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Molecular, cellular, and physiological determinants of bull fertility

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Abstract: Bull fertility, defined as the ability of the spermatozoon to fertilize and activate the ovum and then support embryonic and even fetal development, is a crucial factor influencing animal reproduction and production efficiency. Despite significant influences of fertility on herd genetic improvement and efficient cattle production, the mechanisms regulating this phenotype and reliable biomarkers associated with bull fertility are poorly defined. Specifically, there is a broad lack of knowledge in bull fertility. In this paper, bull fertility is reviewed within the context of animal systems physiology, and system biology markers associated with bull fertility are provided. This mini-review is intended to be a useful resource for students, animal producers, and the general public to help understand and appreciate this economically important trait.

Key words: Bull fertility, sperm, markers, systems biology, systems physiology

1. Introduction

Bull fertility, the ability of the sperm to fertilize and activate the egg and sustain development, is crucial for efficient production of cattle. Bull fertility is measured by the number of viable animals that have been sired by a specific bull (1). The economics of sustainability of animal agriculture depends on obtaining the highest conception rate from genetically superior sires. Commercial artificial insemination (AI) companies continuously strive to develop methods to identify high fertility sires so that beef and dairy producers can capitalize on desired genetics to improve their herd using frozen semen.

During the 50 years since the commercial usage of AI began in the animal breeding industry, it has led to established improved methods and animal recordings. Widespread usage of AI allows semen from one bull to be used to inseminate thousands of females. Thus, bull effects are paramount on herd genetics, dynamics, and production. Use of sperm from a low fertility (or infertile) bull leads to lower pregnancy rates, which then results in greater economic costs of housing these bulls and nonpregnant cows. Today's beef and dairy producers and breeding companies work together to pool information not only on production traits, but also from reproductive outcomes of each breeding. Through widespread use of superior genetic sires, major genetic progress has been made in dairy cattle such that today's cattle produce 346% more milk than their counterparts in 1945 (2).

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Major semen technology companies obtain the fertility records from many well-managed partnering dairy farms. Even though most of the breeding records come from farm management software, the error rate can still be very high, leading to miscalculation of sire fertility. To overcome misidentification, some breeding companies have integrated DNA verification technologies. Thus, sire fertility estimates are computed with DNA verification of the paternity of the offspring and pregnancy diagnoses verified by veterinary palpation or ultrasound, thereby allowing accurate determination of both male and female fertility traits. Typically, fertility evaluations are predicted from a pool of several hundred bulls with thousands of breedings per bull. Breeding events and environmental and herd-management factors that influence fertility performance of sires (i.e. effects of herd-year-month, parity, cow, days in milk, and sire proven status) are all taken into account using sophisticated statistical models to predict fertility scores of each bull so that farmers can select not only good genetic traits but also high fertility sires. Reliable fertility data are essential for research as well as production. For example, the fertility prediction for individual bulls can be obtained using the Probit.F90 software and is expressed as the percent deviation of its conception rate from the average conception rate of all bulls (3,4).

Significant fertility differences exist among bulls producing spermatozoa with apparently normal motility,

morphology, and numbers. This is considered "uncompensable fertility phenotypes", where increasing the number of sperm deposited into a cow's reproductive tract will not increase the fertilizing success because of specific molecular defects in the sperm (5,6). Fertility is a complex trait influenced by many factors such as genetics, epigenetics, environmental factors, and epistasis. As a result, heritability of fertility is low (5). Although genetic factors influence fertility, environmental factors such as climate, nutrition, and management have significant effects on bull physiology and thus fertility. As an example, extremes in climate, long transportation, or unbalanced rations all influence reproductive function of bulls. Precise control of sperm production and maturation through the endocrine system is extremely important to optimize fertility. For example, concentrations of follicle stimulating hormone (FSH) and inhibin are essential for sperm output and viability (7). Balanced nutrition during development of male calves has a significant impact on testicular steroidogenesis as a result of the enhanced Leydig cell function. Because of the complicated, diverse, and sensitive factors involved in bull fertility, low fertility or infertility is prevalent.

Many challenges related to bull fertility exist. Despite huge economic costs associated with low fertility, molecular, cellular, and physiological mechanisms controlling bull fertility are still largely unknown. In addition, no reliable or cost-effective fertility markers exist to predict semen quality and bull fertility. There is a general lack of knowledge of the fundamental biology of how sperm attributes and physiological systems influence bull fertility. Because multiple physiological systems within the animal are involved, physiological mechanisms regulating bull fertility need to be elucidated and understood. In this article, major determinants of bull fertility at the molecular, cellular, and physiological levels are reviewed. This comprehensive synthesis contributes to advances in the science of bull fertility and applications of new knowledge to improve reproductive efficiency of cattle. Because of the significant similarities in physiology and the genome biology among many mammals, this review should help in better understanding male fertility in many other species, among them the human being, horse, swine, and goat.

