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"Development of Genetic and Genomic Predictors of Fertility in Argentinean Holstein Cattle."

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To the Graduate Council:

I am submitting herewith a dissertation written by Fernando Alfonso Di Croce entitled ""Development of Genetic and Genomic Predictors of Fertility in Argentinean Holstein Cattle."." I have examined the final electronic copy of this dissertation for form and content and recommend that it be accepted in partial fulfillment of the requirements for the degree of Doctor of Philosophy, with a major in Animal Science.

Dr. F. Neal Schrick, Major Professor

We have read this dissertation and recommend its acceptance:

Arnold M. Saxton, F. David Kirkpatrick, Gina M. Pighetti

Accepted for the Council:

Carolyn R. Hodges

Vice Provost and Dean of the Graduate School

(Original signatures are on file with official student records.)

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Development of Genetic and Genomic Predictors of Fertility in Argentinean Holstein Cattle

A Dissertation

Presented for the

Doctor of Philosophy

Degree

The University of Tennessee, Knoxville

Fernando Alfonso Di Croce

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DEDICATION

This dissertation is dedicated to my wife Andrea, my son Valentino and my daughter Martina, and my parents Alfonso and Norma Di Croce. From all of you, I draw strength from your love, encouragement, and patience. Andrea, Valentino and Martina are the light of my life. My parents, thanks for your unwavering support no matter what endeavors I embark into.

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ABSTRACT

The overall aim of the studies described herein was to evaluate genetic variation in cattle fertility traits for development of genetic and genomic predictors in breeding strategies. Results from these experiments suggest that improvements in fertility through genetic selection are a possible approach to increase reproductive efficiency. Experiment 1 evaluated the development of genetic parameters associated with multiple ovulation and embryo transfer schemes in an attempt to assist producers in identifying animals with greater genetic merit for these protocols. This study confirmed that genetic selection of donors or sires appears to be a potential approach to improve efficiency of MOET procedures. Although low heritability would slow the progress, results shown in this work suggest that genetic improvement in fertility by selection for embryo transfer traits is possible. Experiment 2 evaluated fertility traits in Argentinean Holstein cattle in order to develop fertility genetic predictors for utilization in breeding strategies. The dollar fertility index ($F) included age to first calving (AFC) as a measure of initial reproductive performance and calving interval (CI) as an indicator of conception rate and success of early insemination. Values for $F ranged from -$76.6 to $139.4 in the current Holstein population. Results indicated substantial variation in fertility traits, suggesting that genetic selection would be highly effective in improving fertility.

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CHAPTER 1 INTRODUCTION AND STATEMENT OF THE PROBLEM

Assisted reproductive technologies (ART; i.e. artificial insemination (AI), multiple ovulation and embryo transfer (MOET), cryopreservation and sperm/embryo sexing strategies) have provided fundamental tools for rapid genetic improvement of livestock, particularly in dairy and beef cattle. These ART protocols have greatly increased efficiency of animal agriculture to provide high quality and low cost products to consumers. Although utilization of ART and genetic selection has resulted in tremendous progress for improving efficiency of cattle production, a significant need still exists to provide economical, "decision-making" tools to improve management and profitability for producers and sustainability to the animal agricultural sector.

Impaired fertility is the primary reason for culling in US (26.5% of all disposals; APHIS-USDA 2007). Even with improved ART strategies, reproductive efficiency in dairy cattle has steadily declined in the United States (US) and other dairy countries (Royal et al. 2000; 2002; Evans et al. 2006) during the past 30 years. Conception rates have been reported to be declining by 0.45 to 1.0% per year (Beam and Butler, 1999; Royal et al. 2000; 2002; Evans et al. 2006). A complex list of factors which impacts reproduction includes management, nutrition, diseases, milk production, genetics, lameness, and environmental stress among others (Hansen and Arechiga 1999; Grohn and Rajala-Schultz 2000; Cartmill et al. 2001; Lucy 2001; Moreira et al. 2001 and Santos et al. 2004). Fertility directly impacts on revenues associated with milk production and offspring born. Moreover, low reproductive performance elevates costs associated with culling, multiple inseminations,

veterinarian expense (pharmaceuticals for estrus synchronization, pregnancy diagnosis, etc.), and replacement animals.

Hence, to mitigate the deterioration of fertility, a strong need exists to identify and select animals according to their future reproductive potential. Therefore, our general approach encompassed understanding of how genotype contributes to phenotypic variation in fertility. In order to accomplish this approach estimation of genetic parameters and development of multi-trait selection indexes for fertility traits were performed. Second, identification of genomic regions and sequence variants (SNPs) affecting phenotypic variation in those traits was performed.

Hence, the **hypothesis** was utilization of breeding values and fertility indexes can predict genetic merit in a bovine population. To test the hypothesis and accomplish the overall objective of this proposal, the research objectives were:

1) Estimate genetic predictors for traits related to multiple ovulation and embryo traits.

2) Development of fertility breeding values and a multi-trait selection index based upon data from milk recording programs in a Holstein population.

CHAPTER 2 LITERATURE REVIEW

2. 1 History of genetic selection in cattle

2.1.1 Introduction

Selection of animals has been performed since domestication over 5,000 years ago. Early selection was solely based on phenotype (outward appearance) of animals that expressed desirable characters. In the last two centuries, producers applied systematic breeding programs to domestic animals (livestock) to select for more specific roles such as meat, milk, and draft. Later during the 1940's, the possibility to identify genetic merit of livestock through quantitative genetic approaches made more effective decisions about selection of replacements; therefore, improving subsequent generations. During the last century, increased accuracy and reduced bias of prediction for additive genetic merit have resulted in more efficient selection strategies and improved profits.

The milestones in selection strategies achieved during the last century were discernible from scientific discoveries in several disciplines (Green 2009). These innovations resulted in technological changes that had salient impacts on improvements in prediction of genetic merit. These improvements involved several interdisciplinary contributions such as animal breeding and genetics, computer science, economics, and statistics (Golden and Garrick 2009). These breakthrough technologies have resulted in milestone enhancement for selection based on genetic merit improvement. An example of statistical technologies is the mixed model (Henderson 1973), which provided best linear unbiased prediction (BLUP) for breeding values, resulting in a substantial increase in the accuracy of genetic prediction. Subsequently, BLUP became widely accepted as the standard approach to predict additive

genetic merit in livestock. Since BLUP is seen as a major breakthrough in dairy and beef genetic evaluation, we will briefly cover the history before (PREBLUB) and after (POSTBLUP) the breakthrough (Figures 1 and 2). Lastly, the connection of these disciplines with molecular biology and discoveries in DNA technologies have shown even greater promise for improving accuracy of prediction of genetic merit, especially for predictions of animals at young ages (Green 2009).

2.1.2 PREBLUP

2.1.2.1 Historical Perspective of Genetic Selection

During the first half of the 20th century, discoveries in the field of genetics and statistics provided enormous contributions to the agricultural sciences. One of the first and more fascinating contributions was made by Gregor Mendel in 1866. Working with peas and other vegetables, he discovered that genes were expressed in a predictable and mathematical progression, now known as Mendelian segregation (Eller 2007). These were the first reports describing basic transmission of hereditary particles from parent to offspring and inheritance of those particles in subsequent generations (Rishell 1997). Subsequently, S. Wright and R.A. Fisher initiated livestock breeding into this new area of science. Developing the statistical and mathematical theory of genetic relationships among relatives, Wright's principals are essential for current day breeding value calculations (Eller 2007).

The concept of population genetics describes and quantifies Darwin's writings during the first decades of the last century. Advances in this area aided in understanding basic genetic concepts such as genes, chromosomes, gene loci, and cellular reproduction (Green 2009). At the same time, a new field known as biometrics was established to apply statistics to biological events. Early statisticians working in this field were pioneers in defining long-term selection and inbreeding in livestock (Green 2009).

During 1940's, J. L. Lush, who many refer to as the modern-day father of animal breeding (Dickerson, 1973; Dickerson and Willham, 1983), made several of the greatest contributions for livestock genetic improvement. He, along with coworkers such as Hazel and Dickerson, defined the concepts of "selection index" and "breeding value" (Hazel 1943; Lush 1947). Furthermore, advances in the biometrics field enabled measurement from experimental populations pertaining to performance and heritability of production traits.

Several of the greatest contributions occurred in the 1950's. For example, Watson and Crick presented for the first time in the scientific literature a structure for Deoxyribonucleic Acid (DNA), later defined as the molecular structure of the genetic code (Crick 1953). This contribution, combined with the theories of genes and heritable variation of traits, suggested the possibility for using these genetic differences at the gene level for future genetic improvement.

Consolidation of the national Dairy Herd Improvement Association (DHIA), which led the first national milk recording program, and continued adoption of artificial insemination

(AI) constituted significant milestones in livestock breeding programs. With these two significant events, the potential for genetic improvement was first understood by dairy breeders. They envisioned the power of combining AI with the quantitative genetic theory resulting in improved genetic evaluations (Green 2009). At the same time, the early experiences of dairy breeders extended to other breed associations which were quickly adopting performance data recording, often at the level of breed registries and societies (Green 2009).

By 1964 in the US, five beef associations had developed performance recording programs with the Red Angus Association of America in 1959 as the pioneer in requiring mandatory input of performance information (Eller 2007).

In 1965, the beef improvement committee of the American Society of Animal Science generated procedures for collecting and recording performance data (Eller 2007). After several years and a great deal of discussion, industry, academic, and government leaders agreed in 1967 to form the Beef Improvement Federation (BIF). The responsibility of BIF is to produce guidelines for performance recording programs and report research result (Eller 2007). Several other organizations were also established to develop guidelines for performance recording programs such as National Swine Improvement Federation and the National Association of Animal Breeders (Green 2009).

The 1960s and 1970s were marked by increased competition in the seedstock industry and the next generation of statistical technology. The first across-herd genetic evaluations using sire-maternal grandsire models, and the initial era of breeding value estimation across

large-scale populations were born. Additionally, statisticians were gaining understanding of statistical approaches and application to large scale populations. As previously proposed in this introduction, what came next is a true time of transition for livestock breeding (the POSTBLUP period) which became a major breakthrough in national dairy and beef cattle genetic evaluation (Golden and Garrick 2009; Green 2009).

2.1.3 POSTBLUP

2.1.3.1 Statistical Models

In 1973, Henderson elucidated statistical approaches that would be widely adopted from that time forward as standard methods for predicting additive genetic merit in livestock (Golden and Garrick 2009). Henderson's major contributions included development of statistical theory related to prediction of random variables from mixed linear models, estimation of variance components, and analyses of unbalanced data (Freeman 1991). Best linear unbiased prediction (BLUP) improved accuracy of prediction in contemporary groups (such as herds or years) and made possible the use of unbalanced data (Henderson 1975). Accordingly, the mixed model procedures (estimating BLUP) represented significant progress in prediction of breeding values. These accomplishments had a major impact on evaluation methods of dairy and beef cattle as well as other livestock species.

2.1.3.2 Mixed Models

Breeding values in dairy and beef cattle are predicted based on linear mixed models using Henderson's mixed model equations. The most common mixed model used in animal breeding and genetics is the "animal" model (Thompson 2008; Di Croce et al. 2009). In this model, all animals are included in the model as random effects, while all other explanatory terms are fixed effects (such as year and herd). Animal models use pedigrees to track relationships among animals, and related individuals will share additive genetic (co)variance. Animals within the same herd will share the herd environmental variance. In this way, variances are separated into components of interest. The advantages of using the animal model are that all additive genetic relationships are utilized and unequal family sizes are correctly accounted for during the analysis (Thompson 2008; Di Croce et al. 2009). In addition, Henderson's mixed model estimation provides the most accurate prediction of breeding values for all animals in the dataset (Henderson 1953; Di Croce et al. 2009).

2.1.3.3 Best Linear Unbiased Prediction (BLUP)

Predictions of individual additive genetic effects or breeding values can be estimated from Henderson's mixed model equations. Best linear unbiased predictions (BLUPs) are breeding values, predictions of the genetic value of that individual as a parent, since additive genetic effects are transmitted to progeny (Di Croce et al. 2009). An individual's breeding value for a given phenotypic trait is the total additive effect of its genes for that

trait (Di Croce et al. 2009). The first BLUP models were univariate and had only contemporary group and sire effects included for predicting additive genetic merit. These models were introduced into dairy (1970) and beef (1974) cattle genetic evaluations (Freeman 1991; Golden and Garrick 2009) and resulted in performance predictions of progeny of a sire. These breeding values were divided in half before publications and the industry gave the name "expected progeny differences" (EPD) in beef and "predicted transmitting ability" (PTA) in dairy (Golden and Garrick 2009).

2.1.3.4 Further Developments (Sire, Animal Models, and Maternal Effects)

Although these linear mixed models represented a breakthrough in animal breeding and genetic improvement, further developments in application of these linear mixed models were needed. For example, early models did not account for the relationships between sires; hence, did not consider the inbreeding of animals. It was not until the 1980s that the matrix which indicates the additive genetic relationships among individuals (A^{-1}) was computed. Several contributions solved this problem in large data set. First, Henderson (1975) and Quaas (1976) elucidated high performance methods for computing the elements of the inverse of A. Later, Golden (1991) and then Meuwissen and Luo (1992) provided faster methods to determine the inbreeding on all animals in large data sets.

Models pertaining to BLUP continued to evolve and improve the prediction capabilities in terms of decreasing bias and increasing accuracy. Also, in conjunction with improvements

in computer power and developments of computational methods, BLUP models evolved from univariate sire models to multivariate animal models. Sire models implies that only sires are included and evaluated in the model using progeny records. Conversely, in animal models, genetic effects of dams and sires are analyzed simultaneously; therefore, sire ratings are adjusted for any non-random mating providing more accurate predictions.These advanced models (animal models) including the direct additive genetic effect for dams along with the direct additive effect of the sires were implemented by 1984 (Golden and Garrick 2009). Including dams into the analyses also allowed for the inclusion of the additive maternal effect described by Willham (1972), who introduced the biometrical aspect of traits having a maternal effect. By definition, this maternal effect is a phenotypic value of a dam measurable only as a component part of her offspring's phenotypic value (Willham 1972). Even more developments in the inclusion of dam effects were made by Pollak et al. (1977) with implementation of a multivariate model including birth weight, weaning weight, and postweaning weight gain to yearling age, with maternal effects for both weaning weight and birth weight.

2.2 Breeding Value Prediction

Prediction of breeding values represents an essential component of most evaluation programs for genetic improvement. The method utilized for predicting breeding values depends on the type and amount of information available to predict these values. Single

and repeated records could be used for predicting breeding values from the animal's own performance. Also, this estimation could be performed from progeny records, commonly used for traits where records can be obtained only from females. This is typical of dairy cattle where predicting breeding values of bulls is evaluated on the basis of their daughters. Furthermore, breeding values for an animal may be predicted from its pedigree and from observations of genetically correlated traits.

As previously shown, multiple equations have been developed over time to aid in accurate prediction of breeding values and must be introduced to assist in understanding this crucial methodology.

2.2.1 The Basic Model

In simplistic terms, the observed or phenotypic trait (P) is described by environmental and genetic factors and may be defined by the model

$$y = m + g + e = m + gA + gD + gE + e,$$

where the

phenotypic observation y is the record of an animal;

environmental effects m refer to fixed environmental effects (i.e., herd management, year and/or month of birth, etc., of the animal); and

genetic effects g are the sum of the additive (gA), dominance (gD) and epistatic (gE) genetic values of the genotype of the animals.

Residual effects e are the sum of the random environmental effects affecting the animal.

2.2.2 Breeding Values

The additive genetic value in the *g* term *g*A represents the average additive effects of genes an individual receives from both parents and is expressed as the breeding value (Mrode 1996, 2005). Each parent contributes half of its genes to its progeny. As stated earlier, these breeding values were divided in half before publication and the industry applied the terminology: Expected Progeny Differences (EPD) in beef and Predicted Transmitting Ability (PTA) in dairy. As the additive genetic value is a function of genes transmitted from parents to progeny, it becomes the only component that can be selected and the primary component of interest (Mrode 1996). Dominance and epistasis are mostly assumed to have small effects and are only included in the residual term of the model (Mrode 1996, 2005).

In most cases, it is assumed that *y* follows a multivariate normal distribution, meaning that the traits are determined by infinitely many additive genes of infinitesimal effect at unlinked loci, known as infinitesimal model (Fisher 1918, Mrode 1996, 2005). Additionally, these models assumed that the variance of *g* A and *e* terms are known and are not correlated (Mrode 1996).

2.2.3 Variance

The genetics of a quantitative character centers on the study of its variation. In the study of genetic variation, the basic understanding is its partitioning into components attributable to different causes or effects. The relative magnitude of these components determines the

genetic properties of the population, specifically the resemblance between relatives (Falconer and Mackay 1996). In simplistic terms, variance measures the range of difference in variables or estimates. For instance in genetics, the genotypic variance is the variance of genotypic values, and the environmental variance is the variance of environmental deviations. The total variance is the phenotypic variance and is the sum of the separate components (genotypic and environmental variance).

2.2.4 Components of Variance

In most genetic models, the amount of variation is measured and expressed as variance. Usually, the values are expressed as deviation of population means. When this occurs, the variance value is simply the mean of the squared values (Falconer and Mackay 1996). The components in which variances are partitioned can be defined as

$$Vp = Va + Vg + Ve,$$

where:

Vp is the phenotypic variance, the total variance;

Va is the additive genetic variance;

Vg is all other genetic variance (i.e., dominance and interaction variance components); and

Ve is the environmental variance.

Thus, the total variance (*Vp*) is the sum of the components (Falconer and Mackay 1996; Di Croce et al. 2009).

2.2.4.1 Heritability

The partitioning of the variance allows for estimating relative importance of various determinants of the phenotype; for instance, the role of heredity versus environment (Falconer and Mackay 1996). The relative importance of heredity in determining phenotypic values is called the heritability of the trait. From this partitioning of the total variance, one can calculate *heritability= Va/Vp* (h^2; Falconer and Mackay 1996; Di Croce et al. 2010). Thus, the ratio, *Va/Vp*, expresses the degree to which phenotypes are determined by the genes transmitted from the parents (Falconer and Mackay 1996). Simply, heritability determines the degree of resemblance between relatives.

2.2.5 Genetic Covariance between Relatives

Genetic relationships among individuals are of fundamental importance for predicting breeding values. For instance, BLUP depends greatly on genetic covariance among individuals for obtaining high accuracy and unbiased estimates (Mrode 1996). The relationships between individuals are usually computed in a matrix that describes additive genetic relationships between individuals of a population, and is usually called the numerator relationship matrix (A), first described by Henderson (1975). This matrix is symmetric and composed of diagonal and off-diagonal elements. From diagonal elements inbreeding coefficients between animals can be computed. Off-diagonal elements (lower triangular elements) present the coefficient of relationships between animals (Mrode 1996, 2005). In other words, these coefficients trace the shared flow of genes from one

generation to the next. The prediction of the breeding values requires the inverse of the relationship matrix to be included in the mixed model equations (MME; Mrode 1996, 2005).

As discussed later in this chapter, variance and covariance "components" are estimated using statistical mixed models, which include fixed and random effects. Fixed effects are constants that only affect the mean of the data. Random effects are terms that introduce variation, such as variation among animals. Maximum likelihood (ML) is the current estimation method of choice; in particular, restricted maximum likelihood (REML). The main advantages of REML include the ability to work with unbalanced data and correctly account for fixed and random effects in the estimation (Saxton 2007; Di Croce et al. 2010).

2.2.6 Best Linear Unbiased Prediction of Breeding Value

In animal breeding, Best Linear Unbiased Prediction (BLUP) is a technique for estimating genetic merit from phenotypic traits (Robison 1991). In general, it is a method of estimating random effects and was developed by Henderson (1949). Henderson developed BLUP so fixed effects and breeding values can be simultaneously estimated (Mrode 2005; Di Croce et al. 2009).

2.2.6.1 Mixed Models

The most general linear model is the mixed linear model. In matrix notation, this model is

$$y = Xb + Zu + e$$

with y containing the observed dependent variable values;

X and Z are the design matrices for fixed and random effects containing observed or known values;

b and u are parameter vectors for fixed and random effects, respectively; and

the term e contains the random component of y that is not explained by the model (Saxton 2007). Understanding the components in *X, b, Z* and *u* is critical to understanding how linear models translate observed data into an explanation of *y* (Saxton 2007).

2.2.6.1.1 Animal Models

The most common mixed model used is the "animal" model. In this model, all animals are included in the model as random effects, while all other explanatory terms are fixed effects (i.e., year and herd; Di Croce et al. 2009). Animal models use pedigrees to track relationships among animals, and related individuals will share additive genetic (co)variance (Di Croce et al. 2009). Animals within the same herd will share the herd environmental variance. As previously shown, variances are separated into components of interest. The advantages of using the animal model are that all additive genetic relationships are utilized and unequal family sizes are accounted for correctly during analysis (Thompson 2008; Di Croce et al. 2009). In addition, Henderson's mixed model estimation provides the most accurate prediction of breeding values for all animals in the dataset (Henderson 1953; Di Croce et al. 2009). Today's computing technology allows one to fit animal models for both univariate and multivariate data.

2.2.6.2 Variance Structure

The mixed linear model, $y = Xb + Zu + e$, is an important equation, but is not a complete description of the model. The general expression of this equation ($y = Xb + Zu + e$) is only describing the effects contained in each observation, commonly referred to as the model for means (Saxton 2007). To complete the model, a model for the variability needs to be included and is usually referred to as the "variance structure". As previously mentioned, the variance of a vector produces a symmetric square matrix, with variances on the diagonal and covariances off-diagonal (Mrode 1996, 2005). Element (*i,j*) is the covariance between the *i*th and *j*th variables of the vector. The variance can be defined as the covariance of something with itself, so element (*i,i*) is the variance of the *i*th parameter (Saxton 2007).

To complete the mixed model ($y=Xb + Zu + e$) with the appropriate variance structure, it is necessary to define the Var(y) that will be some combination of the variances and covariances of the terms on the right hand side (Saxton 2007). Some conventional definitions include Var(Xb)=0, Var(u)=G, Var(e)= R, and Cov (u,e)=0. This is the variance structure which provides variances and covariance among observations (Saxton 2007). Additional to the mean model and the described variance structure, a statistical distribution for *Y* needs to be assumed. Thus, *Y* is assumed usually to be normally distributed.

Putting all of this together gives the "complete" mixed model,

Y is normally distributed

$$y = Xb + Zu + e$$

$$Var\begin{bmatrix} u \\ e \end{bmatrix} = \begin{bmatrix} G & 0 \\ 0 & R \end{bmatrix}$$

or

$$y \sim N = (Xb + Zu, ZGZ' + R)$$

2.2.6.3 Estimation of Parameters: Fixed and Random Terms

Once the mixed model is correctly and completely defined, parameters b and u, the unknown constants used to describe a population, need to be estimated. Several methods are provided by statistical theory for estimating unknown parameters from observed data (Saxton 2007). Least squares estimation is the method of choice for linear models due to its simplicity and the desirable properties of the estimates (Saxton 2007). Currently, the best choice for estimating variance components (the parameters of variance structure) is the restricted maximum likelihood methodology (REML; Saxton 2007; Di Croce et al. 2009).

2.2.6.3.1 Least Squares Estimation for Linear Models

As previously mentioned, least squares estimation is the usual method for calculating unknown parameters from observed data in linear models. This methodology minimizes the sum of squared deviations between observed data and model predictions. In matrix

notation, observed data are *y*, and model predictions are *Xb*+*Zu*. The deviation between these two is obtained directly from the mixed model equations

$$y = Xb + Zu + e, \qquad or\ y - (Xb + Zu) = e$$

One could define least squares estimation by minimizing $e'V^{1}e$ because the residual must be standardized by their variances and covariances (Saxton 2007). To accomplish this estimation in mixed models, Henderson (1953) demonstrated that solving the following matrix equations produces BLUEs and BLUPs.

$$\begin{matrix} X'R^{-1}X & X'R^{-1}Z & b & = & X'R^{-1}y \\ Z'R^{-1}X & Z'R^{-1}Z+G^{-1} & u & & Z'R^{-1}y \end{matrix}$$

These equations are the generalization of the "normal equations" to the mixed model, and are usually referred to as Henderson's mixed model equations (Henderson 1973). Moreover, these equations can be solved by inverting the left hand side (if the inverse exists), and pre-multiplying both sides by the inverse, if the inverse exists. Thus, the estimates are normally distributed, since they are linear functions of *y*, a normally distributed set of variables (Saxton 2007).

Least squares estimates are best linear unbiased estimates (BLUE) with three important properties: are unbiased (for some linear function of the parameters); are linear functions of the data; and have the smallest variance (best) compared to other potential linear unbiased estimators. For random effects, the word "prediction" is used instead of "estimation", giving BLUP (Saxton 2007; Di Croce et al. 2009)

2.2.6.3.2 Non-Full Rank Models

In more complex models (i.e., ANOVA models), the left-hand side of normal equations cannot be inverted (Saxton 2007). Least squares estimation is done without an inverse. If a matrix is full rank, the matrix can be inverted. By definition, rank is the number of linearly independent rows or columns, and a row and column are linearly independent if one is not a linear function of the others. Conversely, if models are non-full rank, the left-hand side of the normal equation (Henderson 1975) cannot be inverted for least squares estimation (Saxton 2007). According to Saxton (2007), two approaches have been developed to solve this problem. The model could be reparameterized to make *X* have full column rank; for example, by deleting the overall mean from the model (Saxton 2007). Conversely, another approach is to use a generalized inverse, which is an inverse that works for non-full rank matrices. The generalized inverse approach is one of the most popular solutions but leaves a problem in which estimates produced by the g-inverse approach are not unique. However, even though estimates are not unique, certain linear functions of the parameters can have unique and unbiased estimates (Saxton 2007).

On the other hand, estimation for random effects is simpler since the mixed model equations are always full rank, so BLUP properties for random effects are not affected by reduced column rank of X (Saxton 2007). This is a result of adding G^{-1} to the random effects section of Henderson's Mixed Model Equations. As usual, the standard errors of the estimates will be the square root of the diagonal elements, and confidence intervals can be calculated.

2.2.6.4 *Best Linear Unbiased Prediction (BLUP)*

In animal breeding, BLUP is a technique for estimating genetic merit for phenotypic traits (Robison 1991). In general, BLUP is a method of estimating random effects developed by Henderson (1949) by which fixed effects and breeding values can be simultaneously estimated (Mrode 2005).

Given the estimates of the variance components, predictions (BLUPs) of individual additive genetic effects or breeding values can be estimated from Henderson's mixed model equations. Best linear unbiased predictions are considered breeding values (predictions of the value of that individual as a parent) since additive genetic effects are transmitted to progeny. An individual's breeding value for a given phenotypic trait is the total additive effect of its genes for that trait. Also of interest, Henderson's equations can produce accuracy measures (ranging from 0.00 to 1.00) for each breeding value, reflecting the amount of information in the data for that prediction.

The properties of BLUP are incorporated in the name:

- Best – means it maximizes the correlation between true and predicted breeding value or minimizes prediction error variance;
- Linear – predictors are linear functions of observations;
- Unbiased – estimation of realized values for a random variable, such as animal breeding values, and of estimable functions of fixed effects are unbiased; and
- Prediction – involves prediction of true breeding value.

These BLUP statistical properties has been widely accepted and used for genetic evaluation in livestock.

As previously shown, mixed model analysis incorporates random effects into the model, allowing random parameters to be predicted. For example, by declaring animals to be random, the animals in the experiment are modeled as a random sample from a population of animals with a particular variance (Saxton 2007). This variance is the variance component used to create the variance structure matrix (V). When the "mean" of a particular animals is being estimated (or predicted), this variance needs to be taken into account (Saxton 2007; Di Croce et al. 2009). The BLUP value produced is the best prediction of the true value of that animal. Thus, the BLUP is giving the expected value of that animals for all possible future offspring. Basically, future performance is being predicted (Saxton 2007). With fixed effects, there is not distribution to take into account (i.e., no variance), so the means are the best possible estimates (BLUE). Therefore, the language distinction is fixed constants are estimated, and random values are predicted (Saxton 2007). Furthermore, BLUP takes into account variability in the random effects and the amount of data, producing the best prediction of the true value for random levels. These results reflect the model definition: the random levels being sampled from a distribution with variance (Saxton 2007).

