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BULL FERTILITY: PAST, PRESENT, AND FUTURE

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Introduction

Reproductive traits are three to nine times more influential for profitability than other production traits, five times more important economically than growth performance, and at least ten times more important than product quality (Trenkle and Willham, 1977). The single most important factor influencing profitability is the percent calf crop weaned, which is largely affected by the number of cows bred during the breeding season. Thus, the breeding capability of a herd bull directly impacts an operations profitability. For instance, if an infertile bull were to be turned out with a group of cows, the result would be the loss of the entire calf crop. Furthermore, a single bull's genetic contribution to a herd can be much greater than that of a female due to an individual sires' ability to service, both naturally and artificially, numerous females. Therefore, it is not only important for a herd bull to be capable of breeding but also provide genetic improvement to the herd. For the previously mentioned reasons and because greater than 90% of beef cows in the United States are bred by natural service, it is important that bulls be managed to optimize breeding performance.

In 2017, the percentage of operations that purchased, leased, or borrowed bulls for the breeding season and performed a semen test, took scrotal measurements, or tested for *tritrichomonas foetus* (trich) was 66.8, 57.0 and 53.6%, respectively. These percentages were greatly decreased when looking at operations that were using bulls that had been on the operation for at least two breeding seasons, such that the percentage of semen tests performed, scrotal measurements taken, and *tritrichomonas foetus* (trich) tested for was 31.4, 22.1, and 20.8%, respectively (NAHMS, 2017). The low percentage of operations implementing reproductive examination procedures on bulls is concerning and thus demonstrates the importance of educating producers on the large effect sires can have on the economics of an operation but also the lack of confidence producers have in the examination procedures available to them. Thus, this proceedings paper will not only discuss the importance of identifying in- and subfertile bulls but also future directions of better identifying said sires.

Attainment of Puberty

A bull is considered to have obtained puberty once an ejaculate collected via electroejaculation contains a minimum of 50×10^6 total sperm with at least 10% progressive motility. When age was used as predictive measurement of the obtainment of puberty in several breeds of bulls, age varied by 62 days. However, the scrotal circumference that was measured at the onset of puberty averaged 27.9 cm and ranged from 25.9 to 30.1 cm indicating its higher accuracy in predicting the obtainment of puberty (Barth and Brito, 2004). Thus, once scrotal circumference reaches 27 to 29 cm a bull is considered pubertal (Lunstra et al., 1978). *However*, *the ability of a bull to produce semen does not indicate good fertility.* The percentage of 12-, 14-, and 16-month-old bulls that were reproductively mature and produce good quality semen is 35, 60, and 95%, respectively (Barth, 2000). This demonstrates that while a bull may have met the minimum requirements to be considered pubertal, sperm quality and quantity will likely continue to increase for several months following initial sperm production.

The Breeding Soundness Exam (BSE)

One of the most commonly used methods to assess male fertility is a breeding soundness exam (BSE), which, when completed correctly, will evaluate semen quality, scrotal circumference, and physical fitness. A BSE is only effective when the bull it is being performed on is pubertal and it only provides a snapshot of that sire's reproductive potential on that given day, meaning that it cannot be reliably used to predict how the bull will continue to perform. This is largely due to the fact that sperm production is a continuous process and thus when a BSE is performed, the sperm production measured is only capturing that of a specific time and thus the classification a bull receives at the completion of one BSE may differ from the classification that same bull receives at the completion of a BSE performed at a later date.

Minimum Requirements

The American Society of Theriogenology indicates that in order for a bull to pass a BSE they must have obtained a minimum scrotal circumference based on their age; Table 1(Chenoweth et al., 1992; Chenoweth and McPherson, 2016), exhibit > 30% motility and have at least 70% morphologically normal sperm (Chenoweth et al., 1992; Chenoweth and McPherson, 2016). Bulls meeting the preceding minimum requirement are classified as ***satisfactory potential breeders***. However, when the minimum requirements are not met, the bull will be classified as either ***deferred*** (indicating that the bull should be tested again at a later date) or as an ***unsatisfactory potential breeder*** (suggesting the bull should be culled). These examinations should occur around 6 to 4 weeks prior to the start of breeding season as this will allow time for bulls that are deferred to be retested or to find a replacement herd bull.

Table 1. Minimum scrotal circumference requirements for bulls to successfully pass a breeding soundness evaluation based on the age of bulls. (Chenoweth et al. 1992)

Age in Months	≤ 15	>15 ≤ 18	> 18 ≤ 21	> 21 ≤ 24	≥ 24
Scrotal circumference (cm)	30	31	32	33	34

Scrotal Circumference

As previously mentioned, scrotal circumference can be used as a predictive measurement of the onset of puberty as it is an indirect estimate of the total mass of testicular tissue and thus related to testosterone production and spermatogenesis. As scrotal circumference increases the production of high-quality sperm will also increase. Scrotal circumference is moderately heritable, meaning that a bull with a larger scrotal circumference will sire sons that have larger scrotal circumference and daughters that obtain puberty at an earlier age and that are more likely to become pregnant.