2. Determinants of bull fertility

2.1. Physiological determinants of bull fertility

Spermatozoa develop within the walls of the seminiferous tubules of the testes by the complex process of spermatogenesis. In the bull, spermatogenesis occurs over a 65-day process in which the spermatogonial stem cells undergo first mitosis, then meiosis, and subsequent physiological and morphological changes to produce mature spermatozoa in the epididymis. Both cellular and molecular integrity of sperm are essential for the sperm to fertilize and activate the ovum, and then sustain early embryonic and subsequent fetal development. Because these processes are influenced by a number of intrinsic and extrinsic factors, the heritability of fertility is low (8,9). If the fertility phenotype is not identified in animal selection, major economic losses can occur due to expenses associated with housing of nonpregnant females as well as bulls that are not of value as they are unable to impregnate females.

In general, a bull can produce 4 to 5 billion sperm in a single ejaculation. If the ejaculate contains a large number of sperm afflicted with any of a variety of abnormalities, fertility of the bull will be expected to be low (10). This low fertility can be improved by increasing the number of sperm used in AI if it is a compensable phenotype, but not in the case of uncompensable fertility characterized by molecular defects in the sperm (3). In general, success of a mating depends on both quality and quantity of semen delivered to the female. However, no correlation exists between number of sperm per AI dose and maximum fertility of a bull (11). Males differ not only in fertility but also in the minimum number of sperm per insemination required to reach maximum fertility (11). Sire fertility estimates are of most value to the producer when they are used as secondary selection criteria after a group of bulls that meet the genetic goal(s) of the herd have been selected (12).

Production of viable sperm is influenced by many systems within the whole animal. The primary organs that produce the necessary hormones and regulators involved in sperm production are the hypothalamus, pituitary gland, and testes. The endocrine system maintains the critical balance between cellular requirements and concentrations of hormones. Key hormones involved in sperm production include gonadotropin-releasing hormone (GnRH), FSH, luteinizing hormone (LH), testosterone, and inhibin. The male reproductive system must maintain the proper balance of these hormones. If the balance is threatened or altered, normal sperm production is then changed and infertility could become an issue (7).

Endocrine disruptors are chemicals that act as agonists or antagonists to hormones and interfere with hormonal balance. Endocrine-disrupting compounds [such as dichlorodiphenyltrichloroethane (DDT), glycol ethers, dibromochloropropane (DBCP), and methoxychlor] modulate endocrine signaling pathways and activate or inhibit estrogen and androgen receptors, and, thereby, negatively affect male fertility, even causing low sperm count (7). Other systems such as the digestive, immune, and cardio-

vascular systems are important for bull health and sperm production. For example, diseases, especially testicular diseases, can alter sperm quality and cause low fertility or infertility. Seminal vesiculitis and epididymitis are common diseases of the secondary sex organs of the bull, in which collected sperm must be discarded if these diseases are present (13). Hence, study of systems physiology is essential in order to examine the reproductive health of the sires to identify changes that could affect bull fertility. The economic impact of maintaining and housing low fertility bulls is a cost that must be studied and overcome to cull these bulls at an earlier age. Economic savings from eliminating low fertility bulls will have a huge impact on a producer's herd (14).

Overcoming environmental challenges (nutrition, climate, and management) is paramount for producers to maximize reproductive efficiency and genetic improvement. A combination of short photoperiod, cold stress, and reduced feed quality will have detrimental effects on semen quality and spermatogenesis in bulls. A well-managed nutrition program should meet nutrient requirements to ensure that animals are not under- or overfed. Overfeeding can have negative effects on reproductive performance as increased scrotal temperatures reduce sperm production and the quality of stored sperm (15). The 3 periods on which to focus nutrition are preweaning nutrition, postweaning nutrition, conditioning prior to breeding season, breeding season, and postbreeding season (16). Nutrition may have a major impact on secretion of gonadotropins and consequently sexual development in bulls (17,18).

Climate (heat, cold, wind, humidity) can affect sperm number, morphology, and physiology. Ambient temperature between 5 °C and 15 °C is optimal for semen production (19). Paying close attention to body condition and providing bedding such as hay and shelter from wind and weather during the winter months helps prevent losses of bull breeding capability.