2.2.7 Variance Component Estimation: Restricted Maximum Likelihood (REML)

As previously mentioned, the variance structure matrix V (V=ZGZ' +R) is a key component of mixed model definition and it is used to estimate random parameters such as BLUPs. These variance structures need to be obtained through variance and covariance component estimation.

Several approaches exist for variance component estimation such as ANOVA based methods and maximum likelihood. However, restricted maximum likelihood (REML), a variation of maximum likelihood approach, outperforms others approach (i.e., ANOVA base method, methods of the moments; Saxton 2007). Some advantages of using REML include the ability to handle unbalanced data and negative estimates are not permitted (Saxton 2007; Di Croce et al. 2009). However, if data are balanced, most of the variance component estimation methods provide the same estimates. Nevertheless, it is a very common problem in biological research to deal with unbalanced data. One example is the genetic parameter estimation for sires that have different numbers of daughters; thus, unbalanced data.

Likelihood is a measure of how likely we are to observe the data, given the variance component value (Likelihood = Prob(obs1) * Prob(obs2) * ...). Maximum likelihood approach starts with initial priors (guesses), and if these priors (guesses) match the data, then the priors are most likely close to the true values. If the priors are bad, they won't fit the data, so iteratively different values are tried. This overall fit to the data is measured by likelihood, with each iteration providing a likelihood value. Once likelihood values do not

change, then convergence on the final estimates will be attained. Therefore, it is important to show that the maximum likelihood estimation algorithm does achieve a true maximum likelihood.

Equation for MME likelihood is

$$logL(G,R) = [\log|V| - \log|X'V^{-1}X| - r'V^{-1}r - (n-p)\log(2\pi)]/2,$$

where: r=residuals; n=number of observations; p=rank (Saxton 2007).

In general terms, the disadvantage of this maximum likelihood approach is the necessity to find the maximum doing an iterative search; thus, computer time is a concern (Saxton 2007). Additionally, the maximum likelihood approach produce estimates which are generally biased; meaning on average they do not estimate the true parameter value. This bias must be accepted to avoid negative variance estimates.

Computer software for these calculations is readily available. Packages used for genetic parameter estimation include ASReml, DFReML (now Wombat), and MTDFREML (Boldman et al. 1995; Di Croce et al. 2009). These programs must be able to model single traits (univariate analysis) to estimate variance components, and model two or more traits (multivariate analyses) for the estimation of covariance components.

2.2.8 Genetic Gain Prediction

Given the predicted breeding values determined by BLUP, genetic improvement is accomplished by selecting superior animals as parents based on those predictions. Predicting rate of genetic gain or response to selection is the rate of genetic change on a population under selection and depends upon three factors: heritability (h^2), selection differential, and generation interval. Selection differential is the amount of improvement in a phenotypic trait of selected individuals compared to contemporaries. This differential is determined by selection intensity (i = standardized mean deviation of selected parents) and phenotypic variation (SD) present in the population. Generation interval is the amount of time required to replace one generation with the next. As an example, utilizing the above methodology (i=0.8)*(SD=49.962)*(h^2=0.029), Di Croce et al. (2008) estimated a genetic gain of 1.16 % per generation in pregnancy rate (PR) following embryo transfer, assuming 50% selection.

2.3 Genetic Selection for Fertility

To achieve a pregnancy in the modern high producing dairy cow has become a challenge for most dairy producers. This issue has motivated the interests of dairy producers, scientists, veterinarians, breed associations and the industry, since dairy cows tend to have lower conception rates, higher days open, and greater probability of culling due to infertility in recent years (Lucy 2001; Weigel 2007, USDA 2007). Genetic evaluation and selection strategies that focus on production traits have led to rapid gains in milk yield and

conformation traits, but performance for traits such as female fertility, longevity, and susceptibility to disease has tended to decline (Weigel 2007). Several factors such as changes in nutrition andreproductive, housing and health management may be contributing to these trends. However, it is clear that functional traits such as fertility, health, and longevity were not a priority in the selection strategies in the past decades. Additionally, the unfavorable genetic correlation with production traits (milk) has led to a decline in fertility in dairy cattle (Berglund 2008). Several studies have shown that selection for production alone causes negative effects on udder health (Heringstad et al. 2003; Miglior et al. 2005) and reproductive performance (Veerkamp et al. 2001; Haile-Mariam et al. 2003; Kadarmideen et al. 2003; Miglior et al. 2005).

Today, the negative trends of fertility traits (functional traits), the crucial economic importance of reproduction, and new genomic technology are a strong motivation for including reproduction in genetic selection programs. In 2006, the World Holstein Friesian Federation (WHFF) initiated a survey on the status of fertility in Holstein populations around the world. In the 19 countries surveyed, eight different fertility traits were used for heifers and eighteen fertility traits were used for cows (Sørensen et al. 2007). Most common traits used in cows were the interval from calving to first insemination (13/19 countries), calving interval (13/19 countries), number of inseminations to achieve conception (11/19 countries), non-return rate 56 days after insemination (9/19 countries), and number of days open (8/19 countries) among others (Sørensen et al. 2007). Furthermore, several countries have implemented genetic evaluation for fertility traits in

recent years. Several different traits are gradually being evaluated and incorporated into the evaluation methods. Berglund (2008) concluded that this implementation of selection for fertility traits leveled out the decline in fertility. However, the low level of reproductive efficiency continues as a major problem in dairy herds (Berglund 2008).

2.3.1 Genetic Selection for Female Fertility

Genetic selection for fertility has been performed for several years in Scandinavian countries to reduce the decline in reproductive efficiency (Weigel 2006). These countries have traditionally recorded and implemented genetic evaluation for a broad range of functional traits including fertility, but in recent years, many other countries have also implemented genetic evaluation for these traits (Berglund 2008). Breeding values for daughter fertility were introduced in Sweden as early as 1972 and have since been used in selection. In the Nordic countries, the integration of cow databases (pedigree, milk recording, artificial insemination and disease data) has facilitated selection for reproductive traits. Three of the Nordic (Denmark, Finland and Sweden) breeding organizations have had a joint genetic evaluation and breeding program since 2005 (Nordic Cattle Genetic Evaluation; http://www.nordicebv.info). Since 1990's, genetic evaluation for fertility has gradually been introduced in several countries, such as the Netherlands (Hoekstra et al. 1994), United Kingdom (Wall et al. 2003) and United States (VanRaden et al. 2004). An international genetic evaluation for fertility traits was introduced for Holstein populations in February 2007 by Interbull and an evaluation of fertility in the other primary breeds of

the Interbull member countries is under development (Jorjani 2007). Thus, the relative emphasis of dairy cattle breeding objectives has gradually changed from production to functional traits such as fertility during the last decade (Miglior et al. 2005). In United States (US) and other leading dairy countries, fertility was ignored until 1993 when VanRaden and Klaaskate (1993) developed the first improvement in female fertility by genetic selection through evaluation in length of productive life.

Weigel (2006) reported that inclusion of length of productive life during selection tended to stabilize genetic decline in daughter pregnancy rate. According to genetic evaluations, indirect selection for length of productive life was useful in improving fertility traits; however, direct selection for improved fertility is more advantageous (Weigel 2004, 2006). In accordance, a US national fertility evaluation was developed in 2004 based on genetic evaluation for daughter pregnancy rate (VanRaden et al. 2004). The daughter pregnancy rate (DPR) measures the percentage of nonpregnant cows becoming pregnant within each 21-d opportunity period. The DPR by definition is calculated as *"the number of cows that became pregnant during a given 21-day period divided by the number of cows that were eligible for breeding at the beginning of the period"* (Weigel 2006). These groups are composed of cows that are not yet pregnant and have completed the voluntary waiting period (VanRaden et al. 2004).

The US genetic evaluation data include measurements for days open, date pregnant, and the success/failure of last breeding (VanRaden et al. 2004). These evaluated measurements were adjusted by parity and calving season within geographic region and time period

(VanRaden et al. 2004). This evaluation was the first report of a predicted transmitting ability for daughter pregnancy rate based on an animal model.

Sire evaluation for DPR can be interpreted as the expected difference in a 21-day pregnancy rate between progeny groups of different sires (Weigel 2006). In other words, the pregnancy rate for an individual daughter indicates the number of 21-day opportunity periods required to achieve pregnancy (Weigel 2006). The significant variation between sires with the highest and lowest differences in daughter pregnancy rate is more than seven percent (Weigel 2006). VanRaden et al. (2004) estimated that a *"1% difference in pregnancy rate is equivalent to approximately 4 days open"*. Therefore, days open between daughters of the highest and lowest sires would differ by approximately 28 days per lactation.

Weigel (2006) reported that daughter pregnancy rate was included in all selection indexes prepared for the US dairy industry. The relative weight of DPR on those indexes was 5–7% of the total economic value (Weigel 2006). Moreover, the genetic negative correlation of milk yield and female fertility was considered relatively small. Thus, this small correlation guarantees the possibility of some sires with high production (milk) and fertility in their daughters. However, there are still many countries that do not include fertility information in their genetic evaluation. Additional improvements need to be made in future genetic evaluation for fertility, such as detailed databases on reproductive performance. These events should include outcomes of pregnancy examinations, dates of

hormonal synchronization for estrus and ovulation, and dates of exposure to natural service sires (Weigel 2006).

2.3.1.1 Genetic Trend for Cow Fertility

VanRaden et al. (2004) reported fertility trends based on predicted transmitting ability (PTA) for DPR. The declining genetic trend for fertility by breed in United States is depicted in Figure 3 across different years (VanRaden et al. 2004). Furthermore, genetic trends were compared across breeds including milking Shorthorn, Jersey, and Ayrshire breeds which had smaller losses of fertility across time; whereas, Guernsey, Brown Swiss, and Holstein had larger losses (VanRaden et al. 2004). VanRaden and coworkers also observed that the genetic trend in Holstein became flat after 1994, suggesting that introduction of productive life, and the subsequent selection for this trait, could be responsible for this change. Furthermore, VanRaden et al. (2004) reported an increased genetic trend in Holstein for days open as well as yearly fluctuations (Figure 4).

As mentioned earlier, pregnancy rate is highly correlated with days open, and a 1% increase in pregnancy rate represents a decrease of 4 days open (VanRaden et al. 2004). Moreover, an unfavorable genetic correlation exists between production (milk) and days open of about 0.35. Additionally, selection for productive life since 1994 apparently has slowed the decline in fertility, but direct selection for fertility should be more profitable.

2.3.2 Genetic Selection for Male Fertility

Two regional systems were developed for evaluating male fertility in the United States (Weigel 2006; Kuhn and Hutchison 2008; Kuhn et al. 2008) consisting of the Animal Improvement Programs Laboratory (AIPL) implemented in May 2006, and the Estimated Relative Conception Rate (ERCR) developed by Dairy Records Management Systems and North Carolina State University (Raleigh, NC). The animal model employed in ERCR adjusted for effects of cows and their ancestors on 70-d nonreturn rates; thus, estimating the effect of the male (Clay and McDaniel 2001). Estimated relative conception rate is an estimate of the difference between an AI service sire and the average AI service sire of herdmates for rate of non-return in 70 d (Clay and McDaniel 2001). The authors evaluated reproductive performance using first service non-return in 70 d (NR70), where a first service was successful when a cow was not reported in heat or rebred within 70 d (Clay and McDaniel 2001).

Other studies (Kuhn et al. 2004; Kuhn and Hutchison 2008; Kuhn et al. 2008) have shown that use of multiple services and use of an "expanded" service sire term improved accuracy of bull fertility. This improvement in use of "expanded" service sire, rather than use of first service exclusively, is due to increasing amount of information used to evaluate each bull. By definition, the term expanded service sire, includes *"fitting factors related to bull fertility separately in the model and then computing a bull's evaluation as the sum of the solutions for each factor"* (Kuhn and Hutchison 2008). Despite improvement in accuracy, heritability estimates for conception rates for artificial insemination are zero. Therefore, an

additive genetic effect does not exist for predicting bull fertility (Kuhn and Hutchison 2008). To evaluate production and conformational traits, evaluation of bull fertility is projected as phenotypic rather than genetic evaluation (Kuhn and Hutchison 2008).

Unlike the AIPL, the Western Bull Fertility Analysis (Agri-Tech Analytics, Visalia, CA) began releasing sire fertility estimates in 2004. Their system uses on-farm data from large herds in the western part of the United States, most of which are located in California. By definition, this system is based on *"75-day veterinary-confirmed conception rate for up to five inseminations per cow per lactation"* (Weigel 2006). In general terms, both regional systems are adjusting estimates similarly by several factors but use different animal populations.

Dairy producers are utilizing results of these genetic evaluations of male fertility when selecting bulls and purchasing semen (Weigel 2006). Differences of 4–5% are reported between the highest and lowest deciles of bulls (Weigel 2006). However, Weigel (2006) reported that most variation in male fertility is removed when bulls are culled for infertility. This culling occurs when ejaculates are discarded for failing to meet laboratory standards.

Although considerable research has now been performed on cow fertility, Weigel (2006) suggested that direct genetic selection for improved female fertility is achievable by exploiting traits such as daughter pregnancy rate or indirect selection such as longevity. The statistical methodologies for analyzing reproductive data are standardized and proven to provide efficient estimates. However, Weigel (2006) reported that enhancements in

collection of fertility data are needed. This improvement would optimize information pertaining to pregnancy diagnosis, hormonal treatments, natural service matings, and "do-not-breed" designations (cows to be culled). As previously mentioned, evaluation of bull fertility is projected as phenotypic rather than genetic evaluation (Kuhn and Hutchison 2008).

Since August 2008, sire conception rate (SCR), an evaluation of the fertility of artificial insemination (AI) service-sire, was available to dairy producers from USDA. As previously mentioned, from 1986 to November 2005, evaluations of bull fertility termed ERCR were provided by Dairy Records Management Systems (DRMS; Raleigh, NC). In May 2006, USDA's Animal Improvement Programs Laboratory assumed responsibility for phenotypic evaluation of U.S. bull fertility. Initially, ERCR evaluations were implemented without change in calculating methods. Since 2006, Kuhn and Hutchison (2008) and Kuhn et al. (2008) have developed an intense research effort developing methods to improve accuracy of bull fertility evaluations. The researchers identified factors associated with the bull effect to obtain a pregnancy, and variables that distorted the fertility measure for the bull providing semen (nuisance variables; Kuhn et al. 2008). Those nuisance variables removed variation and improved accuracy of sire conception rates (Kuhn et al. 2008).

2.3.2.1 Interpretation of Sire Conception Rate

The technical difference between ERCR (70-day nonreturn rate) and SCR (sire conception rate) is based on confirmed pregnancy for SCR (Table 1). However, the two traits are

highly related and provide no differences in interpretation (Norman et al. 2008). For example, a bull with an SCR of 2.0% is expected to produce a conception rate of 32% in a herd that normally averages 30% (historic average of CR in bulls; Norman et al. 2008). The term "expected" implies these results would occur if based on large numbers of matings (Norman et al. 2008). In herds with only two possible inseminations per bull, the potential results for conception rate will represent 0, 50, or 100% (Norman et al. 2008).

2.3.3 Female Fertility Traits and Fertility Index

Several measures of reproductive performance have been proposed for evaluating breeding programs and producing selection indexes. These measurements include traits such as days open (DO), calving interval (CI), days to first service (DFS), number of inseminations per lactation (INS), success in first insemination (SF), interval between first and last insemination (IFL), and pregnancy rates (PR) at 21, 56, 70 days after service (Gonzalez-Recio and Alenda 2005; Caraviello et al. 2006). However, no consensus of which traits should be included in fertility indexes exists (Gonzalez-Recio and Alenda 2005); however, the above mentioned traits have been included in various forms of fertility indexes (Gonzalez-Recio and Alenda 2005).

González-Recio and Alenda (2005) estimated heritability, phenotypic, and genetic correlations among various fertility traits as illustrated (Table 2)

They reported low heritability (0.02 to 0.06) according to previous research available (Dematawewa and Berger, 1998; Veerkamp et al. 2001). In general, genetic correlations were high, ranging from 0.89 to 0.99. Days to first service were an exception with correlations of –0.52 with SF, 0.82 with DO, and –0.82 with PR. Furthermore, P56, P90, and SF had high positive genetic correlations (from 0.92 to 0.97). Conversely, some traits showed negative correlations with the remaining traits.

In general, these results agree with low heritabilities and strong genetic correlations estimated by other researchers. Kadarmideen et al. (2003) and Veerkamp et al. (2001) reported strong (positive and negative) correlations ranging from ± 0.70 to ± 0.98 for fertility traits. However, Wall et al. (2003) reported lower correlations (0.61 and –0.45) for CI with INS and nonreturn rate at 56 d, respectively.

González-Recio and Alenda (2005), using a threshold sire model, reported similar heritabilities for binary traits as Kadarmideen et al. (2000) and Averill et al. (2004). González-Recio and Alenda (2005) suggested that comparison between P56/P90 and nonreturn rates must be carefully considered. They considered these two traits different because nonreturn rates indicate absence of an additional AI within a given period. However, P56 (P90) indicates pregnancy within 56 d (90 d) (achieved with any number of AI) after first insemination (González-Recio and Alenda 2005). This trait indicated that success for P56 and P90 can be achieved with any number of inseminations whereas, only one AI is considered with nonreturn rate.

Gonzalez-Recio and Alenda (2005) proposed six fertility indexes utilizing information from milk recording schemes and insemination records. The fertility index that accomplished the highest genetic gain for reducing fertility cost was composed of DFS and pregnancy within 56 d. Authors remarked on the usefulness of recording insemination data within a dairy population. The usefulness of these indexes is that it achieved 15% higher genetic gain than indexes with information from the milk recording scheme only (calving interval and days open) (Gonzalez-Recio and Alenda 2005). However, the correct application of these fertility indexes is limited to the adequate recording of reproductive data. Although some fertility traits or indexes can be estimates (CI and DO) from the milk recording programs, the remaining traits require insemination and pregnancy records. These data are not frequently recorded in many countries due to the lack of appropriate recording systems (Gonzalez-Recio and Alenda 2005). In that sense, the advantages of recording reproductive data should be emphasized.

Although genetic progress in fertility as previously cited is possible by both direct/indirect selection and fertility indexes, genetic progress in fertility requires an extended time due to low heritabilities. However, most improvement in female fertility is reached with a combination of information from milk recording programs and reproductive performance.

2.4 Economics of Fertility

Fertility is the most economically important trait in dairy and beef herds. Reproductive efficiency impacts directly on revenues associated with milk production per dairy cow per

day and offspring born. Moreover, low reproductive performance directly elevates costs associated with additional inseminations, higher veterinarian costs (drugs for estrus synchronization, pregnancy checks, etc), increased culling, and development cost associated with replacement heifers. Therefore, the profitability of dairy farms depends directly on the reproductive efficiency of the dairy cows (Plaizier et al. 1997; Meadows et al. 2005; De Vries 2006).

Most studies have evaluated the economic impact of impaired reproduction efficiency measured as additional days in which cows are not pregnant beyond the optimal time post-calving (Holmann et al. 1984, Groenendaal et al. 2004; González-Recio et al. 2004; Meadows et al. 2005; De Vries 2006). Furthermore, several factors need to be included into the economic analysis since they have a great impact on the results (i.e., stage of lactation, lactation number, milk production, persistency of lactation, prices, breeding and replacement decisions (Groenendaal et al. 2004; González-Recio et al. 2004; Meadows et al. 2005; De De Vries 2006).

Plazier et al. (1997) defined economic values as the effect of a marginal unit of increase directly associated to a specific trait. This definition measured the economic efficiency of a determined trait without considering changes in other traits. Thus, economic values can be obtained either as the partial derivative of a profit equation or through simulation evaluating the effect of a marginal increase in the trait on production efficiency (Groen 1989; Plazier et al. 1997; Meadows et al. 2005; De Vries 2006). Computer simulation programs for dairy production systems have been used to estimate the economic values of

reproductive traits and the financial implications of different levels of reproductive efficiency (Congelton 1984, , Marsh et al. 1987, Plazier et al. 1997, Meadows et al. 2005; De Vries 2006).

2.4.1 Models for Measuring the Economic Impact of Fertility

Realistic and complete models that represent the economic benefits of improved reproductive efficiency are not simple to estimate (De Vries 2009). Defining real assumptions to imitate currents reproductive management practices as well as to predict probabilities of pregnancies or culling rates are difficult for evaluating economic impact of impaired fertility. However, estimation of the economic impact caused by reduced fertility should provide an incentive for identification and correction of the underlying causes of reduced reproductive efficiency. Several causes are associated with management practices and many others may be associated with selection strategies when reproduction (fertility) is ignored. Reproductive efficiency has continued to decline in dairy herds in spite of the known importance of fertility traits, improved management, and increased knowledge of reproductive biology in the cow (Lucy 2001; Santos et al. 2004).

Improved reproductive performance in a traditional system provides positive changes in cash flow that can be accounted for in the different profit evaluation models (De Vries 2009). In this analysis, realistic estimates of lactation curves, feed intake, risk of involuntary culling, and prices for milk, feed, labor, semen, fertility drugs, calves, replacement heifers and cull cows should be accounted for in the model (De Vries 2009).

In simplistic terms, cows that become pregnant earlier after calving spend more time in the early part of the lactation and have less risk of being culled for reproductive failure (most common involuntary culling reason). Consequently, the earlier that cows become pregnant, the more profit periods these cows will have since they'll spend more time during the more profitable period (high milk production) of the lactation curve. Conversely, when cows become pregnant later after calving, they will spend more time on the tail end (low milk production) of the lactation curve which results in loss of profit. In summary, dairy producers desire pregnant cows as earlier as possible (profitable first period of the lactation curve) to maximize profitability of the herd. Additionally, others important factors such as the voluntary culling policy (defined period to continue inseminating non pregnant cows) and the efficiency for generating replacement heifers have a high economic impact and should be considered in the analysis model (De Vries 2009).

Therefore, to reproduce and integrate these economically important factors, computer programs, spreadsheets, and logic models need to be created to obtain the best possible economic estimates of proven reproductive performance (De Vries 2009). Although researchers have created and used these models, results still differ for several reasons such as variation in prices, management, lactation curves and feed intake, risk of involuntary culling, insemination and voluntary culling policy, and method of calculation among others (De Vries 2009).

2.4.1.1 The Days Open Example

Various studies have evaluated reproductive performance by estimating the additional cost per day open or per calving interval (Table 3; Plazier et al. 1997; Meadows et al. 2005; De Vries 2007, 2009). The term Days Open (DO), is an indicator of reproductive efficiency in dairy cattle that is widely accepted (Plazier et al. 1997; Meadows et al. 2005; De Vries 2007). This useful measure is defined as the interval from calving to conception and is affected by many components of reproductive management including voluntary waiting period, estrus-detection efficiency, and conception rate (CR; Barr, 1975; Esslemont, 1992; Pecsok et al. 1994; Meadows et al. 2005). Estimation of the economic cost of an additional DO has been frequently reported and involves a decrease in profitability associated with reduced milk production and availability of replacements (Plaizier et al. 1997; Meadows et al. 2005; De Vries 2007).

From different approaches that have been developed and used to estimate cost of an extra day open, results (estimates) show variation from a slightly negative cost (which implies a benefit for additional DO) to decidedly greater positive costs (Meadows et al. 2005). Although these variations are published, most of the research implies a negative economic impact regarding the financial consequence of inefficient reproduction as measured by days open (Meadows et al. 2005).

2.4.1.2 Modeling a Dairy Herd: A Tool for Evaluating Days Open and other Fertility Traits

Most tools developed for estimating economic impact of an additional DO simulate life of an average dairy cow on a daily basis in a specific scenario, which is a set of inputs that describe herd characteristics (Meadows et al. 2005). These tools based both on real records and/or simulations require assumptions regarding which revenues and costs will be used (Table 4; Meadows et al. 2005). These assumptions can introduce variability; thus, making interpretation of appropriate comparisons difficult (Schmidt 1989; Plaizier et al. 1997; Meadows et al. 2005). Meadows et al. (2005) simulated each day of the cow's life, estimating a partial cash flow based on these expenses (of inputs) and revenues (from products) that are closely related to reproductive performance (Table 4).

Several outputs and scenarios can be generated with these simulation models (Gonzalez-Recio et al. 2004; Meadows et al. 2005; Hou et al. 2009). As previously shown in Table 4, some input values can generally represent average values across regions, states or countries. Also, a great advantage of these simulation models is the possibility to test variable and input sensitivity; thus, creating diverse scenarios adaptable to most situations in dairy herds. For instance, Meadows et al. (2005) simulated the effect of alternate annual culling rates and days open as shown in Figure 5, which illustrates equivalent daily cash flow (EAC; adaptation for specific evaluation periods from the net present value; NPV; Meadows et al. 2005). This figure shows EAC values estimated for DO from 130 to 190 and culling rates of 25, 34, or 45%. Briefly, as annual culling rate increases, the modeled

dairy herd becomes less profitable. Additionally, for a fixed annual culling rate, profits measured as EAC decline as DO increase (Meadows et al. 2005). In summary, the ideal scenario is to have lower culling rates and shorter days open.

Furthermore, the effect of relating days open with milk and feed prices represent an interesting scenario to evaluate. Meadows et al. (2005) showed that the model inputs led to maximally average daily milk yield near 110 DO, with a marked decline as DO increased (Figure 6).

2.4.2 Estimation of the Cost of Impaired Reproduction Efficiency: The DO Example

In table 3, estimations of costs associated with additional days open are reported and expressed per cow per year. In summary of the table, most of the authors reported that the cost of days open is not constant and each DO becomes more costly as DO increased (Holmann et al. 1984; Dijkhuizen et al. 1985; Schmidt, 1989; Plaizier et al. 1997, Meadows et al. 2005). Meadows et al. (2005) suggested that this conclusion is perhaps more useful to producers and practitioners than any single estimate of the cost of a DO. Meadows and coworkers also suggested that it is important to know greater economic opportunities exist when DO are farther from a target or ideal value. These costs in Table 3 are not adjusted for inflation and reflect the situation at the time of each study. As the cost is not uniform and depends on DO, a column with the average DO used for the analysis was included in this table.

2.4.3 Estimation of the Cost of Impaired Reproduction Efficiency: The Value of a Pregnancy

De Vries (2006) defined the value of a pregnancy for an individual cow as the difference in discounted future cash flows when pregnant compared with when not pregnant. The author also determined that the economic value of a pregnancy could be easily evaluated as the difference in expected future net returns from two identical cows, one pregnant and the other non pregnant. DeVries suggested that the Retention Pay-Off (RPO), also called future profitability or cow value, of the two cows be compared to calculate the economic value of a pregnancy. In simplistic terms, the RPO of a cow is the difference in expected total net returns maintaining the cow until her optimal voluntary replacement compared to immediately culling upon determination of pregnancy failure and replacing her with a heifer (Van Arendonk 1984; De Vries 2006; 2009).

As previously mentioned for days open, estimations of the value of a pregnancy vary for the type of models used, variation in inputs such as prices, management, lactation curves and feed intake, the risk of involuntary culling, insemination and voluntary culling policy, and method of calculation among others (De Vries 2009). Despite these variations, estimations of the value of a pregnancy will be introduced in a summary table (Table 5). However, these estimates are not comparable since most of them represent specific situations. Despite this consideration, these estimates are intended to illustrate the substantial economic impact of a new pregnancy as well as the potential economic loss of abortions (De Vries 2006).

Using a complete model, De Vries (2006) exemplified the value of a pregnancy during the first lactation using average lactation curves (Figure 7). De Vries demonstrated that the dynamic of retention payoff (RPO) for non pregnant and pregnant cows with conception at 61 days in milk (DIM), with the value of pregnancy increasing from $81 during the first month of pregnancy to $841 during the last month of pregnancy (Figure 7; De Vries 2006). Additionally, in this example, non pregnant cows showed that RPO decreased from $993 in the first month after calving to less than $0 after 13 months in milk, suggesting that the cow should then be culled.

