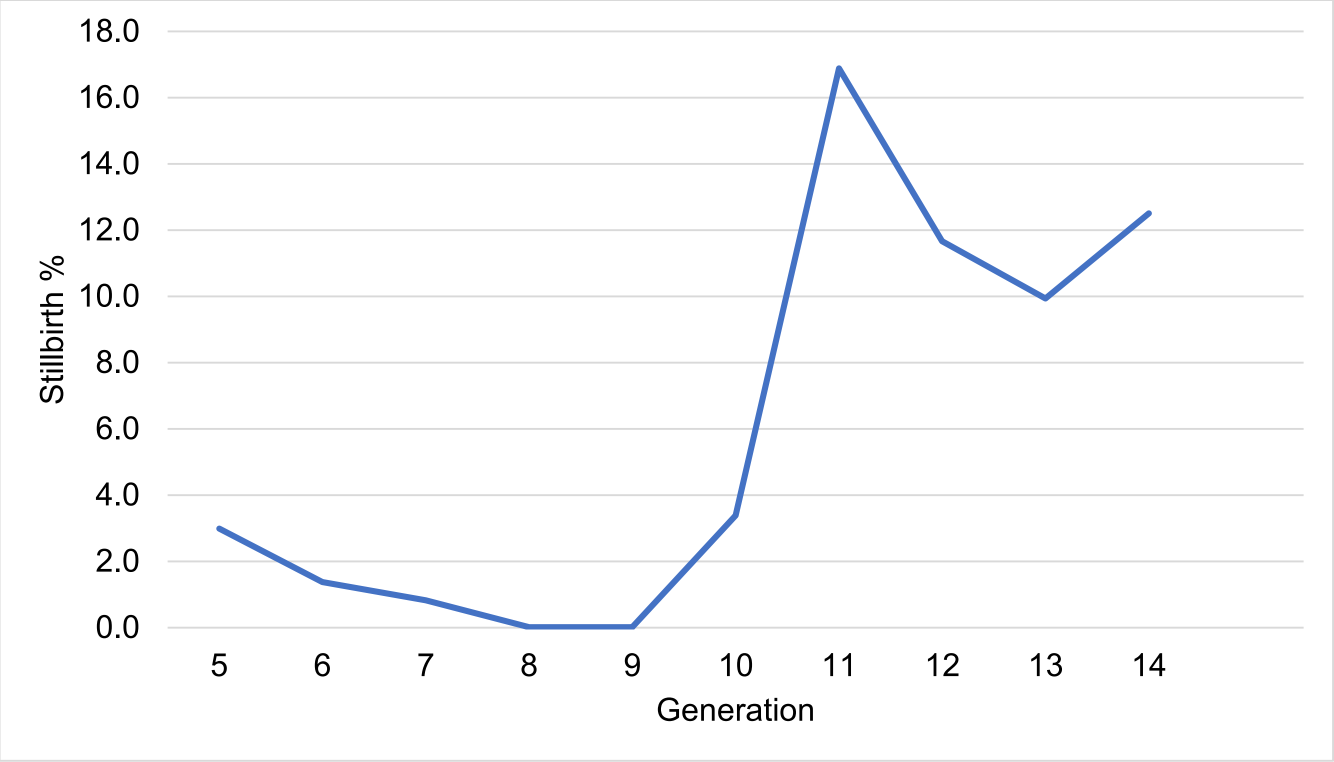

Figure 3. 3. Effect of generation on incidences of stillbirth across generations in South African Holstein dairy cattle

3.3.6. Effect of dam parity on the incidences of stillbirth across the generations in South African Holstein dairy cattle

Figure 3.4. below shows the effect of dam parity on incidences of stillbirth across generations in South African Holstein dairy cattle. Dam parity had a significant effect (*$P<0.05$*) on the incidences of stillbirth, with the 11th and 12th parities having the highest percentage of stillbirth and parities 2nd and 7th having the lowest percentage of stillbirth. The primiparous had less incidences of stillbirth while the multiparous had high incidences of stillbirth.

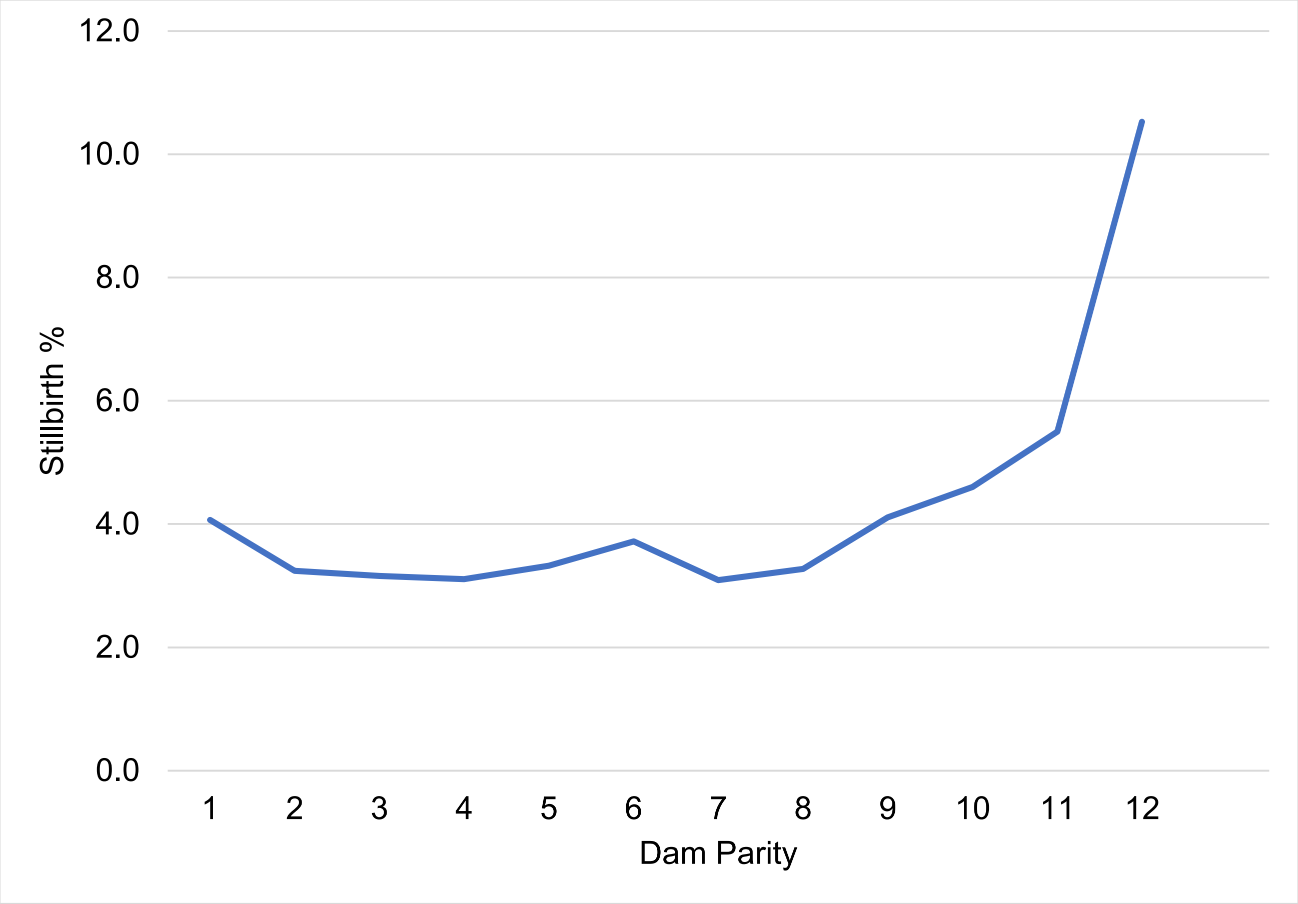

Figure 3. 4. Effect of dam parity on incidences of stillbirth across generations in South African Holstein dairy cattle

3.3.7. Effect of calf sex on the incidences of stillbirth across the generations in South African Holstein dairy cattle

Effect of calf sex on incidences of stillbirth across generations in South African Holstein dairy cattle is shown in Table 3.3 below. Calf sex had a significant effect (*P<0.05*) on the stillbirth incidences, with females (3.516±0.046) having the highest stillbirth percentage followed by males (2.922±0.028) with the lowest stillbirth percentage. The male calf is having less chances of stillbirth than the female calf.

Table 3. 3. Effect of calf sex on incidences of stillbirth across generations in South African Holstein dairy cattle

Calf Sex	Mean Percentage
Female	3.516±0.046^a
Male	2.922±0.028^b

3.3.8. Phenotypic correlation coefficients amongst stillbirth, birth year, generation, dam parity and dam age of South African Holstein dairy cattle

The phenotypic correlations amongst stillbirth, birth year, generation, dam parity and dam age are presented in Table 3.4. The results showed that in total 10 correlations were estimated, 6 was negatively and significantly correlated (*P<0.05*) and 4 were positively and significantly correlated (*P<0.05*) to each other. Birth year was significantly and negatively correlated to dam parity (r= -0.028) and dam age (r= -0.092), however positively and significantly correlated with generation (r= 0.985). The dam parity (r= -0.031) and dam age (r= -0.094) are negatively and significantly correlated to generation. Dam parity was positively strongly and significantly correlated to dam age (r= 0.935), similar result was found between birth year and generation (r = 0.985). Stillbirth were found to have a positive correlation with birth year (r= 0.251) and generation (r= 0.245), but negatively correlated to dam parity (r= -0.009) and dam age (r= -0.024). This implies that incidences of stillbirth increase per birth year and per generation, however young dams in the first parity have high incidences of stillbirth.

Table 3. 4. Phenotypic correlation coefficients amongst stillbirth, birth year, generation, dam parity and dam age of South African Holstein dairy cattle

	Stillbirth	Birth Year	Generation	Dam Parity
Birth Year	0.251***	-	-	-
Generation	0.245***	0.985***	-	-
Dam Parity	-0.009***	-0.028***	-0.031***	-
Dam Age (years)	-0.024***	-0.092***	-0.094***	0.935***

***: significantly different at $p<0.001$; **: significantly different at $p<0.01$; *: significantly different at $p<0.05$; ns: not significant

3.4. Discussion

3.4.1. Herd effect on incidences of stillbirth in South African Holstein dairy cattle

The South African Holstein dairy cattle population on average is on good levels of stillbirth incidences with an average mean of 3.736%. However, at the herd level the stillbirth incidences are not at good level, because stillbirth incidences across herds have a maximum of 100 percent and a minimum of zero percent. In agreement to the current study Bicalho *et al*. (2008), Bleul (2011) and Kayano *et al*. (2016) reported a large variation in the stillbirth incidences across herds. High variation in incidences of stillbirth indicates a high stillbirth incidences problem at the herd level. Different environmental conditions and management practices are the contributing factors to the variation between different herds. In larger herds there is less attention given to management details and this contribute to high stillbirth incidences within herds (Bleul, 2011; Kayano *et al*., 2016).

3.4.2. Seasonal effect on incidences of stillbirth in South African Holstein dairy cattle

The current study showed more stillbirth incidences in autumn than in spring and more in summer than in winter, with autumn and summer having highest stillbirths and spring and winter having the lowest incidences. Variation in different environmental temperatures from different countries, different nutrition, and variations in diseases exposure could be the cause of these difference in the incidences of stillbirth across seasons. In contrary to the current study several schoolers reported significant seasonal effect on incidences of stillbirth (McGuirk, 2004; Al-Samarai, 2012; Ratshivhombela, 2021). This is due to seasonal variation in temperature, disease occurrence rate, feed availability, and gestation period (Fiedlerova *et al*., 2008; Al-Samarai, 2012). High incidences of stillbirth in spring than in autumn were reported by Fiedlerova *et al*. (2008) in the study of Holstein cows in Czech Republic (Europe) and high incidences in winter than in summer were reported by McGuirk (2004) and Del Río *et al*. (2007). Ratshivhombela (2021) in her study on South African Holstein cattle reported similar findings as the current study.

