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The effect of inbreeding on Holstein-Friesian breed

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List of contents:

List of figures:

List of tables:

Introduction:

Cattle are domestic animals which have a special role of providing food for human consumption such as beef and milk. In addition, cattle are often chosen as animal models in biomedical and reproductive research. Holstein-Friesian cattle are known as the world's highest producing dairy breed. The Holstein-Friesian breed is differentiated from other dairy breeds based on its distinct characteristics such as high milk production, high calving survival, physiology, body size, and health. However, in the recent past, an increase in inbreeding has resulted in negative effects such as increased calving mortality, reduced milk production, diminished health quality, and decreased fertility. Inbreeding is the production of offspring from breeding within a population which has the same or closely related genetic makeup. Inbreeding results in increased homozygosity and reduced heterozygosity within the population. Therefore, management of inbreeding is necessary to obtain high value production and increased health and longevity in dairy cattle. Currently, genetic selection programs and assisted reproductive technologies, such as artificial insemination (AI), are utilized to increase genetic variations and decrease the impact of inbreeding depression which improves production, health, reproduction, and longevity of individuals.

Animal breeding:

A breed is defined as a group of domestic animals which have a similar phenotype, behavior, configuration, and physiology that differentiate it from other groups within a species. The main purpose of animal breeding is to improve the production level of farm products (such as milk, meat, and eggs), reduce diseases, increase longevity, and enhance reproductive performance. Outbreeding, outcrossing, crossbreeding, and inbreeding are all propagation methods utilized in breeding domestic amimals.

Outbreeding:

Outbreeding is a breeding system in which the animals are not related. This can be between individuals of the same breed or between different breeds.

Outcrossing:

Outcrossing is a breeding technique which produces offspring from two unrelated parents within the same breed. Outcrossing is the best breeding method to increase milk production and growth rate in cattle as it reduces the effect of inbreeding depression on populations.

Crossbreeding:

Crossbreeds are animals where the offspring contains genetic material from more than one breed. By using crossbreeding, the production and health of domestic cattle is improved.

Crossbreeding advantages in Holstein dairy cows [HDBA. 2009]:

- Fitness traits (i.e. fertility, calving ease, stillbirths, etc.) are enhanced compared to inbred cows: crossbreeding increases the heterozygosity and genetic variation which raises biological fitness.
- Improves health and fertility: genetic variation is increased, which leads to enhanced genetic quality within the population.
- Easier management: when production and health are improved, feeding and animal care management is more straight forward compared to inbred populations.

Crossbreeding disadvantages:

- Reduces milk production in some species: milk production is reduced when an animal from a high producing breed is crossbred with an animal from a lower producing breed.
- Breeding choices become more difficult after the first generation of crossbreeding: increased heterogeneity yields increased variability in offspring.
- Loss of overall production in weight of milk, fat and protein: may reduce milk components such as fat, which is important for marketing purposes.
- Farm management difficulties: offspring produced with different genetic material display different performance within the population making farm management more problematic.

Inbreeding:

Inbreeding is a breeding system which produces offspring from closely related animals. Inbreeding results in increased homozygosity and reduced heterozygosity, which means that inbreeding decreases an allele's variation from parental genes causing a decrease in genetic variation across the population [Nabulsi, M. 2003]. Increased inbreeding within a population reduces phenotypic value and biological fitness. This phenomenon is termed inbreeding depression [Wang, S. 2003].

Impact of inbreeding on cattle productions:

Advantages:

Uniformity:

Inbreeding increases homozygosity and decreases heterozygosity within a population. Increased homozygosity leads to further uniformity and maintains that specific genotype within the population.

Disadvantages:

Fertility:

Inbreeding exhibits a negative correlation with fertility in a population. When inbreeding is increased in small populations fertility is reduced in the next generation. According to González's research with inbred Spanish cattle, fertility was reduced by 3% in the most inbred animals with the rate of pregnancy also being diminished. In addition, the results confirmed that increased inbreeding triggered a decline in reproductive fitness [González-Recio O., 2007] (Figure 1).

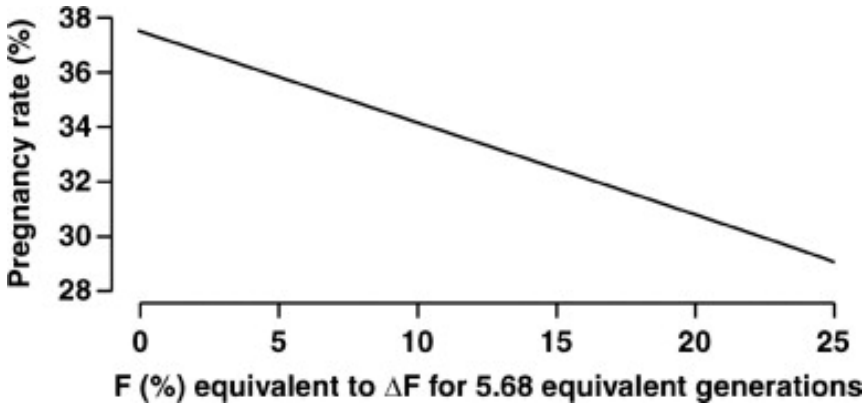

Figure 1: Regression line for inbreeding rate (ΔF) on pregnancy rate as a fertility trait [González-Recio O., 2007].