Other authors have evaluated the economic value of a pregnancy using the value of a marginal increase in pregnancy rate (i.e., the economic value of a 1 percentage point increase in pregnancy rate). For instance, Pecsok et al. (1994) showed that the value of a 1 percent increase in pregnancy rate was worth about $0.86 per cow per year when the herd's pregnancy rate was approximately 45%. However, if one decreases this pregnancy rate to 13%, the 1 percent value increases up to $16.60. Plaizier et al. (1998) reported values of a 1-percentage-point increase in estrus detection rate had been estimated from a loss of $2 to a gain of more than $16 (1998 US dollars). Their results also showed that improving poor reproductive performance was the most valuable. United States Department of Agriculture (USDA), in its revision of the net merit as a measure of lifetime profit in 2010, reported a change of 1% in daughter pregnancy rate was worth $ 27 lifetime (Cole and VanRaden 2010).

2.4.4 Other Economic Values for Fertility

Interestingly, González-Recio et al. (2004) simulated fertility costs of dairy herds through number of inseminations per service period (ISN; Table 6). According to their results, doses of semen, hormonal treatment, and culling costs in cows with poor fertility lead to lower lifetime production, shorter productive life, and lower profit. They evaluated female fertility using a specific reproductive recording scheme. However, they stated that if insemination records are not recorded, genetic correlation of CI or DO with INS could be used to develop a selection index (González-Recio et al. 2004). Also, they suggested to include traits from milk recording schemes (such as CI or DO) could be included in the selection index by relating them to economic value of inseminations per service period (Gonzalez-Recio et al. 2004).

2.5 Fertility Selection Index

2.5.1 The Selection Index

Hazel and Lush (1943) introduced the concept that net merit of the individual, considering several traits of economic importance, outperforms other forms of selection including single trait selection. The fundamental concept of aggregate genotype described by Hazel and Lush (1943) introduces a linear function (selection index) of observations providing that the observations of each trait are weighted by the relative economic value of that trait. The aggregate genotype permit obtaining a single value for each animal; thus, representing an objective valuation integrated by net genetic merit of an animal and the profit associated

to these traits (Weaber 2010). Incorporation of breeding values to selection indexes, motivated by Hazel (1943) and Henderson (1951), definitely improved genetic prediction of economically important traits.

2.5.2 Index Basics

In simplistic terms, the selection index equation (Hazel 1943) defines the economic merit of an animal as a parent since it incorporates breeding values:

$$Hi = a1BVi1 + a2BVi2 + \ldots + anBVin.$$

Where:

Hi = the aggregate economic merit of animal *i*, as a parent;

aj = the relative economic weight of trait j, j = 1...n, where *n* = the total number of traits: and

BVij = the breeding value of animal *i* for trait *j*.

Usually, breeding values are represented by PTAs or EPDs. Thus, animals (as parents) are ranked on a prediction of H called (I), the index value defined as (Henderson 1963):

$$Ii = a1PTAi1 + a2PTAi2 + \ldots + anPTAin.$$

Where:

Ii = the predicted aggregate economic merit of an animal, *i*, as a parent.

aj = the relative economic weight of trait *j, j* = 1…n, where *n* = the total number of traits; and

PTAij = the predicted transmitting ability of animal *i* for trait *j*.

As previously discussed in genetic parameter estimation section, the index is unbiased as the genetic predictions themselves are unbiased since they were predicted with Best Linear Unbiased Predictions (BLUP) procedures.

2.5.3 Relative Economic Values

Several studies discussed earlier in this section presented relative economic values (weighting factors) for traits in potential development of fertility indexes. However, the adoption and implementation of indexes of aggregate economic merit have been limited by the absence of economic values in different areas (primarily in functional traits), and as such, the actual genetic evaluation could be improved (Golden et al. 2000).

Economic values or weights (the *a*'s in the previous equations) reflect the change in profit when a trait is changed by a single unit. Several examples were previously introduced that could be apply to fertility indexes. To illustrate the selection index theory, an example, including different fertility indexes developed by Gonzalez-Recio and Alenda (2005), will be briefly presented.

2.5.4 Fertility Indices in Dairy Herds

Gonzalez-Recio and Alenda (2005) studied which traits should be included in a selection index to reduce fertility costs. They proposed six different selection indices, the first 2 fertility indices (FI1 and FI2) were calculated with only information from milk recording

scheme (CI and PR, respectively). Using data from insemination records, the next 4 indices (FI3, FI4, FI5, and FI6) included days to first service (DFS), interval from first to last insemination (IFL), nonreturn rate (P56; P90), and number of inseminations (ISN) per service period, respectively.

The fastest genetic gain was achieved with DFS (as a trait to indicate beginning of reproductive performance), and P56 (as a trait to measure conception rate; Gonzalez-Recio and Alenda 2005). The fertility index (DFS + P56) lowered DFS (–1.31 days), and reduced ISN (–0.03) per generation. This genetic progress would increase profits by $8.60 per cow per generation (Gonzalez-Recio and Alenda 2005).

2.6 Genomic Technologies

2.6.1 The Era of the Genomes

Starting in the 1980s, genomics technology described the scientific discipline of mapping, sequencing, and analyzing genomic level DNA information (Green 2009). Along with genomics, another technology, polymerase chain reaction (PCR), allowed one to understand the structure of DNA (Mullis and Faloona 1987). Polymerase chain reaction along with other complementary technologies, provided the opportunity to investigate the genetic code of plants and animals, and to identify locations on the chromosomes that might contain genes affecting economically important traits (Green 2009). In 1990's microsatellite markers, found readily throughout the genome, were used to develop linkage mapping. Bishop et al. (1994) reported one of the first genetic linkage maps in US cattle.

From these early efforts and accompanying technological advances (e.g., radiation hybrid and bacterial artificial chromosome maps), more defined and better maps continue to be developed (e.g., "composite bovine map"; Snelling et al. 2007). These linkage maps motivated researchers to start searching for regions of the genome harboring genes containing polymorphisms, which cause differences in phenotypic performances in most economically important traits (Green 2009). Thus, identification of quantitative trait loci (QTL; specific region of the genome linkage with different performance of a trait) was an important advance in genome technology, motivated in part by its possible application in marker-assisted selection (MAS; Dekkers 2004; Green 2009). However, application of this technology (MAS) in the industry was overstated and later considered as only an important step towards practical technology (Dekkers 2004; Green 2009).

Taking advantage of the highly funded research associated with diseases on human genome, livestock genomics rapidly advanced due to similarities in genomes (85% or greater) between mammalian species. Despite this interesting and aggressive approach, only a few genes have been mapped to date through the comparative mapping approach (Cockett et al. 1994; Casas et al. 2000; Green 2009).

Due to the slow, expensive and inefficient advance of linkage maps, QTL searches, comparative mapping, and some fine mapping, the National Institutes of Health, through its National Human Genome Research Institute, designed a plan for sequencing the human genome (Human Genome Project). The Human Genome Project refers to international studies to discover the estimated 20,000-25,000 human genes and make them accessible

for further biological study (Collins et al. 2003; http://www.ornl.gov/sci/techresources/Human_Genome/project/hgp.shtml; Green 2009). Utilizing the created infrastructure of the National Human Genome Research Institute, this organization supported the sequencing of several other genomes chosen based on comparative mapping and use as medical models. The draft sequence for the bovine (Gibbs et al. 2002; Kappes et al. 2006) was assembled and has been placed in a public domain through the National Center for Biotechnology Information (http://www.ncbi.nlm.nih.gov/). Additionally, other species have been sequenced and/or are expected to be assembled to the same level (Green 2009).

Beside development of the draft sequence, another major challenge was development of tools that would allow bovine genomics research to move forward quickly (Kappes et al. 2006; Green 2009). This genomic technology has permitted detection of large pools of new SNP, and full-length complementary DNA sequences have been developed for multiple tissue systems for the study of gene expression (Green 2009). These efforts and findings are being annotated; thus, providing a gene atlas for various species (Green 2009).

Van Tassell et al. (2008) aided development of high-throughput genotyping platforms to determine large numbers of markers (56,000 bovine panels) in a single run in a cost effective manner. Additionally, Meuwissen et al. (2001) developed methods to predict genetic parameters with a limited number of phenotypic records, based on information provided by these high-throughput genotyping platforms, using marker haplotypes. The authors suggested that selection based on breeding values predicted from markers could

substantially increase rate of genetic gain, especially in traits where accuracy of selection is low. Low accuracy is due mostly to limited number of phenotypic records, primarily in traits with low heritability (Meuwissen et al. 2001). In dairy cattle, VanRaden et al. (2009) reported significant enhancements in accuracy of breeding values of young bulls when obtained by the 56,000 bovine SNP panel compared with pedigree estimates.

2.6.2 DNA, Genes and Markers

The bovine genome consists of 30 pairs of chromosomes that contain a fundamental component know as deoxyribonucleic acid (DNA). Chromosomes and DNA (excluding mitochondrial DNA) are stored inside the cell nucleus. DNA encodes information for all proteins needed to create and maintain an organism. Information for protein's synthesis is contained within genes. These genes, a DNA segment that contributes to phenotype/function, represent only a small portion of a cell's entire DNA (the genome). DNA consists in a double helix of simple units or building blocks know as nucleotides. These building blocks are constituted of sugar molecules and organic bases. There are four types of bases: adenine (A), guanine (G), cytosine (C), and thymine (T). The sequence of these building blocks (nucleotides) provides the order of amino acids required to develop proteins. Mutations, changes in the nucleotide sequence, can occur that result in altered proteins, contributing to the development of different phenotypes (coat color or height). These changes or mutations of single base pairs represent polymorphisms that can be used as DNA markers. Many mutations do not result in amino acid and protein changes;

however, these changes in single base pairs could still be associated with an altered phenotype or increased disease risk.

2.6.2.1 DNA Markers

DNA markers are sequences of nucleotides with a known location on the genome and associated with a particular gene or trait (i.e. increased marbling). Variations in this location can be used to identify specific alleles or forms of a particular gene. Genotyping (DNA tests) determines which alleles an animal is carrying for a specific DNA marker. Although several types of DNA markers exist, one of the most popular used are called single nucleotide polymorphisms (SNPs).

2.6.2.1.1 Single Nucleotide Polymorphism (SNPs)

Single nucleotide polymorphisms (SNPs) are a change in a single base (nucleotide) pair in the genome and usually generate two alleles for a specific SNP marker. For example, two pieces of DNA sequences from two different calves represented by AAGGC**C**TA and AAGGC**T**TA contain a difference in a single nucleotide. For this specific location, there are two alleles (C and T). Thus, these different alleles for each SNP can be associated with traits or phenotypes (i.e. increased tenderness).

A simply way to understand SNP markers is to think that they are "tags" across the genome (DNA) that allows one to identify a piece of DNA and track its inheritance from parent to offspring (Weaber 2009). As discussed previously, these markers can be

genotyped with modern equipment (SNPs panels). Thus, DNA marker test determine which DNA sequence variant (allele) an animal is carrying for a specific DNA marker (SNP; Van Eenennaam 2009). These DNA markers, based on SNPs, allow for tracking inheritance of "simple" traits controlled by a single gene or "complex" traits controlled by several genes (Weaber 2009).

2.6.2.1.2 DNA Markers for Single Traits

The fundamental point in any DNA marker test is to find the variations (SNPs) in DNA sequences that cause variation in the phenotype (weight, height, etc). For example, a wide spectrum of genetic tests are used today and available for producers for different genetic abnormalities (Arthrogryposis Multiple, Tibial Hemimelia, Pulmonary Hypoplasia with Anasarca, etc), coat color (red vs black) and horn status (Table 7). As previously mentioned, most of these traits are controlled by a single gene, and a marker associated with this gene can predict the phenotypic response of these simple traits. These tests cover a variety of anatomic and metabolic genetic defects in cattle where producers can identify if their animals are free or carriers of most common genetic defects. These tests are highly reliable in identifying genetic diseases since most are single gene mutations. Thus, a simple mutation explains most of the variation (if not all) of the trait. These genetic panels constitute a group of DNA markers tests that are being actively marketed for the past few years and are readily available for producers.

2.6.2.1.3 DNA Markers for Complex Traits

Test for DNA markers of complex traits (most economically important traits) need to track several genes in order to explain a large part of the genetic variance. Furthermore, these traits are highly influenced by environment and management. Typically hundreds of genes are needed to explain most of the genetic variance; although, in some cases, a single gene may have a large effect (Goddard 2009). Thus, several markers are need in a DNA test for complex traits since each single marker is associated with only one of the many genes that control those traits. For instance, a complex trait such as milk production is estimated to be affected by more than 1000 genes (Goddard 2009). In general, DNA marker tests with only a few markers explain a small fraction of the variation of these traits. Currently, DNA tests for complex traits involve a small numbers of SNPs (<100) and explain only a small proportion of the genetic variation of the traits (<10%; Van Eenennaam 2009). One novel approach to identify and discover markers (SNPs) in single and complex traits is the genome wide association study.

2.6.3 *Discovering Markers: Genome Wide Association Studies (GWAS)*

The possibility to identify genes for complex or quantitative traits (measurable traits affected by many genes and the environment) would greatly enhance the understanding of these genes and their genetic prediction for representing substantial benefits for producers. As previously shown in genetic estimation for quantitative traits, the genetics of these traits

have been studied without identifying genes involved (Dekkers and Hospital 2002; Goddard and Hayes 2009). Thus, the estimated breeding values and genetic parameters (heritability), predicted from phenotypic records and pedigrees, have been leading successful selection strategies for quantitative traits. However, the progress is slow, essentially if traits can only be measured in one sex (i.e., milk yield, fertility), after death or late in the life (i.e., meat quality, longevity), or are expensive (i.e., feed requirement or disease resistance; Goddard and Hayes 2009). One fresh alternative to improve and speed the rate of genetic gain would be to identify genes associated with these traits and select animals carrying the desirable alleles (Meuwissen et al. 1996).

2.6.3.1 *Candidate Gene vs. GWA Studies*

Both candidate gene and genomic wide association studies (GWAS) have been extensively used to discover genes and polymorphisms associated with variation of complex traits (McCarthy et al. 2008; Goddard and Hayes 2009). The basic idea behind the candidate gene approach is that a major component of genetic variation of the phenotype under study is caused by functional mutation of specific genes (Zhu and Zhao 2007). These genes have been selected based on their known role in the physiology of the trait. This candidate gene approach allows one to confirm if effects of the causative genes are associated with variation in the phenotype. This approach has been extensively applied to gene-disease research, genetic association studies, biomarker and drug target selection in many mammalian species (Tabor et al. 2002; Zhu and Zhao 2007). Although many candidate

genes of economically important traits or disease resistance/susceptibility were detected, one of the primary disadvantages is the requirement of extensive physiological, biochemical or functional knowledge (Tabor et al. 2002; Zhu and Zhao 2007). The absence of background knowledge (phenotype) in some traits, low replication of results, limited ability to include all possible causative genes, and the complex genetic structure and physiology of the quantitative trait have limited this approach and further application (Tabor et al. 2002; Zhu and Zhao 2007; Goddard and Hayes 2009). However, with the recent availability of large panels of SNPs in cattle, the GWAS approach now has the possibility to search for mutations underlying variation in complex traits (Goddard and Hayes 2009). The genome-wide association approach can best be defined as an association study that surveys the genome for causal genetic variants. In contrast with the candidate gene approach, association studies can be performed without knowing the causal genes. The basic idea with GWAS is to detect statistical association between the trait (phenotype) recorded and the markers analyzed with a genome-wide panel.

2.6.4 Principles and Tools of Genomic Wide Association Studies

The basic design in GWAS include definition of parameters, number of animals involved, and size of the genome wide panel (number of markers; Goddard and Hayes 2009). Typically, linear models are used to analyze data from GWA studies where models fit one SNP at a time, including the effect of a SNP as fixed effects (Goddard and Hayes 2009).

Until now, genome wide association studies have not been feasible due to cost and labor. However, recent advances in SNP discovery by sequencing and re-sequencing the genome of several domestic species have moved GWAS from futuristic to realistic (Hirschhorn and Daly 2005; Goddard and Hayes 2009). Most of these discoveries have been implemented with commercial SNPs arrays or SNPs chips. For instance, commercial arrays are available in cattle (54,000 SNPs; Illumina BovineSNP54 BeadChip), dogs (22,362 SNPs; Illumina CanineSNP20 BeadChip), sheep (56,000 SNPs), pigs (60,000 SNPs; Illumina PorcineSNP60 BeadChip), and horses (54,602 SNPs; Illumina EquineSNP50 BeadChip; Goddard and Hayes 2009).

Several strategies have been used to avoid and minimize false positive associations in studies that involve thousands of markers. Hirschhorn and Daly (2005) classified the source of false positive associations into three categories: statistical fluctuations in testing multiple hypotheses resulting in low p-values, biases due to study design, and technical artifacts. For the first source of bias (testing multiple hypotheses), strong and conservative criteria for declaring significant associations should be taken. For instance, the Bonferroni correction is a preventative strategy to avoid these false positive associations when examining multiple hypotheses. Another important source of bias in the study design (false positive associations) is population stratification due the admixture in samples of individuals used. By definition, admixture occurs when samples of individuals are derived from more than one breed or race, and have not undergone random mating (Hirschhorn and Daly 2005; Goddard and Hayes 2009). Population stratification occurs when multiple

subgroups exist within a population that differs in average trait value (phenotype). This can lead to differences in one or more subgroups since false positive associations can result if a genetic marker has different frequencies in different subgroups (Hirschhorn and Daly 2005). When information is available, this problem can be easily avoided by including breed in the statistical model (Goddard and Hayes 2009). Conversely, admixture associated to the existence of relationships among the animals requires a more elaborate solution. The ideal scenario should be to sample individuals unrelated; however, cattle are usually bred in half sibling families. For this issue, Goddard and Hayes (2009) suggested inclusion in the statistical model of a term for the effect of all other genes affecting the trait (the polygenic term). Finally, as in any array test where large numbers of markers are typed, source of potential bias and false positive association exists due to technical artifacts.

2.6.5 Number of Single Nucleotide Polymorphisms in Genome Wide Association Studies

The amount of markers (SNPs) in GWAS depends on the distance between the QTL and the marker measured as the potential association by linkage disequilibrium. By definition, linkage disequilibrium is the non-random association of alleles at two or more loci (Goddard and Hayes 2009). For instance, if markers are separated by a long distance, the QTL may not be in linkage disequilibrium with the markers, making it necessary to increase the density of markers to detect the QTL (Goddard and Hayes 2009).

In cattle, Goddard and Hayes (2009) stated that significant associations were found within a breed using only 10,000 SNPs, but estimated that 300,000 SNPs would be required for between-breed analyses in *Bos taurus* cattle. They estimated that SNPs need to be spaced less than 10 kb apart to show association between the marker and the QTL across breeds. To illustrate these scenarios, the mutation in the DGAT1 gene that affects fat percentage in milk segregates only in *Bos Taurus* cattle (Goddard and Hayes 2009). Conversely, the mutations in calpastatin and calpain, associated with meat tenderness, segregate in both *Bos taurus* and *Bos indicus* breeds (Tantia et al. 2006; Goddard and Hayes 2009).

2.6.6 Number of Animals in Genome Wide Association Studies

In GWA studies, the estimation of the number of animals involved in the study is crucial and depends on the size of the effects that one expects to detect. One of the more important parameters to be reported is the proportion of variance explained by the SNP (Goddard and Hayes 2009). The explained proportion of variance can be described by combining the allele frequency with the mean difference between the SNP genotypes. In simplistic terms, Goddard and Hayes (2009) defined how this number (animals required) needs to be estimated. For instance, they reported that the correlation (r) between the marker and the trait, r(t,m), is equal to r(m,q) x r(q,g) times r(g,t), in which *m* is the marker genotype (usually scored 0, 1 or 2), *q* is the QTL genotype, *g* is the genetic value of the animal, and *t* is the phenotypic value of the animal. From these equations, the number of animals necessary for completion of GWAS can also be estimated by r2(m,q), which is the

conventional r2 defined measure of linkage disequilibrium, r2(q,g) is the proportion of genetic variance explained by the QTL, and r2(g,t), that is the heritability of the trait (Goddard and Hayes 2009). In an example and using several assumptions, Goddard and Hayes (2009) estimated the number of animals required by r2(m,q) = 0.50, r2(q,g) = 0.04, and r2(g,t) = 0.25; then r(t,m) = 0.07 and a standard error equal to 0.33; then expected number of animals required was 1,800 (Goddard and Hayes 2009). As most of the SNPs explain less than 4% of the genetic variance, more than 1,800 animals would be needed for GWAS (Visscher et al. 2008; Goddard and Hayes 2009). In the above example, the 1,800 animals represent both genotypes and phenotypes that must be measured. However, if used animals have been progeny tested (using the mean of their progeny instead of their own phenotypic value), the number of animals could be notably reduced (Goddard and Hayes 2009). The formula above still applies but now r2(g,t) is the reliability of the progeny test and *t* is the progeny mean (Goddard and Hayes 2009).

2.6.7 Results of Genome Wide Association Studies

2.6.7.1 Result for Single Traits

One of the biggest impacts of GWAS has been identifying genes for single traits. For instance, Charlier et al. (2008) reported three genes harboring mutations resulting in three recessive abnormalities (Table 8). The number of animals used in the study was low because calves suffering from a fatal recessive disorder are homozygous for a large chromosome segment containing the causative gene, allowing this segment to be detected

using moderately dense markers (Goddard and Hayes 2009). Larger numbers of animals are required for GWAS for complex traits, but the strategy to find genes for those traits is similar.

2.6.7.2 Results for Complex Traits with Emphasis on Fertility

Lately, several mutations have been found through GWAS for various complex traits. The characteristics that these traits have are represented by many SNPs significantly associated with small effects (explaining a low percentage of the variations; Goddard and Hayes 2009). In other words, complex traits have contribution of a large numbers of genes with small additive effects (Cole et al. 2009).

As illustrated in Table 9, various GWAS have found SNPs that affect complex traits. Emphasizing fertility in the Holstein breed, GWAS are listed according to the phenotypic traits used, breed, number of animal's genotyped, number of SNPs significantly associated, and authors.

2.6.8 Validation Studies

Once a SNP-trait association has been found in a discovery population, this association needs to be validated in a different population representing the population where the marker or test will be applied. Thus, validation studies from these SNP associations are very important and necessary. Goddard and Hayes (2009) suggested three reasons for these validation studies; first, the size of the effect of each association is small. The second

reason is the linkage disequilibrium between the SNP and the QTL may be present in the original sample of animals but not in other samples from a different breed or even from different families within the same breed. The final reason is the false discovery rate is high in these GWAS, meaning most of the significant associations are expected by chance when large numbers of SNPs are tested (Goddard and Hayes 2009). Furthermore, SNPs associations are more likely to be validated when GWAS use large numbers of animals, when only one breed is utilized, and when selected SNPs are highly significant. Also, GWAS are most likely to be validated when the analyses include appropriate protection from false discovery rate (Goddard and Hayes 2009).

In summary, the ideal design of a GWA study depends on the genetic architecture of complex traits (Goddard and Hayes 2009). As previously described, many polymorphisms of different types affect a typical complex trait and result in small effects having low minor allele frequency. Knowledge of this architecture is needed for GWAS involving complex traits. Thus, large numbers of individuals are required to have the necessary power to find the genes explaining most of the genetic variance (Goddard and Hayes 2009).

2.6.9 Marker Assisted Selection

Marker assisted selection (MAS) is a selection method utilizing results of DNA tests (discovered markers) to select parents for subsequent generations. Basically, this selection approach improves accuracy of the selection and increases the rate of genetic progress by including genotypic information provided by DNA tests. Thus, MAS improves accuracy

by identifying animals carrying desirable genetic variants (SNPs) for a given trait, even at the time of birth.

It is possible to categorize use of MAS in two possible alternatives. For the first alternative, once a causative mutation has been identified in a gene or regulatory region (i.e. mutations with major effects on the trait; single traits), the objective is to eliminate the abnormal allele from the population (Goddard and Hayes 2009). Some examples of this alternative are numerous recessive abnormalities such as bovine leukocyte adhesion deficiency in cattle (Shuster et al. 1992). Although the idea of this alternative is eliminating a potential abnormal allele from the population, it can also be used to increase the frequency of a specific allele such as introgressing the booroola gene from Merino sheep into Border Leicester sheep (Davis 2004).

The second alternative use of MAS is for SNPs that are in linkage disequilibrium with appropriate QTLs (Goddard and Hayes 2009). This approach estimates the effect of the significant marker or allele in a different population that is independent from the discovery population. Thus, using a combination of pedigree, marker and phenotype information breeding values for selection candidates can be estimated. This type of MAS has been applied to improve reproduction rate, feed intake, growth rate and body composition in various livestock species, meat quality in commercial lines of pigs, muscle development in sheep, and milk yield in dairy cattle (Dekkers 2004; Goddard and Hayes 2009). Despite great benefit of MAS, improvements in accuracy and genetic gain will depend on the amount of additive genetic variation accounted for by the markers. Thus, one of the key

criticisms of MAS is the limited ability to predict breeding values, since a low number of markers with validated associations will typically explain a small proportion of the genetic variance of the trait (Goddard and Hayes 2009; Van Eenennaam 2009).

2.6.10 Genomic Selection

A better approach to MAS, know as Genomic Selection, was reported by Meuwissen et al. (2001). They suggested using genome wide panels of dense markers which included all QTLs that are in linkage disequilibrium with at least one marker. This approach differs from MAS which concentrates on small number of QTLs that are tagged by markers with validated associations.

One advantage of this approach is that all genetic variance for a trait can be tracked by a marker panel. The marker effect does not need to reach a significant threshold to be used in predicting breeding values or phenotypes (Goddard and Hayes 2009). An additional advantage is that the effect of marker alleles can be estimated on a population basis rather than within each family (Goddard and Hayes 2009).

Thus, genomic selection refers to selection decisions based on molecular or genomic breeding values estimated by high density panels. For estimating genome breeding values for genomic selection, a large sample of animals needs to be measured (phenotype) for the trait and genotyped for markers. This population is usually referred to as a training or reference population (Figure 8; Goddard and Hayes 2009). The genotypes coming from high density panels, which can be represented by a variable (x), usually has values as 0 or

1 or 2 corresponding to the homozygotes, the heterozygote or the other homozygote, respectively (Goddard and Hayes 2009). The statistical analysis of the reference or training population estimates effects for each marker (w), and a prediction equation can be generated that combines all marker genotypes with their effects to predict the breeding value of each animal (Goddard and Hayes 2009). These predicted breeding values from marker genotypes (i.e. the genomic breeding value) take into account the effect of each marker simultaneously with the other markers (Goddard and Hayes 2009). This prediction equation can then be applied to a group of animals that have genotypes but not phenotypes, usually referred as selection candidates (Figure 8), and the estimated breeding values calculated can be used to select the best animals for breeding (Goddard and Hayes 2009).

Thus, the possibility to include traits that are difficult to record at a young age and the achievement of high accuracies for animals at birth has huge implications for animal breeding improvement. For instance, Schaeffer (2006) suggested that genomic selection of dairy bulls at 1 year of age could greatly reduce the generation interval and speed up rate of genetic improvement compared with the traditional selection strategy (progeny test), where bulls are 5 years old by the time they can be accurately evaluated on the basis of their daughters' milk yields. The simulation results suggest that accuracy of the GEBV for a bull calf can be as high as the accuracy of an EBV after a progeny test (König et al. 2009). Reliable GEBV for both sexes with accuracies greater than 0.70 can be calculated at an early stage of an animal's life (e.g., even for embryos). This implies a huge change from BLUP-animal models based on pedigree information toward SNP-based BLUP or other

models, but placing less weight on information provided by relatives. This change may generate an extreme reduction in generation intervals, thus increasing the annual economic genetic gain.

2.6.11 Accuracies in Genomic Selection

In the original introduction of the genomic selection concept performed by Meuwissen et al. (2001), the accuracy of the genomic breeding value was 0.85 with simulated data. The concept of accuracy for genomic selection differs from the traditional one. For genomic breeding values, accuracy indicates the correlation between the genomic breeding value and the true breeding value. Simulation on real data found less accuracy than the analysis performed by Meuwissen et al. (2001). For instance, Van Raden et al. (2009), using 3,576 bulls genotyped for 38,416 SNPs, indicated a correlation of 0.71 in American Holstein dairy bulls, averaged across a number of traits. These bulls were used as reference or training population and phenotypes for these bulls were the averages of their daughters' production records. Other authors such as Harris et al. (2008) and Hayes et al. (2008) have reported similar accuracies of genomic breeding value in Holstein and Jersey dairy cattle located in New Zealand and Australian. It is important to recall that the accuracy of the traditional breeding value based on its pedigree (based on the average of their parents' breeding value) is only 0.50 (Goddard and Hayes 2009).

