BULL GENOMICS AND FERTILITY

R. Kasimanickam, BVSc, DVSc, DACT,
V. Kasimanickam, DVM, MS, DACVPM,
Department of Veterinary Clinical Sciences, Washington State University, Pullman WA, USA
V. Koziy, doctor of vet.sci.
Bila Tserkva National Agrarian University
V. Ladyka, doctor of agr. sci.,
A. Kraevskiy, doctor of vet. sci.
Sumy National Agrarian University

Reproductive efficiency is the primary economic factor for the commercial success of a cattle operation. In a management system using natural service or artificial insemination, bull fertility is more important than fertility of any individual cow because a sire will produce higher number of calves in his lifetime compared to a cow. Thus, bull selection is an important decision as they contribute to future genetics of the farm beyond their lifetime. Genetic make-up of all bulls is not equal - there are high performance bulls with excellent genetic merit and there are some others recorded as poor performers. This paper provides with important tactic and criteria of bull selection by employing clinical and laboratory approaches and details how genomics could be applied in selection of bulls for desired productive and reproductive traits.

To effectively select sires, producers must use selection tools and understand within and between breed differences. In addition, producers must also accurately and objectively assess their current genetics, nutritional resources, and management. This will help producers with decision making. The selection and addition of bulls must not only meet revenue improving priority traits but also compliment other important production traits. The recent advances in DNA/genomic technology and decision support tools will enhance selection accuracy.

Key words: *bull, reproduction, genomics, fertility, selection*

Introduction. Reproductive efficiency is the primary economic factor for the commercial success of a cattle operation. Many factors may influence reproductive efficiency and may broadly be classified as (1) the bull (2) the cow (3) the method of insemination (4) the conditions of herd management (5) chance. In a management system using natural service or artificial insemination, bull fertility is more important than fertility of any individual cow because a sire will produce higher number of calves in his lifetime compared to a cow. Thus, bull selection is an important decision as they contribute to future genetics of the farm beyond their lifetime. Genetic make-up of all bulls is not equal - there are high performance bulls with excellent genetic merit and there are some others recorded as poor performers. This paper provides with important tactic and criteria of bull selection by employing clinical and laboratory approaches and details how genomics could be applied in selection of bulls for desired productive and reproductive traits.

Key for determination of sire fertility. Finding a sperm population with attributes for fertilization and ability for embryonic development by a quick screening of multi-parametric methods would allow for a better estimation of fertility, provided the particular bull produces this sperm population in a repeatable manner.

There are numerous parameters used to determine sire fertility outcome. It is advisable to select the parameter which accounts for other factors which could potentially influence the sire fertility.

1. *Non-return rate:* the proportion of cows not seen to come back into estrus within a specified period after breeding, and are thus considered pregnant. They can be specified as 28, 35, 60 or 90 day non-returns depending on the interval since mating.

2. *Estimated relative conception rate (ERCR):* is a measure of conception rate of a service sire relative to service sires of herd-mates. ERCR is a phenotypic predictor of bull fertility, expressed as a relative conception rate.

3. *Sire conception rate (SCR):* the deviation of mean conception rate of an A.I. bull of interest from the mean conception rate of all published A.I. sires of same breed is

4. *Competitive or heterospermic index:* ranking of sires based on their reproductive performance following heterospermic insemination. Heterospermic indices were calculated to express the relative ability of sires to father offspring after heterospermic insemination with semen from two or more males mixed with an equal number of spermatozoa from each male.

Approaches to determining sire fertility. Bull fertility can be estimated by applying following modalities: breeding soundness evaluation; application of testes specific to sperm organelles and its association with reproductive outcome; correlation of mRNA expression of genes those are important for sperm structural and functional parameters with fertility outcome and application of genomics.

These approaches have advantage and disadvantages. Even though these methods have merits over one another when applied individually (Fig. 1), it is advisable to use combination of these tests to predict sire fertility.