Milk production:

The long-term effects of inbreeding have been shown to negatively impact milk production. Smith (1998) indicated the inbreeding influence on milk production performance of dairy cattle. This study demostrated that inbreeding has a negative effect on milk production as well as an adverse effect on the health of the offspring. The results of Vleck's, L.D. (1984) study confirm that inbreeding leads to reduced milk production in cattle. The negative coefficient between inbreeding and milk production is indicated in Table 1.

Table 1: Regression coefficients of milk and fat production and calving interval on percent inbreeding [Vleck's L.D., 1984].

	Milk	Fat	Stayability	Calving
No. of records	**20430**	**20430**	**14894**	**14435**
Regression	**-2.3**	**-1.02**	**-0.0081**	**-0.095**
Standard error	**3.3**	**0.13**	**0.0017**	**0,2**

Genetic disorders:

Inbreeding increases the incidence of genetic disorders within and between populations. A genetic disorder is caused by an inherited abnormality in the genetic sequence and resulting phenotype of an individual. Genetic disorders trigger many diseases such as diabetes, brain diseases, and cancer. Genetic illnesses may occur when a mutation in the genome results in alterations to the DNA and proteins. Mutations are caused by inappropriate replication of DNA or environmental exposure to harmful substances such as cigarette smoke or radiation, which produce changes in the genomic sequence. DNA encodes genetic information used to create proteins in the body. When mutations occur in the DNA sequence, protein and enzyme structures are changed, as is the case in many diseases. If the genetic disorder is located in germ cells (spermatozoa or oocytes), it is transmitted to subsequent generations. However, if the genetic disorder is rooted in somatic cells only, the genetic disorder's influence on health and development is not inherited by future generations [Chial H., 2008].

Single gene disorders:

Single gene disorders are illnesses which cause alteration of gene functions [Chial H., 2008].

- Dominant diseases: occur when one allele in an individual contains a mutation caused by the disease-associated gene. For example, Huntington's disease.
- X-linked disorders: the mutation occurs in a gene on the X chromosome such as muscular dystrophy.
- Recessive diseases: occur if an individual has mutations on two alleles from the disease-associated gene. For example, cystic fibrosis is a type of recessive mutation [Chial H., 2008].

When inbreeding is increased in small populations the homozygosity is higher, which boosts the probability of allelic mutations in one or two alleles of a gene. Many studies indicate that inbreeding increases the risk of caring diseases and also significantly enhances the chance of mutation [Caballero et al. 1992; Wang & Hill 1999; Wang et al. 1999]. In another study, researchers showed that homozygote animals, compared to heterozygote animals, have more opportunity to pass on their mutations [Wiener., 2002].

Offspring survival:

Inbreeding and homozygosity influence offspring survival and produce reduced survival rates. According to Mattey's (2013) study involving chickens, inbreeding causes a significant decrease in offspring survival; however, no difference between body size in inbred and outbred offspring is indicated (Table 2). The results indicated that inbreeding diminishes offspring survival in chickens with no effect on the body size of surviving offspring.

Table 2: Direct effects of inbreeding on offspring traits. The P values are based on comparisons between outbred and inbred offspring [Mattey S. 2013].

Trait	Par	SE	t/Z value	P value
Number of eggs	**2.83**	**2.1**	**1.35**	**0.184**
Hatching success (%)	**-0.821**	**0.22**	**-3.83**	**0.0001**
Time to dispersal (days)	**0.014**	**0.081**	**0.18**	**0.855**
Survival to dispersal (%)	**-0.43**	**0.126**	**-3.42**	**0.0006**
Size at dispersal (g)	**-0.004**	**0.008**	**-0.462**	**0.647**
Overall offspring survival (%)	**-0.535**	**0.116**	**-4.611**	**<0.0001**

Craig A Walling (2011) showed that in inbred red deer calves the calving survival rate and birth weight were reduced compared to the control groups. An inbreeding

coefficient of 0.25 showed the first year survival of offspring was decreased by 77% compared to offspring with an inbreeding coefficient of 0. In another study, inbreeding had a negative impact on juvenile mortality in Austrian Brown Swiss cattle [Waltl B., 2011]. Østerås (2007) indicated the role of dairy farm management for calf and heifer mortality in inbred animals. Rollins (1949) mentioned inbreeding increased offspring mortality in Holsteins and Jerseys compared to outbred cattle.

Growth rate:

Inbreeding in animals is associated with smaller size at birth (MacNeil et al., 1989; Pariacote et al., 1998) and also leads to low birth weights (Nelms and Stratton, 1967; Keller and Brinks, 1978; MacNeil et al., 1989) and mature weights (McCurley et al., 1984). Rollins (1949) showed inbreeding leads to low birth weight and rate of growth was slower up to about sixth months of age compared to outbred animals.

Lifespan:

Sewalem (2006) indicted the negative effect of inbreeding on lifespan in Canadian dairy cattle. Three different cattle species, Jerseys, Holsteins and Ayrshires, were used. They determined the rate of inbreeding from 1980 to 2004 using inbreeding coefficients. The results indicated an increase of inbreeding. The inbred cattle had significant differences of ($P<0.001$) functional longevity in all three species compared to the control group.