3.4.3. Year effect on incidences of stillbirth in South African Holstein dairy cattle

In the current study animals from 2005 until 2018 are at high risk of stillbirth, while those before 2005 are at less risk of stillbirth. In agreement to the current study Atashi (2011) and Ratshivhombela (2021) reported birth year to have a significant effect to incidences of stillbirth. Birth year was one of the important factors affecting stillbirth. Generally, in the current study stillbirth incidences were less than 5% from 1985 to 2004 and from 2005 the stillbirth incidences were ranging from 10 to 30.4%. Mahnani *et al*. (2018) in the study of stillbirth in Holstein dairy cattle reported an average of 4.2% incidence of stillbirth per year. The birth year influence can be attributed to biological variation within the population over a period and the different in environmental conditions from year to year (Al-Samarai, 2012; Ratshivhombela, 2021). Environmental conditions are different from year to year the same as the seasonal conditions (Fiedlerova *et al*., 2008).

3.4.4. Generational effect on incidences of stillbirth in South African Holstein dairy cattle

The study shows that before the 10th generation the stillbirth incidences was below 3 percent, then start increasing from 10th generation with the range from 3.4% to 16.4%. The increase in the incidences of stillbirth from the 10th generation could be attributed to the genetic contribution from both the maternal and the paternal side to the next generation. The genetic diversity loss could be a contributing factor to the increases in the incidences of stillbirth (Stachowicz *et al*., 2011). Generation intervals (GI) include the four paths of selection, which are the sire to sire (L_{ss}), sire to dam (L_{sd}), dam to sire (L_{ds}) and dam to dam (L_{dd}), as the average age of parents when their offspring were born (Menezes *et al*., 2015). Generation intervals is longer when older sires and dams are used and shorter when young sires and dams are used (Decker, 2014).

3.4.5. Dam parity effect on incidences of stillbirth in South African Holstein dairy cattle

The current study indicates that cows in later parity are having high incidences of stillbirth as compared to cows at the first parity. A significant effect of dam parity on stillbirth incidences has been reported by several researchers (Fiedlerova *et al*., 2008; Szücs *et al*., 2009; Ratshivhombela, 2021). Several schoolers reported results in contrary to the current study (Berry *et al*., 2007; Bicalho *et al*., 2007; Fiedlerova *et al*., 2008; Szücs *et al*., 2009). This may be due to disproportion between the pelvic area of the cows at first calving and the calf size, which result in dystocia and high incidence of stillbirth (Szücs *et al*., 2009). In the current study the 11th and 12th parities have high stillbirth, and the 2nd parity has the lowest stillbirth incidences. The difference in the incidences of stillbirth in cow in different parity could be due to the health status associated with cows in different parities (Citek *et al*., 2011).

3.4.6. Calf sex effect on incidences of stillbirth in South African Holstein dairy cattle

The female calves shown a high incidence of stillbirth in the current study while the male calves had low incidences of stillbirth. In disagreement with the current study Steinbock *et al*. (2006), Hickey *et al*. (2007) and Ratshivhombela (2021) reported male calves to have higher incidence of stillbirth and female calves having low incidences of stillbirth. However, Ratshivhombela (2021) in the study done on South African Holstein only reported the calf sex effect from 2014 to 2018 only. The high stillbirth incidences in male calves than in female calves could be attributed to the high birth weight of male calves as compared to female calves (Adamec *et al*., 2006; Steinbock *et al*., 2006).

3.4.7. Phenotypic correlation coefficients amongst stillbirth, birth year, generation, dam parity and dam age of South African Holstein dairy cattle

Stillbirth were found to have a positive correlation with birth year and generation, but negatively correlated to dam parity and dam age. This implies that incidences of stillbirth increase per birth year and per generation, however young dams in the first parity have high incidences of stillbirth. The high incidences of stillbirth are associated with dams in first parity (Atashi, 2011) and young dams (Mee *et al*., 2014). Stillbirth had positive correlation with birth year and generation, this could be described by the proportion of the calf weight and the dam weight and pelvic area (Hossein-Zadeh, 2013). Birth year is directly associated with generation, but inversely associated to dam parity and dam age. The high negative correlation between the stillbirth and the dam parity could be attributed to the body weight proportion of the dam and the calf (Szücs *et al*., 2009).

3.5. Conclusion and recommendation

In conclusion, dam parity, calf sex, birth season, birth year, herd, and generation are important factors affecting stillbirth incidences. It was evident that there is a great variation in the incidences of stillbirth across herds of South African Holstein dairy cattle, which could be due to different environmental conditions, different herd size, genetic variation, and management practices. Multiparous and female calves are associated with high incidences of stillbirth as compared to primiparous and male calves. Study provided understanding on the effect of dam parity, calf sex, birth season, birth year, herd, and generation on the incidences of stillbirth in South African Holstein dairy cattle. However, there is very high variation in the incidences of stillbirth across the herds in small scale faming. Thus, warrant a need for further investigation of the inbreeding rate and the effective population size within the herds of South African Holstein dairy population.

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CHAPTER FOUR

Inbreeding rate and the effective population size within herds of South African Holstein dairy cattle

Abstract

Inbreeding rates within a population contribute to the reproductive performance of that population. Pedigree records of Holstein dairy cattle of South Africa born between 1945 to 2020 were extracted from INTERGIS database and used to evaluate population structure and the genetic diversity. Inbreeding coefficients, inbreeding rate, effective population size, relatedness, and average Discreet Generation Equivalents (DGE) per generation were estimated from the population. Parameter estimation and pedigree analysis were done based on the entire population using ENDOG V4.8 (Gutiérrez *et al.*, 2010). CFC software was used to estimate the effective population size (N_e), inbreeding coefficient (F) and Inbreeding rate (ΔF). From the entire population, 0.48% of the progenies were inbreds with an average F ranging from 2.48% to 24.60%. Only 42.59% of the population were founders while 57.41% were non-founders. Average DGE ranged from 0.226 to 1.256, with most generations having average DGE approximating one, indicating that majority of the generations had complete generation. Average generational inbreeding coefficient ranged from 0.0020% to 0.1099% and relatedness was mostly less than 0.0002 from the 1st to the 12th generation. Inbreeding rate increased per generation with the 13th generation recoding the lowest inbreeding rate of 0.0479 while the 2nd generation recorded the highest inbreeding rate of 0.5536. The 14th generation was more related to the 13th and 12th generation than the 1st generation. The 2nd generation had the lowest number of breeding males of 23 and the lowest N_e of 90.3, while the 13th generation had the highest number of breeding males of 264 and the highest N_e of 10447.7. The effective population size depends mainly on the number of breeding males than the number of breeding females.

Key words: Founders, Inbreeding coefficients, Pedigree information, Discreet Generation Equivalents, Relatedness

4.1. Introduction

Intensive selection in South African dairy industry for high yields and improved type has resulted in more use of biotechnologies for breeding purposes (Adamec *et al*., 2006; Makanjuola *et al*., 2020) resulting in the reduction of genetic diversity (Maiwashe *et al*., 2006; Melka *et al*., 2013). Population structure and genetic diversity monitoring within breeds of any species is vital for maintenance of genetic resources and conservation of future breeding options (Melka *et al*., 2013; Mandal *et al*., 2022). Genetic enhancement and breed conservation in livestock population are facilitated by conservation and maintenance of low levels of inbreeding and high levels of genetic variability (Mandal *et al*., 2022). Rate of inbreeding accumulation in a population is affected by incorporation of strategy of technologies such as genomic selection (Makanjuola *et al*., 2020), artificial insemination (AI) and embryo transfer (Melka *et al*., 2013) into the breeding program routine, depending on rate of recurrence and strategy used to implement (Stachowicz *et al*., 2011; Baes *et al*.,2019). Introduction of AI technologies significantly increased number of progenies produced by superior sires and lowered the effective population size, as a result genetic diversity is reduced in the subsequent generations (Melka *et al*., 2013; Rodríguez-Ramilo *et al*., 2015; Baes *et al*., 2019). Stachowicz *et al*. (2011) reported an estimated effective population size to approximately 115 in Canadian Holstein and 100 in Jersey cattle, Rodríguez-Ramilo *et al.* (2015) also reported similar results in Spanish Holstein population.

No significant change is expected in the effective population size if generation intervals remain the same, as rates of increases in inbreeding and coancestry show decreasing trends (Stachowicz *et al*., 2011; Rodríguez-Ramilo *et al*., 2015). Inbreeding increases and effective population size deceases due to intensive selection and mating using few sires (Adamec *et al*., 2006; Makanjuola *et al*., 2020). This led to only alleles of selected parents being represented in the next generation, as a result there is a loss of genetic diversity which could have been contributed by the unselected individuals (Melka *et al*., 2013). However main causes of genetic diversity loss can be determined by analysing the pedigrees of the South African Holstein dairy cattle to approximate current and past inbreeding rate and the genetic diversity (Stachowicz *et al*., 2011).

Inbreeding within a population reduces genetic variability and performance mainly in traits associated with individual fitness such as fertility (Maiwashe *et al*., 2006; Melka *et al*., 2013). Hence, minimizing inbreeding and maximizing genetic diversity are the two main objectives of any conservation program, and this requires a detailed knowledge of population structures within breeds to set conservation priorities (Caballero and Toro, 2000; Melka *et al*., 2013). Stachowicz *et al*. (2011) reported that an overall population size that is large it does not imply that the population is problems free typical for small, endangered populations. Given the increase in the inbreeding rate there is necessity to implement means and measures to control the inbreeding rate per year, this will help in managing and maintaining farm animal genetic resources (Makanjuola *et al*., 2020). Loss in genetic diversity in South African dairy animals can be due to increase in genetic drift over the non-founder generations, this takes place due to small effective population size (Stachowicz *et al*., 2011; Melka *et al*., 2013; Mandal *et al*., 2022). The objectives of this study were to use the pedigree data to determine the effective population size, inbreeding coefficient, and the inbreeding rate of the South African Holstein dairy cattle population.

4.2. Material and Methods

4.2.1. Study site, Study animals and Management

The study site was the same as described under chapter 3, section 3.3.1. This goes the same for the study animals and management as described under the same chapter section 3.3.2.

4.2.2. Data set, pedigree information and editing

Data set of 1 million dairy Holstein cattle born from 1945 to 2020 were extracted from INTERGIS database of registered South African Holstein cattle and used in this study. No sampling was done because this is a population study which use animal relationships of which removing some animals voluntarily may remove some common ancestors critical in calculating the inbreeding coefficients thus negatively affecting the generations to be studied. The information that was collected included the pedigree information containing breeding males and females, animal ID, birth date, the season of birth, breeder, and farm region.

4.2.3. Statistical methods

4.2.3.1. Pedigree analysis

Data for pedigree analysis included 1 357 200 dairy Holstein cows born between 1945 and 2020. Parameter estimation and pedigree analysis were done based on the entire population using ENDOG v4.8 (Gutiérrez *et al*., 2010) and CFC v1.0 (Sargolzaei *et al.,* 2006).