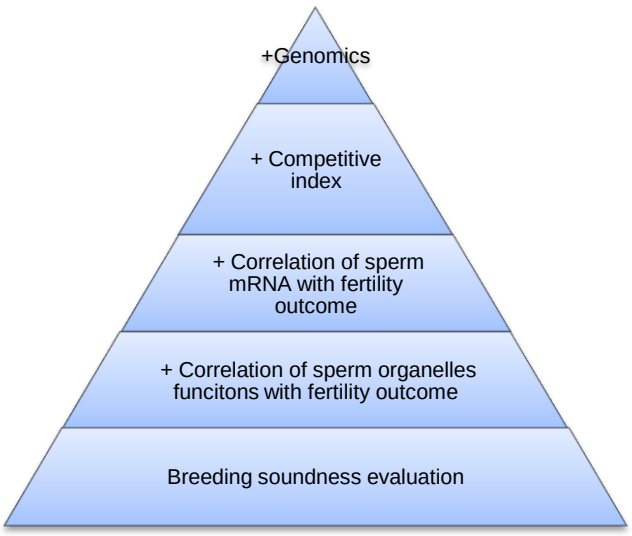

Fig. 1. **Hierarchy of sire fertility evaluation methods**

Application of breeding soundness evaluation. Breeding soundness evaluations (BSE) are commonly used for identifying bulls that have potential for satisfactory fertility and those that are clearly unsatisfactory. Bulls should be evaluated for structural and physical soundness, breeding soundness and for venereal diseases. The bulls should have good structural and physical soundness, meet the Society for Theriogenology breeding soundness evaluation recommendations and be with no venereal diseases. Based on physical examination, scrotal circumference, and semen parameters, bulls are classified into three categories: satisfactory, questionable and unsatisfactory for breeding potential [1]. Bulls that pass a BSE have 6% higher fertility than untested bulls. It is estimated that 20% of bulls in an unselected population are subfertile, emphasizing the importance of selecting bulls that are satisfactorily potential breeders. It is important that BSE of bulls be done in a highly professional manner. Errors, lack of repeatability in evaluation methods and lack of agreement between clinicians on the classification of bulls have resulted in dissatisfaction by some producers. Even though most commonly used method in the clinical field the test the determination of fertility is limited to the test day.

Application of laboratory methods to determine the association of sperm organelles function and fertility. A spermatozoon is a multi-compartment cell and must possess several attributes for a successful fertilization. It should have motility and morphologically normal. In field conditions, evaluation of motility and morphology of a semen sample are common methods for estimating breeding potential. However, the usefulness of these parameters to accurately measure fertility of a semen sample is limited.

The ultimate goal of semen evaluation is to predict the fertilizing capacity of an ejaculate. It is generally accepted that conventional sperm characteristics are not well correlated with the fertilizing capacity of sperm and that both inter- and intra-assay variability of these characteristics are high. Hence, it is challenging to predict fertilizing capacity, as there is no single sperm parameter that accurately predicts fertility in vivo. Therefore, advanced evaluation techniques of semen are needed to increase the odds of achieving an accurate prediction. Researchers have used additional laboratory assays to accurately predict the fertilizing potential of a semen sample [2-4]. Among these are assays that evaluate sperm DNA Fragmentation Index (DFI), sperm membrane integrity and other sperm organelles. It should be noted that individual laboratory assays, which evaluate a single parameter, are not effective predictors of the fertility; however, a combination of several assays may provide a better prediction of fertility.

We conducted several studies [5, 6] to determine the association of intactness of sperm organelles with fertility outcome, competitive index. The results indicate that: (i) the chance of siring calves was low for a bull with higher sperm lipid peroxidation; (ii) the chance of siring calves was low for a bull with higher DFI; (iii) the chance of siring calves was high for a bull with a higher PMI and (iv) the bulls with higher sperm lipid peroxidation were more likely to have a high DFI and low PMI.

Application of sperm mRNA expression. Proteins present in sperm have distinctive functions and are essential in preparing sperm for fertilization in a timely manner. Understanding the function of individual sperm protein may explain male infertility. Selection of bulls with these biomarkers may lead to improved fertility. We conducted several studies [7-10] to determine the association of intactness of sperm mRNA expression of genes with functions related to male fertility with sire conception rate. The results indicated that these mRNAs were expressed abundantly in high fertile bulls than low fertile bulls (Table 1).

Table 1 − **mRNA abundances of sperm functional and structural biomarkers and their association to fertility**

Protein	Function	Association to fertility*
CRISP2	Sperm capacitation and sperm-egg fusion	Positive
PEBP1	Sperm capacitation and sperm-egg fusion	Positive
CCT8	Indicator for the presence of immature cells	Negative
AK1	Motility	Positive
IB5	Fertilization and early embryo development	Positive
Doppel	Acrosome function and fertilization	Positive
TIMP2	Acrosome function and fertilization	Positive
AQP7	Membrane water channel	Positive
Adiponectin	Fatty acid oxidation; membrane integrity	Positive

Notes: *CRISP2 − Cysteine-Rich Secretory Protein 2; CCT8 − Chaperonin containing T complex protein 1, sub unit 8; PEBP1 − Phosphatidylethanolamine binding protein 1; AK1 − Adenylate kinase 1; IB5 − Integrin beta 5; TIMP2 − Tissue inhibitors of metalloproteinases 2; AQP7 – Aquaporin 7.*

*******High fertile bulls showed increased expression.*

Interestingly, these genes are regulated by following miRNAs – miR-17-5P, miR-20A, miR-20B, miR-106A, miR-106B, miR-410miR-432, miR-452, miR-519A, miR-519B, miR-519C, miR-519D, miR-520D. It should be noted that miRNA regulation for CRISP2, SNRPN and PLCz1 genes were not identified yet.