Measurement of inbreeding:

Coefficient method:

One common method used to measure levels of inbreeding is an inbreeding coefficient, denoted by f. The inbreeding coefficient gives a statistical parameter to measure the percentage of homozygosity based on individual pedigree. DNA contains genes which occur at particular locations called loci. Each gene has two alleles. If the alleles are the same at a specific locus they are called homozygous. If the alleles are different at that locus they are termed heterozygous. The differences between genes are based on the loci. The coefficient of inbreeding calculates the probability of alleles at randomly chosen loci by pedigree. Each form of allele has an equal chance of transfer to the next generation at a randomly chosen location in the DNA, which is distinguished by descent. One of the disadvantages of the inbreeding coefficient method is in determining pedigrees, as the coefficients are not accurate if the pedigrees are unavailable [Wright, 1922]. The level of inbreeding coefficient F_x is measured by:

$$F_x = \sum 0.5^{n+\acute{n}+1}\,(1 + F_a),$$

$\sum$: sum over mean to common ancestor

n: number of generations from sire to common ancestor

$\acute{n}$: number of generations from dam to common ancestor

F_a : inbreeding coefficient of common ancestor

Coancestry coefficient method:

The probability that $F_{AB,}$when they are two homologous genes, one from individual A and the other from individual B, are identical by descent. The coancestry coefficient method measures the relationship between F_A and F_B which are two individuals'

homologous genes (F_{AB}). The advantage of this method is it calculates the inbreeding of individual animals which contain closely related genetic material [Hered. J. 1949].

When parents X, Y have ancestor A in common with individual *I*, and when there are n individuals we have: $F_I = \theta XY = {}^1\!/_2\, n(1 + F_A)$

Determining the level of inbreeding using molecular markers:

Utilization of molecular markers involves laboratory methods to determine the level of inbreeding and genetic variation in a population. Molecular markers ascertain inbreeding by marking of allele's loci, which are used to indicate heterozygosity and homozygosity within populations. Common types of genetic markers are microsatellites and single nucleotide polymorphisms (SNPs).

Microsatellite markers:

One of the most common molecular markers are microsatellites. Microsatellites are powerful DNA markers used to determine genetic variations within and between populations of a species. They detect the allele's size and loci which convey information for inbreeding and genetic variation. Microsatellites use a number of tandem repeats (1-6 bp). The number of repeats is variable within populations and alleles of an individual [Miah. G. 2013]. There are four different types of microsatellites based on the number of repeated base pairs:

- Mono (CCCCCC or AAAAAA)
- Di (CA CA CA CA)
- Tri (CCA CCA CCA CCA)

- Tetra (GATA GATA GATA)

Advantages and disadvantages of microsatellites:

Advantages:

- Highly polymorphic: microsatellites are able to determine the level and quantity of polymorphisms and homozygosity.
- PCR-based: the level of polymorphisms can be ascertained by mixing microsatellite markers and tiny amounts of tissue or "ancient" DNA with PCR reagents.
- Codominant: heterozygotes can be distinguished from homozygotes.
- Less expensive compared to other markers.

Disadvantages:

- Multiple bands in gel electrophoresis make it challenging to analyze the data: the analysis of microsatellites is difficult when sample numbers are high.
- Little information to interpret in terms of loci and alleles: microsatellites determine polymorphisms based on size.

Application:

Microsatellite markers have multiple applications including determining the level of inbreeding and crossbreeding within a population, distinguishing specific genes or pathways in a genomic map, and uncovering genes or pathways associated with diseases.

The role of microsatellite markers in inbreeding detection:

The level of inbreeding in a population is determined as follows:

If both alleles are determined to be the same length and size during gel electrophoresis the sample would be classified as homozygous. If the alleles are different lengths the sample would be identified as heterozygous [Vignal A. 2002]. Figure 2 illustrates gel electrophoresis of microsatellite markers amplified by mixing sample DNA with PCR components. The 1/2, 2/3 and 1/3 DNA samples showed two different lengths of amplicons which indicated heterozygosity; however, other samples contained one size of amplicon which illustrated homozygosity [CCMD].

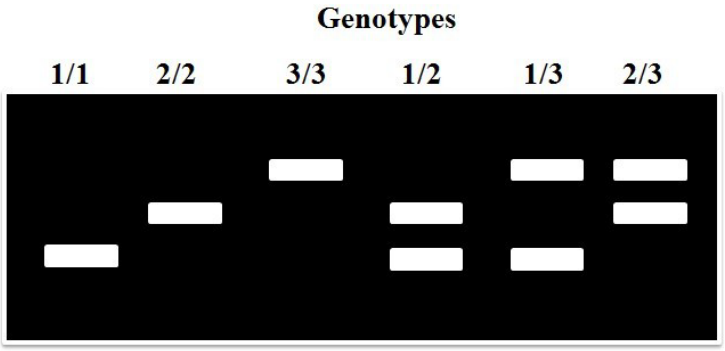

Figure 2: Stylized examples of microsatellite data. Data were produced by gel electrophoresis.

http://genomics.cafs.ac.cn/ssrdb/index.php?do=about

Single nucleotide polymorphisms:

In general, a DNA sequence is composed of four different nucleotide bases: A, C, G, and T. A SNP is a variation in a single nucleotide that occurs at a specific position in the genome. Single nucleotide variations may be classified as SNPs if >1% of a population possesses a different nucleotide in that position. Each SNP is present to some appreciable degree within a population. SNPs arise within coding (gene) and noncoding regions of DNA and create genetic variation between individuals.. When SNPs occur within genes,

different variations of amino acids lead to altered proteins. Some SNPs lead to genetic disorders, such as disease susceptibility or alteration of drug response. Experiments have been designed to discern these SNPs in order to determine the genes associated to specific diseases [Picoult-Newberg L., 1999]. Additionally, if certain SNPs are known to be related to a disease, regions of DNA near these SNPs can be examined to identify the gene(s) responsible for the genetic disorder [Vignal.A., 2002].