2.6.12 Challenges for Genomic Selection

One of the major challenges for genomic selection includes assembling a large reference or training population to accurately estimate the SNPs effects (Goddard and Hayes 2009). Few projects have achieved appropriate levels of accuracy. One of these projects is led by the USDA, who used a reference population of more than 6,700 dairy bulls and reached levels of accuracy in their genomic breeding values of greater than 0.80 (Dalton 2009; Goddard and Hayes 2009). This project allowed some US breeding companies to market semen from young bulls with breeding values only estimated on the basis of their DNA and pedigree information, not from its progeny.

Another challenge for these genomic selection technologies is utilization in multiple breeds and /or species, primarily in beef cattle and sheep industries. Due to the limited extent of the linkage disequilibrium across breeds, genomic breeding values generated for a specific breed (reference population) cannot be applied to different breeds. It will be necessary to use genomic breeding values within breed (designated to the breed used as reference population), or larger multi-breed references population need to be created before genomic selection can be applied across the breeds (Goddard and Hayes 2009). Additionally, Goddard and Hayes (2009) indicated several unknowns in implementation of genomic selection, as frequency with marker effects need to be re-estimated and new markers need to be identified.

2.7 Appendix: Figures and Tables

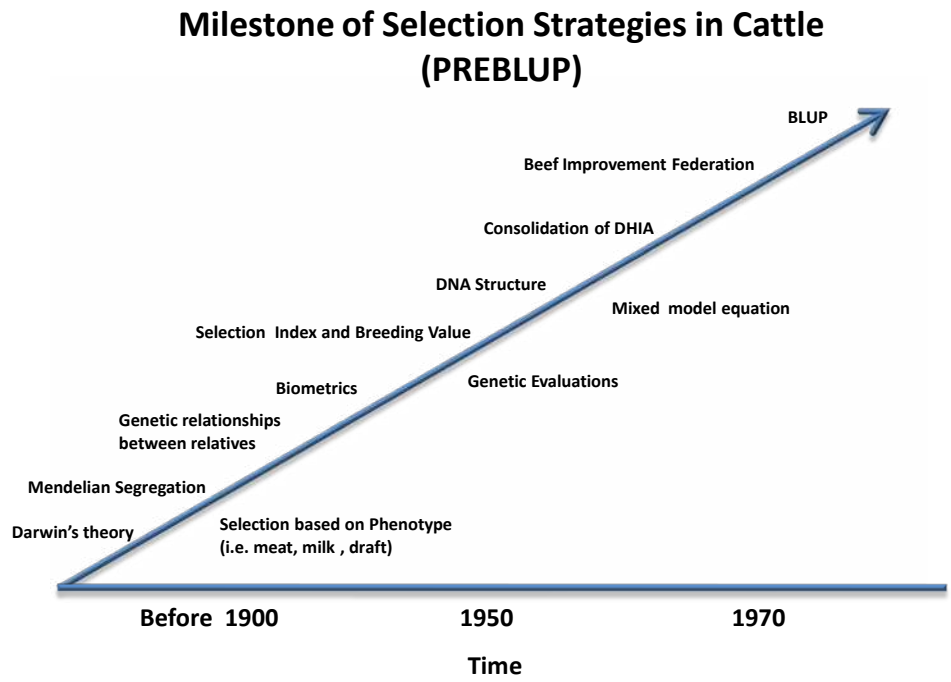
Milestone of Selection Strategies in Cattle
(PREBLUP)
BLUP
Beef Improvement Federation
Consolidation of DHIA
DNA Structure
Mixed model equation
Selection Index and Breeding Value
Genetic Evaluations
Biometrics
Genetic relationships
between relatives
Mendelian Segregation
Darwin's theory
Selection based on Phenotype
(i.e. meat, milk , draft)
Before 1900
1950
1970
Time

Figure 2.1.

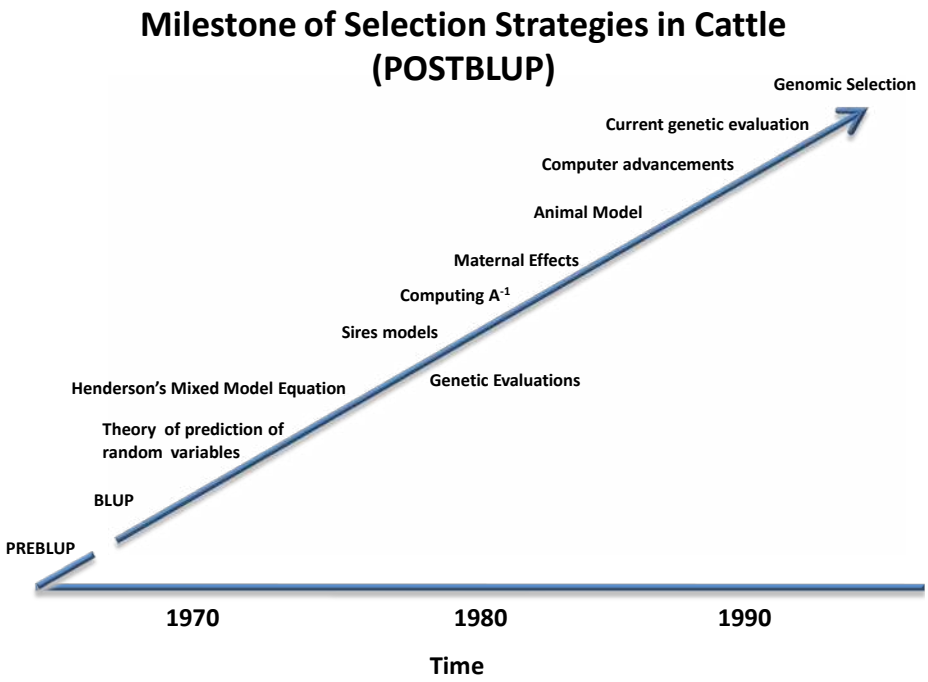
Milestone of Selection Strategies in Cattle
(POSTBLUP)
Genomic Selection
Current genetic evaluation
Computer advancements
Animal Model
Maternal Effects
Computing A-1
Sires models
Genetic Evaluations
Henderson's Mixed Model Equation
Theory of prediction of
random variables
BLUP
PREBLUP
1970
1980
1990
Time

Figure 2.2.

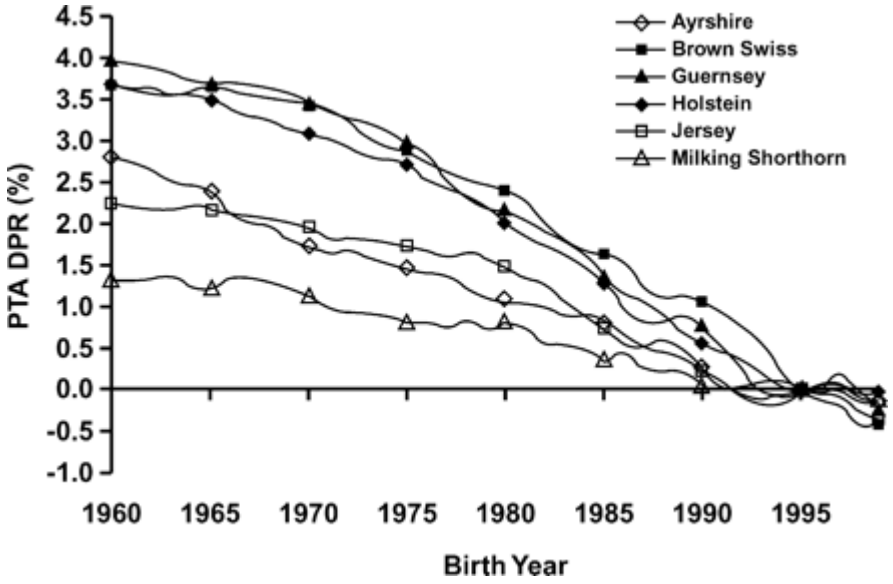

Figure 2.3. Trend in PTA daughter pregnancy rate (DPR) for bulls born from 1960 to 1999 by breed (VanRaden et al. 2004).

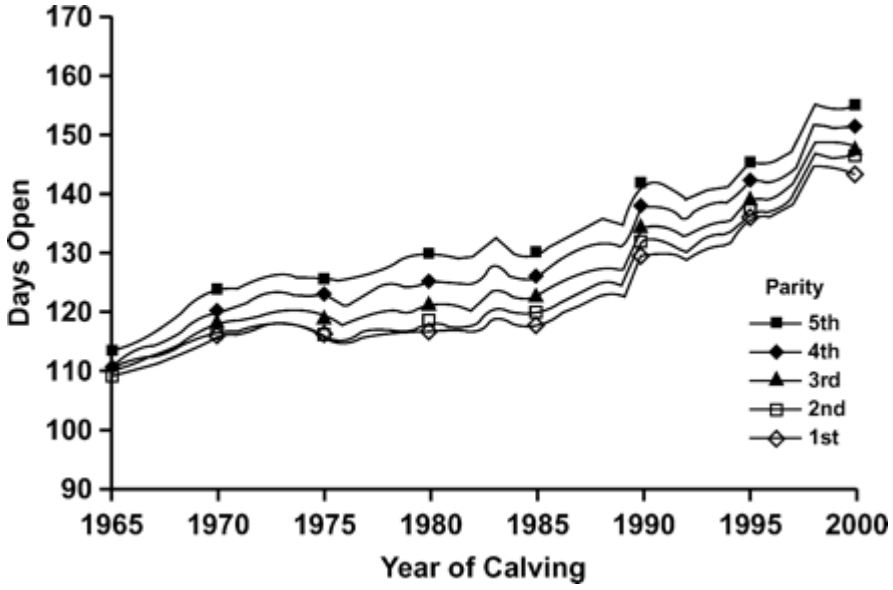

Figure 2.4. Phenotypic trend of days open for Holsteins by parity (VanRaden et al. 2004).

Table 2.1. Comparison between estimated relative conception rate (ERCR) and sire conception rate (SCR)

Category	**ERCR**	**SCR**
Trait evaluated	First service 70-day nonreturn rate	Conception rate
Breeds evaluated	Holstein, Jersey	Ayrshire, Brown Swiss, Guernsey, Holstein, Jersey, Milking Shorthorn
Lactation numbers included	1st through 6th; >6th	1st through 5th
Service numbers included	1st	1st through 7th
Bulls included	AI (active; inactive), <12 years old	AI (active) <13 years old
Minimum number of matings	≥300 first services	≥300 services in the last 4 years and ≥100 in the last year for Holsteins; somewhat fewer services for other breeds
Minimum number of herds	None	10 for Holsteins and Jerseys, somewhat fewer for other breeds
Fertility expression	Deviation from mean (nearest 1%)	Deviation from mean (nearest 0.1%)
Base assigned	Published bulls sum to 0	Published bulls sum to 0
Participating Dairy records processing centers	AgSource Cooperative Services, DRMS, Minnesota Dairy Herd Improvement Association	AgriTech Analytics, AgSource Cooperative Services, DRMS

(Norman, et al. 2008; AIPL research report scr1 (7-08), http://www.aipl.arsusda.gov/reference/arr-scr1.htm)

Table 2.2. Heritability (diagonal, in bold), genetic (above diagonal), and phenotypic (below diagonal) correlations among fertility traits[1] with standard errors[2] in parentheses (González-Recio and Alenda 2005).

	CI	DO	PR	IFL	DFS	INS	P56	P90	SF
CI	**0.04**	$0.99_{(0.01)}$	$-0.99_{(0.01)}$	$0.98_{(0.01)}$	$0.80_{(0.03)}$	$0.89_{(0.02)}$	$-0.95_{(0.002)}$	$-0.95_{(0.001)}$	$-0.59_{(0.01)}$
DO	0.91	**0.04**	$-0.99_{(0.01)}$	$0.99_{(0.01)}$	$0.82_{(0.03)}$	$0.94_{(0.01)}$	$-0.95_{(0.001)}$	$-0.93_{(0.02)}$	$-0.94_{(0.002)}$
PR	–0.91	–1.00	**0.04**	$-0.99_{(0.01)}$	$-0.82_{(0.03)}$	$-0.94_{(0.01)}$	$0.95_{(0.001)}$	$0.93_{(0.02)}$	$0.94_{(0.002)}$
IFL	0.79	0.88	–0.88	**0.03**	$0.50_{(0.05)}$	$0.91_{(0.02)}$	. . .	. . .	. . .
DFS	0.38	0.42	–0.42	–0.07	**0.05**	$0.11_{(0.06)}$	$-0.44_{(0.03)}$	$-0.18_{(0.02)}$	$-0.52_{(0.03)}$
INS	0.68	0.75	–0.75	0.87	–0.08	**0.02**	$-0.90_{(0.01)}$	$-0.54_{(0.03)}$	. . .
P56	–0.64	–0.75	0.75	–0.85	0.04	–0.75	**0.05**	$0.97_{(0.02)}$	$0.94_{(0.02)}$
P90	–0.59	–0.74	0.74	–0.84	0.03	–0.69	0.76	**0.06**	$0.92_{(0.02)}$
SF	–0.54	–0.61	0.61	–0.71	0.06	–0.76	0.63	0.48	**0.04**

[1]CI = Calving interval, DO = days open, PR = pregnancy rate, IFL = interval between first and last inseminations, DFS = days to first service, INS = number of inseminations per service period, P = pregnancy within 56 (P56) or 90 d (P90) after first insemination, SF = success in first insemination.

Table 2.3. Various reports estimating cost of extra days open.

Authors	**The cost per extra day open per cow per year** ***($, value at time of publication)***	**Average DO considered in the analysis (days)**
Speicher and Meadows (1967)	$0.78	beyond 117
Louca and Legates (1968)	$0.25 to $0.70	NR
lds et al. (1979)	$0.71-$1.18	40-140
Holmann et al. (1984)	$0.04 to $0.23	120
Dijkhuizen et al. (1985)	$1.16	165
Schmidt (1989)	$0.18 and $0.60	85-175
Plaizier et al. (1997)	$0.96, $2.72, and $4.56	85-145
Meadows et al. (2003)	$1.71	190
French and Nebel (2003)	$4.95	175
Meadows et al. (2005)	$1.37	160

Table 2.4. Example of inputs and values used in the general model (Meadows et al. 2005).

Input	Value
Herd characteristics	
Cull rate	34%/yr
Heifer calf survival to lactation	80%
Average age at first calving	26.6 mo
Services per conception for virgin heifers	2.12
Days to first service for cows	96
First-service conception rate for cows	45%
Heat detection rate for cows	45%
Services per conception for all cows	2.33
Average weight of cull cow	544 kg
Interest rate for alternative use of capital	8%/yr
Expenses	
Cost to raise heifers	$1.70/d
Cost of replacement heifer ready to calve	$1600
Feed cost for dry cow maintenance	$1.50/d
Feed cost (above maintenance) for milk	$0.169/kg
Semen cost	$15/service
Additional veterinary cost, $/d	Not used
Additional management cost, $/d	Not used
Revenues	
Salvage value of cull cow	$0.736/kg
Market price of milk	$0.306/kg
Heifer calf value	$200
Bull calf value	$50

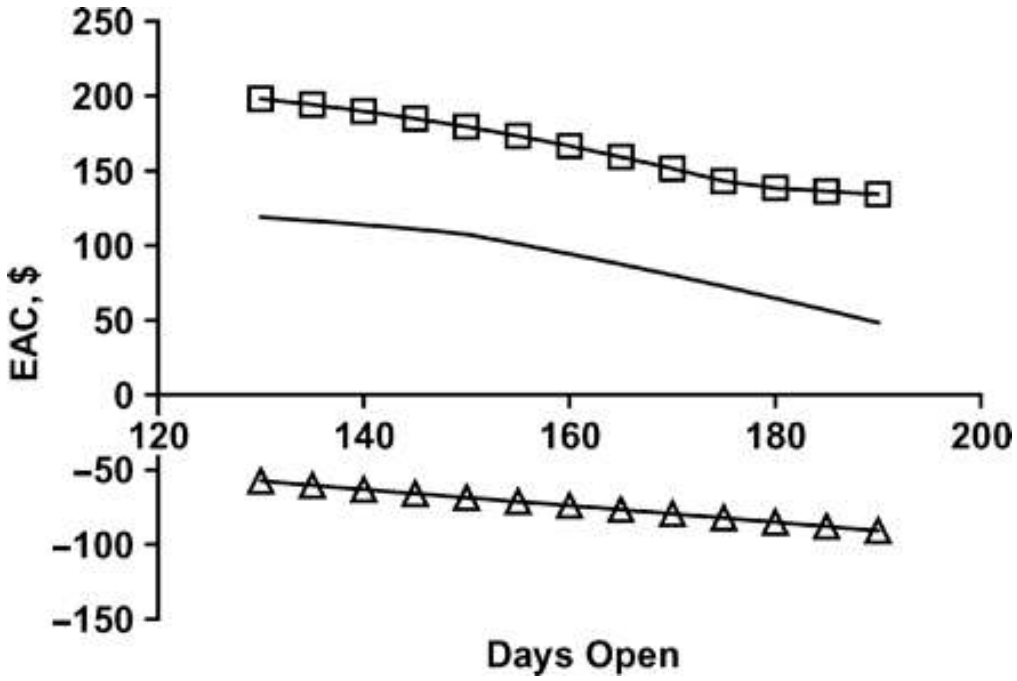

Figure 2.5. Effect of days open on equivalent annual cash flow (EAC) values estimated by the model for different annual culling rates: (□) 25% annual culling rate; (—) 34% annual culling rate, and (▩) 45% annual culling rate (Meadows et al. 2005).

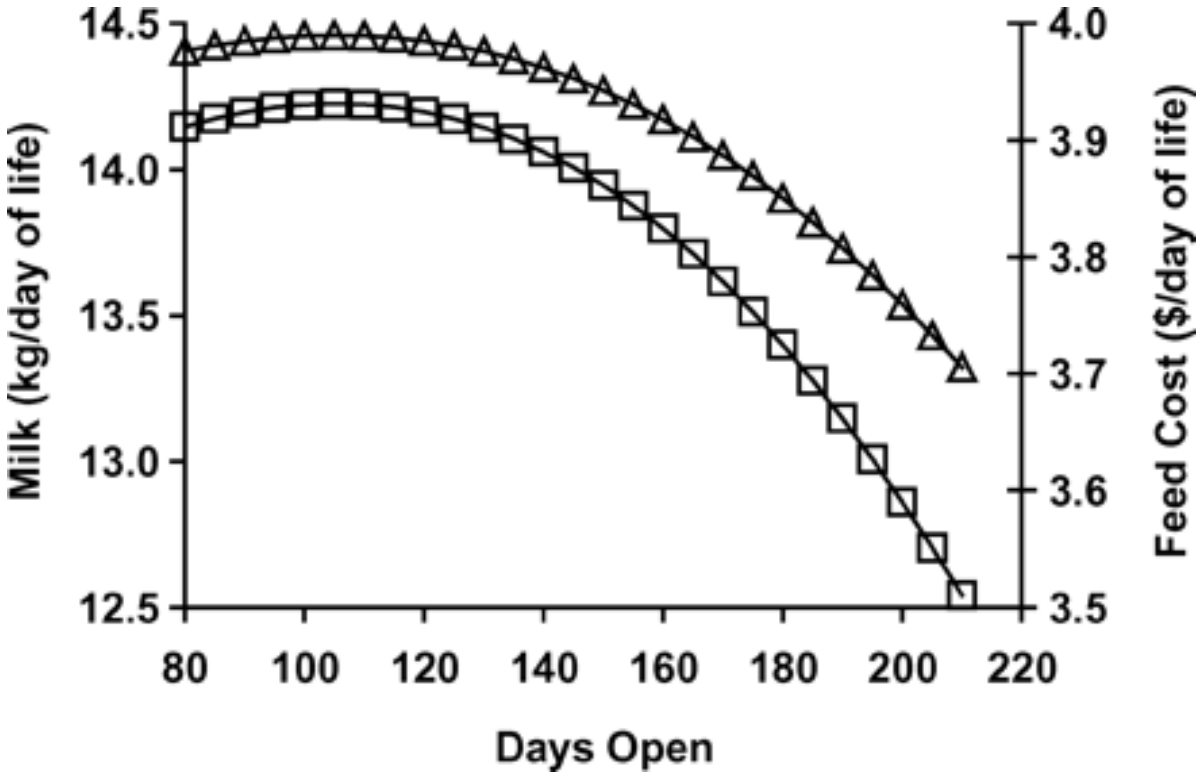

Figure 2.6. Effect of days open (DO) on milk yield (□), plotted on the left axis, and feed cost (▩), plotted on the right axis, per day of life (Meadows et al. 2005).

Table 2.5. Various reports estimating the value of a pregnancy.

Authors	**The value of a pregnancy**	**Evaluation of new pregnancy or pregnancy loss (abortion)**
Stevenson (2001)	$253 -$274	New pregnancy
Eicker and Fetrow (2003)	$200	New pregnancy
De Vries (2006)	$278	New pregnancy
Thurmond and Picanso, (1990)	$640	Pregnancy loss (abortion)
Eicker and Fetrow (2003)	$600 - $800	Pregnancy loss (abortion)
Pfeiffer et al. (1997)	$624	Pregnancy loss (abortion)
Peter (2003)	$600 - $1,000	Pregnancy loss (abortion)
Weersink et al. (2002)	$1,286	Pregnancy loss (abortion)
De Vries (2006)	$555	Pregnancy loss (abortion)

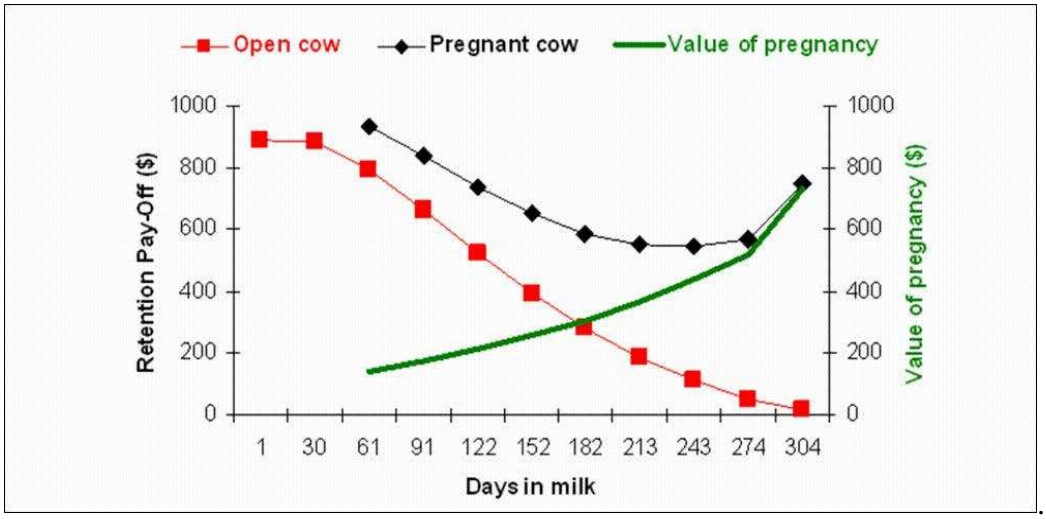

Figure 2.7. Retention payoffs (RPO) for a nonpregnant cow (○) and a cow that became pregnant on d 61 after calving (■) by day after calving. Cows are in their first lactation with average lactation curves. By definition the value of pregnancy is equal to the difference between the RPO of the pregnant and nonpregnant cow (De Vries 2006).

Table 2.6. Economic values and economic importance relative to protein for actual kilograms of milk, fat, and protein (PROT); productive life (THL); mature BW; age at first calving (AFC); number of inseminations per service period (INS); calving interval (CI); DIM; and dry period (DP; Gonzalez-Recio et al. 2004).

	Economic value	
	($/yr per cow)	**($/yr per cow)/ SD unit[1]**
Milk (kg)	$0.13	$0.95
Fat (kg)	$1.02	$0.30
PROT (kg)	$4.04	$1.00
THL (days)	$0.22	$0.35
BW (kg)	–$0.67	$0.10
AFC (d)	–$0.28	$0.08
INS	–$67.32	$0.24
CI (d)	–$4.90	$0.64[2]
DIM	$1.19	$0.22
DP (d)	–$4.90	$0.40

[1]Economic value per phenotypic standard deviation (SD) unit (from lactating cows from 1998 to 2001) relative to actual protein.

[2]Corresponding standard deviation was calculated considering 300 <CI <500. An economic value per SD of 49 and 89% would have been estimated considering 300 <CI <450 and 300 <CI <600, respectively

Table 2.7. List of companies and web site with associated genetic test.

COMPANIES	**DNA tests**
AgriGenomics, Inc	Tibial Hemimelia (TH), Pulmonary Hypoplasia with Anasarca (PHA), Idiopathic Epilepsy (IE), Arthrogryposis Multiplex (AM; or Curly Calf Syndrome analysis), Dilution (DL), Black/Red Coat Color (CC)
Biogenetic Services	BSE resistance, Johne's disease, Bovine Viral Diarrhea (BVD-PI), freemartin, leptin, meat quality, parentage, coat color,
GeneSeek	AM, BVD-PI, Seek-Black, Seek-Tender, identity tracking, parentage, coat color, 50 K SNP CHIP genotyping
Genetic Visions	Prolactin *(CMP),* BLAD, Citrullinemia, DUMPS, Kappa-Casein, Beta-lactoglobulin, Complex Vertebral Malformation (CVM), Calpain 316/530, Freemartin, Coat color,
Igenity	AM, Neuropathic Hydrocephalus (NH), IE, Osteopetrosis (OS), PHA, TH, BVD-PI, Igenity Profile Analysis (tenderness, marbling, quality grade, fat thickness, ribeye area, hot carcass weight, yield grade, heifer pregnancy rate, stayability, calving ease, weaning weight, docility, residual feed intake, and average daily gain), DoubleBLACK coat color, dilution (DL), horned-polled, identity tracking, parentage, Myostatin
MMI Genomics	AM, NH, OS, Parentage, Tru-Marbling™, Tru-Tenderness™, MMIG Homozygous Black, polled/horned
Pfizer Animal Genetics	AM, NH, OS, GeneSTAR® MVP™ (feed efficiency, marbling, tenderness), GeneSTAR® Elite Tender, GeneSTAR® Quality Grade, GeneSTAR® Tenderness 2, GeneSTAR® Feed Efficiency, GeneSTAR® BLACK, parentage, identity tracking
Quantum Genetics	Leptin
Repro Tec Inc.	Fertility Associated Antigen (FAA)
Veterinary Genetics Laboratory (UC Davis)	Parentage, freemartin, coat color, Dexter Cattle
Viagen	Breed identification (AnguSure™)

This list is provided, updated and maintained at the following web address http://animalscience.ucdavis.edu/animalbiotech/Biotechnology/MAS/index.htm (Van Eenennaam 2009).

Table 2.8. Genes with identified mutations affecting single traits cattle discovered by genome-wide association studies

	Population			Mapping		
Defect	**Breed**	**Cases**[a]	**Controls**[a]	**Chrom.**	**Interval**	**Gene**
Congenital muscular dystonia 1 (CMD1)	Belgian Blue	12 (81)	14 (2,000)	25	2.12 Mb	*ATPA2 A1*
Congenital muscular dystonia 2 (CMD2)	Belgian Blue	7 (21)	24 (2,000)	29	3.61 Mb	*SLC6A 5*
Ichthyosis fetalis (IF)	Chianina	3 (3)	9 (96)	2	11.78 Mb	*ABCA1 2*
Crooked tail syndrome (CTS)	Belgian Blue	8 (36)	14 (2,000)	19	2.42 Mb	–
Renal lipofuscinosis (RL)	Holstein Friesian Danish Red	6 (16) 6 (27)	24 (141) 14	17	0.87 Mb	–

[a]Numbers correspond to sample sizes used to perform the genome-wide scan, whereas the numbers in brackets correspond to the total number of samples available.

Adapted from Charlier et al. (2008)

Table 2.9. Various reported GWAS have found SNPs that affect complex traits in cattle.