4.2.3.2. Generation interval

Generation intervals (GI) were computed across the four paths of selection, sire to sire (L_{ss}), sire to dam (L_{sd}), dam to sire (L_{ds}) and dam to dam (L_{dd}), as the average age of parents when their offspring were born (Menezes *et al*., 2015). The average generation interval was computed as follows:

$$GI = \frac{\mathrm{Lss} + \mathrm{Lsd} + \mathrm{Lds} + \mathrm{Ldd}}{4}$$

The above calculation was carried out using ENDOG Software (Ver 4.8) (Gutiérrez and Goyache, 2005).

The completeness, quality and depth of the pedigree crucially affect estimates of inbreeding coefficients, relationships among animals, and, to a lesser extent, generation intervals and effective numbers of founders and ancestors. Number of equivalent complete generations was used to assess pedigree quality. The number of equivalent complete generation for individual *i* (EqG_i) was computed as $EqGi = \Sigma\ (1/2)^n$ where *n* is the number of generations separating the individual from each known ancestor (Maignel *et al*., 1996). The average numbers of equivalent complete generations for the whole and reference populations were computed by averaging individual EqG (Mokhtari *et al*., 2015). This was done using CFC Software 1.0 (Sargolzaei *et al.,* 2006)

4.2.3.3. Measures of population genetic variability

Inbreeding coefficient (F), Inbreeding rate (ΔF) and Effective population size (N_e) was calculated using the fallowing formulas by (Wright, 1931).

4.2.3.3.1. Inbreeding coefficient

Inbreeding coefficient of an individual (F_x) is the probability that two alleles at the same locus are identical by decent (Wright, 1922). This Fx was calculated per generation as.

$$F_x = \Sigma\ [(\frac{1}{2})^{n+1}\ (1+F_A)]$$

Where: F_x = coefficient of inbreeding of an individual *X*;

n = number of connecting links between the two parents of *X* through shared ancestors; and

F_A = coefficient of inbreeding of the shared ancestor *A*.

4.2.3.3.2. Inbreeding rate

Inbreeding rate (ΔF) was calculated per generation as:

$$\Delta F = \frac{1}{8 * \mathrm{Nm}} + \frac{1}{8 * \mathrm{Nf}}$$

Where: ΔF = inbreeding rate;

N_m = The number of breeding males and

N_f = The number of breeding females.

4.2.3.3.3. Effective population size

Effective population size (N_e) is the number of breeding animals that would lead to the observed increase in inbreeding if they contributed equally to the next generation (Gutiérrez *et al*., 2003). This N_e, referred to as the realized effective size by Cervantes *et al*. (2011), was calculated per generation as:

$$N_e = \frac{(4 * \mathrm{N}m * \mathrm{N}f)}{\mathrm{N}m + \mathrm{N}f}$$

Where: N_e = Effective population size;

Nm = The number of breeding males; and

N_f = The number of breeding females.

4.3. Results

4.3.1. Population demography

Statistical summary from the pedigree analysis (shown under Table 4.1 below) indicated that 6 575 (0.48%) of the progeny from the total population of 1 357 200 animals were inbreds. Total number of sires and dams were 16 946 (1.25%) and 509 753 (37.56%) respectively, while sire and dam progeny contribution were 49,60 and 37.56%, respectively. However, individuals with progeny were 38.81% while 61.19% where individuals with no progeny. Number of founders were 578 047, which is 42.59% of the total number of individuals, while founding sires and dams were 0.84% and 25.84%, respectively. Progeny contribution from founders were 46.35% of the total number of individuals while founding sires and dam’s progeny contributions were 29.96% and 32.76% respectively. However, 15.91% of the total individuals were founders without progeny.

Number of non-founders were 57.41% of the total number of individuals, while non-founding sires and dams were 0.41% and 11.72%, respectively. Progeny contribution from non-founding sires were 19.64% while non-founding dams’ contribution were 15.67%. Non-founders with known parents were 40.62% of the total number of individuals, while non-founders with only known sire were 8.99% and those with only known dam were 7.81%. Full sibling groups were 1.16% of the total number of individuals and average family size was 2.1 although family siblings were ranging from 2 to 12 individuals.

Table 4. 1. Statistics summary from the pedigree analysis in South African Holstein cattle

Attribute	**Count**	**Proportion (%)**
Individuals in total	1357200	100
Inbreds in total	6575	0.48445
Evaluated individuals	1357200	100
Inbreds in evaluated	6575	0.48445
Progeny		
Sires in total	16946	1.24860
Sire Progeny	673200	49.60212
Dams in total	509753	37.55917
Dam Progeny	657205	48.42359
Individuals with progeny	526699	38.80777
Individuals with no progeny	830501	61.19223
Population Founders		
Founders	578047	42.59114
Progeny	629103	46.35301
Founding Sires	11364	0.83731
Progeny	406585	29.95763
Founding Dams	350701	25.84004
Progeny	444563	32.75589
Founders with no progeny	215982	15.91379
Non-founders		
Non-founders	779153	57.40886
Non-founding Sires	5582	0.41129
Progeny	266615	19.64449
Non-founding Dams	159052	11.71913
Progeny	212642	15.66770
Non-founders only with known sire	121948	8.98526
Non-founders only with known dam	105953	7.80673
Non-founders with known sire and dam	551252	40.61686
Siblings		
Full-sib groups	15770	1.16195
Average family size	2.10108	0.00015
Maximum	12	0.00088
Minimum	2	0.00015

4.3.2. Equivalent complete generation, and generation interval

Average Discreet Generation Equivalents (DGE) and DGE per generation of South African Holstein dairy cattle population is represented in Table 4.3. The minimum DGE was zero across generations and maximum per generation ranged from 2.625 to 5.285, while the average DGE ranged from 0.225 to 1.256. The 10th generation had the highest DGE of 1.256 while the 1st generation had the lowest DGE of 0.256. This implies that the 1st generation was not complete, and the 10th generation was having more than 1 complete generation. Inbred animals (6 575) account for only 0.48% to the whole population. From the 6575 of inbreds, DGE ranged from 1.250 to 5.285 with standard deviation of 0.7359. The 1st generation had the lowest number of inbreds of 2 and the lowest number of DGE.

4.3.3. Inbreeding and average relatedness

Inbreeding rates and coefficients and average relatedness are represented in Table 4.2 and 4.3. An increase in average F was observed until the 8th generation, with a peak of 0.001099 in the 4th generation. However, there was a drop in average F in the 5th generation and from the 9th to the 12th generation. The 1st generation recorded the lowest average F of 0.000020. A decrease in average F in inbreds was observed across the generations with a minimum F record of 0.0248 in the 10th generation. An increase was observed in the 3rd, 11th, 13 and 14th generations. The 3rd generation recorded the highest F in inbreds of 0.2460. A decrease in the inbreeding rate per generation was observed with the 13th generation recoding the lowest inbreeding rate of 0.0479.

In the 7th ,9th and 10th generation inbreeding rate increased to 0.0615, 0.1473 and 0.2342 percent, respectively. The 2nd generation recorded the highest inbreeding rate of 0.5536. Inbred animals (6 575) account for only 0.48% to the whole population. From the 6575 of inbreds F ranged from 0 to 0.375 with standard deviation of 0.0915. Average relatedness of individuals across generations of Holstein population in South Africa is represented in Figure 4.1. The relatedness was less than 0.0002 from the first generation until the 12th generation. Thereafter, relatedness increased picking in the 14th generation.

4.3.3. Effective population size (N_e) per generation

The effective population size (N_e) per generation is shown on Table 4.2 below. A steady increase in N_e was observed from the 2nd until the 8th generation. Thereafter there was a drop in N_e in the 9th and 10th generation. Another increase in N_e was observed from the 11th generation with a pick of 1044.7 in the 13th generation and dropped to 867 in the 14th generation. The lowest N_e of 90.3 was observed in the 2nd generation. Table 4.2 also shows the number of breeding males and females per generation. It was observed that the higher the number of breeding males the higher the effective population size. This was evident in the 2nd and the 13th generation; the 2nd generation had the lowest number of breeding males of 23 while the 13th generation had the highest number of breeding males of 264. However, the 2nd generation also had the lowest N_e of 90.3, while the 13th generation had the highest N_e of 10447.7.

Table 4. 2. Effective population size and inbreeding rate per generation of South African Holstein dairy cattle

Generation	Breeding Males	Breeding Females	Ne	∆F (%)
1	68	17486	269.6	0.1855
2	23	25105	90.3	0.5536
3	28	39414	113.3	0.4412
4	81	57481	324.3	0.1542
5	115	109973	460.5	0.1086
6	211	140487	843.5	0.0593
7	203	168286	812.6	0.0615
8	236	146530	942.5	0.0531
9	85	108762	339.3	0.1473
10	53	79396	213.5	0.2342
11	91	42203	363.0	0.1377
12	207	31744	822.8	0.0608
13	264	22171	1044.7	0.0479
14	219	21600	867.0	0.0577

Table 4. 3. Average inbreeding coefficient, Average inbreeding coefficient in inbreds and average Discreet Generation Equivalents per generation of South African Holstein dairy cattle

Generation	Number of Individuals	Number of Inbreds	Number of Founders	Number of individuals with Known	No progeny	Unique ancestors	Average F	Average F in inbreds	Maximum F in inbreds	Minimum F in inbreds	R (Reciprocals & Self R)	R (Reciprocals)	Average Dis. Gen. Equivalents)	Max (DGE)	Min (DGE)
1	18839	2	12967	1919	14576	6834	0.000020	0.1875	0.2500	0.1250	0.000064	0.000011	0.225	2.625	0
2	25557	3	15900	2054	21843	10581	0.000022	0.1875	0.2500	0.0625	0.000089	0.000050	0.257	2.813	0
3	39981	121	22167	6578	32547	14444	0.000744	0.2460	0.2500	0.0156	0.000744	0.000719	0.360	2.844	0
4	59105	285	29426	14893	43472	20745	0.001099	0.2279	0.3750	0.0313	0.001454	0.001437	0.466	3.125	0
5	112278	263	51923	41088	75163	47540	0.000358	0.1527	0.2500	0.0039	0.001217	0.001208	0.615	3.313	0
6	144711	613	32923	82999	107602	94903	0.000475	0.1122	0.3750	0.0010	0.003800	0.003794	1.029	3.781	0
7	172354	1099	38983	98899	137031	120418	0.000540	0.0847	0.3125	0.0010	0.003092	0.003086	1.072	4.172	0
8	151250	1536	23647	89472	119912	120218	0.000572	0.0563	0.2813	0.0005	0.003858	0.003851	1.167	4.492	0
9	110460	1272	14868	67713	90671	104252	0.000475	0.0412	0.2500	0.0002	0.002258	0.002249	1.210	4.789	0
10	80476	930	9038	50947	70042	83367	0.000287	0.0248	0.2817	0.0001	0.001754	0.001742	1.256	4.781	0
11	44022	280	7794	27463	38425	45330	0.000256	0.0403	0.2626	0.0001	0.001111	0.001088	1.088	5.285	0
12	35885	92	5870	24185	31441	35307	0.000099	0.0387	0.2500	0.0001	0.000936	0.000908	1.048	4.359	0
13	27457	50	2290	21540	24025	28560	0.000172	0.0944	0.2500	0.0001	0.001716	0.001680	1.118	4.168	0
14	25979	29	1405	21502	23751	25858	0.000143	0.1277	0.2500	0.0008	0.001228	0.001189	1.188	3.566	0
Total/mean	**1357200**	**6575**	**578047**	**551252**	**830501**	**0**	**0.000356**	**0.0735**	**0.3750**	**0.00006**	**0.000485**	**0.000485**	**0.747**	**5.285**	**0**