Disadvantages of SNPs:

- Fewer alleles (most SNPs are bi-allelic) equals less information: however, SNPs can be bi-, tri-, or tetra-allelic. Although, in humans, tri- and tetra-allelic SNPs are extremely rare.
- More expensive compared to other markers: microsatellites are less expensive to utilize.
- Difficult to interpret pooled data: not good for multiplexing as of yet.

Advantages of SNPs:

- Discover new genes associated with diseases: SNPs can be used to track the inheritance of genetic disorders within families along with complex diseases such as heart disease, diabetes, and cancer.
- Easy to determine the number of polymorphisms: by using SNPs with real time PCR or microarray, the number of polymorphisms and homozygosity can be determined.

Applications of SNP detection:

- Disease diagnosis: many SNPs are associated with genetic disorders. Based on the relationship between SNPs and disease genes, researchers can use SNPs to ascertain the gene(s) which corresponds to a particular disease [Lai E., 2001].
- SNPs have been advantageous in drug discovery: approximately 12 million true SNPs have been identified in the human genome. However, most have yet to be linked to disease susceptibility or drug response. Patients could receive individualized therapy through testing for the appropriate drug response SNPs.
- SNPs are important in identifiying mutations.
- SNPs are used in next generation sequencing to detect diseases and map genomes.
- SNPs are an accurate method for detection of genetic variation and inbreeding.
- SNPs are used to determine parentage in animals and humans.

The role of SNPs in determining inbreeding:

SNPs are used to determine the level of inbreeding in animals [Gazal S., 2014]. Gazal used different SNP panels to measure homozygosity against several estimators such as single-point estimates. This method is based on (ROHs). Figure 3 shows the results of the coefficient of inbreeding of individuals in populations utilizing SNPs.

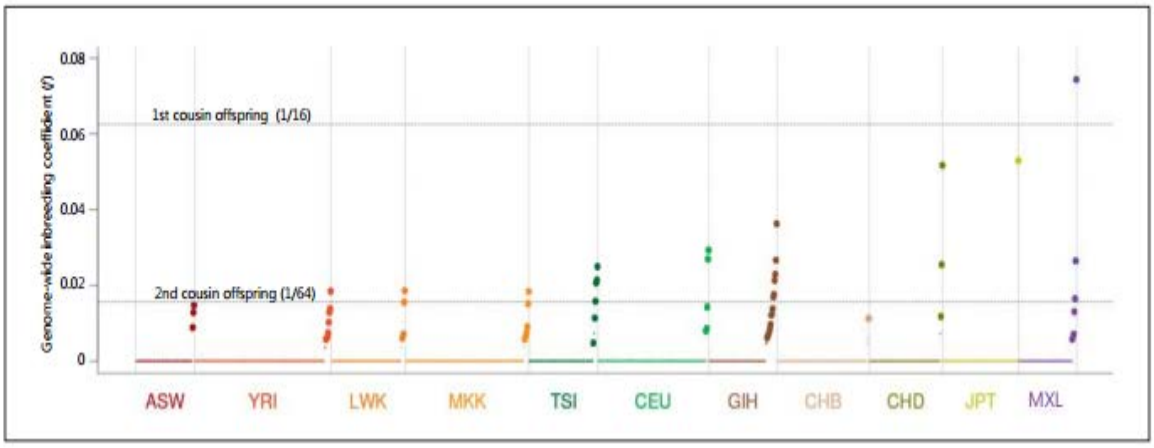

Figure 3: Inbreeding estimation and detection on HapMap III. Each point represents the f estimation for one individual [Gazal S., 2014].

Inbreeding depression

Inbreeding leads to decreased biological fitness. This phenomenon is called inbreeding depression and reduces the production, reproduction, survival and lifespan in populations [Charlesworth D., 2009]. Inbreeding depression is the result of three mechanisms:

- Inbreeding increases homozygotes in a population. Homozygotes occur when two dominant alleles or two recessive deleterious alleles are present in an individual. The probability of receiving recessive deleterious alleles is increased in inbred populations. In addition, the biological fitness of recessive deleterious alleles is zero, so inbreeding depression is enhanced in populations.
- The second mechanism of inbreeding depression occurs when over-dominance is increased in populations. The raising of over-dominance is called heterozygote advantage and leads to a reduction in biological fitness in homozygote genotypes,

when they are not recessive deleterious alleles. In addition, over-dominance causes higher fitness in offspring compared to the parent genotypes.

- Mutations are the third mechanism which causes inbreeding depression. In natural selection, mutations increase the presence of recessive deleterious alleles in populations and the likelihood of those alleles being passed to offspring, thus raising the inbreeding depression.

Figure 4 represents the role of an inbred individual to reduce fitness and heterozygotes in a population. If different alleles are located at the same loci of a gene over-dominance and biological fitness are increased (first box). When the population contains an over heterozygote genotype the recessive deleterious mutant alleles are increased, which raises homozygote frequencies for recessive deleterious mutant alleles within the inbred population (second box). Over-dominance exhibits a reduction in the biological fitness of homozygotes compared to outbred individuals (third box) [Charlesworth. D. 2009].