Application of genomics. Genomic predictions combine genotypic, phenotypic, and pedigree data to increase the exactitude of estimates of genetic merit and to decrease generation interval. Traditional genetic evaluations combine only phenotypic data and probabilities that genes are identical by descent from pedigree data instead of tracing the inheritance of individual genes. Widely spaced markers could indicate the sharing of long chromosome segments within closely related family members, but could not detect the many minor genetic effects shared by distant relatives. Marker genotypes for thousands of loci across the genome can measure genetic similarity more precisely. Markers that are identical in state may be shared through common ancestors earlier than those in the known pedigree.

A genetic marker (single nucleotide polymorphisms or SNPs) is the difference in the DNA sequence at same point between two animals. Genomic selection involves identifying which SNPs are linked to important functional traits and selecting bulls with DNA patterns to produce superior priority traits. This genomic information has many advantages, including: potential to speedy genetic improvement; enables bull selection from a much wider genetic pool; provides earlier information about genetic differences between siblings; predicts genetic merit of young animals with more accuracy; improves the reliability of current progeny testing results for low-heritability; cost effective. For example identifying elite dairy sires relied on a tedious progeny-testing scheme took 6 to 7 years and cost approximately $35,000 per bull. It is now possible to evaluate the genetic merit of a preimplantation embryo with comparable accuracy for less than $100.

This tool has advantage not only providing information for selection of bulls with genetic make up for superior priority traits but also providing information for elimination of bulls with low genetic make-up for the same priority traits.

Its disadvantages have also to be taken into account − intense selection may lead to detrimental erosion of domestic diversity, cost and the fact that they are more advantageous for front users.

Recently, genomic selection has been adopted globally by cattle industries to accelerate genetic gains. To meet projected global demands for milk and meat, rates of genetic gain must be further accelerated without disquieting animal health and welfare. Improved accuracy of genomic predictions and rapid identification and management of genetic defects could be achieved if genome sequence data were available for large numbers of cattle phenotyped for traits of interest. However, given the genetic architecture of production traits in cattle, in which large numbers of loci individually explain relatively little genetic variation, the number of individuals required with both phenotype and genomic sequence would be cost prohibitive.

1000 bull gnome project. The 1000 bull genomes project supports the goal of accelerating the rates of genetic gain in domestic cattle while at the same time considering animal health and welfare by providing the annotated sequence variants and genotypes of key ancestor bulls.

The aim of the 1000 bull genomes project is two-fold: (i) to build a database of sequence variant genotypes of individuals, ideally key ancestors, from modern cattle breeds that enables sequence-based genome-wide association studies (GWAS) and genomic prediction and (ii) to enable the use of these same data to rapidly identify mutations that compromise animal health, welfare and productivity.

In the first phase of the 1000 bull genomes project, the whole genomes of 234 cattle were sequenced to an average of 8.3-fold coverage. This sequencing includes data for 129 individuals from the global Holstein-Friesian population, 43 individuals from the Fleckvieh breed and 15 individuals from the Jersey breed. A total of 28.3 million variants, with an average of 1.44 heterozygous sites per kb for each individual were identified. The use of this database in identifying a

recessive mutation underlying embryonic death and a dominant mutation underlying lethal chrondrodysplasia was demonstrated. Currently more data of more than 1000 bulls are added.

Sequence based genomic selection: Genomic selection generating prediction equations from the joint analysis of 10 to 50K SNP genotypes and phenotypes recorded in a large reference populations (>20000 individuals). These equations can then be used to predict genomic breeding values of test animals from their SNP genotypes aline. The orange ares ahsoe=ws that sequence database of the 1000 bull genome project allows for imputations of genotype of millions of additional DNA variants for both reference and test animals to generate more robust prediction equation and genomic breeding value (Adopted from D. Maizels/Nature).

Recently a genome-wide association study by Penagaricona et al. [11] identified eight SNPs that showed significant association with SCR. Some of these SNPs are in the genes with functions related to male fertility, such as the sperm acrosome reaction, chromatin remodeling during the spermatogenesis, and the meiotic process during male germ cell maturation (Table 2).