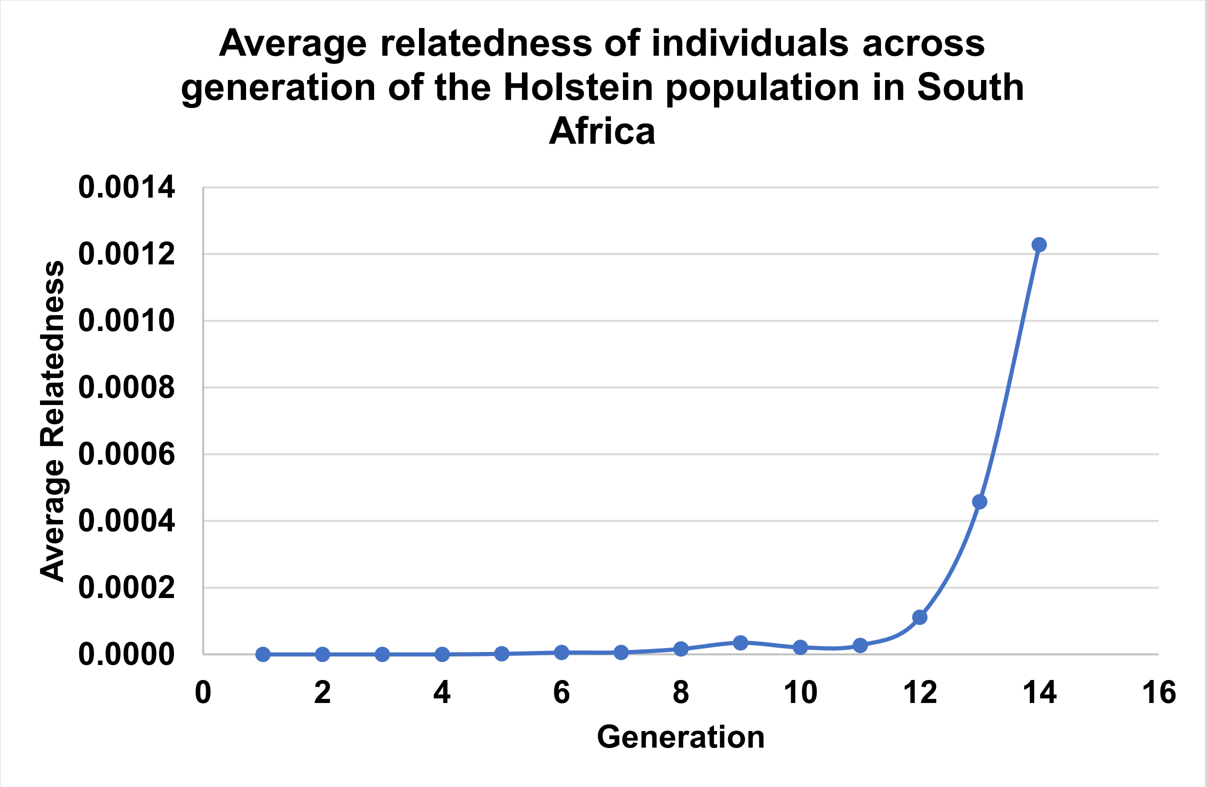

Figure 4. 1. Average relatedness of individuals across generations of the Holstein population in South Africa

4.4. Discussion

4.4.1. Equivalent complete generation, and generation interval

In this study, minimum DGE was zero across generations and maximum per generation ranged from 2.625 to 5.285, while the average DGE ranged from 0.225 to 1.256. The highest average DGE of 1.256 and the lowest of 0.256 was observed in the 10th and the 1st generations, respectively. Inbred animals (6 575) account for only 0.48% to the whole population. From the 6575 of inbreds DGE ranged from 1.250 to 5.285 with standard deviation of 0.7359, while F among inbreds ranged from 0 to 0.375 with standard deviation of 0.0915. It was reported that average DGE in 2006 reached about 15 to 10 for Canadian Holstein and jerseys respectively (Stachowicz *et al*., 2011). However lower values were reported in Danish Holstein and Jersey in 1999 to 2003 (7.20 and 7.36, respectively) by Sørensen *et al*. (2005). Germany reported average number of DGE approximately 6 in Holstein cows born between 1993 to 1999 (Stachowicz *et al*., 2011). Hammami *et al*. (2007) reported average DGE in Holstein cattle of 6.3 in Luxembourg and 8.2 in Tunisia during the year 2000. The issue is linked to the overlapping generation brought about by the AI and other biotechnology protocols in dairy production. A high number in those other countries mean a lot of overlapping happened.

4.4.2. Inbreeding and average relatedness

Inbreeding coefficient (F) indicates the chances that an animal receives the same allele from both parents due to their relatedness (Kor and van der Waaij, 2015). In this study, average F and F in inbreds ranged from 0.0020% to 0.1099% and 2.48% to 24.60% respectively. Rokouei *et al*. (2010) on their study on dairy Gyr cattle reported that dams with inbreeding coefficient of 0-12.5% and 12.5-25% had 1% and 3% greater incidence of dystocia, respectively, over non-inbred dams. The high average F can be attributed to having more inbreds as breeding animals and use of biotechniques such as AI with perhaps superior sires repeatedly. Increase in inbreeding coefficient can lead to reduction in milk production, fat and protein and losses in reproductive traits (Mc Parland *et al*., 2007; Reis Filho *et al*., 2015).

Inbreeding rate (ΔF) expresses the increase in average inbreeding level in a population from one generation to the next and due to the fact that a rise in inbreeding is non-linear. The rate of inbreeding is expressed relative to how much the population is away from full inbreeding (Kor and Van der Waaij, 2015). The study showed that high inbreeding rate is associated with lower number of breeding males. This was well evident in the 2nd and 13th generation. The 2nd generation had the highest ΔF with lowest breeding males, while the 13th generation had the lowest inbreeding rate with the highest breeding males. High inbreeding rate in the 2nd generation can be attributed to the contribution of few breeding males in that generation. An increase in inbreeding rate can result in decline in phenotypic performances of livestock animals such as body weight and milk yield (Makanjuola *et al*., 2020a).

Average relatedness indicates genetic contribution of founders to the population and can be used as an index to maintain the initial genetic stock as well as to compare inbreeding among subpopulations (Goyache *et al*., 2003). Relatedness in the current study was less than 0.0002 cross generations and started to increase from the 13th generation, reaching a pick of 0.0012 in the 14th generation. This indicate that the 14th generation is more related to the 13th and 12th respectively, than the 1st generation. Reis Filho *et al*., (2010) reported higher average relatedness of 2.10% in their study in Brazilian Gyr dairy cattle.

4.4.3. Effective population size (N_e) per generation

Effective population size (N_e) refers to the number of individuals that effectively participates in producing the next generation (Sbordoni *et al*., 2012) and is sensitive to changes in census population size over time (Kliman *et al*., 2008). In this study, it was observed that the higher the number of breeding males the higher the N_e. This was evident in the 2nd and the 13th generation. The 2nd generation had the lowest number of breeding males and the lowest N_e, while the 13th generation had the highest breeding males and the highest N_e. However, Maiwashe *et al* (2006) reported that N_e depends largely on number of breeding males and females contributing an offspring to the next generation within a population. Small N_e can result in inbreeding depression, which is the noticeable decline in the phenotypic mean of economically traits of importance within a population (Makanjuola *et al*., 2020a).

Lower N_e is also associated with less genetic variability within the population (Maiwashe *et al*., 2006). The low N_e in generations such as the 2nd generation with small number of breeding males can be attributed to the reduction in variation as few sires contribute towards the next generation. The low N_e can be attributed to the intensive selection in dairy industry for high yields resulting the improved type having decreased effective population sizes and increased average inbreeding coefficients within all pure breeds (Adamec *et al*., 2006; Makanjuola *et al*., 2020).

4.5. Conclusion and Recommendation

In conclusion, it was evident that effective population size of the Holstein dairy cattle depends mainly on the number of breeding males than breeding female. Due to the observation of higher population size always associated with higher number of breeding males. Ne is having a reverse relationship with the inbreeding rate, which was well shown in the 2nd and 13th generation. The Ne was lowest but inbreeding rate was highest in the 2nd generation, while the opposite was observed in the 13th generation. Average DGE across generations ranged from 0.226 to 1.256, with most generations having average DGE of close to 1. This indicated that most generations were complete. Inbreeding rate is one of the main factors contributing to the production performance of the Holstein dairy cattle population.

Current study shown instability in the effective population size and inbreeding rate across generations of South African Holstein dairy cattle. The result also indicated a need for proper animal management practice to regulate inbreeding rate of population. Application of proper management in dealing with levels of inbreeding will increase effective population size and maintain genetic variation in South African Holstein dairy cattle population. The low effective population size and the relatively high inbreeding rate across generations of South African Holstein dairy cattle, indicated the need to further investigate the inbreeding effect on reproductive performance such as stillbirth incidences.

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CHAPTER FIVE

Effect of inbreeding on the incidences of stillbirth across the generations in South African Holstein dairy cattle

Abstract

The study determined the effect of inbreeding rate, effective population size, average inbreeding coefficient in across generation on the incidences of stillbirth in South African Holstein dairy cattle population. Inbreeding coefficient (Fx), effective population size (N_e) and inbreeding rate (ΔF) were calculated using formulas by Wright (1931). The effect of inbreeding rate on incidence of stillbirth per generation were modelled in SAS software (SAS, 2021). Generation intervals were determined using ENDOG Software (Ver 4.8) (Gutiérrez and Goyache, 2005). The regression model was observed to be statistically significant ($P<0.05$) with R^2 value of 88.61%, however the evaluated factors; number of individuals, no of inbreds, no of founders, average F, average F in the inbreds, effective population size and inbreeding rate were not significant on incidences of stillbirth. Inbreeding rate had no significant effect on the incidences of stillbirth across generations. Generations had a significant ($P<0.05$) effect on the incidences of stillbirth, average inbreeding coefficients in the inbreds, ΔF and N_e of South African Holstein dairy cattle. Stillbirth incidences across generations of South African Holstein dairy cattle population was significantly ($P<0.05$) affected by N_e and average inbreeding coefficients in the inbreds. Inbreeding rate was significantly and negatively correlated to generations (r= -0.653) and stillbirth (r= -0.553). Generation was observed to be positively and significantly correlated with effective population size (r= 0.653) and stillbirth (r= 0.873), however negatively and significantly correlated with average inbreeding coefficient in the inbreds (r= -0.700). This means an increase in generation is associated with an increase in effective population size and stillbirth, and a decrease in average inbreeding coefficient in the inbreds. Stillbirth was positively and significantly correlated with effective population size (r= 0.553) and negatively and significantly correlated with average inbreeding coefficient in the inbreds (r= -0.656). Number of inbreds had a positive and significant correlation with average inbreeding coefficient (r= 0.732). This means stillbirth incidences decreases with increasing inbreeding rate in the South African Holstein population.

Key words: Effective population size, inbreeding, generation, inbreds, regression

5.1. Introduction

Inbreeding on average in Holstein dairy cows appears to be increasing and its increase is of serious concern to dairy breeders and the industry (Mc Parland *et al*., 2007; Atashi *et al*., 2012). Increase in inbreeding by 1% is associated with an increase in the incidences of stillbirth by 0.25% and 0.20% for male and female calves respectively for first parity births (Adamec *et al*., 2006) and associated with lengthening calving interval (Mc Parland *et al*., 2007; Hossein-Zadeh, 2013; Morek-Kopeć *et al*., 2021). Influence of inbreeding on stillbirth and dystocia decline with parity and is mostly small in later parities (Adamec *et al*., 2006). In dairy cattle, increase in inbreeding is associated with major economic losses such as calve loss and high veterinary services (Maiwashe *et al*., 2008).