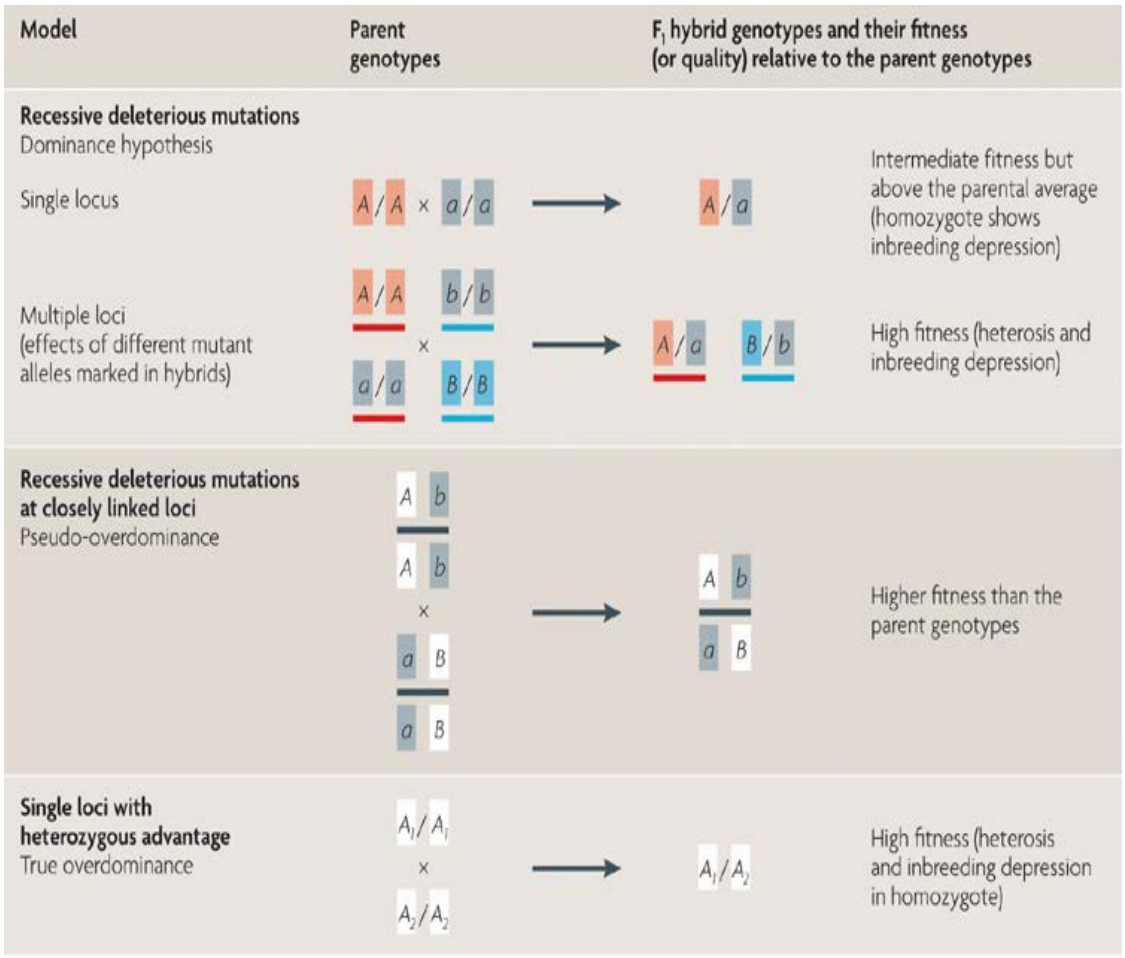

Figure 4: Role of an inbred individual to reduce fitness [Charlesworth. D. 2009].

Holstein-Friesian breed

History:

One of the most famous cattle breeds in the world is the Holstein-Friesian breed. Holstein cows exsisted in the Netherlands about 2,000 years ago. Near 100 BC, the people from Hesse migrated with their cattle to the island of Batavia. These cattle were black, and the Friesian cattle were white. The Holstein-Friesian breed was produced by crossbreeding the black cattle of the Batavians with the white cows of the Friesians**.** This

crossbreeding led to increased milk production and a reduction in the feed necessary to produce more milk [Elischer. M., 2014].