Traits	Breed	# genotyped animals	# SNPs found	Authors
Conformation, mammary system, feet and legs, dairy strength overall rump, udder texture, median suspensory, foot angle, bone quality, stature, angularity, herd life, **daughter fertility**, milking speed, milking temperament, direct calving ease	Canadian Holstein Bulls	462	196	Kolbehdari et al. 2009
Somatic cell score, **daughter pregnancy** rate, type, stature, strength, body depth, dairy form, rump angle, thurl width, rear legs side view, rear legs rear view, foot angle, feet leg score, fore udder attachment, rear udder height, rear udder width, udder cleft, udder depth, front teat placement, teat length, Calving ease, productive life, **male fertility**	Holstein Bulls	6	8	Schnabel et al. 2005
milk, fat, and protein yields; fat and protein percentages; productive life; SCS**; daughter pregnancy rate**; sire and daughter calving ease; final score; stature; strength; body depth; dairy form; foot angle; rear legs (side and rear views); rump angle and width; fore udder; rear udder height; udder depth and cleft; front teat placement; teat length; and net merit	Holstein Bulls	5,360	3 (larger effects)	Cole et al. 2009

Table 2.9. Continued.

Traits	Breed	# genotyped animals	# SNPs found	Authors
305-d lactation milk, fat, and protein yield; somatic cell score; herd life; **interval of calving to first service**; and **age at first service.**	Holstein bulls	427	144	Daetwyler et al. 2008
Feed intake (residual food intake; RFI); daily gain and body-weight measurements	Angus, Brahman, Belmont Red, Hereford, Murray Grey, Santa Gertrudis, and Shorthorn	1472 animals	161	Barendse et al. 2007
Conception rate	Holstein bulls	20	97	Feugang et al. 2009
Fertilization rate and blastocyst rate	Holstein cows	233 cows and 34 bulls	27	Huang et al. 2010
Twinning rate	Holstein Bulls	200	13	Kim et al. 2009
Non return rate	Holstein bulls	926	N/A	Druet et al. 2008
Milk yield	Holstein and Jersey bulls	1615	23	Hayes et al. 2009

Table 2.9. Continued.

Traits	Breed	# genotyped animals	# SNPs found	Authors
Number of inseminations; 56 day non-return rate; interval from first to last insemination; interval from calving to first insemination; veterinary treatments of reproductive disorders; fertility index	Danish and Swedish Holstein bulls	2531	74	Sahana et al. 2010
Milk, fat, and protein yields, fat and protein concentration (as a percentage) in milk, and **female fertility**	Holstein bulls	780	1573	Pryce et al. 2010

Figure 2.8

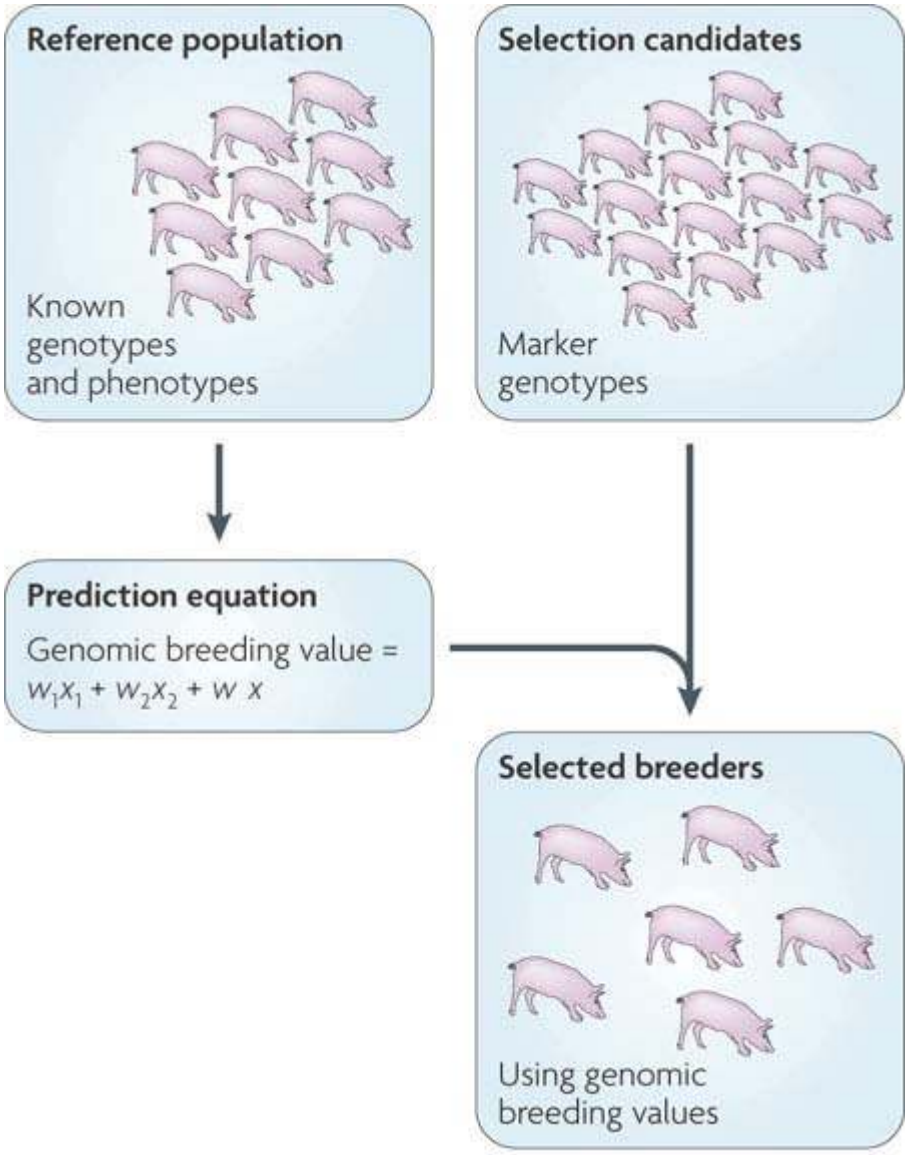

Figure 2.8. Diagram illustrating how prediction equations are used to generate genomic breeding values (Goddard and Hayes 2009).

CHAPTER 3 VALUES OF EXPECTED PROGENY DIFFERENCE AND HERITABILITY FOR EMBRYO TRANSFER TRAITS

7. Abstract

Genetic selection has made tremendous progress on economically important traits in the beef industry. Most of the progress has been from quantitative genetics through use of expected progeny differences (EPD). These values allow prediction of differences in progeny of a sire compared to progeny of other sires. Development of EPD for male and female reproductive traits has largely been ignored because of low heritability of reproductive traits, even though reproduction plays a vital role in the economics of beef operations. Therefore, continued research in the area of genetic selection for fertility is becoming increasingly important. Critical limiting factors for animal breeding programs using MOET nucleus schemes include variability in superovulatory response of donor animals and resulting pregnancy of transferred embryos. Thus, the overall objective of this research was to develop genetic parameters associated with MOET to assist producers in identifying animals with greater genetic merit for these protocols. Records were examined from a large-scale MOET system in beef cattle that contained data only for cows in which at least one transferable embryo was obtained. Data on these animals were extracted and analyzed on 10 425 transferred embryos (2900 collections) from 611 donor animals (Angus, Brangus, and Charolais) utilizing semen from 215 bulls. Phenotypic traits examined included pregnancy status of the recipient following transfer (ET-preg; determined by rectal palpation at 60 days post-transfer and/or confirmed calving date of recipient), number of transferable embryos per collection (ET-trans), and number of unfertilized ova at collection (ET-UFO). Basic statistical analysis and pedigree/trait files

were developed using procedures in SAS (SAS Institute, Cary, NC). Genetic parameters were estimated for a single-trait animal model using restricted maximum likelihood (REML) procedures in Wombat (Meyer 2007). Wombat also computed EPD and standard errors for each trait evaluated. The model included fixed effects of year as well as random animal and residual effects. The EPD for ET-preg ranged from –6.1 to 4.4% (SE = 2.2 to 4.2) for semen sires (sires of the transferred embryos) and –5.3 to 3.8% (SE = 3.2 to 4.2) for donor animals. Additionally, the heritability estimated for ET-preg was 0.03. Heritability estimated for ET-trans was 0.00, indicating minute genetic variation and thus, EPD were not presented. Heritability estimated for ET-UFO was 0.05 with EPD values (deviation of the number of UFO from the mean) ranging from –0.6 to 0.8 (SE = 0.3 to 0.6) for semen sires and –0.4 to 1.1 (SE = 0.5 to 0.6) for donor cows. As previously shown for reproductive traits, heritability of ET-preg, ET-trans, and ET-UFO was low. Genetic improvement in fertility by selection on embryo transfer traits is possible, but progress would be slow. Further studies are underway on a larger dataset to refine these estimates and to examine repeatability.

3.2. Introduction

The use of statistical parameters for genetic improvement of multiple ovulation/embryo transfer (MOET) technology in cattle, primarily through quantitative genetics and selective breeding, is an area of rapidly developing research. Although significant genetic variation for fertility is generally accepted, development of breeding values for fertility traits has mostly been ignored due to low heritability and unfavorable genetic correlation with production characteristics (Olds et al. 1979; Berger et al. 1981; Clay and McDaniel 2001; Lucy 2001; VanRaden et al. 2004; De Vries and Risco 2005; Melendez and Pinedo 2007). Fertility traits are the most economically important traits in the livestock industry. However, fertility has declined over the past decades, especially in the dairy industry, due to the steady increase in milk production and the associated increased metabolic demands. Since it is assumed that declining fertility cannot be arrested by improving management alone, elevating fertility in cattle by means of genetic (genomic) selection will become increasingly important. Genetic gain of fertility traits may be accelerated by combining genomic selection with advanced reproductive technologies to increase genetic contributions of superior animals.

Multiple ovulation/embryo transfer (MOET) technology and artificial insemination (AI) have had a major impact on livestock breeding over the past several decades (Mapletoft and Hasler 2005). MOET technology rapidly increases genetic progress, reduces risk of disease transmission, eliminates cost/difficulty of animal transport, and expands number of progeny that can result from genetically superior parents. However, critical limitations of

animal breeding programs using MOET include variability in superovulatory response of donor animals and pregnancy success from transferred embryos (Mapletoft et al. 2002). These unpredictable responses create tremendous logistical problems that reduce availability of embryos and profitability. Therefore, to mitigate the variability in superovulatory response of donor animals and resulting pregnancy of transferred embryos, continued research in the area of genetic selection for fertility is becoming increasingly important. Thus, the overall objective of this research was to develop genetic parameters associated with MOET to assist producers in identifying animals with greater genetic merit for these protocols.

3.3. Materials and methods

Records were examined from a large scale MOET system in beef cattle which contained data only for cows in which at least one transferable embryo was obtained. Data on these animals were extracted and analyzed on 10,425 transferred embryos (2,900 collections) from 611 donor animals (Angus, Brangus, and Charolais) utilizing semen from 215 bulls. Standard superovulation procedures included FSH injected twice daily for 4 days in decreasing doses. Donor animals were artificially inseminated 2-4 times at estrus, 12, and 20 hours later. Phenotypic traits examined included pregnancy status of the recipient following transfer (ET-Preg; determined by rectal palpation at 60 days post transfer and/or confirmed calving date of recipient), number of transferable embryos per collection (ET-Trans) and number of unfertilized ova at collection (ET-UFO). Basic statistical analysis

and pedigree/trait files were developed using procedures in SAS. Genetic parameters were estimated for a single-trait animal model using Restricted Maximum Likelihood (REML) procedures in Wombat (Meyer 2007). Wombat also computed EPDs, standard errors and heritability for each trait evaluated. The model included fixed effects of year as well as random animal and residual effects.

The statistical model was:

$$Y\ (PREG) = \mu + year + Animal\ effect + e$$

where:

$Y\ (PREG)$ was the trait of interest in this example pregnancy rate after embryo transfer;

$year$ was year of the embryo transfer;

$Animal\ effect$ was the random genetic effect;

e was the residual error term.

Heritability was estimated using the variance components by the following formula:

$$h^2 = \frac{\sigma_a^2}{\sigma_p^2}$$

Where:

h^2 = heritability;

σ_a^2= genetic variance component; and

σ_p^2= phenotypic variance component (Tonhati et al. 1999).

Following the rationale of Falconer and Mackay (1996) and Saxton (2004), rate of genetic gain was estimated using

$$\Delta G = i * h^2 * \sigma_p \div L$$

where the expected change in the mean of a trait per unit of time is a function of:

i= selection intensity;

h^2= heritability;

σ_p= phenotypic standard deviation; and

L= the length of a generation interval.

3.4. Results

Phenotypic means and descriptive statistics are summarized in Table 1. Results for EPD values and standard errors (SEM) for ET-Preg, ET-Trans and ET-UFO, for sires and donors cows respectively, as well as heritabilities are introduced in Table 2. Expected predicted distribution for sire pregnancy rate (ET-Preg), donor pregnancy rate (ET-Preg) and sire unfertilized oocytes (UFO) are illustrated in Figures 1, 2, and 3, respectively. Moreover, the heritability estimated for number of transferable embryos (ET-Trans) was 0.00, indicating minute genetic variation and thus, EPD distribution and maximum and minimum values are not presented. A summary of the genetic parameter estimated for the different embryo transfer traits is given in Table 2.

As previously mentioned for reproductive traits, heritability of ET-preg, ET-trans, and ET-UFO was low. Given the predicted breeding values determined by BLUP and expressed in this manuscript as EPD (expected progeny difference), genetic improvement would be accomplished for breeders by selecting superior animals as parents based on those predictions. For any trait, estimating rate of genetic gain depends upon three factors: heritability (h2), selection differential and generation interval. Selection differential is determined by selection intensity (i = standardized mean deviation of selected parents) and phenotypic variation (SD) present in the population. Utilizing the above methodology, we estimated a genetic gain of 1.16 % per generation in pregnancy rate (PR) following embryo transfer assuming 50% selection for PR, estimated by the formula (i=0.8)*(SD=49.962)*(h2=0.029).

3.6. Discussion

Estimates of genetic parameters for MOET traits in Angus, Brangus, and Charolais breeds utilizing relatively large datasets are restricted to a few publications. Veerkamp and Beerda (2007) indicated that low heritability of MOET traits is a consequence of large environmental variation, and does not necessarily indicate a lack of genetic variation. Generally, studies have reported heritability for number of transferable embryos ranging from 0.01 to 0.59 (Liboriussen et al. 1998; Tonhati et al. 1999; Peixoto et al. 2004; König et al. 2007). The calculated heritability in this study for number of transferable embryos was much lower (0.00) compared with values reported previously. Although not an

uncommon value of heritability (very close to zero) for fertility traits (Khun et al. 2008), differences with the reported studies could be explained by the variation in the experimental population used. For instance, Liboriussen et al. (1998), Tonhati et al. (1999) and König et al. (2007) used Holstein data for the MOET genetic parameter estimation; whereas, Peixoto et al. (2004) utilized Nellore data for the analysis. As previously mentioned, this study estimated genetic parameters from records of Angus, Brangus, and Charolais cattle. Additionally, variation in the genetics models, number of records utilized (collections, donors, and sires) and rules for validating MOET data could explain the differences. Similar to the genetic evaluation of sire conception rate (Khun et al. 2008), the number of transferable embryos estimated in the present research is a phenotypic prediction (not a genetic evaluation) of the bull's or donor's number of transferable embryos. Variation in phenotypic values is large enough to suggest that culling of low performing cows or sires should result in higher performance in the remaining herd.

Although statistical models and heritability values for transferable embryos differ from previous reports, heritability values for number of unfertilized ova and pregnancy rate were in the same range and consistent with data previously reported in the literature. To date, just one research study has reported direct heritability for recipients to establish pregnancy after transfer of viable embryos (König et al. 2007) in Holstein cattle. Thus, the present work is the first reported heritability value for pregnancy status of recipients following embryo transfer in beef breeds. Despite differences models and breeds (Holstein vs Angus,

Brangus, and Charolais), heritability values for pregnancy status of the recipient following embryo transfer procedure were very similar between values from the present work (0.049) and from König et al. (2007; 0.056). Additionally, König et al. (2007) indicated similar results when comparing heritability results for pregnancy status of the recipient with the general range for nonreturn rates in dairy cattle reported in the literature (Jamrozik et al. 2005; Di Croce et al. 2009). Therefore, pre-selection of recipients according to a recipient's fertility status must be taken into account to improve efficiency of MOET schemes.

3.6. Conclusions

Multiple ovulation embryo transfer schemes have great potential for enabling rapid genetic change by increasing reproductive capacity of animals. However, growth of this technology has become limited by high variability in donor response as well as the high percentage of donors which do not produce any progeny. According to the current study, genetic selection of donors or sires appears to be a potential approach to improve efficiency of MOET procedures. Although low heritability would slow genetic progress, results shown in this work suggest that genetic improvement in fertility by selection for embryo transfer traits is possible. Moreover, genetic gain estimate was 1.16% per generation suggesting a useful tool for genetic improvement and the feasibility of including MOET traits in future breeding strategies. However, further studies to estimate

genetic parameters would give more reliable estimates and allow more accurate assessment of the possibility for genetic improvement.

Additionally, the use of molecular genetics in parameter estimation may aid in identifying individuals that improve efficiency of MOET traits. Schaffer (2006) reported that a genome-wide selection scheme using genome-wide estimated breeding value could produce a genetic change two times greater than current progeny testing schemes. However, to apply this scheme, it will be necessary to identify informative markers (SNPs), especially in the case of MOET and fertility. Further studies for identifying markers for MOET traits, along with available technology (e.g., Bovine SNP Chip), may create an even more effective approach for improving efficiency of MOET schemes and overall fertility of the livestock industry.

8. Appendix: Figures and Tables

Table 3.1. Descriptive statistics of analyzed traits of embryo donor and recipient cows including phenotypic means, standard deviations, minimum and maximum for all traits.

Traits	Mean	Standard Deviation	Minimum	Maximum
Pregnancy (%)	52.005	49.962	0	100
Transferable (*n*)	7.243	7.214	0	63
UFO (*n*)	2.516	5.192	0	55

Pregnancy (%) = calving rate of fresh and frozen embryos**; Transferable (n)** = number of transferable embryos per collection (Grade 1 to 3 embryos, IETS classification); **UFO (n)** = number of unfertilized oocytes per collection.

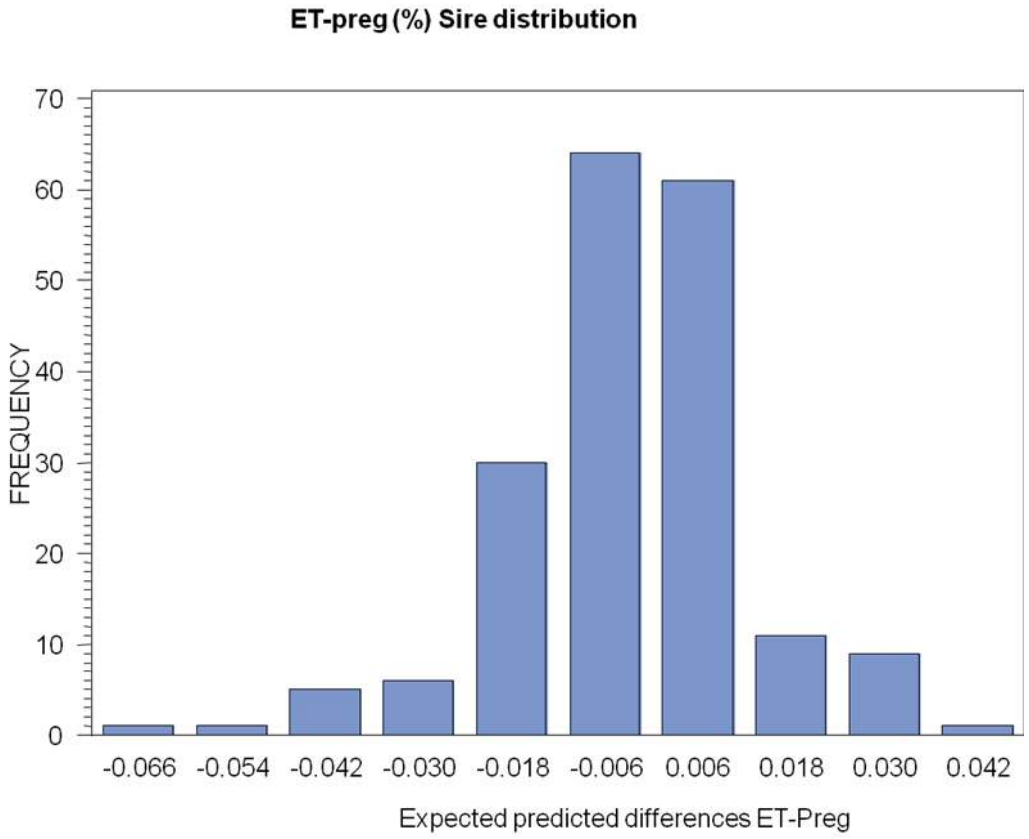

Figure 3.1. Expected predicted differences distribution for pregnancy rate trait for sire

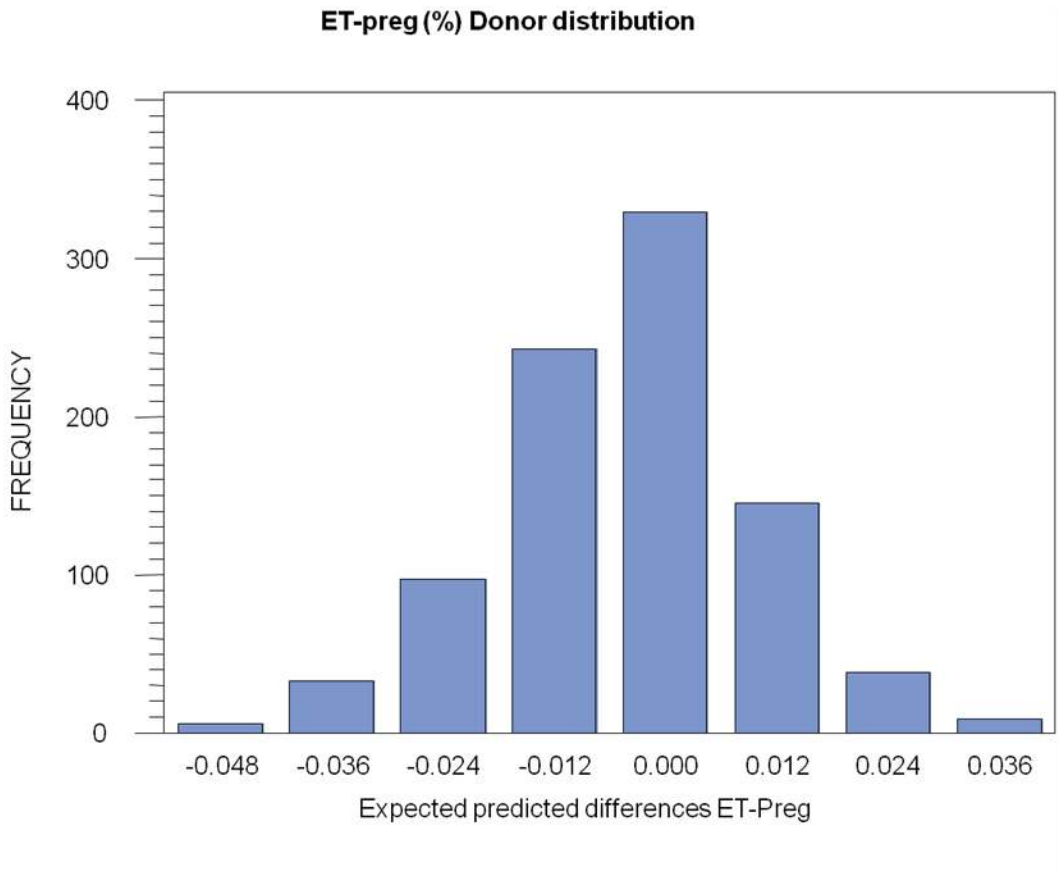

Figure 3.2. Expected predicted differences distribution for pregnancy rate trait for donor cows

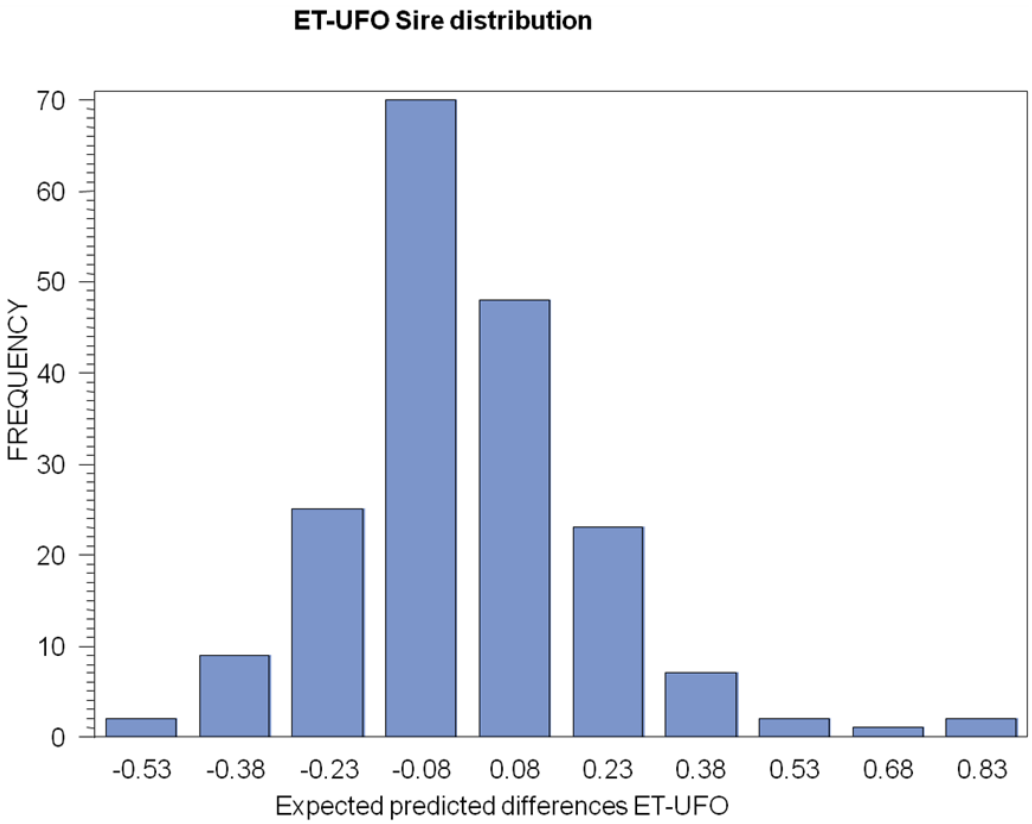

Figure 3.3. Expected predicted differences distribution for unfertilized oocytes (UFO) trait for sire

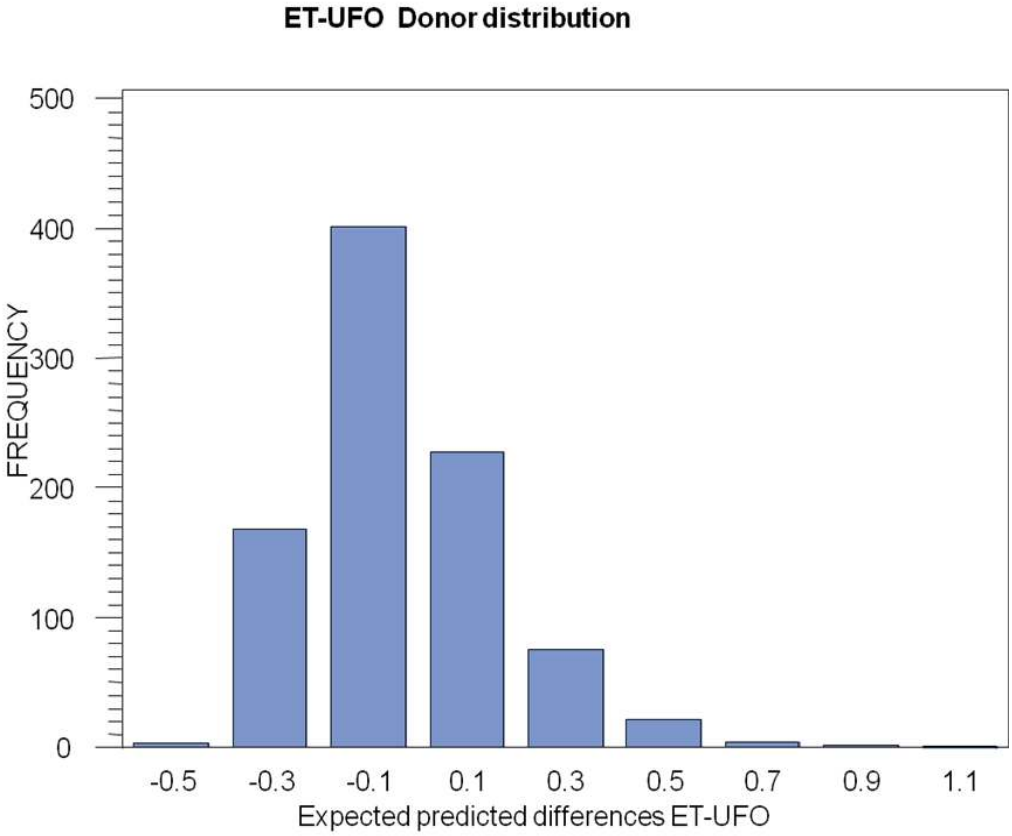

Figure 3.4. Predicted progeny difference distribution for unfertilized oocytes (UFO) trait for donor cows

Table 3.2. Maximum and minimum EPD values and standard errors (SEM) for ET-Preg, ET-Trans and ET-UFO, for sires and donors cows respectively, as well as heritabilities.