Table 2 − **Single nucleotide polymorphism and genetic markers associated with bull fertility and their functions**

SNP	Gene	Function
Hapmap38225-BTA-43804	ZNF541	Chromatin remodeling
ARS-BFGL-NGS-4009	CACNA1H	Calcium channel
ARS-BFGL-NGS-31020	LOC521021	Lipid metabolism
ARS-BFGL-NGS-13272	ROGDI	Cell proliferation
ARS-BFGL-NGS-13853	LOC617302;PRSS21	Male germ cell maturation
BTB-01354898	-	Scrotal circumference and sperm production
Hapmap44380-BTA-46707	DYNC1I2	Nuclear migration; postmeiotic spermatid development
(ARS-BFGL-NGS-116417	LOC784935; cpb-1	Spermatogenesis

These results could contribute to the identification of genes and pathways associated with male fertility in dairy cattle and subsequent use of marker-assisted selection for male fertility in commercial breeding schemes.

Conclusions. Bull selection is one of the most important decisions because it offers an opportunity to enhance the genetic merit and profitability of the farm. To effectively select sires, producers must use selection tools and understand within and between breed differences. In addition, producers must also accurately and objectively assess their current genetics, nutritional resources, and management. This will help producers with decision making. The selection and addition of bulls must not only meet revenue improving priority traits but also compliment other important production traits. The recent advances in DNA/genomic technology and decision support tools will enhance selection accuracy. Producers who utilize these advances in cattle genetics in the selection process should not only gain profit from improved revenue and reduced production costs but to best match genetics for their farm's production demands. Also it is important to use all available tools such as phenotypic, genetic information, information from close relatives and individual performance.

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Касіманікам, Р., Касіманікам, В., Козій, В., Ладика, В., Краевский, А. ГЕНОМІКА ТА ЗАПЛІДНЮВАЛЬНА ЗДАТНІСТЬ БУГАЇВ-ПЛІДНИКІВ

Репродуктивна ефективність є важливим економічним чинником для комерційного успіху скотарства. Багато факторів можуть впливати на репродуктивні показники стада. За використання як природного так і штучного осіменіння, репродуктивна здатність бугая є більш важливою, ніж плодючість будь-якої окремої корови, так як від плідника, впродовж його використання, отримують значно більшу кількість телят, ніж від корови. Тому, вибір якісного бугая-плідника є важливим завданням фермера. Бугаї відрізняються за своєю генетичною цінністю. В статті розглядаються особливості тактики і критеріїв відбору бугая з використанням клінічних та лабораторних підходів і докладно описано, яким чином геноміка може бути використана при відборі плідників для отримання бажаних продуктивних і репродуктивних якостей.

Було встановлено, що для ефективного вибору бугаїв-плідників, виробники повинні розуміти особливості генетичних характеристик в межах і між різними породами тварин. Вони також повинні уміти точно і об'єктивно оцінити поточний генетичний потенціал стада з урахуванням особливостей кормової бази і системи управління. Вибір і додавання биків повинні не тільки удосконалювати виробничі характеристики, а й доповнювати інші важливі виробничі риси. Останні досягнення в області ДНК / геномних технологій і напрацювання відповідних інструментів підтримки дозволяють підвищити точність і якість вибору.

Ключові слова: *бугай, репродукція, геноміка, заплідненість, селекція.*

Касиманикам, Р., Касиманикам, В., Козий, В., Ладыка, В., Краевский, А. ГЕНОМИКА И ОПЛОДОТВОРЯЮЩАЯ СПОСОБНОСТЬ БЫКОВ-ПРОИЗВОДИТЕЛЕЙ

Репродуктивная эффективность является важным экономическим фактором для коммерческого успеха скотоводства. Многие факторы могут влиять на репродуктивные показатели стада. При использовании как естественного, так и искусственного оплодотворения, репродуктивная способность быка является более важной, чем плодородие любой отдельной коровы, так как производитель, в течение его использования, дает значительно большее количество телят по сравнению с коровой. Поэтому, выбор качественного быка-производителя является важной задачей фермера. Быки отличаются своей генетической ценностью. В статье рассматриваются особенности тактики и критериев отбора быков с использованием клинических и лабораторных подходов и подробно описано, каким образом геномика может быть использована при отборе быков для получения желаемых качеств.

Было установлено, что для эффективного выбора производителей, фермеры должны понимать особенности генетических характеристик в пределах одной и между разными породами животных. Они также должны уметь точно и объективно оценивать текущий генетический потенциал стада с учетом особенностей кормовой базы и системы управления. Выбор и добавление быков должны не только совершенствовать производственные характеристики животных, но и дополнять другие важные хозяйственные черты. Последние достижения в области ДНК/геномных технологий и наработки соответствующих инструментов поддержки позволяют повысить точность и качество выбора.

Ключевые слова: *бык-производитель, репродукция, геномика, оплодотворение, селекция.*