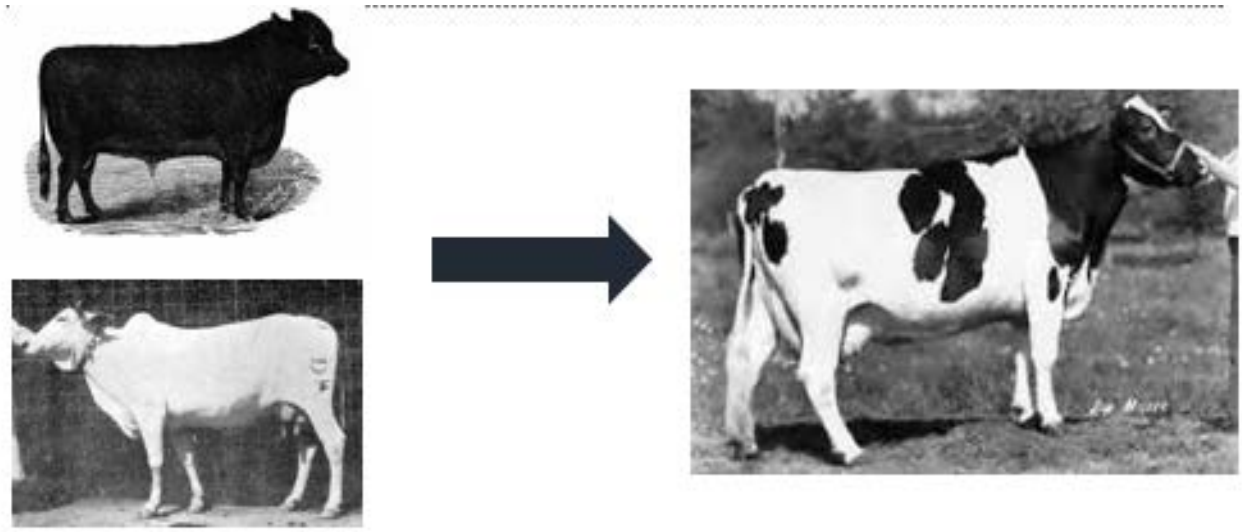

Figure 5. Crossbreeding of Batavia with Friesian [Elischer. M., 2014].

Between the 13th and 16th centuries, the Netherlands produced massive amounts of butter and cheese. Holstein cows were bred to produce as much milk and beef as possible. Based on these traits, Holstein cows were known throughout the world. The Holstein-Friesian breed was imported to the United States in 1985 by a Massachusetts man named Winthrop Chenery. In the late 18th century, the United States created the new breed of Holstein.

In general, Holsteins have the following characteristics:

- Mature cow: weighs about 1,500 pounds and is 58 inches long.
- Holstein calves: weigh 80 to100 pounds as new borns.
- Milk production: average cow produces about 25,000 pounds.

Holstein-Friesian breed production:

Milk:

One of the best products produced by cattle is milk. In Holsteins, the average production for 2008 was 23,022 pounds (10,443 kg) of milk, 840 pounds (380 kg) of butterfat and 709 pounds (322 kg) of protein [Britt J.S. 2003]. In general, Holsteins are known for high milk production, especially in comparison to the Jersey breed. However, they have less butterfat and protein based on percent composition in milk compared to other breeds [Areias M., 2003]. Table 3 compares the average milk production between Holstein and Jersey breeds and also shows the percentages of butterfat and protein.

Table 3: Breed-specific performance data inputs to the model. (USDA-National Agricultural Statistics Service, 2000, 2009; Khanal et al., 2010).

Performance characteristic	Holstein	Jersey
Daily milk yield,1 kg	29.1	20.9
Milkfat,1%	3.8	4.8
Milk protein,1%	3.1	3.7
Cheese yield,2 kg/kg of milk	0.101	0.125
Calving interval,1 mo	14.1	13.7
Dry period length,2 d	60	60
Annual turnover,1%	34.5	30
Expected number of lactations2	2.54	3
Age at first calving,1 mo	26.1	25.3
Heifer:cow ratio1	0.86	0.83
Heifers aged 0–12 mo,3%	46.2	48
Heifers aged >12 mo,3%	53.8	52
Prorated rbST response,6 kg/d	3.4	3.4

Beef:

Another important cattle product is meat. One of the best breeds for beef production in the United States is Holstein. Beef from Holstein cattle is a main source of the U.S. meat supply. Based on the full-term pregnancy rate [Nahms, 2002], gender distribution, dairy beef placement (80%) and survival to market (94%), 2.35 million Holstein steers are marketed annually. This population constitutes about 8.0-8.5% of the 28 million cattle harvested for beef in the U.S. [Mckenna et al., 2002]. According to Siemend's (1914) research, a comparison between Holstein and other beef cattle breeds showed the carcass of Holsteins is more efficient in the production of beef [Siemend G., M. 1914].

Table 4: Comparison of Holstein vs beef breed carcass yields [Siemend G., M. 1914]

Sub-Primal	Yield,% of carcass			Sub-Primal price/lb ($)	Value differences for Holstein per cwlof carcass
	Beef breed Choice	Holstein Choice	Difference		
Ribeye	3.4	2.9	-0.5	3.25	-1.63
Brisket	2.7	2.6	-0.1	1.03	-0.10
3-Way Chuck	23.7	21.8	-1.9	1.02	-1.94
Knuckle	2.9	3.0	0.1	1.40	0.14
Top Round	5.8	5.5	-0.3	1.55	-0.47
Gooseneck	7.3	7.0	-0.3	1.3	-0.39
Strip	3.9	3.0	-0.9	2.55	-2.30
Top Butt	3.4	3.3	-0.1	1.5	-0.15
Miscellaneous (5 items)	4.2	3.6	-0.6	2.13	-1.28
total	57.3	52.7	-4.6		-8.10

The impact of inbreeding on production in Holstein dairy cows:

Jiri Bezdicek's (2008) study showed the role of inbreeding on milk production in Holstein cattle. Their results indicated that inbreeding led to decreased milk production; however, fat and protein were increased. The effect of inbreeding and outbreeding on

milk production is shown in Figure 6 which indicates the reduction of milk yield in inbred animals.