Trait	EPD Minimum	EPD Maximum	SEM Minimum	SEM Maximum	h^2
ET-Preg (%) Sire	**-6.1**	**4.4**	**0.022**	**0.042**	**0.029**
ET-Preg (%) Donor	**-5.3**	**3.8**	**0.032**	**0.042**	
ET-Trans (*n*) Sire	**-0.000**	**0.000**	**0.001**	**0.001**	**0.000**
ET-Trans (*n*) Donor	**-0.000**	**0.000**	**0.001**	**0.001**	
ET-UFO (*n*) Sire	**-0.564**	**0.801**	**0.342**	**0.570**	**0.049**
ET-UFO (*n*) Donor	**-0.420**	**1.053**	**0.447**	**0.570**	

CHAPTER 4 GENETIC APPROACH TO IMPROVE FERTILITY IN CATTLE

4.1. Abstract

In spite of the economic importance of fertility and increased knowledge of reproductive biology in the cow, efficiency of reproductive performance has continued to decline in dairy cattle. Improvements in fertility through genetic selection may be a possible approach to increase reproductive efficiency. While progress may be slow due to low heritability of fertility traits, ignoring fertility in genetic improvement programs has contributed to the current reproductive inefficiency. The objectives of this study were to 1) estimate genetic parameters and breeding values for fertility traits based on data from milk recording programs in a Holstein population, located in Argentina and 2) develop a multi-trait selection index (dollar fertility; $F) based on estimated breeding values. Data representing 3,282,843 lactations and 1,622,088 animals (cows and bulls from 1936 to 2007) were used for analysis and obtained from official records maintained by the Argentinean Holstein Association (ACHA). Data were collected from official milk records, and lactations were standardized to 305 DIM for milk, protein and fat. Gestation period was considered to be 282 days and restrictions were applied to ensure the quality of data (days open <40 and >350 days; calving interval <300 and >600 days; and contemporary groups with <25 lactations were eliminated). Days open (DO), calving interval (CI), age to first calving (AFC), and daughter pregnancy rate (DPR) were traits chosen for genetic parameter estimation. Daughter pregnancy rate was calculated from DO as PR = 0.25 x (233 – DO; Kuhn et al. 2004). Animal models by lactation for each fertility trait included contemporary group (dairy herd and calving year), calving month, animal effect, milk

production as a covariate and error term. Multiple Trait Derivative-Free Restricted Maximum Likelihood (MTDFREML) was used to estimate covariance components (Boldman et al. 1995). Solutions for fixed effects, breeding values, and sampling variances (accuracies) were obtained for each trait. The proposed selection index (dollar fertility; $F) included AFC and CI with economic weights from $-0.28 and -4.90/day per cow for AFC and CI, respectively (Gonzalez-Recio et al. 2004). Heritability for DO and DPR ranged from 2% to 7%; 3% to 8% for CI; and 16% for AFC. Predicted transmitting ability (PTA) values across different lactations ranged from -16.3 to 11.4 days, -24.7 to 15.1 days, -2.4 to 4.0% and -120.3 to 76.2 days for DO, CI, DPR and AFC, respectively. Values for $F ranged from -$76.6 to $139.4 in the current Holstein population. Results indicate substantial variation in fertility traits, suggesting that genetic selection may be effective in improving declines in fertility.

4.2. Introduction

Impaired fertility is the primary reason for culling dairy cattle in United States (US; 26.5% of all disposals; USDA 2007; Norman et al. 2007). Even with improved advances in reproductive technologies (ART), reproductive efficiency in dairy cattle has steadily declined in the US (Lucy 2001; VanRaden 2004; Hare et al. 2006; Norman et al. 2009) and other dairy countries (Royal et al. 2000; 2002; Evans et al. 2006) during the past 30 years. Conception rates have been reported to be declining by 0.45 to 1.0% per year (Beam and Butler 1999; Royal et al. 2000; 2002; Evans et al. 2006). A complex list of factors that

impacts reproduction includes management, nutrition, diseases, milk production, genetics, lameness, and environmental stress among others.

Many studies (Olds et al. 1979; Berger et al. 1981; Clay and McDaniel 2001; Lucy 2001; VanRaden et al. 2004; De Vries and Risco 2005; Melendez and Pinedo 2007) have consistently reported a negative association between fertility and milk production in dairy cattle. The Animal Improvement Programs laboratory (2009) reported that the relationship between milk production and reproductive performance appears to explain most of the decline in fertility, since genetic merit for milk has increased by 120 kg/year. However, a few studies have not observed the negative correlation between milk yield and fertility and/or reported a positive association between both traits (Rothschild et al. 1981; Hansen et al. 1983; Hillers et al. 1984).

Fertility directly impacts revenues associated with milk production and offspring born. Decreased or delayed reproductive efficiency reduces the percentage of cows in their peak production period, which decreases herd milk production (Norman et al. 2009). Moreover, low reproductive performance increases involuntary culling (USDA 2007) and elevates costs associated with multiple inseminations, veterinarian expense (pharmaceuticals for estrus synchronization, pregnancy diagnosis, etc), and replacement animals.

Hence, to mitigate the economic impact and the high prevalence/incidence of infertility, a strong need exists to identify and select animals according to their reproductive potential (daughter pregnancy rate, calving interval, etc). Improvements in fertility through genetic selection may be a possible approach to increase reproductive efficiency in dairy cattle.

While progress may be slow due to low heritability of fertility traits, ignoring fertility in genetic improvement programs has contributed to the current reproductive problems. The objectives of this study were to 1) estimate genetic parameters and breeding values for fertility traits based on data from milk recording programs in a Holstein populations located in Argentina 2) develop a multi-trait selection index (dollar fertility; $F) based on estimated breeding values.

4.3. Material and Methods

4.3.1 Data

Data representing 3,282,843 lactations and 1,622,088 animals (cows and bulls from 1936 to 2007) were obtained from official records maintained by the Argentinean Holstein Association (ACHA; http://www.acha.org.ar/). Pedigree information (genealogy) included animals (bulls and cows), sires and dams for these animals, calving year, sex and origin. Lactation data included entity of official milking test, owner, dairy, birth month and year of the cows, calving month and year of the cow, calving age, lactation number, days in milk, days open considering last and current calving, calving interval, and milk, protein and fat standardized to 305 days on milk.

4.3.2 Description of herd management and dairy industry

Milk production in Argentina is from 15,520 dairy farms with a total of 3,510,318 head of which 1,495,551 are milking cows (Castignani et al. 2008). Argentina's dairy industry is

concentrated in the central and east-central parts of the country (Buenos Aires, Santa Fe and Córdoba provinces). The states/provinces with the highest number of dairy farms includes Santa Fe with 4,020 dairies and 1,012,356 dairy cows, Córdoba with 3,835 dairies and 1,247,729 dairy cows, and Buenos Aires with 3,117 dairies and 900,968 dairy cows. Mean size of dairy farms and herds is 271 hectares and 157 head, respectively (Castignani et al. 2005).

Argentina ranks 12th among countries in milk production, with a production of nearly 9.5 million tons, and registered growth of 8% between 1995 and 2005 (Meirelles de Souza Filho et al. 2008). Dairy pastures compete with soybeans, corn, and wheat production for land. Many dairy farms are diversified operations devoting between 10% and 50% of their land to crop production. Alfalfa, tall fescue, rye grass and clover are typically used as dairy pastures and are included in rotational systems to preserve soil fertility. In general terms, dairy farms in Argentina are pasture-based operations, and cows are not confined when lactating. Producers supplement cattle diets with corn silage and some grain concentrates. Typically, diet composition across the year includes 67% grazing pastures, 22% concentrate, and 11% of silage (Zehnder and Gambuzzi et al. 2003).

In each province, production practices range from the least capital intensive (100 percent pasture) to the most capital intensive (100 percent confinement) with a shift from pasture to confinement occurring with increase in herd size. Most small and medium dairy farms in Argentina use pastures extensively, with animal confinement limited to twice a day during milking. Supplemental feeding tends to increase with size of the operation, ranging from

600 kg/head per year on small farms to 2,500 kg/head per year on larger operations in the Buenos Aires province. The primary dairy cattle breed in Argentina is Holstein, and milk output per animal averages 7099 kg of milk adjusted to 305 days in milk (Casanova et al. 2007). The genetic source (semen) of the Holstein population comes primarily from United States (59%), Argentina (24%) and Canada (13%; Casanova et al. 2007).

The official milking test is available since 1981 and lead by the Argentinean Holstein Association, which is a member of the International Committee for Animal Recording (ICAR). The current milking test description is shown in Table 1.

Table 1. Official milking test in February 2010

Entities by region	Owners	Dairies	Totals cows	Milking cows	Milk in liters
89	**1,543**	**2,050**	**512,602**	**343,150**	**6,992,563**

(http://www.acha.org.ar/)

4.3.3 Traits definition and rules for validations

Days open (DO), calving interval (CI), age to first calving (AFC), and daughter pregnancy rate (DPR) were traits selected for genetic parameter estimation. Days open was considered the period between calving and conception in cows and was estimated by subtracting 282 days from the total days of calving interval. Calving interval included the

time between one calving and the next. Age to first calving was defined as the time from birth to calving for the first time. Data were collected from official milk records, and lactations were standardized to 305 DIM for milk, protein and fat. Records with <305 DIM were adjusted as fixed effects by four fixed periods. Gestation period was considered to be 282 days and restrictions and rules for validations were applied to ensure quality of data. For instance, records with days open less than 40 days or longer than 350 days were eliminated. Calving interval between 300 and 600 days were considered acceptable (Gonzalez-Recio and Allenda 2005). Similarly, appropriate contemporary groups (CG) were defined base on the structure of the population to remove variation due to changes in herd environmental conditions over time, and CG with <25 lactations were eliminated. This restriction differs from Gonzalez Recio et al. (2004) where only 5 records were required for inclusion in the statistical model.

4.3.4 Daughter Pregnancy Rate

Daughter pregnancy rate (DPR) measures how quickly cows become pregnant after calving (VanRaden et al. 2004). The DPR measures the percentage of eligible cows becoming pregnant within each 21-d opportunity period. The DPR by definition is calculated as *"the number of cows that became pregnant during a given 21-day period divided by the number of cows that were eligible for breeding at the beginning of the period"* (VanRaden et al. 2004; Weigel 2006). These groups are integrated by cows that are not yet pregnant and have completed the voluntary waiting period (VanRaden et al.

2004). In other words, the pregnancy rate for an individual daughter indicates the number of 21-day opportunity periods required to achieve pregnancy (Weigel 2006). In recent years, many reproductive specialists have recommended this measure of reproductive success over the more traditional measure of days open. Furthermore, calculations of pregnancy rate are more current, cows that do not become pregnant are included in calculations more easily, and larger rather than smaller values are desirable; thus, simplifying selection by producers.

VanRaden et al. (2004) defined pregnancy rate as:

$$Pregnancy\ rate = 21/(days\ open - voluntary\ waiting\ period + 11).$$

The voluntary waiting period, the initial phase of lactation during which no inseminations occur, may vary across herds or seasons but would not affect genetic evaluations unless it differed for cows within the same herd-year-season (VanRaden et al. 2004). The constant factor of 11 centers the measure of possible conception within each 21-d time period such that cows conceiving during the first 21-d period receive 100% credit on average and so on. As an example (assuming a voluntary waiting period of 60 days), a herd that averages 154 DO has a pregnancy rate of 20%, while a herd averaging 133 DO has a pregnancy rate of 25%.

Similar to the Animal Improvement Programs Laboratory (AIPL; USDA), daughter pregnancy rate was one of the fertility trait defined for this study. Records of days open were transformed to pregnancy rate using the simple linear function:

$$Pregnancy\ rate = 0.25 \times (233 - days\ open)$$

(Khun et al. 2004; VanRaden et al. 2004). Genetic evaluations are expressed as deviations from a base pregnancy rate within each breed.

4.3.5 Animal Model

Animal models allow simultaneous genetic evaluation for male and female dairy animals with all relationships included. Data were processed by lactation for the genetic evaluation through the animal model.

The statistical model was:

$$Y\,(DO) = \mu + CG\,(E + O + D + C) + CM + DIM + Animal\ effect + CMP + e$$

where:

$Y\,(DO)$ was the trait of interest in this example days open;

$CG\,(E + O + D + C)$ was contemporary group which included E=entity, O=owner, D=dairy and Yr=year of calving;

CM was calving month;

DIM was days in milk;

$Animal\ effect$ was the random genetic effect;

CMP was milk production as a covariate; and

e was the residual error term.

4.3.6 Variance component estimation and software

Multiple Trait Derivative-Free Restricted Maximum Likelihood, denoted as MTDFREML, was used to estimate covariance components (Boldman et al. 1995). Solutions for fixed effects and breeding values were obtained for each trait. MTDFREML is a set of programs used to estimate (co)variance components involving animal models and derivative-free REML (Boldman et al. 1995). These programs can be used for single trait, bivariate, and multiple trait animal models with repeated records including traits with sex-limited expression (Boldman et al. 1995). Solutions for fixed effects, breeding values, and uncorrelated random effects, sampling variances of solutions (accuracies) can also be obtained (Boldman et al. 1995). The programs were developed by Drs. Keith Boldman and Dale Van Vleck and available at the Multiple Trait Derivative Free REML home page (http://aipl.arsusda.gov/curtvt/mtdfreml.html).

The MTDFREML software package consists of three executable programs: MTDFNRM, MTDFPREP and MTDFRUN. The first program (MTDFNRM) (1) calculates the inverse of the relationship matrix to be used in mixed model equations and makes use of the Henderson (1975) and Quaas (1976) rules to calculate the inverse of the relationship matrix directly from a list of animals and their parents (forms non-zero elements of A-1 using an ASCII pedigree; reorders animal, sire and dam identification), (2) provides individual identification for matching phenotypic records to individuals, (3) determines inbreeding coefficients, and (4) calculates the logarithm of the determinant of the relationship matrix needed to calculate the log of the likelihood function (Boldman et al.

1995). The second program of the set (MTDFPREP) prepares coefficients for mixed models equations based on the statistical model (fixed and random factors) for single and multiple trait analyses (Boldman et al. 1995). The third (MTDFRUN) and last element program solves mixed model equations and finds variance component estimates (using the SIMPLEX algorithm) from coefficients of MME formed in MTDFPREP, which maximize the restricted likelihood given the phenotypic data. Additionally, MTDFRUN finds solutions for covariates, fixed and random effects, and sampling variances (accuracies; Boldman et al. 1995).

Additionally, SAS (SAS Institute Inc. 2008) was used for recoding the original files, validating data, and creating files that MTDFREML requires for the estimation (pedigree and trait/production file).

Computer System (language), operations and other tools

The operations were computed on a Linux cluster computer system operated by the UNIX Systems Group of Office Information Technology (OIT), designed for use by researchers at the University of Tennessee, Knoxville (http://hpc.usg.utk.edu/bin/view/Main/WebHome).

The OIT Newton Linux Cluster serves as a high performance computational resource for the UT research community. The 69-node cluster consists of 290 Xeon Intel 64-bit compute cores, 407.4 GB of total memory and 10 TB of disk space. Each node runs a 64-bit x86_64 Linux 2.6 kernel. Nodes are interconnected with gigabit Ethernet and

InfiniBand interconnects. Each processor has from approximately 4 GB to 8 GB of random access memory and 100 GB of local disk space (Note: description of OIT Newton Linux Cluster serves was extracted from http://hpc.usg.utk.edu/bin/view/Main/WebHome for the purpose of this dissertation).

Additionally, a free (MIT-licensed) software and SSH (secure shell protocol) client, PuTTY, is a terminal emulator application which can act as a client for the SSH, allowing the connection of Windows computers with UNIX (LINUX) systems. PuTTY was utilized to open the UNIX account on Newton Linux Cluster from a Windows computers and PuTTY FTP (Secure File Transfer Protocol) allowed the transferring of files from Windows to UNIX accounts. (http://www.chiark.greenend.org.uk/~sgtatham/putty/docs.html).

4.3.7 Multi-trait selection index ($ Fertility)

Hazel and Lush (1943) introduced the concept that the net merit of the individual considering several traits of economic importance, outperforms other forms of selection including single trait selection. The fundamental concept of aggregate genotype described by Hazel and Lush (1943) introduces a linear function (selection index) of observations permitting that the observations of each trait were weighted by the relative economic value of that trait. Thus, the aggregate genotype allows calculating a single value for each animal; representing an objective evaluation integrating the net genetic merit of an animal and the profit associated with these traits (Weaber 2010). The incorporation of the breeding

values in the selection indexes motivated by Hazel (1943) and Henderson (1951) definitely improved the genetic prediction of the economically important traits.

4.3.8 Index Basics

In simplistic terms, the selection index (Hazel 1943) defines the economic merit of an animal as a parent which also incorporates breeding values

$$Hi = a1\ BVi1 + a2BVi2 + \ \ldots + anBVin.$$

Where:

Hi = the aggregate economic merit of an animal, i, as a parent;

aj = the relative economic weight of trait j, j = 1...n, where n = the total number of traits; and

$BVij$= the breeding value of animal i for trait j.

Usually, breeding values are substituted by PTAs or EPDs as in the index for dollar fertility ($F). Thus, animals (as parents) are ranked on a prediction of H called (I), the index value defined as:

$Ii = a1\ PTAi1 + a2PTAi2 + \ \ldots + anPTAin$ (Henderson 1963).

Where:

Ii = the predicted aggregate economic merit of an animal, i, as a parent;

aj = the relative economic weight of trait j, j = l…n. where n = the total number of traits; and

$PTAij$ = the predicted transmitting ability of animal I for trait j.

As previously discussed, the index is unbiased as genetic predictions themselves are unbiased since predictions are the Best Linear Unbiased Predictions (BLUP).

The relative economic values or weights (the a's in the above equations) reflect the change in profit when a trait is changed by single unit.

The proposed selection index included calving interval (CI) and age to first calving (AFC) with their corresponding economic weights. These economic models are available in the literature for those traits (Gonzalez-Recio et al. 2004; Gonzalez-Recio and Allenda 2005) and their economic estimations were used for the $F index.

The $F index was estimated with the following model:

$$\$Fi = a1AFCi1 + a2CI2i.$$

Where:

$\$Fi$ was the proposed $F value;

$aj1$ = the relative economic weight of trait AFC and CI, respectively; and

$PTAij$ = the predicted transmitting ability of animal AFC and CI.

Traits for $F were selected from available data in an attempt to combine genetic merit of animals that reach early puberty (precocity; measured as AFC) and develop efficient overall fertility (shorter anestrous postpartum; measured as DO, DPR and CI). In the

second term of the $F, three traits are valid options to integrate the index (CI, DPR and DO), but only CI was used. Calving interval was selected because it showed high correlation with other fertility traits such as interval between first and last inseminations (.98), days to first service (.80), number of inseminations per service period (.89), pregnancy within 56d (-.95), pregnancy within 90d (-.95), success in first insemination (-59), days open (.99) and daughter pregnancy rate (-.99; Gonzalez-Recio et al. 2005). Furthermore, CI to 1st lactation showed 5% heritability; thus, outperforming DO and DPR, achieving requirements for the index, and had available economic information.

Additionally, SAS (SAS/STAT® 9.2 User's Guide, Cary, NC, USA: SAS Institute Inc. 2008) was used for creating the proposed index ($F) and ranking bulls from the pedigree file. Pearson correlation coefficients were used to determine the strength of the relationship between fertility traits, index and lactations.

4.5.3 Results and Discussion

Fertility traits are presented by partitioning a specific trait into 6 sub-traits according to corresponding lactation or parities. Lactations were analyzed individually for days open, calving interval and daughter pregnancy rate except lactations 6 through 10 that were considered as one (lactation group 6th-10th). Hence, 19 fertility traits are introduced and analyzed separately by lactation (six lactation groups per traits) including days open, calving interval, daughter pregnancy rate, and age to first calving. For estimation of fixed effects (milk testing entities, dairies, owners and calving years), a total of 35,610

contemporary groups (CG) were created to remove variation due to changes in herd environmental conditions. However, due to different restrictions applied, total number of CG was reduced to 25661. Additional solutions for fixed effects included grouping for calving months and days in milk with a total of 12 and 6 levels, respectively.

4.4.1 Descriptive statistics of fertility data

Descriptive statistics of fertility data utilized in the present study are illustrated in Tables 1 through 4. These tables are organized by the four major traits (DO, CI, DPR and AFC) including lactations, number of records, mean, and SD.

Phenotypic means for days open by lactation number ranged from 144.35 to 147.18 days (Table 1). The highest mean value for DO was observed during the first lactation. These mean values were somewhat higher compared with those reported in other studies (US, 135 d, Pszczola et al. 2009; Spain, 117 d, Gonzalez-Recio and Alenda 2005). Furthermore, phenotypic means for calving interval by lactation ranged from 403.32 to 409.54 days (Table 2). Similar to DO, first lactation means were higher than subsequent lactations. Also, mean values for calving interval were slightly higher than some reported in the literature.

For instance, phenotypic means of 385 and 400 days were reported in United Kingdom and Spain, respectively (Pryce et al. 2000; Gonzalez-Recio and Alenda 2005). However, Norman et al. (2009) reported CI values ranging from 410 to 428 days for Holstein breed.

In 2006, the US value for CI was 422 days, slightly higher than phenotypic means found in the present study (Norman et al. 2009).

Daughter pregnancy rate showed phenotypic means ranging from 21.50% to 22.21% (Table 3). Third lactation had the highest DPR. Norman et al. (2009) reported an average DPR for US Holstein of 24.9% utilizing data from nearly 8 million lactations from 5 million cows in over 23,000 herds. Furthermore, Animal Improvement Program Laboratory (AIPL; US Department of Agriculture; USDA) reported an average DPR value of 28.14% for 2010. Similarly, Gonzalez-Recio and Alenda (2005) observed a 29% DPR for Holstein herds in Spain.

Phenotypic means for age to first calving averaged 985.72 days (32.32 months) on 965,137 Holstein heifers. This value was higher than those (839.36 days or 27.52 months) reported by Powell (1985) in an extensive study evaluating AFC in US Holstein herds from 1960 to 1982. Moreover, more recent data from US Holsteins herds showed that phenotypic mean for AFC averaged 788 days (Cole and Null 2010). Interestingly, Gonzalez-Recio et al. (2004) showed 854 days from 1988 and 2001 in Holstein herds in Spain.

4.5.4 Breeding values of fertility data

Predicted transmitting abilities (PTA's) for fertility data including 19 fertility traits are depicted in Tables 5-8 corresponding to DO, CI, DPR, and AFC, respectively. Solutions for fixed effects, breeding values, and sampling variances (accuracies) were obtained for each trait and presented by lactation, including number of animals with predicted

transmitting ability (PTA), mean PTAs, SD, PTA minimum and maximum, heritability (h^2) and standard error.

Heritability for DO and DPR ranged from 1 to 3% across different lactations (Tables 5 and 7). Heritability for CI ranged from 3 to 6% across different lactations (Table 6). The highest heritability values among fertility traits were those for AFC which reached 16% (Table 8). In general, low heritability for these fertility traits is consistent with reports in the literature (Dematawewa and Berger 1998; Veerkamp et al. 2001; VanRaden et al. 2004; Gonzalez-Recio et al. 2004; Gonzalez-Recio and Alenda 2005; Veerkamp and Beerda 2007).

Furthermore, values for predicted transmitting ability (PTA) across different lactations ranged from -16.3 to 11.4 days for DO (Table 5). Mean PTA values for DO fluctuated between 0.12 and -1.34 (Table 5). For CI, PTA values ranged from -24.7 to 15.1 days with means PTAs of -3.11 and -0.72 days across different lactations (Table 6). The PTA values for DPR varied from -2.4% to 4.0% with mean PTA values ranging from -0.02% and 0.33% across lactations (Table 7). These values for DPR are consistent with the literature (VanRaden et al. 2004). Age to first calving presented PTA values ranging from -120.3 to 76.2 days with a genetic mean of -5.81 days (Table 8).

Results of these different fertility traits across lactations indicate substantial genetic variation, suggesting that genetic selection may be effective in improving declines in fertility.

Fertility index

The predicted selection index (Dollar Fertility Value; $F) included AFC and CI with economic weights of $-0.28 and -4.90/day per cow, respectively (Gonzalez-Recio et al. 2004; Gonzalez-Recio and Allenda 2005). Using predicted transmitting ability from age to first calving and calving interval during first lactation, values for dollar fertility ($F) ranged from -$76.61 to $139.47 (Figure 1) and -$67.36 to $105.08 (Figure 2) for bulls and cows, respectively. These results indicate considerable genetic variation in the proposed fertility index. Selection based on this fertility index would have considerable influence on genetic gain; hence, reducing fertility costs. These results suggest that dollar fertility ($F) benefits by including AFC as a measure of initial reproductive efficiency followed by the inclusion of CI which measures success of inseminations.

4.5.5 Genetic correlation of fertility data

Table 9 presents heritability and Pearson genetic correlations among fertility traits and the fertility index ($F). First lactation/parities were utilized for estimating correlations among BLUPs for DO, CI, DPR, AFC and $F. Tables 9 - 11 display Pearson correlation statistics for pairs of analyzed variables. The Pearson coefficients are a parametric measure of association between two continuous random variables. By definition, Pearson correlation measures both the strength and direction of a linear relationship. Negative genetic correlation means that an inverse, linear relationship exists between these two variables. For instances, as days open increases (days), daughter pregnancy rate (percentage)

decreases. Same is true for positive correlations where a positive, linear relationship exists between two variables (i.e., days open with calving interval).

In general, moderate and high genetic correlations were observed (from 0.238 to 0.999; P<0.001; Table 9; Figure 3). Days open and calving interval showed high negative associations with DPR (-0.999, -0.648) and $F (-0.612, -0.981; Table 9; Figure 3). Age to first calving showed moderate correlations with all other traits (from 0.238 to 0.522; P<0.001; Table 9; Figure 3).

Results from the correlation analysis suggest that CI and $F are qualified indicators of fertility in lactating dairy Holstein and relate well with other traits (Table 9; Figure 3). For instance, $F showed high correlations (both positive and negative) with days open (-0.612), calving interval (-0.981), daughter pregnancy rate (0.614) at first lactation, and age to first calving (-0.676; Table 9; Figure 3). In simplistic terms, producers should reduce DO, CI, and AFC and increase DPR to improve $F.

In general, these results agree with low heritability and strong genetic correlations estimated by other researchers (Dematawewa and Berger 1998; Veerkamp et al. 2001; VanRaden et al. 2004; Gonzalez-Recio et al. 2004; Gonzalez-Recio and Alenda 2005; Veerkamp and Beerda 2007). Gonzalez-Recio and Alenda (2005) suggested that CI, DO and DPR had genetic correlations near 1.00 with each other, suggesting that analyzing one of them would be sufficient. Results from present research agree with Gonzalez-Recio and Alenda (2005) except for CI which showed a genetic correlation of 0.647 with days open and -0.648 with daughter pregnancy rate (Table 9; Figure 3). According to the present

study, CI represents a different trait compared with DO and DPR. Figure 3 shows a symmetric matrix plot for the fertility traits and $F analyzed. First lactation/parities were utilized for estimating correlations among BLUPs for DO, CI, DPR, AFC and $F.