2.2. Cellular determinants of bull fertility

Sperm are produced in the testes, which consist of Leydig cells and seminiferous tubules. The seminiferous tubules contain the somatic Sertoli cells that nurse germ cells where sperm are produced. Leydig cells produce the sex hormones, primarily testosterone. The male reproductive system also includes other components that are vital for production of viable sperm. The scrotum surrounds the testes and provides temperature control, support, and protection. The spermatic cord supports the testes and aids in temperature control. The epididymis functions to concentrate, store, mature, and transport spermatozoa, while the vas deferens transports sperm to the penis. Accessory glands empty their seminal plasma into the urethra, which helps transport semen. In most species, the vesicular glands contribute fluid, energy substrates, and buffers to semen. In bulls, these glands contribute well over half of the total fluid volume of semen (20).

Several methods can be used to examine quality of spermatozoa: microscopy, computer-assisted sperm analysis, and flow cytometry (21). These 3 methods help analyze different sperm characteristics including motility, membrane integrity, viability, and morphology. Abnormal spermatozoa lack the morphometric shape and/or size of the sperm characteristic of the species. Abnormal sperm often are associated with subfertility or sterility, depending on the type or frequency of the morphological abnormalities. Origins of abnormal sperm morphology can be determined by looking at the localization of the abnormality, reactive oxygen species (ROS), environment, DNA methylation, and chromatin structure (10). Prediction of fertilizing ability is still largely a mystery owing to the fact that abnormal sperm coexist along with normal sperm cells. Economic impact of this is so important that conducting sperm evaluations and breeding soundness exams (BSEs) is essential for establishing fertility levels in advance (16). The BSE consists of a general physical soundness examination, a genital tract examination of both the external and internal genitalia (including scrotal circumference), and a semen quality evaluation. A BSE is a quick and cost-effective way of evaluating bulls for fertility phenotype (22).

Size and shape of a bull's scrotal circumference are associated with bull fertility. A bull's scrotal circumference provides estimate of testes volume and thus the quantity of sperm-producing tissue that producers can measure. Scrotal circumference has a moderate correlation between both fertility and early puberty in the bull's daughters. Heifers from sires with larger than average scrotal circumference tend to reach puberty earlier than those from bulls with smaller circumferences. Thus, increased scrotal circumference in sires is closely correlated to their daughter's age at first breeding, pregnancy rate, and days to rebreeding after calving. Because of low heritability, direct selection for female fertility has not been successful. The strong genetic relationship between scrotal circumference and positive female reproductive traits therefore provides an alternative selection method (23,24).

2.3. Molecular determinants of bull fertility

Sperm DNA is tightly packed around mostly protamines and some histones within the tiny volume in the sperm head. This feature is vital for sperm function during the sperm's progress through the female reproductive tract

and subsequent fertilization and activation of the zygotic/embryonic genome. Integrity of sperm DNA is critical for reproduction because, depending on the correct functions of a damaged region, embryonic gene expression or chromatin structure might be disrupted (25). A change in the conformation of the sperm chromatin can cause a decrease in fertility, so stable and correct chromatin structure is essential for sperm function (26). Sperm DNA is under the constant pressure of oxidative stress because of excessive generation of ROS, which oxidize DNA and interfere with capacitation, hyperactivation, and sperm–oocyte fusion (10). They are produced internally within the cell as well as exogenously by atmospheric oxygen and other environmental factors including pollution and radiation (27). Methods measuring ROS and sperm chromatin structure assay can be used to assess molecular and cellular characteristics of sperm. However, caution should be taken because the feasibility and reliability of these approaches have limitations (28). Researchers screened DNA from high versus low fertility bulls using high density single nucleotide polymorphism (SNP) microarrays and demonstrated specific SNPs associated with bull fertility (5). In another genomics study, an SNP in the *itgb5* gene was shown to be associated with bull fertility (14). Using a proteomics approach, Peddinti et al. (1) showed 125 proteins differentially expressed in sperm from bulls with varied fertility.

Recently, researchers have demonstrated that sperm contain mRNAs as well as small noncoding RNAs. Although sperm are transcriptionally silent, the significance and functions of these small noncoding RNAs are not totally clear (29). Possibly, sperm transcripts can be a mirror of spermatogenesis and, thus, can reveal the health of the sperm. For example, Govindaraju et al. (30) showed that top miRNAs from bull sperm are involved in the expression of genes. These include highly important genes such as *DALRD3*, which is essential to ATP and nucleotide binding, and *IFT80*, which is required by the cell to maintain functional cilia. Sperm transcripts might also be transferred into the oocyte during fertilization and play important roles in zygotic and embryonic gene expression. Feugang et al. (31) demonstrated differentially expressed sperm transcripts that exhibited functions for biological processes critical for embryonic development. Some of these biological processes include transport, signaling pathways, and cell protein modifications. Liu et al. (32) discovered that the first embryonic cleavage in mice is directly dependent on the presence of miRNA-34c, which is found mostly in mammalian sperm. Sperm transcripts can be detected using RNA sequencing (also known as deep sequencing or next generation sequencing) and by real-time reverse-transcriptase polymerase chain reaction. Special attention needs to be given to RNA isolation because sperm contain large amounts of DNA and only minute amounts of RNA. Thus, it is essential to use techniques to ensure isolation of RNA, free from DNA.