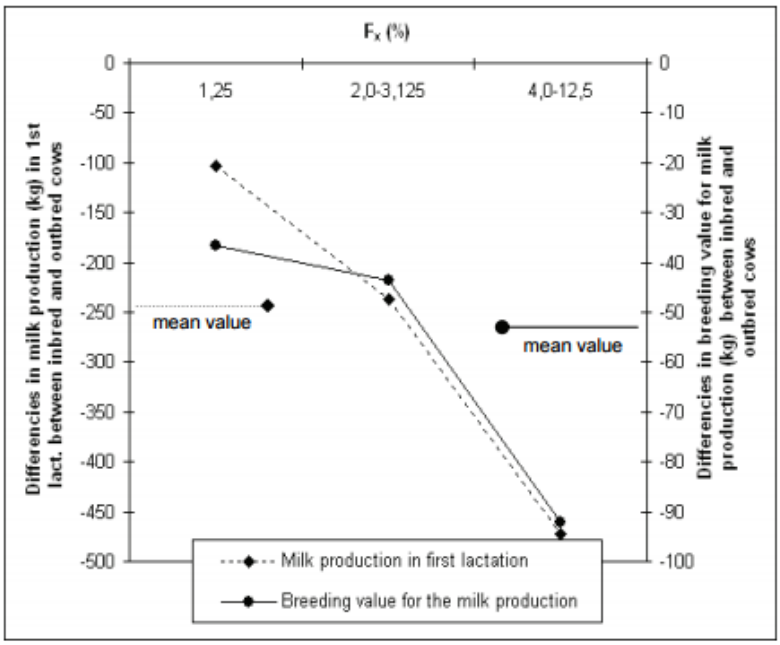

Figure 6: Differences between inbred and outbred cows on milk production [Bezdicek .M.2008].

According to Parland's (2007) research, inbreeding has a negative impact on health, milk quality, fertility, survival, and calving performance in Irish Holstein-Friesians. Results were analyzed for milk, fat, and protein yields. All milk productions were significantly reduced ($P<0.01$) with inbreeding. The results also indicated an association between increased calf inbreeding and a rise in the proportion of male births.

Management of inbreeding on dairy cow farms:

Inbreeding depression results in the reduction of biological fitness, health, milk production and its components, fertility, and calving survival. Increasing genetic variability reduces the impact of inbreeding depression within the population. Loss of

genetic variation increases homozygotes and decreases genetic fitness [Charlesworth. D., 2009]. Therefore, management of inbreeding depression is necessary to obtain high value productions and improve health and longevity in dairy farm cattle. Recently, genetic selection programs and assisted reproductive technologies, such as AI, have been employed to increase genetic variations and diminish the impact of inbreeding depression, thus enhancing production, health, reproduction, and longevity [Blair Murray, 2012].

Genetic selection programs in dairy cattle:

Genetic selection programs are used to increase production based on genomic information and genomic evaluations. One such genetic evaluation is genomic predicted transmitting abilities (GPTA) obtained from genome information. GPTAs contain important information allowing for selection of the best genetics based on individual sequencing differences (SNPs), thus improving production, health, reproduction and longevity on the farm. The level of inbreeding determined by GPTA is based on pedigree information [Rogers. G. W. 2008]. Genetic information is obtained by progeny testing, whole genome selection (SNPs), and genomic selection of males and/or females.

Progeny testing:

Progeny testing is a pedigree-based genetic selection program in dairy cattle [Robertson and Rendel, 1950]. Progeny testing functions by using AI to improve milk production and composition, fertility, lifespan, calving survival, disease resistance, and physiology of body. Progeny testing plays a role in dairy farm management and economics. It increases production on dairy farms by genetically selecting bulls, but is

limited to the lifespan of the animal [Normal et al., 2001]. For instance, when researchers used progeny testing in North American Holsteins their production was raised by roughly 90 kg of milk. A limitation of this method is cost. Progeny testing is not an economical method for improving traits.

Whole genome selection

Whole genome selection is based on the premise that genomic sequences of individuals differ within a population (SNPs). This may be used to estimate economic factors including milk production, health, fertility, and lifespan [Meuwissen et al., 2001]. Whole genome selection provides information regarding the phenotype and genotype of populations, which may be used as a reference for other populations. Information from other populations is compared with this reference control to determine and estimate SNPs and measure GPTA. GPTA provides performance and genomic information. It is used to select a reference population of bulls with high reliability (REL). REL correlates with genetic deviation in that a higher REL represents increased genetic variation within a population.

Genomic Selection of Males

A common method employed in genetic selection is selection of dairy bulls. The potential sire is chosen based on GPTA. The candidate bull is tested against progeny-tested bulls to determine GPTA, with high GPTA values being preferred. These bulls are used for AI to improve the genetic variation and production of the dairy farm. In addition, semen from selected bulls can be used for subsequent generations [Schefers and Weigel, 2012].

Genomic Selection of Females

Genomic selection of females involves choosing heifers based on family production records. However, the accuracy of this selection is low. Candidate mothers are then used for embryonic transfer or AI.

Assisted reproductive technologies in breeding of dairy farm cows:

Cattle are economic domestic animals which have a special role of providing food for human consumption such as beef and milk. In addition, cattle are utilized in biomedical and reproductive research. Biotechnology applications are used to propagate genetic material during breeding which reduces inbreeding depression in populations. Reproduction biotechnology (assisted reproductive technologies) is a management method for improving the efficiency of production and development on dairy cattle farms. Assisted reproductive technologies are common technologies which include AI, superovulation and embryo transfer (MOET), in vitro fertilization (IVF), and cloning, such as somatic cell nuclear transfer (SCNT). Genetic variation is increased by introducing new genetic material into the population. Assisted reproductive technologies reduce the impact of inbreeding depression in populations and increase the economic production in dairy cows, such as milk production, milk components, reproduction, health, and longevity [Faber et al 2003, Gardner & Seidel 2008, Seidel 2009].