Reproductive traits such as stillbirth and dystocia are economically and ethically important and it is of important interest to the public and the farmers that they occur at the lowest possible levels. Stillborn calves are of increasing concern to dairy producers (Atashi *et al*., 2012; Piwczyński *et al*., 2013). Inbreeding affects other reproductive aspects including interval to estrus and number of services per conception, with an increase of 5 days in age at first calving and 3.3 days calving interval extension in young cow with 12.5% inbreeding (Adamec *et al*., 2006). There are less publications on the effect of inbreeding on dairy cattle calving performance in terms of dystocia, stillbirth, twinning, and sex ratio. This indicates a need to investigate the inbreeding rate and how it affects the incidences of stillbirth. The objective of this study was to determine the effect of inbreeding rate on the incidences of stillbirth across a generation in South African Holstein dairy cattle population.

5.2. Material and Methods

5.2.1. Study site, Study animals and Management

The study site was the same as described under chapter 3, section 3.3.1. This goes the same for the study animals and management as described under the same chapter section 3.3.2.

5.2.2. Study design and data collection

The description of the data done in Chapter 3. The information that was gathered included the calculated inbreeding coefficient, inbreeding rate, and the effective population size per generation. They were calculated using formulas by Wright (1931), inbreeding coefficient as $Fx = \Sigma\ [(\frac{1}{2})^{n+1}\ (1+F_A)]$; effective population size as $N_e = (4^*N_m^*N_f)/\ (N_m + N_f)$ and inbreeding rate as $\Delta F = 1/\ 8^*N_m + 1/8^*N_f$. Generation intervals were computed across the four paths of selection, sire to sire (L_{ss}), sire to dam (L_{sd}), dam to sire (L_{ds}) and dam to dam (L_{dd}), as the average age of parents when their offspring were born (Menezes *et al.*, 2015). These were calculated from the pedigree information which included breeding males and females, animal ID, birth date, the season of birth, breeder, sex, and age of dam at kidding.

5.2.3. Statistical methods

The effect of inbreeding rate on incidence of stillbirth per generation were modelled by the regression model given below, and SAS (SAS, 2021) statistical analysis software was used for regression analysis.

$$Y = a + b_1X_1 + b_2X_2 + b_nX_n + e$$

Where:

Y = The response variable (the incidences of stillbirth).

a = The regression constant;

$b_1 – b_n$ = regression coefficient of 1st to *n*th independent variables, and $x_1 – x_n$ = score of the 1st to *n*th independent variables (Inbreeding rate, effective population size, no of inbreds, inbreeding coefficient in inbreds, no of individuals and no of founders); and

e = The residual error.

5.3. Results

5.3.1. Regression analysis

Table 5.1. below represent the ANOVA table for Regression analysis determined from generations, no of individuals, no of inbreds, no of founders, average inbreeding coefficients, average inbreeding coefficients in the inbreds, effective population size and inbreeding rate. The regression model was observed to be statistically significant (*P<0.05*) with R square (R^2) value of 88.61%, however all the factors were not significant.

Table 5. 1. ANOVA table for Regression analysis

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Regression	8	394.528	49.3160	4.86	0.049
Generations	1	16.891	16.8915	1.66	0.253
No of Individuals	1	0.104	0.1036	0.01	0.923
No of inbreds	1	22.655	22.6548	2.23	0.195
No of founders	1	0.004	0.0041	0.00	0.985
Average F	1	2.603	2.6026	0.26	0.634
Average F in the inbreds	1	11.483	11.4833	1.13	0.336
Effective population size	1	0.959	0.9588	0.09	0.771
Inbreeding rate	1	0.618	0.6184	0.06	0.815
Error	5	50.732	10.1465		
Total	13	445.260			

5.3.2. The inbreeding effect on the incidence of stillbirth across generations of South African Holstein dairy cattle

The inbreeding effect on the incidences of stillbirth across generations of South African Holstein dairy cattle is represented in Figure 5.1. Inbreeding rate had a significant effect ($P<0.05$) on the incidences of stillbirth across generations. Inbreeding rate had a negative relationship with stillbirth with R square of 13.6%, meaning that an increase in inbreeding rate result in the reduction in the stillbirth.

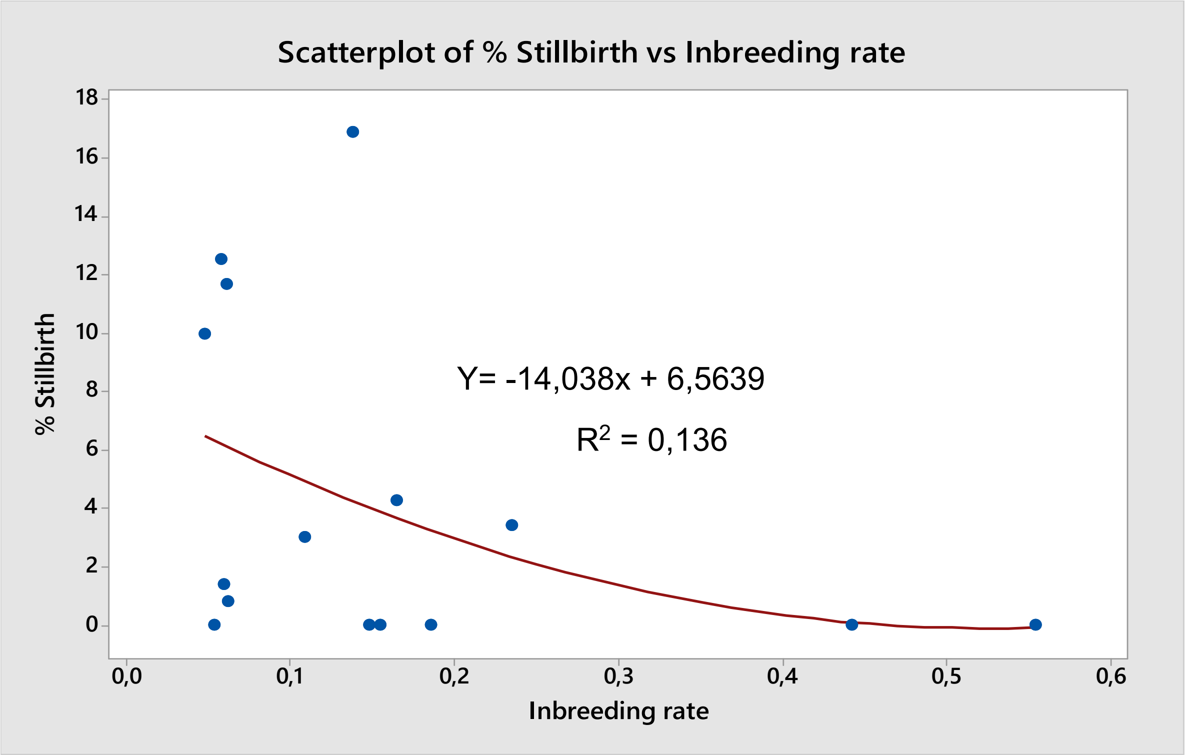

Figure 5. 1. Inbreeding rate effect on the incidences of stillbirth across generations of South African Holstein dairy cattle

5.3.3. Generational effect on the incidences of stillbirth, average inbreeding coefficients in the inbreds, inbreeding rate and effective population size of South African Holstein dairy cattle

The effect of generations on incidences of stillbirth across generations of South African Holstein dairy cattle is represented in Figure 5.2. Generations had a significant ($P<0.05$) effect on the incidences of stillbirth, average inbreeding coefficients in the inbreds, inbreeding rate and effective population size of South African Holstein dairy cattle. Generational effect on effective population size is represented in Figure 5.7. Generations had a positive relationship with stillbirth with R square of 60% and effective population size was fluctuating across generations with R square of 92%. However, generation had a negative relationship with inbreeding rate and average inbreeding coefficients in the inbreds with R squares of 35% and 53% respectively. This means an increase in generations of South African Holstein dairy cattle population is associated with an increase in stillbirth and effective population size, and a reduction in inbreeding rate and average inbreeding coefficients in the inbreds. Inbreeding rate result in an increase in average inbreeding level in a population from one generation to the next. Generational effect on inbreeding rate across generations of South African Holstein dairy cattle is represented in Figure 5.3. The effect of generation on average F in the inbreds of South African Holstein dairy cattle is represented in Figure 5.4.

5.3.4. The effect of effective population size and average inbreeding coefficients in the inbreds on stillbirth incidences across generations of South African Holstein dairy cattle

The effect of effective population size on stillbirth incidences across generations of South African Holstein dairy cattle is represented in Figure 5.5. Stillbirth incidences across generations of South African Holstein dairy cattle population was significantly ($P<0.05$) affected by effective population size and average inbreeding coefficients in the inbreds. Stillbirth incidences had a positive relationship with effective population size, with an R square value of 12%. However, stillbirth had a negative relationship with inbreeding rate and average inbreeding coefficients in the inbreds, with R square values of 13% and 20% respectively. Therefore, an increase in stillbirth incidences is associated with an increase in effective population size and a reduction in average inbreeding coefficients in the inbreds. The effect of average inbreeding coefficients in the inbreds on stillbirth incidences across South African Holstein dairy cattle is represented in Figure 5.6.

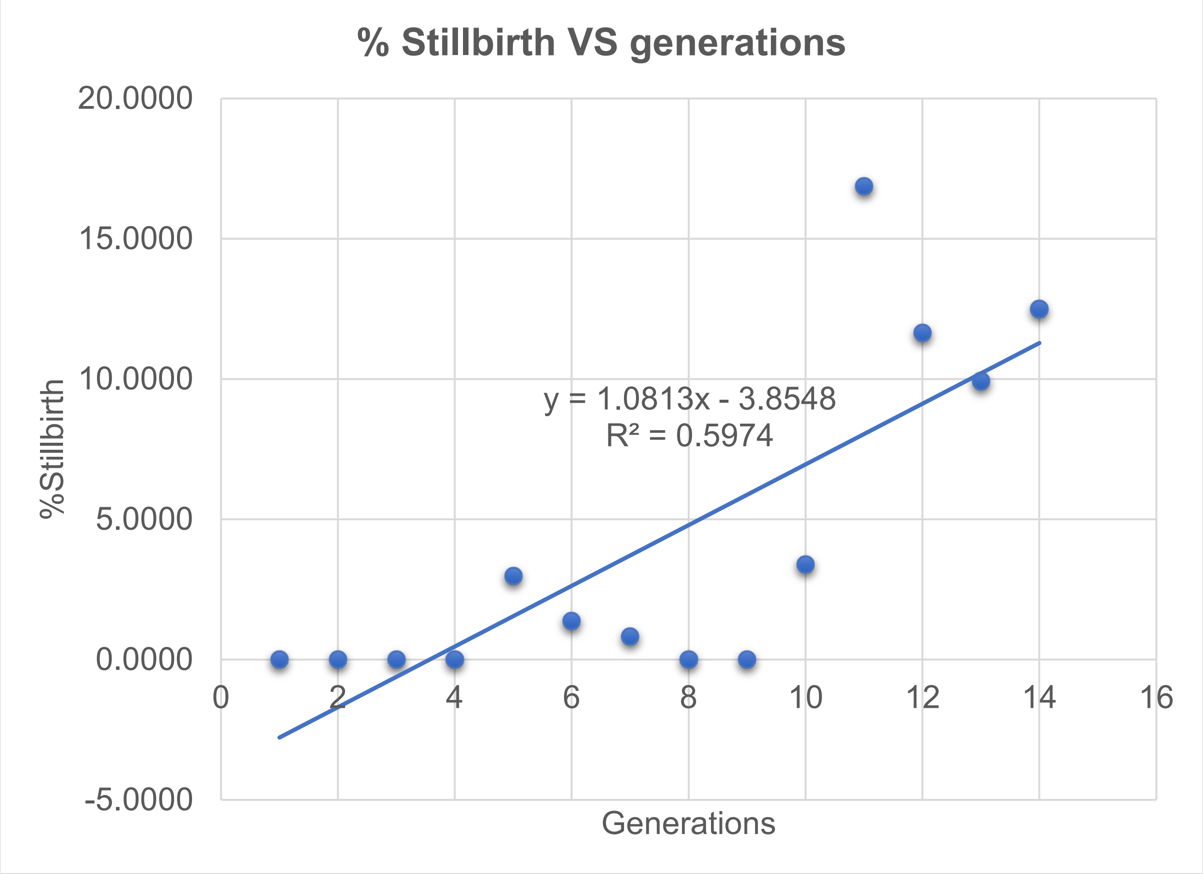

Figure 5. 2. The effect of generation on stillbirth incidences of South African Holstein dairy cattle