Spermatozoa contain diverse proteins that are essential for sperm structure and function. For example, AQP7, an integral membrane aquaporin protein essential for aqueous movement, is found in the sperm tails, providing motility, and is often found lacking in sperm from patients or animals suffering from infertility (33). Due to the important nature of these sperm proteins, their expression can/should be monitored to determine male infertility. Additionally, some sperm proteins play important roles in fertilization and embryonic development. As an example, PLC Zeta and PAWP regulate egg activation and embryonic development, respectively (34). Still, some other sperm proteins regulate sperm chromatin structure; these are protamines and histones. Concentrations of sperm protamines are associated with male fertility (35). Oliveira et al. (3) demonstrated that bull sperm contain histones that may play important roles in sperm chromatin structure associated with bull fertility. Two of the histones involved in the structure of chromatin, specifically H2B and H3, often are associated with gene activation. Another histone, H4, is important for proper chromatin remodeling during spermatogenesis and is necessary for the zygote to inherit the correct chromosomal structure. These examples show that paternal histones play important roles in early embryonic development and can be analyzed to evaluate male fertility (3).

Other macromolecules contained in the membranous sperm are polyunsaturated fatty acids, which are highly susceptible to oxidative damage and can interfere with the ability of the sperm to fertilize the ovum. Any toxic lipid peroxides cause membrane damage and reduce motility. Bulls with lower sperm lipid peroxidation have higher chances of siring calves. This is attributed to the deleterious effects of lipid peroxidation on sperm plasma membrane integrity and sperm DNA, which may reduce fertilizing potential of spermatozoa (36). The lipid content of a spermatozoon is very precise and provides functions for maintaining proper cell structure and physiology. Spermatozoa contain polyunsaturated fatty acids, sterols, plasmalogens, and sphingomyelins in great quantity. Lipid composition supplies a necessary flexibility to the cell. Lipids regulate other cellular functions, such as spermatogenesis and capacitation (37). However, high levels of lipids cause the sperm cell to be susceptible to

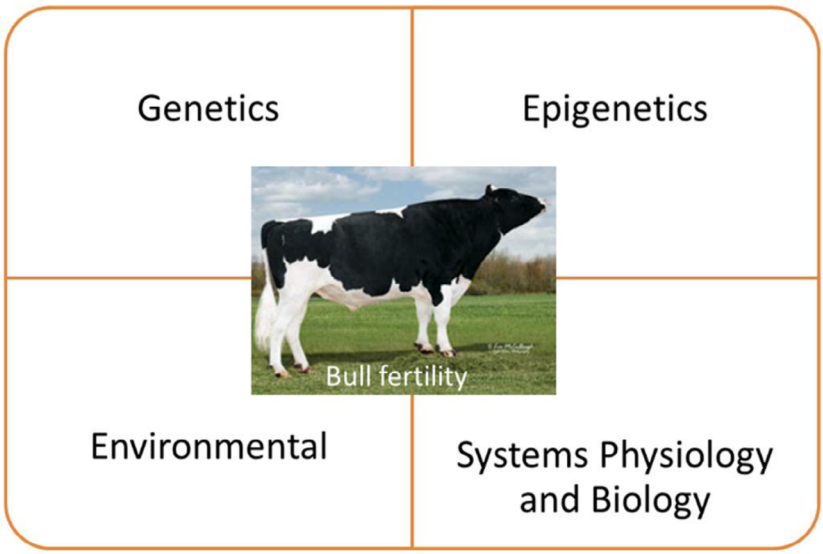

Figure. Bull fertility, the ability of the sperm to fertilize and activate the egg and support embryo development, is an economically important trait that is crucial for efficient reproduction of cattle. Influenced by many factors including genetics, epigenetics, and the environment (climate, nutrition, and management), bull fertility should be studied within the systems physiology concept using a systems biology approach.

damage by reactive oxygen species. Amounts of sperm lipids can be measured using lipidomics approaches (38).

3. Conclusions

Bull fertility plays a crucial role in controlling reproductive efficiency in cattle. In addition to genetics, other conditions such as environmental factors can influence this economically important phenotype. The concept of systems physiology should be considered in order to fully understand and improve bull fertility (Figure). A systems biology approach is required to determine physiological, cellular, and molecular determinants of semen quality and bull fertility. The economic impact of low fertility rates has a critical influence on beef and dairy cattle production. Therefore, new research is essential to eliminate this problem and increase production.

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