Artificial insemination:

AI is a common method employed by farms to introduce good quality sperm to multiple females and improve production and reproduction. This application may increase genetic variation by 50%, and the semen may be preserved in liquid or deep

frozen [Vishwanath. R. 2003]. AI utilizing frozen sperm with high records of production, such as beef quality and milk production, is used all over the world. The efficiency of AI depends on the quality of the sperm with the effectiveness of frozen semen being reduced. However, the efficiency of fertility in fresh-liquid conserved semen increases to >80% [Garner & Seidel. 2008]. If selection of sperm is based on milk production, not reproductive traits, the reproductive success is reduced. For example, American Holsteins focus on milk production; therefore, there is a reduction in reproductive success. [García. 2011].

Figure 7: Artificial insemination technique [Vishwanath. R. 2003].

Advantages and disadvantages of AI:

Advantages:

- No extra cost to maintain bulls for breeding (cost reduction)
- Reduction of genital diseases
- Increased genetic variation and diminished negative impact of inbreeding depression

- Easy to do progeny testing at an early age
- Ability to introduce good quality sperm to many females
- Improvement in production, reproduction, health, and longevity
- Easy breeding of different sized animals

Disadvantages of AI:

- Training of personnel is required
- Quality of sperm, equipment, and technique influence AI success
- If the bull is not properly tested, genital diseases may increase

Embryo transfer:

Embryo transfer (ET) is a technology used to distribute genetic material and variations. Like AI, ET allows the selected genetic material to be transferred with a reduced risk of disease transmittance, thus providing a healthy environment for the new embryo. The quality and size of oocyte, environmental factors, such as temperature, secretion hormones, such as luteinizing hormone (LH), management of the estrous cycle, follicle development, and superovulation influence the efficiency of ET [Bo. 2003]. A cow normally produces only one egg per estrus cycle, and the gestation period is 40 weeks. On average, a cow produces only 2-3 calves in her lifetime. By using ET reproduction is increased and genetic traits improved. Both estrus synchronization and superovulation techniques are typically used in ET. Embryo transfer is done by synchronization of donor and recipient cows to set the same time point in their cycle. The donor cows undergo superovulation and then AI to produce an embryo. Embryos are collected by surgical and non-surgical flushing and cultured in a lab until the blastocyst

stage of development. Embryos are then transferred to recipient cows. By using this method, a cow can produce 10-20 embryos a year [Presicce et al., 2011].

Advantages and Disadvantages of ET [Flossie Sellers. 2011]**:**

Advantages:

- More than one embryo produced per donor per season
- Cows possessing desirable traits but have sustained a physical injury can reproduce with ET
- Aged donor cows can continue to reproduce
- Risk of transmitting diseases to embryo is reduced

Disadvantages:

- Procedure is expensive
- Special training and equipment are necessary
- Efficiency depends on environmental conditions, such as quality of oocyte

Estrus synchronization

Estrus synchronization is one method used to regulate and manage the estrus cycle. Estrus synchronization is critical in animals bred at the beginning of the breeding season. Synchronization allows for increased use of AI. The three most common methods of synchronization are:

1) PGF2a injection

2) GnRH-PGF2a- GnRH protocol: GnRH is injected on days 1 and 10 with PGF2a being injected on day 8. Insemination is recommended on day 11.

3) CIDR (Controlled Internal Drug): The CIDR is placed into the vagina for 7 days. PGF2a is injected on day 6 after CIDR implantation. Estrus is observed on day 8. The CIDR is then removed and a GnRH shot administered before timed AI [Chakravarthi. V. 2010].

Superovulation:

Superovulation, also known as controlled ovarian hyper-stimulation, is the process of inducing a cow to release more than one egg per month. It is different from ovulation induction where the goal is to release one egg a month. This is especially important in monoovulatory animal species such as cows. Common superovulation protocols involve injection of follicle stimutlating hormone (FSH) every day for 4 days followed by an injection of PGF2alpha on the last day [Chakravarthi. V. 2010].

Impact of AI and genetic selection on the Holstein-Friesian breed:

Coleman (2009) used three different Holstein-Friesian breeds (LowNA, average-genetic-merit North American Holstein-Friesian; HighNA, high-genetic-merit North American Holstein-Friesian; HighNZ, high-genetic merit New Zealand Holstein-Friesian) to measure genotypes and performance, such as fertility traits and survival, after first calving. The results indicated that fertility and rate of pregnancy are highest in the HighNA and HighNZ genotypes, which received AI earlier in the breeding season, compared to the LowNA group. The results suggested genetic differences in the groups influenced fertility performance [Coleman. J. 2009].

Summary:

Inbreeding influences the performance of animals. Heterozygosity and inbreeding depression are increased within inbred populations. Inbreeding depression leads to a reduction in biological fitness which decreases milk productions, milk components, fertility, calving survival, and lifespan and increases genetic disorders and diseases. The Holstein-Friesian breed is known as one of the highest performers compared to other breeds in milk and beef production and plays a significant role in providing food throughout the world. Inbreeding depression reduces biological fitness, milk production and milk components in the Holstein-Friesian breed. Outbreeding leads to an increase in biological fitness, health, fertility, production, and longevity. On dairy farms, breeding management and controlled inbreeding is necessary to obtain the highest economic performance. Genetic selection and assisted reproductive technologies are the most common methods to maintain genetic variation and improve performance and health in the Holstein-Friesian breed.

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