Table 10 illustrates Pearson genetic correlations among different lactations for calving interval traits. CI-1, CI-2, CI-3, CI-4, CI-5, CI-6+ represent BLUP values for calving interval at 1^{st} , 2^{nd} , 3^{rd} , 4^{th} , 5^{th} and 6^{th}-10^{th} lactations, respectively. Calving interval at first lactation showed moderate and high genetic correlation with second, third, fourth, fifth and sixth to tenth lactations (0.753, 0.688, 0.613, 0.593, 0.494, respectively; Table 10; Figure 4). These results indicate that CI-1 has a high positive, linear relationship with CI-2, suggesting CI-1 as a good predictor of subsequent lactation. However, this relationship observed between CI-1 and CI-2 decreases across subsequent lactations (3^{rd}, 4^{th}, 5^{th} and 6^{th}-10^{th}) at an average rate of 6.4%.

The highest correlation among different lactations in calving interval trait was 0.881 between fourth and fifth calving interval. Interestingly, the highest correlations were observed between subsequent lactation averaging 0.814 (Table 10; Figure 4). Moreover, these relationship values increase for each additional and subsequent lactation (Table 10) where values increased from 0.753 to 0.881.

Table 11 depicts Pearson genetic correlations among different lactations for daughter pregnancy rate. DPR-1, DPR-2, DPR-3, DPR-4, DPR-5, DPR-6+ represent BLUP values for daughter pregnancy rate at 1^{st} , 2^{nd} , 3^{rd} , 4^{th} , 5^{th} and 6^{th}-10^{th} lactations, respectively. Daughter pregnancy rate at first lactation had moderate and high genetic correlation with

second, third, fourth, fifth and sixth to tenth lactations (0.593, 0.492, 0.322, 0.309, 0.208, respectively; Table 11; Figure 5). Similar to calving interval, this relationship between DPR-1 and DPR-2 decreases across the subsequent lactations (3^{rd}, 4^{th}, 5^{th} and 6^{th}-10^{th}) at an average rate of 9.6%. The highest correlation among different lactation in daughter pregnancy rate trait was 0.814 between fourth and fifth lactations. The highest correlations values were found between subsequent lactation averaging 0.691 (Table 11; Figure 5). Moreover, these relationships increase for each additional and subsequent lactation (Table 11) where values increased from 0.593 to 0.814.

Figure 5 illustrates a symmetric matrix plot for calving interval traits analyzed. BlupDPR2, blupDPR3, blupDPR4, blupDPR5, blupDPR6 represent BLUP values for calving interval at 1^{st} , 2^{nd} , 3^{rd} , 4^{th} , 5^{th} lactations, respectively.

Figure 6 illustrates a scatter plot among calving interval at first lactation and age to first calving. This figure is separated in 4 squares according to the "0" means of BLUPs and the minimum and maximum values for both traits. Each blue dot/circle represents the BLUP value for each individual animal (*n*= 1,628,844).

From the fourth squares created on the scatter plot among calving interval and age to first calving (Figure 6), the top left square represents those animals which have negative values of CI (desired) but positive values of AFC (undesired). The top right square characterizes those animals that have positive values for both CI and AFC (undesired; low fertility group). These animals are not desired because more time is needed to become pregnant as heifers but also take longer to become pregnant again. The bottom right square depicts

those animals that perform well for AFC (negative values; desired) but have longer calving intervals (positive values; undesired). Finally, the bottom left square represents those animals that have desired reproductive attributes indicated by shorter CI and AFC (high fertility group).

4.5.6 Genetic trends for fertility traits

Genetic trends for fertility traits and $F are depicted in Figures 7 through 11 for CI, AFC, DO, DPR and $F, respectively. These figures show genetic trends for Holstein sires and dams born from 1970 to 2000.

Figure 7 depicts the genetic trend for calving interval in sires; indicating an initial increase in CI BLUP values until the middle 1980's, then decreasing CI BLUP values through the 1990's and 2000's (increasing fertility values). On average, genetic trends for CI resulted in decreases of 0.45 days per year between 1970 and 2000 for sires. In dams, Holstein genetic trend for calving interval became nearly flat before 1993, where CI values decreases steadily. On average, CI Holstein dams decreased at a slower rate than sires, falling by 0.08 days per year. Results found in the present study agreed with investigations performed by Norman et al. (2009) where CI increased throughout the early years but have stabilized or are declining in recent years. In the present study, these decreasing trends start earlier and reduce CI at faster rates. However, other studies in United States and United Kingdom revealed a steady increase of genetic trend in calving interval associated with increased milk yield (Royal et al. 2000; Lucy 2001).

The genetic trend for AFC showed a decrease of 2.76 and 0.73 days per year (Figure 8; P-value <.0001) between 1970 and 2000 for sires and dams, respectively. Similar to CI, the genetic trend for AFC had become nearly flat before 1980 and 1993 in Holstein bulls and cows, respectively. After these years the genetic tendency for AFC in both sires and dams steadily decrease. These results suggest an increased genetic merit for heifers to attain early sexual maturity (reduced AFC). Moreover, these results agree with conclusions made by Makgahlela et al. (2008) and Cole and Null (2010). Estimating by regression, Cole and Null (2010) reported a decreasing US genetic trend for sire PTA for AFC of 0.09 days per year.

Figures 9 and 10 depict genetic trend for DO and DPR during first lactation for sires and dams. Due to similarities in estimation and high correlation between DO and DPR, they are discussed together. Sire genetic trend for DO shows an initial increased in BLUP values until the late 1980s where values start steadily decreasing (Figure 9). In dams, DO genetic trend had become nearly flat before 1997 where this tendency sensible decreased (Figure 9). In general, the genetic trend for DO of sires and dams decreased slightly at a rate of 0.07 and 0.02 days per year, respectively. During the same period, the genetic trend for DPR, estimated by regression, increased by 0.02 percent per year in sires (Figure 10). However, in cows, DPR had a slight decrease of 0.006 percentage by year (Figure 10). Similar to other fertility traits, DPR showed an initial decline but progressively recovered during the late 1980s in the sires and early 1990s in dams (Figure 10). Similar to other traits (CI and DO), the analyzed Holstein population showed increases after implementation of genetic evaluations for productive life in 1994 (VanRaden and Wiggans

1995; Norman et al. 2009) and DPR in 2003 (VanRaden et al. 2004; Norman et al. 2009). Norman et al. (2009) suggested that the use and implementation of productive life evaluations helped reverse the initial genetic decline in DPR because of the negative relationship between DO and productive life (genetic correlation of −0.59; VanRaden et al. 2004).

As shown in Figure 11, the genetic trend for $F of sires and dams increased $2.99 and $0.60 per year for sires and dams, respectively. In summary, results indicate progress in the genetic trend for fertility traits and fertility index ($F) in the Holstein population of Argentina with higher improvement in sires compared to dams. Not surprisingly, genetic trends in sires are preceded by dam's trends. Trends visually showed faster improvement after 1980, possibly due to selection for productive life introduced in 1993, and extensive importation of US and Canadian bulls (72% of bulls born after 1986).

The amount of change from 1980 and 2000 in unit traits for calving interval (CI-1); age to first calving (AFC); daughter pregnancy rate (DPR-1); and dollar fertility ($F) for sires and dams is shown in Table 12.

In conclusion, results indicate substantial variation in fertility traits, suggesting that genetic selection may be effective in improving declines in fertility. Furthermore, evaluation of data tends to suggest improvement in certain fertility traits over the past decade that may be the result of selection strategies using productive life.

4.5 Appendix: Figures and Tables

Table 4.1. Descriptive summary and phenotypic mean values for days open across lactations

Traits	**Lactations**	**No. of Records**	**Mean**	**SD**
DO (d)	1st	545,663	147.18	81.85
DO (d)	2nd	401,956	144.49	81.47
DO (d)	3rd	275,153	144.35	81.32
DO (d)	4th	173,299	144.97	81.05
DO (d)	5th	99,605	145.61	80.67
DO (d)	6th - 10th	90,623	145.27	79.57

DO (d): days open expressed in days; No. of records: number of animals utilized in the analysis. Mean: phenotypic mean per this trait. SD: standard deviation.

Table 4.2. Descriptive summary and phenotypic mean values for calving interval across lactations.

Traits	**Lactations**	**No. of Records**	**Mean**	**SD**
CI (d)	1st	642,393	409.54	69.49
CI (d)	2nd	490,038	404.43	66.79
CI (d)	3rd	344,968	403.32	66.93
CI (d)	4th	223,219	404.06	67.23
CI (d)	5th	131,669	405.78	67.91
CI (d)	6th - 10th	126,509	408.20	68.58

CI (d): calving interval expressed in days; No. of records: number of animals utilized in the analysis. Mean: phenotypic mean per this trait. SD: standard deviation.

Table 4.3. Descriptive summary and phenotypic mean values for daughter pregnancy rate across lactations.

Traits	**Lactations**	**No. of Records**	**Mean**	**SD**
DPR (%)	1st	545,663	21.50	20.47
DPR (%)	2nd	401,956	22.17	20.38
DPR (%)	3rd	275,153	22.21	20.34
DPR (%)	4th	173,299	22.05	20.27
DPR (%)	5th	99,605	21.90	20.18
DPR (%)	6th - 10th	90,850	21.97	19.90

Daughter pregnancy rate (%): daughter pregnancy rate expressed in percentage; No. of records: number of animals utilized in the analysis. Mean: phenotypic mean per this trait. SD: standard deviation.

Table 4.4. Descriptive summary and phenotypic mean values for age to first calving.

Traits	**Lactations**	**No. of Records**	**Mean**	**SD**
AFC (d)	1st	965,137	985.72	146.01

AFC (d): age to first calving expressed in days; No. of records: number of animals utilized in the analysis. Mean: phenotypic mean for this trait. SD: standard deviation.

Table 4.5. Mean values and ranges for predicted transmitting ability (PTA) of days open across lactations.

Traits	**Lactations**	**No. of Records**	**Mean**	**SD**	**PTA Min.**	**PTA Max.**	**h^2**	**SE**
DO (d)	1^{st}	1,209,991	0.12	1.58	-16.09	9.27	0.02	0.002
DO (d)	2^{nd}	1,144,030	-0.06	1.84	-16.36	11.45	0.02	0.002
DO (d)	3^{rd}	1,072,363	-0.55	1.85	-14.31	8.09	0.02	0.003
DO (d)	4^{th}	1,003,261	-1.34	2.34	-15.03	9.63	0.03	0.004
DO (d)	5^{th}	943,626	-0.99	1.78	-12.53	6.95	0.02	0.006
DO (d)	6^{th} - 10^{th}	910,594	-0.54	0.91	-5.51	4.11	0.01	0.004

No. of records: number of records (animals) with predicted transmitting ability (PTA), Mean: Mean PTAs for trait, SD: standard deviation, PTA minimum: minimum PTA value across lactation, PTA maximum: maximum PTA value across lactations, h^2: heritability (h^2), SE: Standard Error

Table 4.6. Mean values and ranges for predicted transmitting ability (PTA) of calving interval across lactations.

Traits	**Lactations**	**No. of Records**	**Mean**	**SD**	**PTA Min.**	**PTA Max.**	**h^2**	**SE**
CI (d)	1st	1,263,058	-0.72	3.09	-24.75	15.16	0.05	0.003
CI (d)	2nd	1,197,520	-1.00	2.61	-23.59	13.16	0.03	0.002
CI (d)	3rd	1,120,682	-1.46	2.71	-18.18	10.53	0.03	0.003
CI (d)	4th	1,043,127	-2.55	3.14	-19.18	9.00	0.04	0.004
CI (d)	5th	972,604	-2.72	3.05	-20.13	7.52	0.04	0.007
CI (d)	6th - 10th	931,628	-3.11	3.04	-19.05	11.27	0.06	0.005

No. of records: number of records (animals) with predicted transmitting ability (PTA), Mean: Mean PTAs for trait, SD: standard deviation, PTA minimum: minimum PTA value across lactation, PTA maximum: maximum PTA value across lactations, h^2: heritability (h^2), SE: Standard Error

Table 4.7. Mean values and ranges for predicted transmitting ability (PTA) of daughter pregnancy rate across lactations.

Traits	**Lactations**	**No. of Records**	**Mean**	**SD**	**PTA Min.**	**PTA Max.**	**h^2**	**SE**
DPR (%)	1^{st}	1,209,627	-0.02	0.39	-2.31	4.00	0.02	0.002
DPR (%)	2^{nd}	1,143,706	0.03	0.35	-2.36	3.33	0.02	0.002
DPR (%)	3^{rd}	1,072,108	0.14	0.46	-1.97	3.56	0.02	0.003
DPR (%)	4^{th}	1,003,049	0.33	0.58	-2.41	3.76	0.03	0.004
DPR (%)	5^{th}	943,451	0.25	0.44	-1.70	3.16	0.02	0.006
DPR (%)	6^{th} - 10^{th}	910,519	0.14	0.22	-1.03	1.35	0.01	0.004

No. of records: number of records (animals) with predicted transmitting ability (PTA), Mean: Mean PTAs for trait, SD: standard deviation, PTA minimum: minimum PTA value across lactation, PTA maximum: maximum PTA value across lactations, h^2: heritability (h^2), SE: Standard Error

Table 4.8. Mean values and ranges for predicted transmitting ability (PTA) of age to first calving.

Traits	**No. of Records**	**Mean**	**SD**	**PTA Min.**	**PTA Max.**	**h^2**	**SE**
AFC (d)	1,396,894	-5.81	13.35	-120.33	76.23	0.16	0.003

No. of records: number of records (animals) with predicted transmitting ability (PTA), Mean: Mean PTAs for trait, SD: standard deviation, PTA min: minimum PTA value across lactation, PTA max: maximum PTA value across lactations, h^2: heritability (h^2), SE: Standard Error

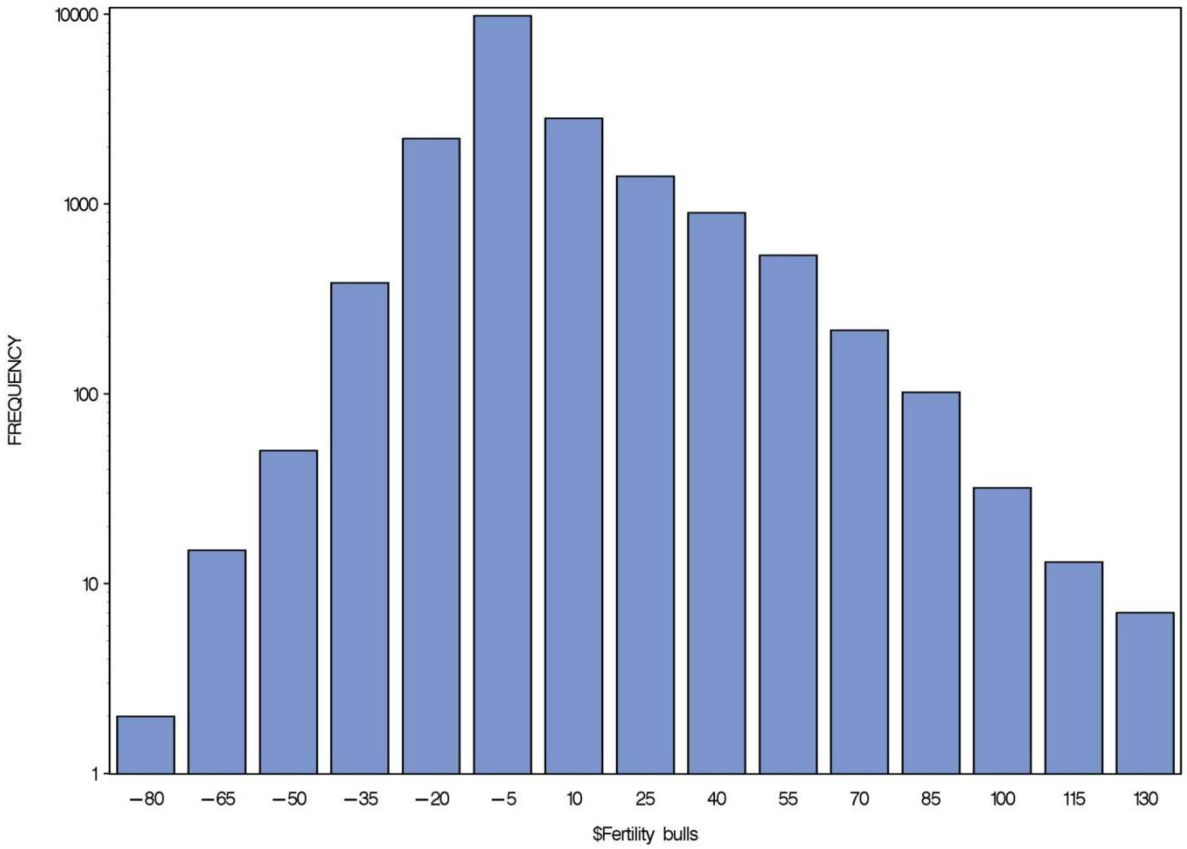

Figure 4.1. Distribution of the Dollar Fertility PTA values for bulls.

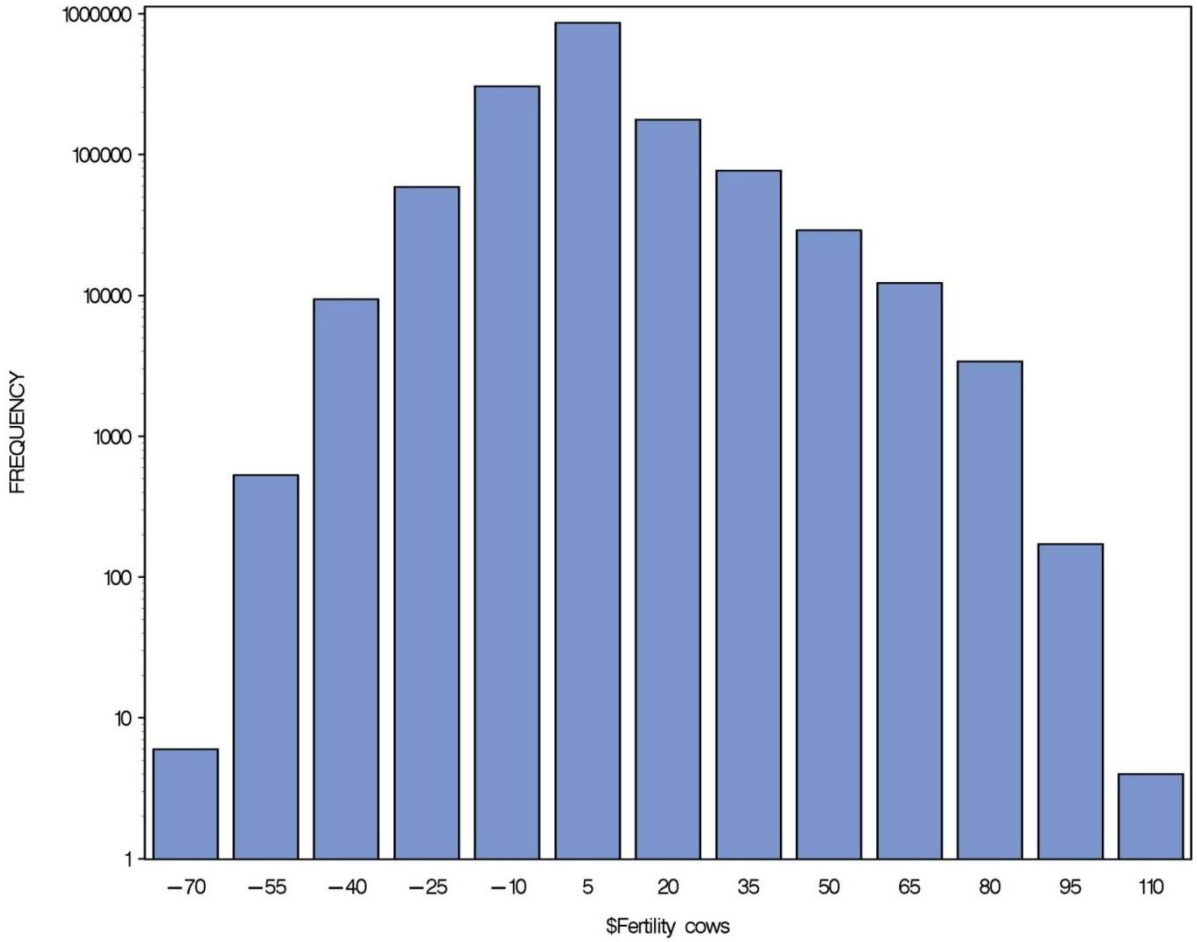

Figure 4.2. Distribution of the Dollar Fertility PTA values for cows

Table 4.9. Heritabilities (diagonal, in bold), Pearson correlation coefficients (above diagonal) among first lactation BLUPs values of fertility traits[1] and fertility index values[1] ($F) with p-value[2] immediately below coefficients

	DO-1	CI-1	DPR-1	AFC	$F
DO-1	**0.02**	0.64710	-0.99958	0.23872	-0.61256
		<.0001	<.0001	<.0001	<.0001
CI-1		**0.05**	-0.64829	0.52253	-0.98130
			<.0001	<.0001	<.0001
DPR-1			**0.02**	-0.24121	0.61414
				<.0001	<.0001
AFC				**0.16**	-0.67688
					<.0001

[1] DO-1: days open at first lactation; CI-1: calving interval at first lactation; DPR-1: daughter pregnancy rate at first lactation; AFC: age to first calving; $F: dollar fertility.

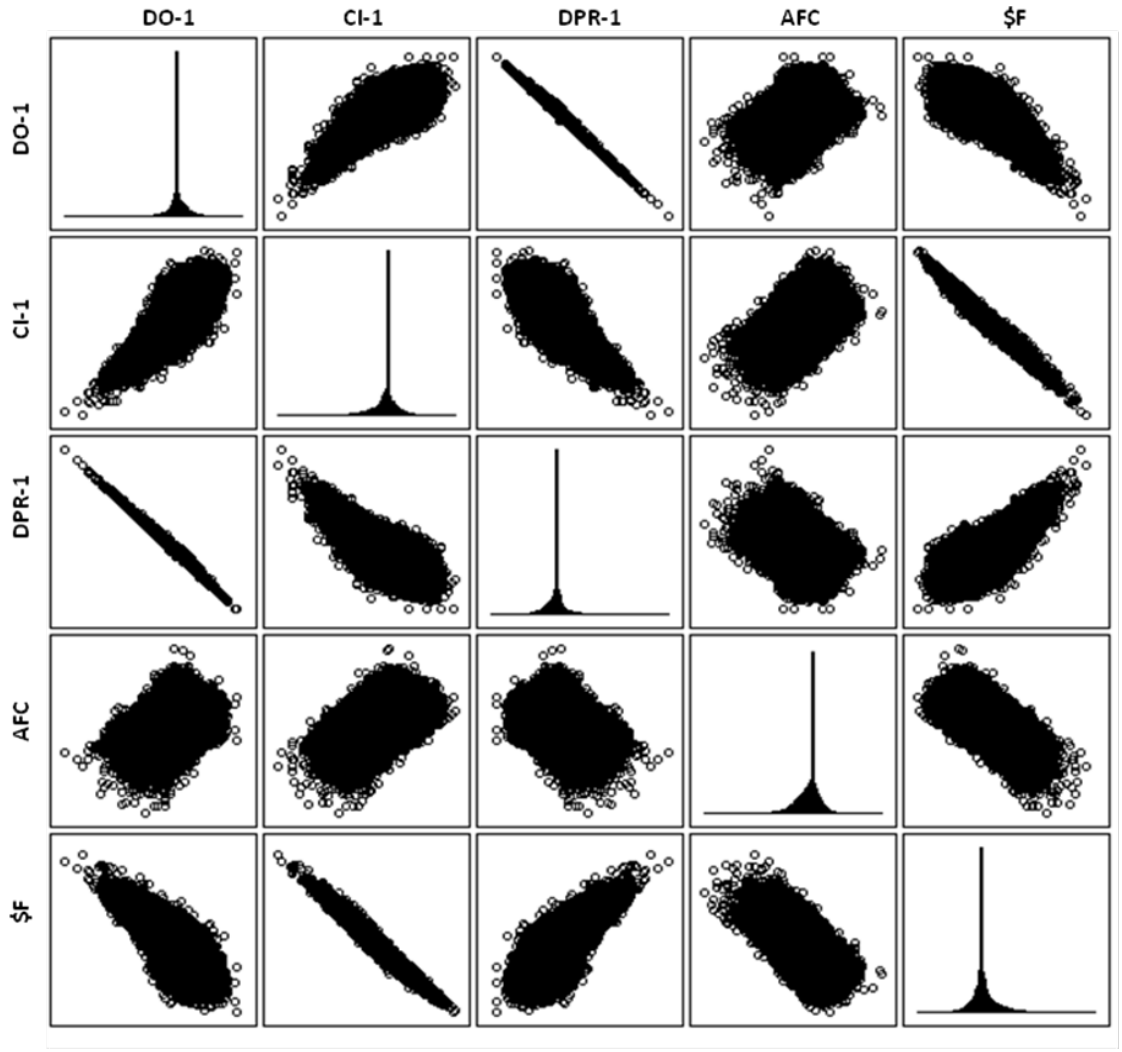

Figure 4.3. Scatter plot matrix for first lactation BLUPs of fertility traits (DO, CI, DPR, and AFC) and fertility index ($F). DO-1: days open at first lactation; CI-1: calving interval at first lactation; DPR-1: daughter pregnancy rate at first lactation; AFC: age to first calving; $F: dollar fertility.