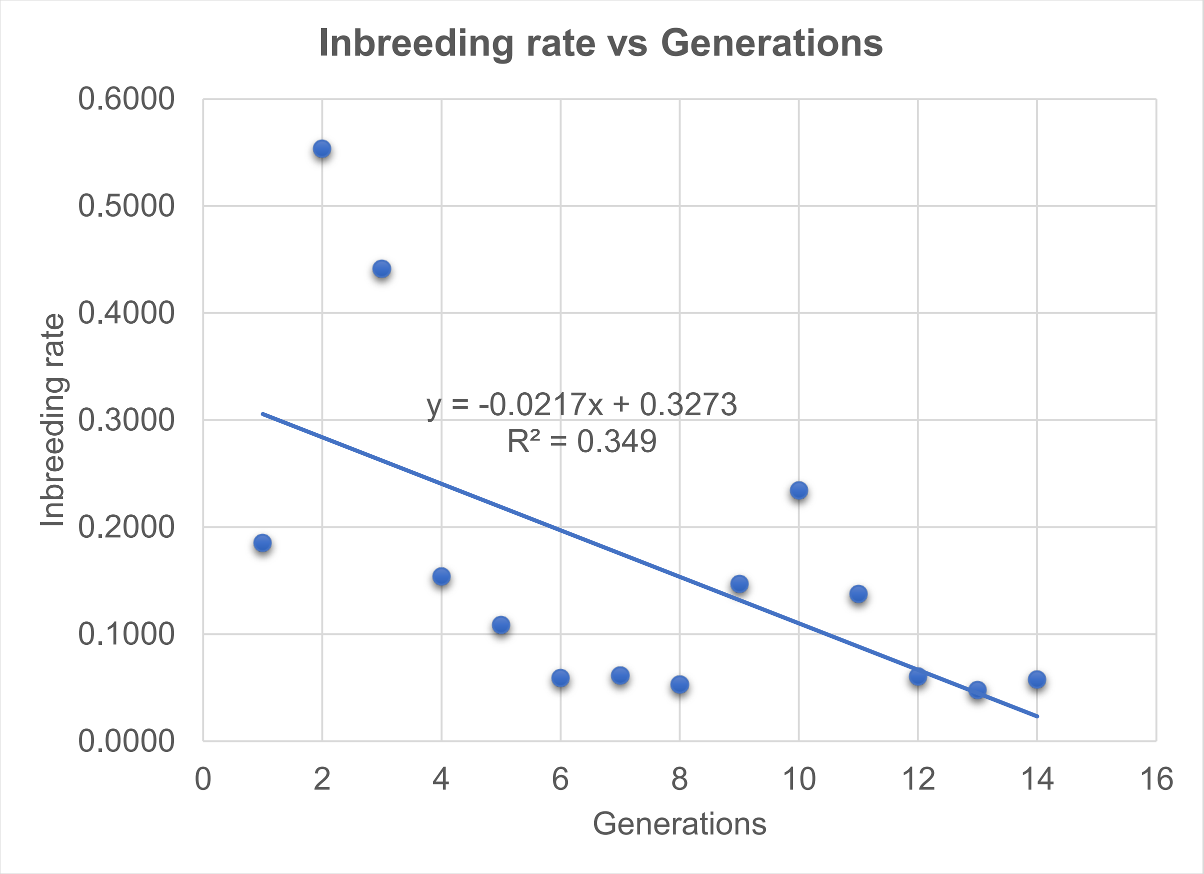

Figure 5. 3. The effect of generation on inbreeding rate of South African Holstein dairy cattle

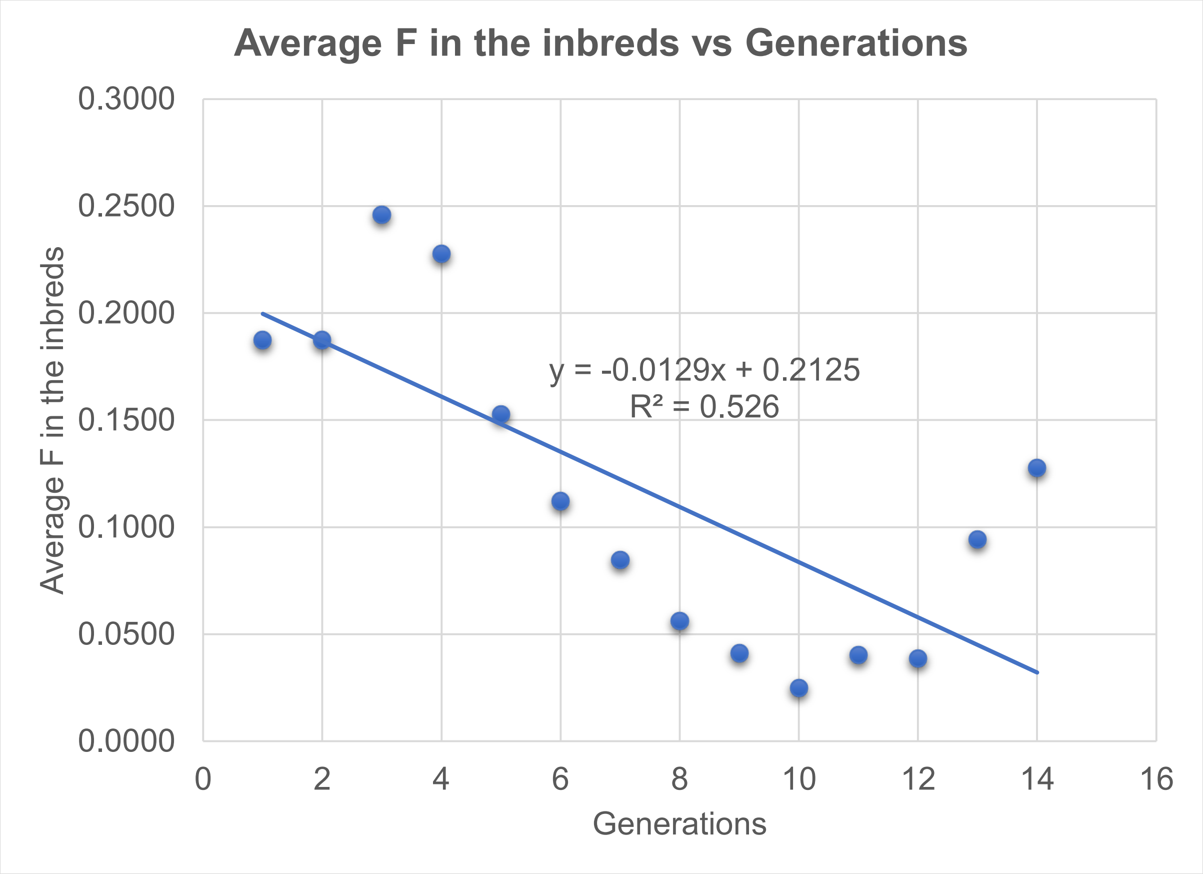

Figure 5. 4. The effect of generation on Average F in the inbreds of South African Holstein dairy cattle

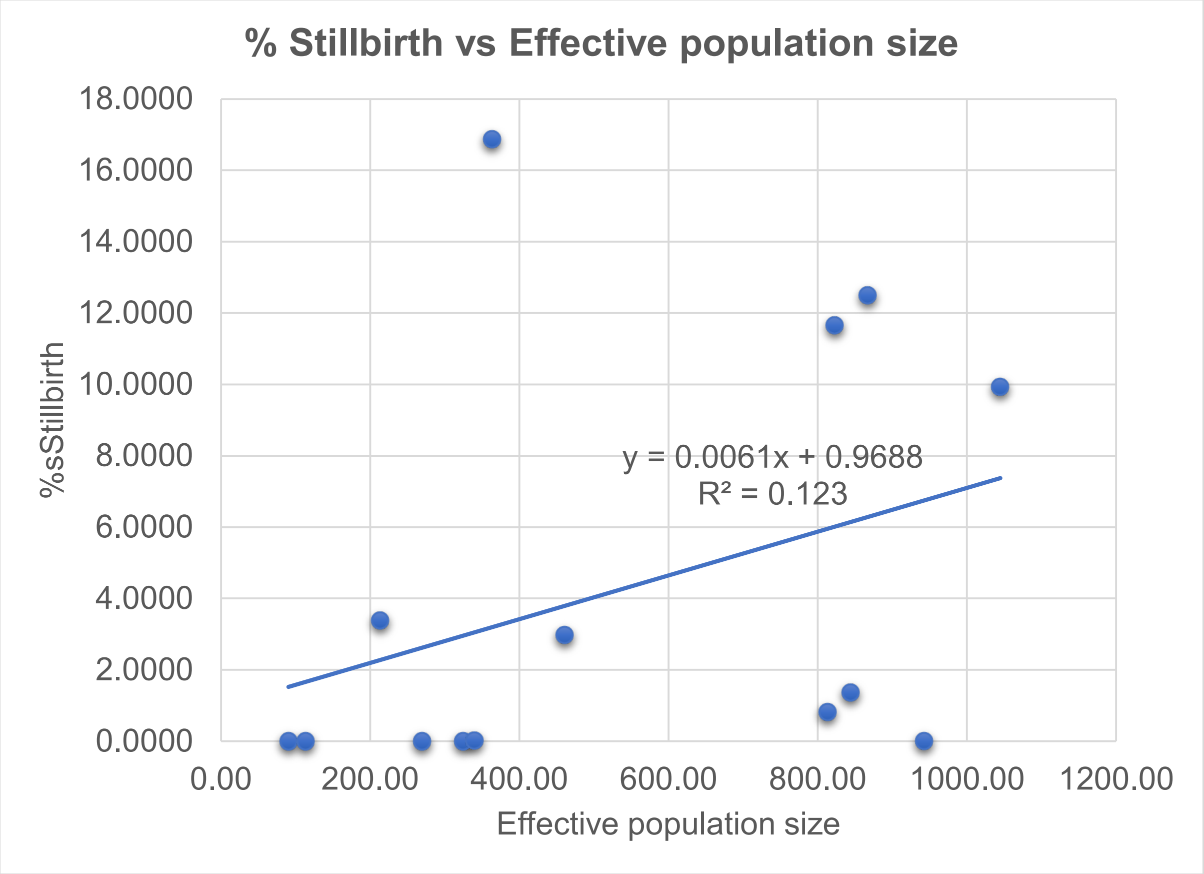

Figure 5. 5. The effect of effective population size on stillbirth of South African Holstein dairy cattle