Table 4.10. Heritability (diagonal, in bold), Pearson correlation coefficients (above diagonal) among BLUPs values of calving interval traits[1] with p-value[2] immediately below coefficients

	CI-1	CI-2	CI-3	CI-4	CI-5	CI-6+
CI-1	**0.05**	0.75389	0.68809	0.61391	0.59364	0.49435
		<.0001	<.0001	<.0001	<.0001	<.0001
CI-2		**0.03**	0.78280	0.72743	0.69043	0.60441
			<.0001	<.0001	<.0001	<.0001
CI-3			**0.03**	0.81879	0.79292	0.70253
				<.0001	<.0001	<.0001
CI-4				**0.04**	0.88199	0.81959
					<.0001	<.0001
CI-5					**0.04**	0.83289
						<.0001
CI-6+						**0.06**

[1] CI-1: calving interval at first lactation; CI-2: calving interval at second lactation; CI-3: calving interval at third lactation; CI-4: calving interval at fourth lactation; CI-5: calving interval at fifth lactation; CI-6+: calving interval at sixth to tenth lactations. [2] p-value

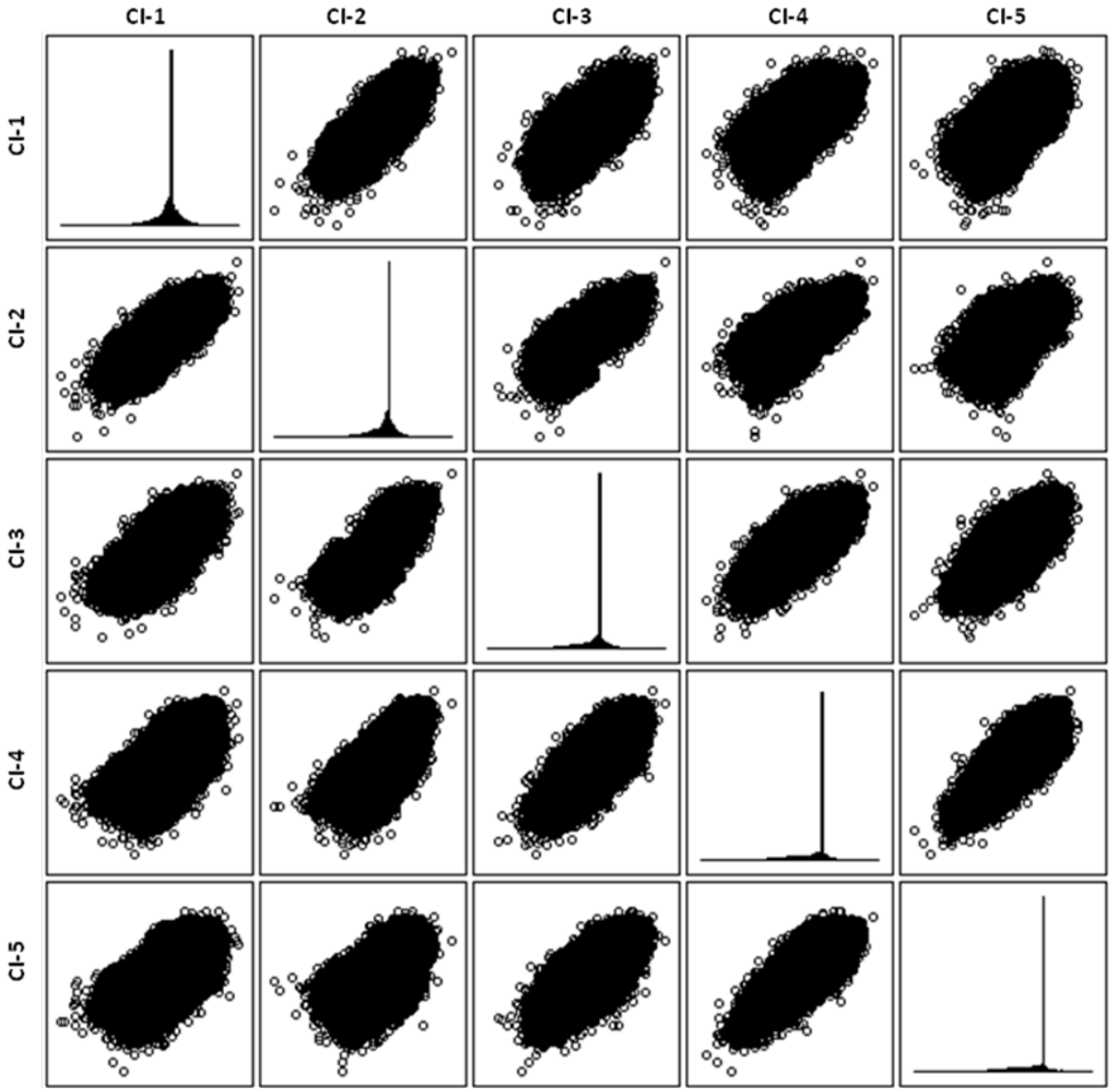

Figure 4.4. Scatter plot matrix for calving interval BLUP values across lactations. CI-1: calving interval at first lactation; CI-2: calving interval at second lactation; CI-3: calving interval at third lactation; CI-4: calving interval at fourth; CI-5: calving interval at fifth lactation.

Table 4.11. Heritability (diagonal, in bold), Pearson correlation coefficients (above diagonal) among BLUPs values of calving interval traits[1] with p-value immediately below coefficients.

	DPR-1	DPR-2	DPR-3	DPR-4	DPR-5	DPR-6+
DPR-1	**0.02**	0.59322	0.49225	0.32267	0.30950	0.20853
		<.0001	<.0001	<.0001	<.0001	<.0001
DPR-2		**0.02**	0.62319	0.54148	0.47556	0.36225
			<.0001	<.0001	<.0001	<.0001
DPR-3			**0.02**	0.72337	0.71799	0.53905
				<.0001	<.0001	<.0001
DPR-4				**0.03**	0.81436	0.72376
					<.0001	<.0001
DPR-5					**0.02**	0.70208
						<.0001
DPR-6+						**0.01**

[1] DPR-1: daughter pregnancy rate at first lactation; DPR-2: daughter pregnancy rate at second lactation; DPR-3: daughter pregnancy rate at third lactation; DPR-4: daughter pregnancy rate at fourth lactation; DPR-5: daughter pregnancy rate at fifth lactation; CI-6+: daughter pregnancy rate at sixth to tenth lactations.

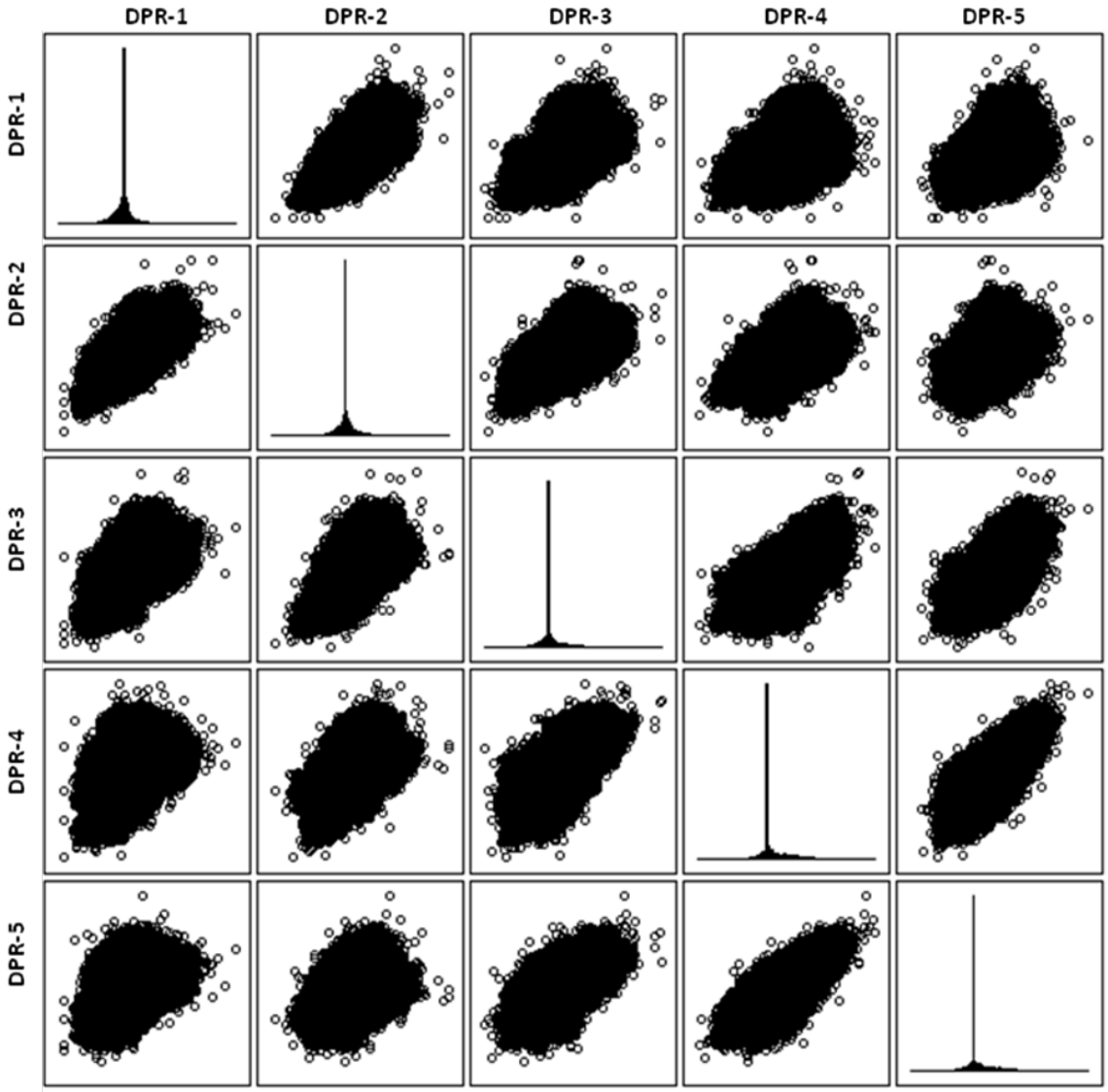

Figure 4.5. Scatter plot matrix for daughter pregnancy rate BLUP values across lactations. DPR-1: daughter pregnancy rate at first lactation; DPR-2: daughter pregnancy rate at second lactation; DPR-3: daughter pregnancy rate at third lactation; DPR-4: daughter pregnancy rate at fourth; DPR-5: daughter pregnancy rate at fifth lactation.

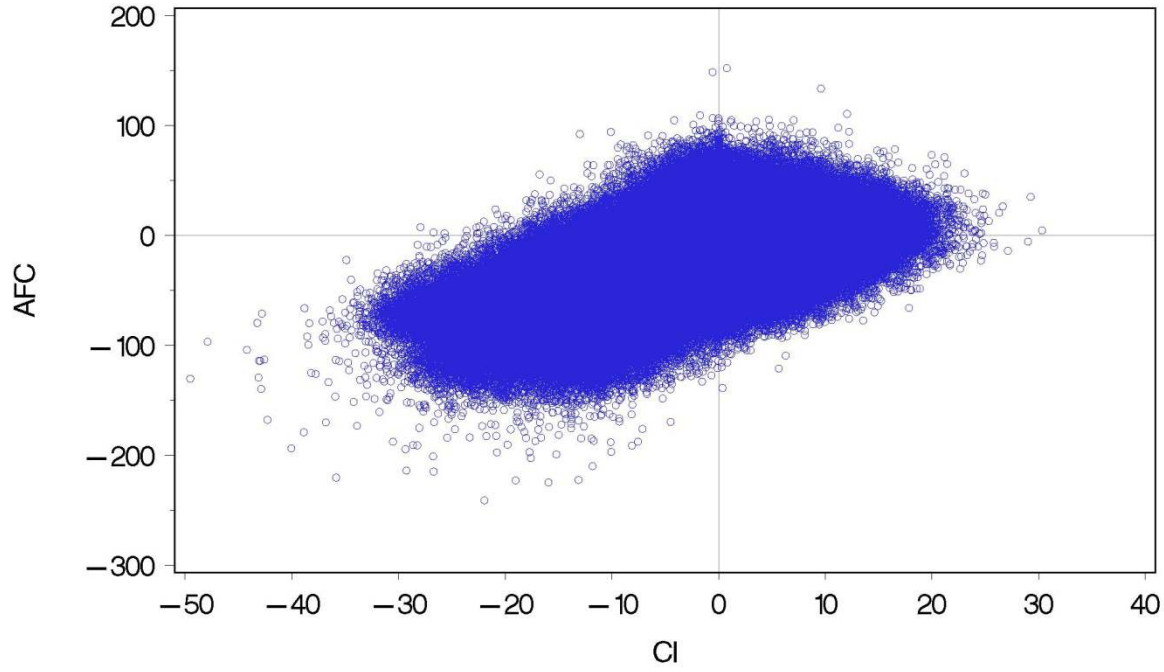

Figure 4.6. Scatter plot of BLUP values between calving interval at first lactation and age to first calving. AFC: age to first calving; CI-1: calving interval at first lactation

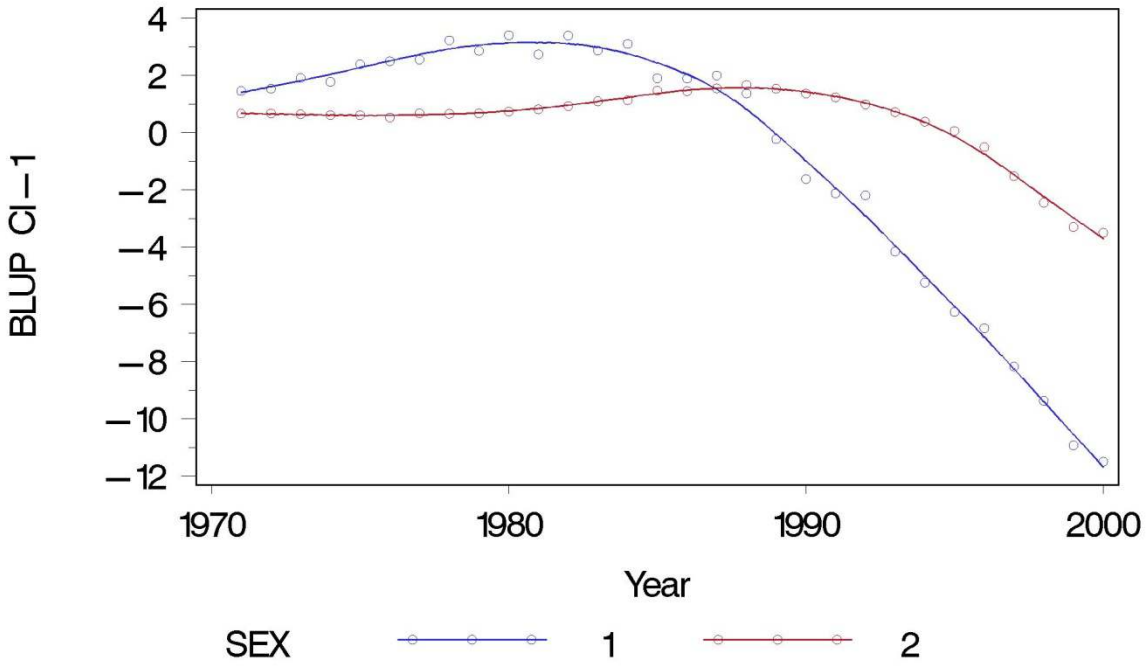

Figure 4.7. Trend in BLUP calving interval at first lactation (BLUP CI-1) for sires (SEX 1-blue line) and dams (SEX 2-red line) born from 1970 to 2000 in Argentinean Holsteins.

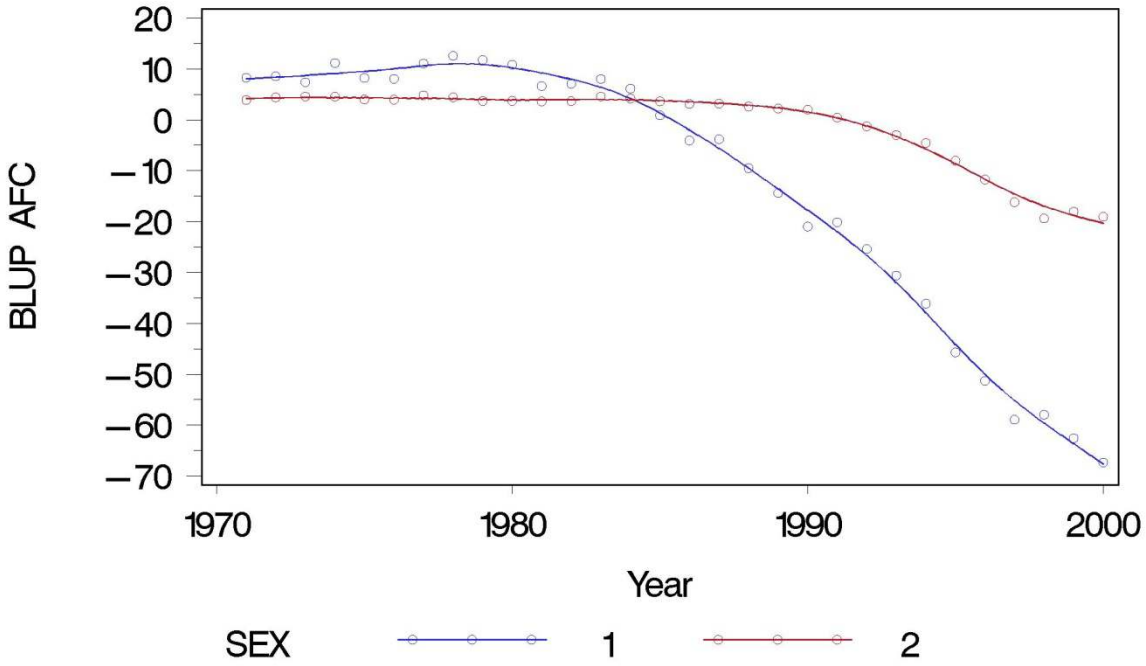

Figure 4.8. Trend in BLUP age to first calving (BLUP AFC) for sires (SEX 1-blue line) and dams (SEX 2-red line) born from 1970 to 2000 in Argentinean Holsteins.

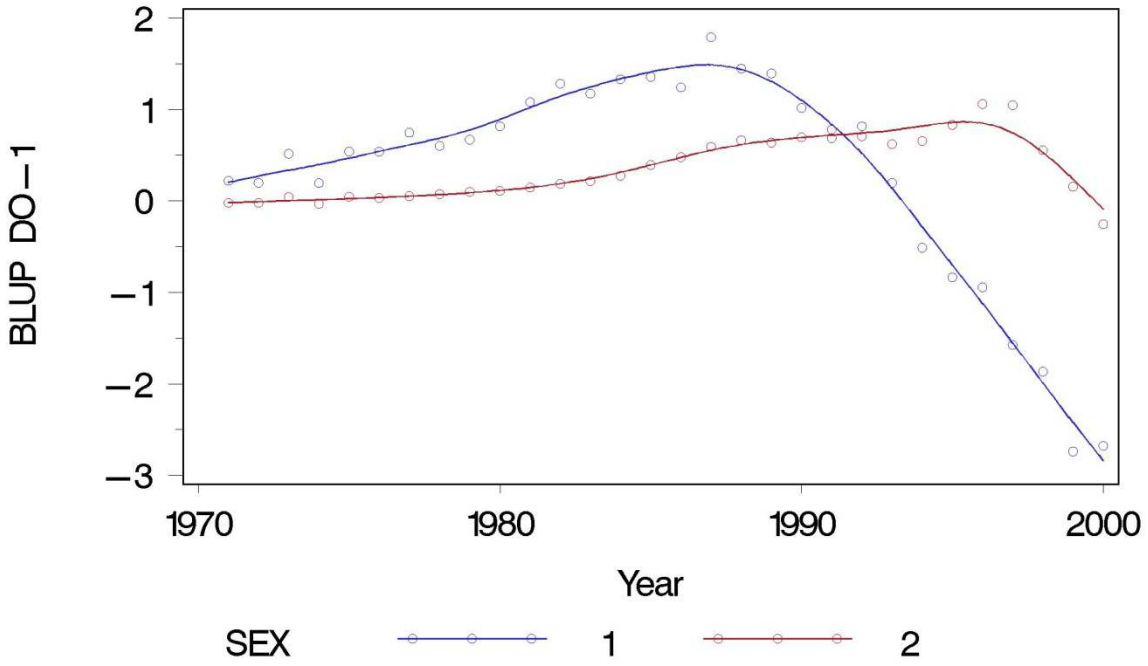

Figure 4.9. Trend in BLUP days open at first lactation (BLUP DO-1) for sires (SEX 1-blue line) and dams (SEX 2-red line) born from 1970 to 2000 in Argentinean Holsteins.

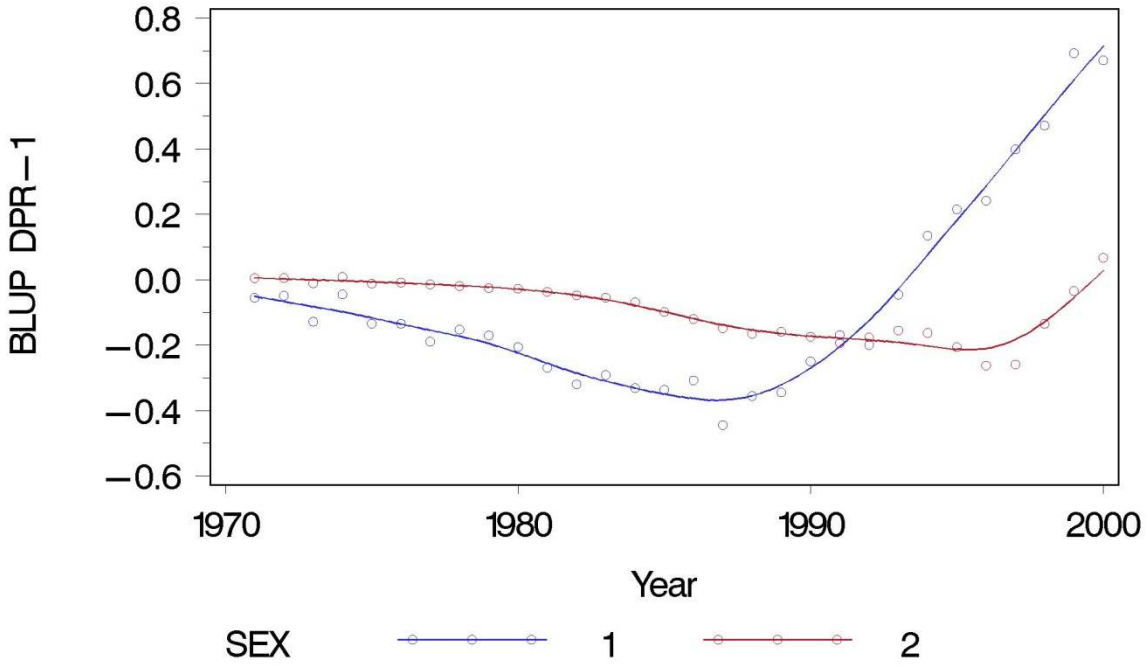

Figure 4.10. Trend in BLUP daughter pregnancy rate at first lactation (BLUP DPR-1) for sires (SEX 1-blue line) and dams (SEX 2-red line) born from 1970 to 2000 in Argentinean Holsteins.

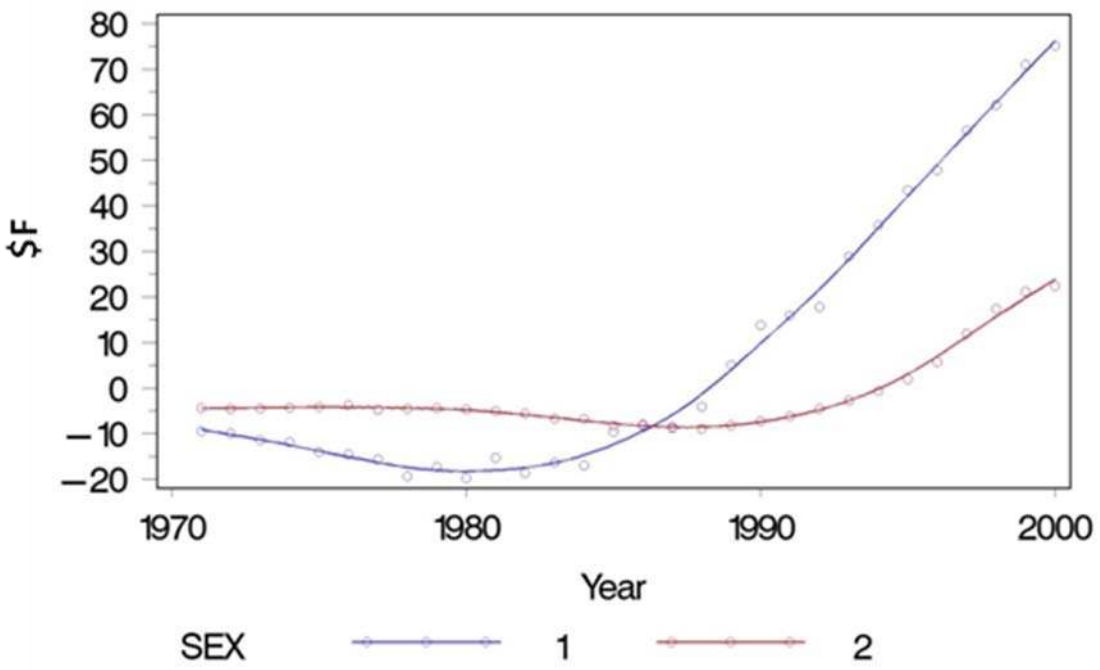

Figure 4.11. Trend in BLUP Dollar Fertility ($F) for sires (SEX 1-blue line) and dams (SEX 2-red line) born from 1970 to 2000 in Argentinean Holsteins.

Table 4.12. Amount of change in mean BLUP values of Argentinean Holstein sires and dams born from 1980 to 2000 for fertility traits[1] and dollar fertility.

	Mean 1980	**Mean 2000**	**Difference 1980-2000**
CI-1 Sires	3.401	-11.498	**14.899**
CI-1 Dams	0.739	-3.495	**4.235**
AFC Sires	10.843	-67.331	**78.175**
AFC Dams	3.741	-19.026	**22.768**
DPR-1 Sires	-0.205	0.671	**0.876**
DPR-1 Dams	-0.027	0.067	**0.094**
$F Sires	-19.702	75.194	**94.896**
$F Dams	-4.673	22.456	**27.129**

[1] CI-1: calving interval at first lactation; AFC: age to first calf; DPR-1: daughter pregnancy rate at first lactation; $F: dollar fertility. Means 1980 and 2000 are the average BLUP value for bulls and dams born in 1980 and 2000, respectively.

CHAPTER 5 SUMMARY AND CONCLUSION

Numerous assisted reproductive technologies (i.e. artificial insemination (AI), multiple ovulation and embryo transfer (MOET), cryopreservation and sperm/embryo sexing strategies) have provided fundamental tools for rapid genetic improvement of livestock, particularly in dairy and beef cattle. These reproductive technologies protocols have greatly increased efficiency of animal agriculture to provide high quality, low cost to consumers. However, significant needs still exist to provide economical, "decision-making" tools for producers and sustainability to the animal agricultural sector.

Improvements in fertility through genetic selection are a possible approach to increase reproductive efficiency. While progress may be slow due to low heritabilities, ignoring fertility in genetic improvement programs has contributed to the current fertility problems. Hence, to mitigate the deterioration of fertility and variability in superovulatory response of donor animals, a strong need exists to identify and select animals according to their future reproductive potential. Therefore, our general approach encompassed understanding of how genotype contributes to phenotypic variation in fertility. In order to accomplish this approach, estimation of genetic parameters and development of multi-trait selection indexes for fertility traits were performed. Hence, it was hypothesized that utilization of breeding values and fertility indexes can predict genetic merit in a bovine population.

As a first step, we developed genetic parameters associated with multiple ovulation and embryo transfer schemes in an attempt to assist producers in identifying animals with greater genetic merit for these protocols. Our study confirmed that genetic selection of donors or sires appears to be a potential approach to improve efficiency of MOET

procedures. Although low heritability would slow the progress, results shown in this work suggest that genetic improvement in fertility by selection for embryo transfer traits is possible. Moreover, genetic gain estimate was 1.16% per generation suggesting a useful tool for genetic improvement and the feasibility of including MOET traits in future breeding strategies. Further studies for identifying markers for MOET traits, along with available technology (e.g., Bovine SNP Chip), may create an even more effective approach for improving efficiency of MOET schemes and overall fertility of the livestock industry.

In our second experiment, we developed a genetic evaluation for fertility traits in Argentinean Holstein cattle. In order to develop fertility genetic predictors for utilization in breeding strategies, we estimated genetic parameters, breeding values, and developed a multi-trait selection index (dollar fertility; $F). We developed a $F that included age to first calving (AFC) and calving interval (CI), with economic weights of -0.28 and -4.9 $/day per cow for AFC and CI, respectively. Thus, $F benefits by including AFC as a measure of initial reproductive performance as well as CI, which measures conception rate and an early successful insemination. Also, $F showed high correlations (both positive and negative) with days open (-0.612), calving interval (-0.981), daughter pregnancy rate (0.614) at first lactation, and age to first calving (-0.676). Heritability for days open (DO) and daughter pregnancy rate (DPR) ranged from 2% to 3%; 3% to 6% for CI; and 16% for AFC. Predicted transmitting ability (PTA) values across different lactations ranged from -16.3 to 11.4 days, from -24.7 to 15.1 days, -2.4% to 4.0% and -120.3 to 76.2 days for DO, CI, DPR and AFC, respectively. Values for $F ranged from -$76.6 to $139.4 in the current

Holstein population. Results indicated substantial variation in fertility traits, suggesting that genetic selection may be effective in improving declines in fertility. To our knowledge, this is the first report of genetic evaluation for fertility traits in Argentinean Holstein cattle.

In conclusion, this research provided evidence of substantial genetic variation in cattle fertility traits. First, we developed genetic parameters associated with multiple ovulation and embryo transfer schemes to assist producers in identifying animals with greater genetic merit for MOET traits, suggesting a potential approach to improve efficiency of MOET procedures in cattle. Second, using a large Holstein population we were able to develop genetic predictors of fertility traits for utilization in breeding strategies. Consistently, we observed substantial genetic variation suggesting that genetic selection is highly effective in improving fertility.

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