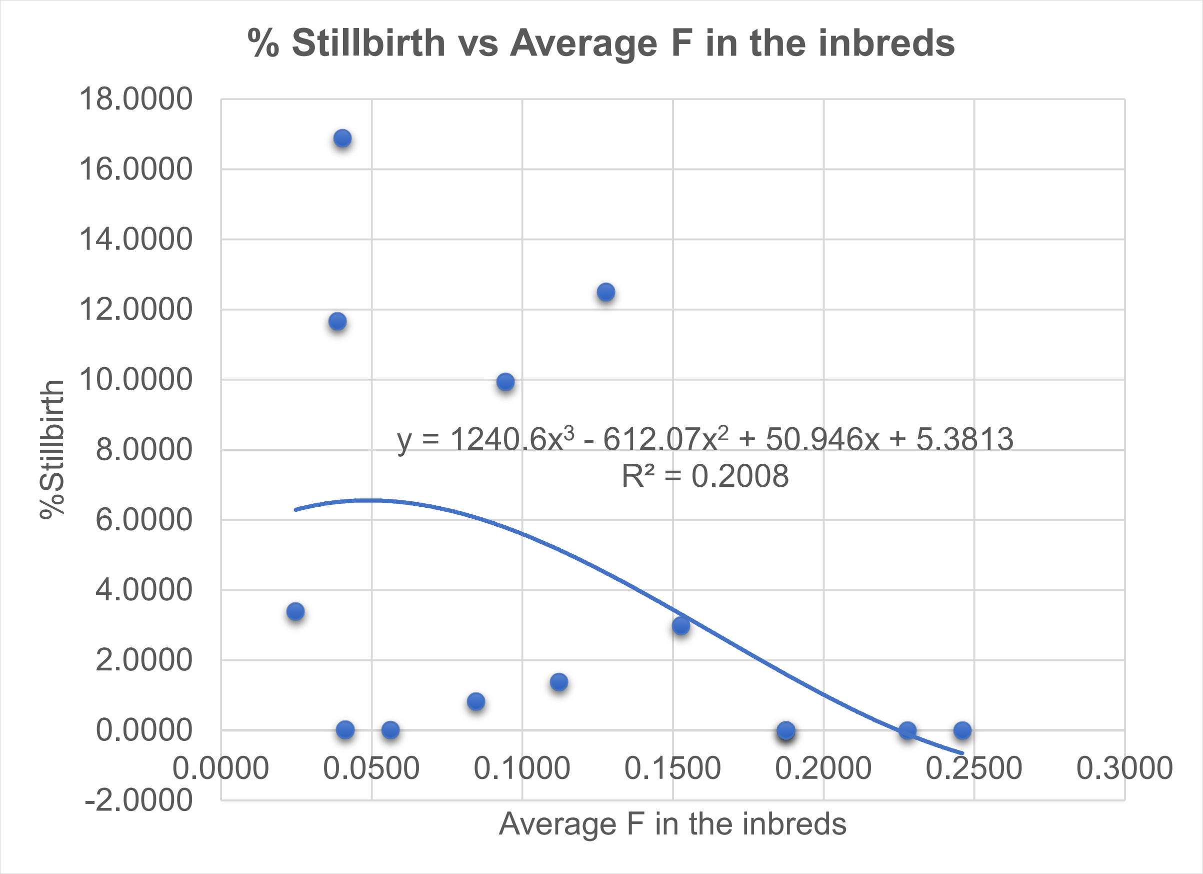

Figure 5. 6. The effect of average F in the inbreds on stillbirth of South African Holstein dairy cattle

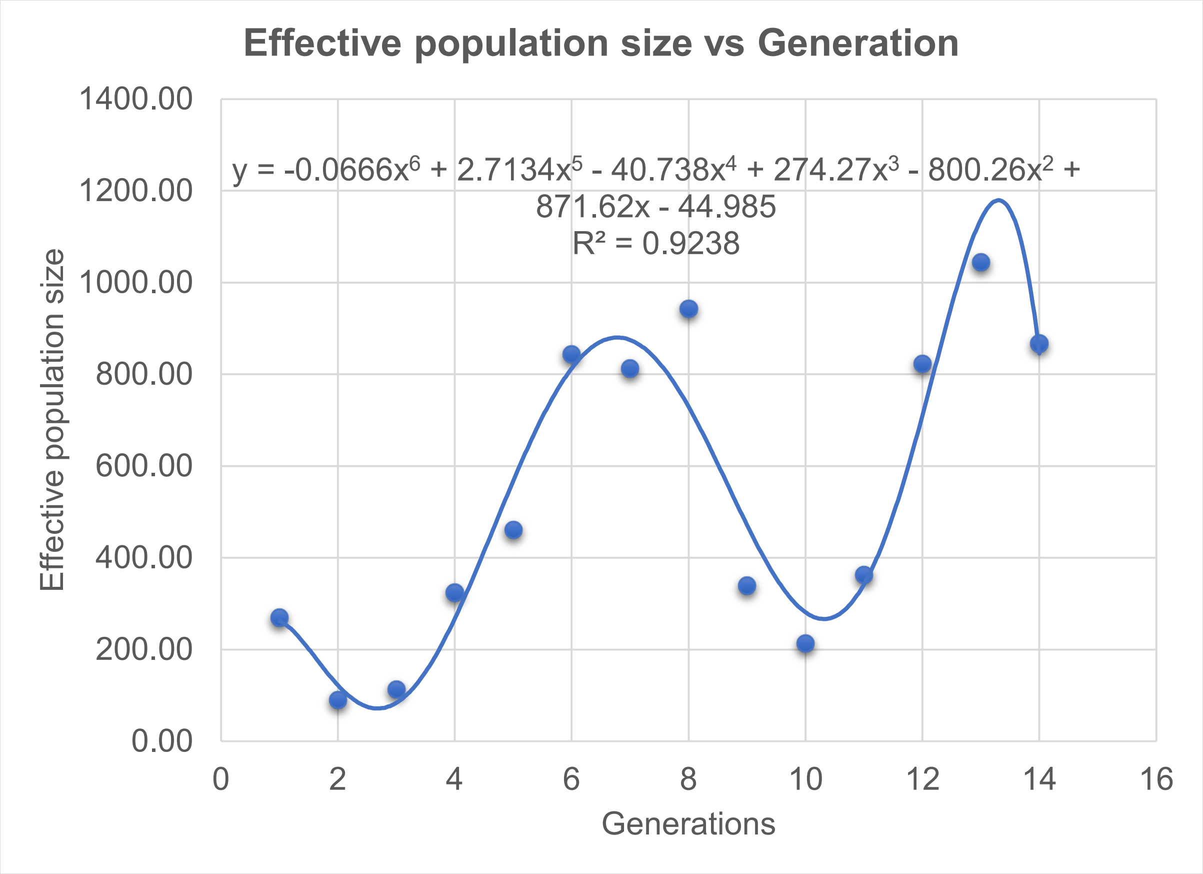

Figure 5. 7. The effect of generation on effective population size of South African Holstein dairy cattle

5.3.5. Spearman Rho correlations amongst average inbreeding coefficients in the inbreeds, generations, effective population size, inbreeding rate, and stillbirth percentages across generations of South African Holstein dairy cattle

Spearman Rho correlations amongst average inbreeding coefficients in the inbreeds, generations, effective population size, inbreeding rate and stillbirth percentages are presented in Table 5.2. The results showed that in total 21 correlations were estimated using Spearman Rho, 8 was significantly correlated (*P<0.05*) and 13 were not significantly correlated. However, from the 8 significantly correlated 5 were negatively correlated while 3 were positively correlated to each other. Inbreeding rate was significantly and negatively correlated to generations (r= -0.653) and stillbirth (r= -0.553) but had no significant correlation with average inbreeding coefficient in the inbreds (r= 0.334), effective population size (r= -1.000), number of inbreds (r= -0.178) and average inbreeding coefficient (r= -0.037). Generation was observed to be positively and significantly correlated with effective population size (r= 0.653) and stillbirth (r= 0.873), however negatively and significantly correlated with average inbreeding coefficient in the inbreds (r= -0.700). This means an increase in generation is associated with an increase in effective population size and stillbirth, and a decrease in average inbreeding coefficient in the inbreds.

Stillbirth was positively and significantly correlated with effective population size (r= 0.553) and negatively and significantly correlated with average inbreeding coefficient in the inbreds (r= -0.656). This implies that an increase in stillbirth is associated with an increase in effective population size and a decrease in average inbreeding coefficient in the inbreds. Stillbirth had no significant correlation with average inbreeding coefficient (r= -0.322) and number of inbreds (r= -0.011). Average inbreeding coefficient in the inbreds had no significant correlation with number of inbreds (r= -0.475), effective population size (r= -0.33) and average inbreeding coefficient (r= 0.154). Number of inbreds had a positive and significant correlation with average inbreeding coefficient (r= 0.732), however had no correlation with effective population size (r= 0.178). There was no significant correlation between effective population size and average inbreeding coefficient (r= -0.037).

Table 5. 2. Spearman Rho correlation coefficients amongst average inbreeding in the inbreeds, no of inbreds, generations, effective population size, inbreeding rate, and stillbirth percentages of South African Holstein dairy cattle

	% Stillbirth	Generations	No of inbreds	Effective population size	Average F	Average F in the inbreds
Generations	0.873***					
No of inbreds	-0.011^{ns}	0.138^{ns}				
Effective population size	0.553*	0.653**	0.178^{ns}			
Average F	-0.322^{ns}	-0.178^{ns}	0.732**	0.037^{ns}		
Average F in the inbreds	-0.656**	-0.700**	-0.475^{ns}	-0.334^{ns}	0.154^{ns}	
Inbreeding rate	-0.553*	-0.653**	-0.178^{ns}	-1.000^{ns}	-0.037^{ns}	0.334^{ns}

***: significantly different at $p<0.001$; **: significantly different at $p<0.01$; *: significantly different at $p<0.05$; ns: not significant

5.4. Discussion

5.4.1. The inbreeding effect on the incidence of stillbirth

In the current study inbreeding rate had a significant effect on the incidences of stillbirth. In line with the current study stillbirth was reported to be significantly affected by inbreeding in Holstein (Piwczyński *et al*., 2013; Mahnani *et al*., 2018; Morek-Kopeć *et al*., 2021). A significant relationship between inbreeding and stillbirth were also reported in German Holstein cattle (Hinrichs and Thaller 2011; Atashi *et al.,* 2012). Inbreeding rate was observed to have a negative relationship with incidences of stillbirth in the current study, meaning an increase in inbreeding rate result in a reduction in incidences of stillbirth. This reduction in incidences of stillbirth with increasing inbreeding rate, shows that stillbirth was affected more by the environment in the current study. In disagreement with the current study an increase in inbreeding was reported to be associated with an increase in the incidences of stillbirth (Piwczyński *et al*., 2013; Mahnani *et al*., 2018; Morek-Kopeć *et al*., 2021). Inbreeding result in high risk of stillbirth and reduces productivity and reproductive performance (Mellado *et al*., 2017) and it is correlated to rising cow culling rate due to poor reproductive performance and lower production of milk. In dairy cattle increase in inbreeding is associated with major economic losses such as calve loss (stillbirth) and high veterinary services (Maiwashe *et al*., 2008). Inbreeding reduces the embryo survival and conception rate and result in high risk of stillbirth (Hinrichs and Thaller, 2011).

5.4.2. Generational effect on the incidences of stillbirth, average inbreeding coefficients in the inbreds, inbreeding rate and effective population size

In the current study generations had a positive relationship with stillbirth and effective population size but had a negative relationship with inbreeding rate and average inbreeding coefficients in the inbreds. This means an increase in generations of South African Holstein dairy cattle population is associated with an increase in stillbirth and effective population size, and a reduction in inbreeding rate and average inbreeding coefficients in the inbreds. Stachowicz *et al*. (2011) and Rodríguez-Ramilo *et al*. (2015) reported no significant change in the effective population size if generation intervals remain the same. Genetic diversity loss could be a contributing factor to the increases in the incidences of stillbirth (Stachowicz *et al*., 2011). However, the current study showed that factors contributing to genetic diversity such as inbreeding rate and average inbreeding coefficients in the inbreds had an inverse relationship with the incidences of stillbirth in the South African Holstein dairy cattle.

5.4.3. The effect of effective population size and average inbreeding coefficients in the inbreds on stillbirth incidences

Stillbirth incidences were observed to have a positive relationship with effective population size and a negative relationship with average inbreeding coefficients in the inbreds. Therefore, an increase in stillbirth incidences is associated with an increase in effective population size and a reduction in average inbreeding coefficients in the inbreds. Effective population size is defined as the number of individuals that effectively participates in producing the next generation (Kliman *et al*., 2008; Sbordoni *et al*., 2012). Effective population size is a key parameter in conservation genetics and normally can be estimated from the increase in average relativeness (Gutiérrez *et al*. 2003). Inbreeding increases and effective population size deceases due to intensive selection and mating using few sires, resulting in high stillbirth incidences (Adamec *et al*., 2006; Makanjuola *et al*., 2020). In contrary to the current study Szücs *et al*. (2009) and Morek-Kopeć *et al*. (2021) reported that accurate animal selection to achieve genetic improvement and reduce inbreeding coefficients as a result can reduce stillbirth within the Holstein dairy cattle population, but this will depend on a sound genetic evaluation programme.

5.4.4. Spearman Rho correlations amongst average inbreeding coefficients in the inbreeds, generations, effective population size, inbreeding rate, and stillbirth percentages

Inbreeding rates in the current study were found to have a negative correlation with generations and effective population size. This implies that an increase in the inbreeding rate within the South African Holstein dairy population result in the reduction in the generations and effective population size. Adamec *et al*. (2006) and Makanjuola *et al*. (2020a) reported that increase in inbreeding is associated with effective population size deceases this increases in the inbreeding can be because of intensive selection and mating using few sires (Adamec *et al*., 2006; Makanjuola *et* *al*., 2020b; Makanjuola *et al*., 2020b). Inbreeding coefficient indicates chance that an animal receives the same allele from both parents because they are related and the inbreeding level in a particular animal is expressed in inbreeding coefficient (Kor and van der Waaij, 2015). In the current study generation was observed to have a positive correlation with effective population size and stillbirth incidences, but negatively correlated to average inbreeding coefficients in the inbreds.

Stillbirth incidences in the current study were passively correlated to effective population size and negatively correlated to inbreeding rate and average inbreeding coefficients in the inbreds. This means that an increase in inbreeding rate and average inbreeding coefficients in the inbreds result in a decrease in stillbirth incidences. In agreement with the current study Reis Filho *et al*. (2015) reported that an increase in inbreeding coefficient causes reduction in the effective population size. Maiwashe *et al*. (2006) in their study on Holstein reported that a lower Ne is associated with less inbreeding coefficients within the population.

5.5. Conclusion and Recommendation

In conclusion the stillbirth incidences of the South African Holstein dairy cattle population were decreasing with an increase in the inbreeding rate and average inbreeding coefficients in the inbreds, however it was increasing with an increase in the effective population size and generation. In the population with high effective population size and increasing number of generations the incidences of stillbirth in that population are expected to be high. This means that there are other factors such as management practices that contribute to the high incidences of stillbirth other than the genetic factors such as inbreeding, which should be studied to deal with the high incidences of stillbirth within the Holstein dairy cattle population. Generation, average inbreeding coefficients in the inbreds and effective population size were the factors contributing to the incidence of stillbirth. The environmental factors were the main contributor to the incidences of stillbirth than the genetic factors in the South African Holstein population. There is a need to investigate the environmental factors that contribute to the incidences of stillbirth.

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CHAPTER SIX

General Conclusion, and Recommendation

6.1. General conclusion

Stillbirth is a trait of economic importance in the dairy industry, as it contributes to the profitability of the dairy farm. Generation, dam parity, calf sex, birth season, birth year and herd are the main factors affecting the incidences of stillbirth. Incidences of stillbirth were different within seasons, dam parities, birth year, calf sex and generation. With the multiparous, female calves, autumn and summer were associated to high incidences of stillbirth, as compared to primiparous, male calves, spring, and winter. High incidences of stillbirth in primiparous and male calves is due to the proportion of the pelvic area and calf size. There was a great variation in the incidences of stillbirth across the herds, which is because of different environmental conditions, different herd size, genetic variation, and management practices. High variation showed a need to investigate the inbreeding rate and effective population size.

Effective population size increases with an increase in the number of breeding males and is having an inverse relationship with inbreeding rate and the average DGE was approximately one across generations. Stillbirth incidences were decreasing with an increase in the inbreeding rate and average inbreeding coefficients in the inbreds, however it was increasing with an increase in the effective population size and generation. This indicate that stillbirth in the South African Holstein population was affected by environmental factor than the genetic factors such as the inbreeding. South African Holstein population high effective population size and increasing number of generations result in high incidences of stillbirth. Generation, average inbreeding coefficients in the inbreds and effective population size are the main factors contributing to the incidence of stillbirth.

6.2. General Recommendation

Current study shown instability in the effective population size and inbreeding rate across generations of South African Holstein dairy cattle. The result also indicated a need for proper animal management practice to regulate inbreeding rate of population. Application of proper management in dealing with levels of inbreeding will increase effective population size and maintain genetic variation in South African Holstein dairy cattle population. Study provided understanding on the effect of dam parity, calf sex, birth season, birth year, herd, and generation on the incidences of stillbirth. Due to the negative correlation between stillbirth and dam parity and age, it is advisable to not keep the cows for many parities or too old cows.

Older cows are associated with high stillbirth incidence same as the cows in later parities. Autumn and summer were associated to high incidences of stillbirth, as compared to spring, and winter. Therefore, it is recommended that the breeding program of the Holstein dairy cattle be designed in a way that the animals will give birth during the spring season of winter to reduce the chances of high stillbirth. Inbreeding had les effect on stillbirth, indicating that it is more affected by the environmental factors than the genetic factors such as inbreeding. Then it is recommended that environment and management practices be investigated as a way to address the occurrence of stillbirth within the South African Holstein population.

CHAPTER SEVEN

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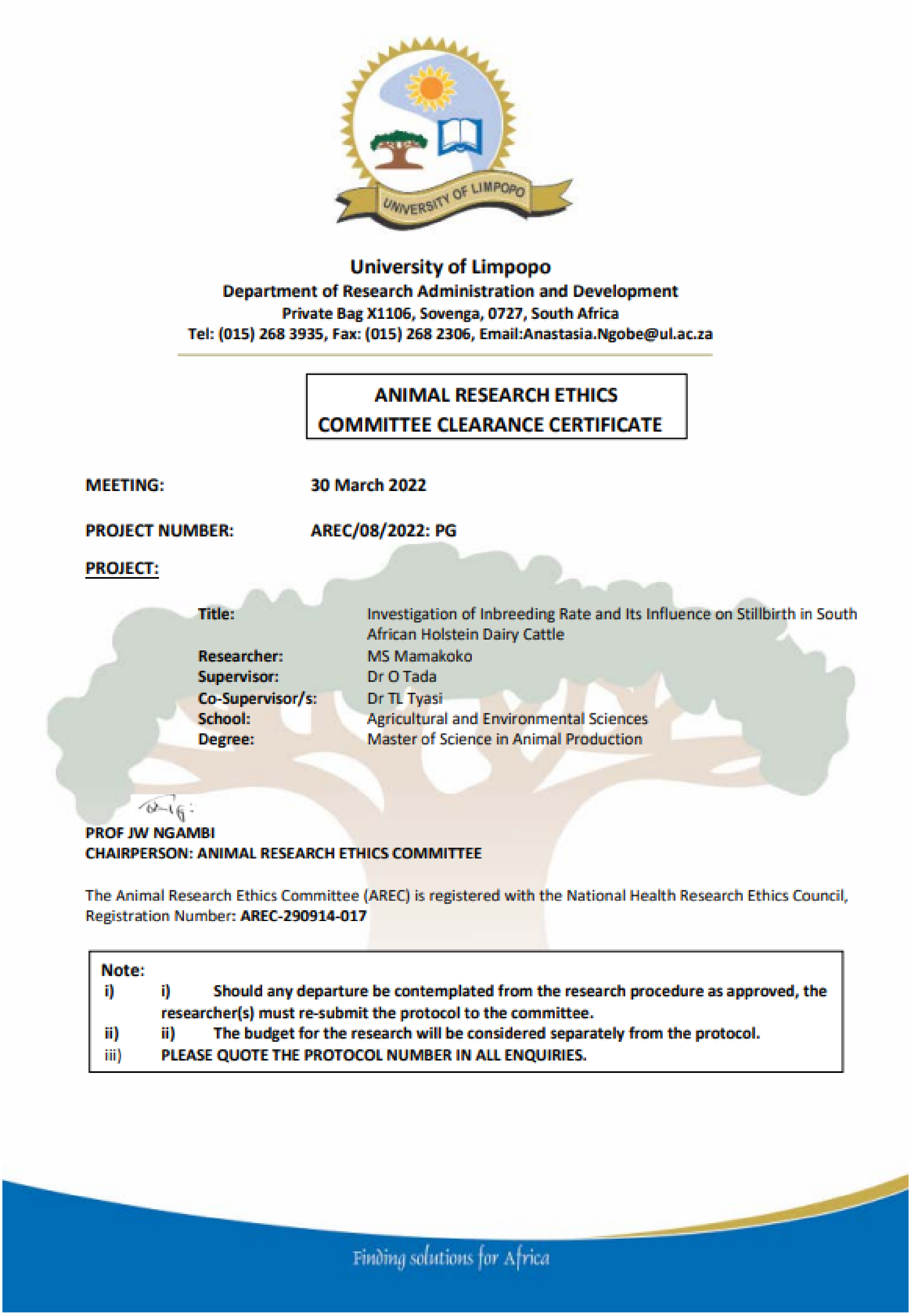
7.2. Appendix

Appendix A

Animal Research Ethics Committee Clearance Certificate



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ANIMAL RESEARCH ETHICS COMMITTEE CLEARANCE CERTIFICATE

MEETING: **30 March 2022**

PROJECT NUMBER: **AREC/08/2022: PG**

PROJECT:

Title:	Investigation of Inbreeding Rate and Its Influence on Stillbirth in South African Holstein Dairy Cattle
Researcher:	MS Mamakoko
Supervisor:	Dr O Tada
Co-Supervisor/s:	Dr TL Tyasi
School:	Agricultural and Environmental Sciences
Degree:	Master of Science in Animal Production

PROF JW NGAMBI
CHAIRPERSON: ANIMAL RESEARCH ETHICS COMMITTEE

The Animal Research Ethics Committee (AREC) is registered with the National Health Research Ethics Council, Registration Number: **AREC-290914-017**

Note:
i) i) Should any departure be contemplated from the research procedure as approved, the researcher(s) must re-submit the protocol to the committee.
ii) ii) The budget for the research will be considered separately from the protocol.
iii) **PLEASE QUOTE THE PROTOCOL NUMBER IN ALL ENQUIRIES.**

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