Semen Quality

Components that make up the semen quality portion of a BSE are ejaculate volume, sperm cell motility, and sperm cell morphology. Both gross and individual motility are accessed, with gross motility assessing mass motion and individual motility determining the percentage of sperm that have progressive motility, or in other words sperm that travel headfirst in a linear fashion. The second component of the semen quality portion of a BSE is sperm cell morphology. Compensable abnormalities are of particular interest when artificial insemination is utilized as the low pregnancy rates that are often associated with these abnormalities can be compensated for by adding additional sperm numbers to the dose. Uncompensable traits are those commonly associated with DNA damage and thus regardless of the number of sperm added to the dose, pregnancy rates will not improve.

There are several factors that contribute to sperm production, four factors that mainly decrease sperm production are injury, disease, fever, and extreme environmental conditions with environmental conditions being the most common cause of abnormal sperm production. Thus, if a bull is injured, stressed, or experiences extreme environmental conditions prior to or during the breeding season, a precautionary step may be to perform a BSE or add an additional sire to the breeding group to prevent any extremely low pregnancy rates due to the hindered sperm production/quality of the first sire.

Physical Fitness

Satisfactory potential breeders should be of good health and appropriate body condition at the start of the breeding season (BCS of 6-7 on a 1-9 scale). This will ensure that as the bull progresses through the breeding season and begins to lose weight, the reserves that are being lost are fat and not muscle. Bulls that are not of appropriate fitness may experience reduced libido or fertility. A herd bull’s vision should not be impaired as vision is essential to recognize estrous behavior. Structurally a bull should be sound and capable of supporting his weight on his hind legs and feet when mounting a female(Koziol and Armstrong, 2018).

Factors not evaluated in a BSE but affect fertility

Libido, or the desire/willingness to mate, is heritable and has a positive effect on pregnancy rates to natural service; however, it is not commonly measured. Scrotal circumference, semen quality, and mating ability are not correlated to libido and thus while a bull may be classified as a satisfactory potential breeder, his libido could be low. Observing bulls when they are exposed to estrual females could be advantageous to producers especially in single sire pastures.

Social dominance should be taken into consideration when deciding which bulls to pair together in a multiple sire breeding pastures as social rankings exist among bulls and thus may influence the number of females a particular sire is willing/able to breed. Producers should pay special attention to sires that have poorer semen quality (smaller scrotal circumference, borderline motility and morphology) but are socially dominant as this dominance could prevent a more fertile, but less dominant, bull from being able to breed as many females.

Variation among satisfactory potential breeders

A BSE examination and its minimum parameters help producers cull bulls that have suboptimal reproductive qualities; however, research has shown there is significant variations in fertility among bulls who met the minimum requirements of a BSE. An example is the significant differences in pregnancy per timed artificial insemination (P/TAI) that existed between three bulls that all passed a BSE where Bull A (48.1%[a]) and B (47.7%[a]) had greater P/TAI then Bull C (40.7%[b]). In another study, two bulls that had similar semen characteristics, such as sperm plasma membrane viability, DNA stability, and percent total and progressively motile sperm, resulted in differing pregnancy rates after AI with Bull A obtaining 71.2% P/TAI and Bull B obtaining only 27.8%[b]. The previously mentioned results indicate that while a BSE is definitely a tool that should continue to be used in selection against infertile bulls, other tools will need to be developed to identify sires that are subfertile.

When thinking about new tools that could help identify subfertile bulls it is important to keep in mind that male fertility is often correlated with sperm motility, abnormalities, DNA status, mitochondria function, and membrane integrity and thus are used to determine the potential fertility of individuals (Alves et al., 2019) therefore it would be preferable if the tools developed to evaluate fertility would be noninvasive while identifying bulls capable of producing sperm that can (1) reach the fertilization site; (2) fertilize the ovum; and (3) contribute to early embryonic development (Krawetz, 2005).

Future Directions

Previously it was believed that sperm only delivered the paternal genome to the oocyte, however; research has provided evidence that in addition to the sperm’s centrosome for reactivation of meiosis II (Albertson, 1984) and sperm specific phospholipase C-ζ which plays a role in activation of embryonic development (Saunders et al., 2007), approximately 5-10 fg of paternal RNA can be delivered to the oocyte (Boerke et al., 2007). Among the paternal RNA is coding and noncoding RNA (Lalancette et al., 2008) which includes microRNAs (miRNAs; (Yuan et al., 2016).

microRNAs

microRNAs are highly conserved among species and are small non-coding RNA molecules that are approximately 22 nucleotides that alter protein translation post transcriptionally (O'Brien et al., 2018).

Once it was discovered that miRNAs from sperm enter the oocyte, the question then became, are they important to early embryo development? With the use of intracytoplasmic sperm injection (ICSI) oocytes were able to be fertilized by sperm with partially depleted miRNA profiles. The resulting embryo had reduced developmental potential compared to embryos that were fertilized with sperm that had complete miRNA profiles (Yuan et al., 2016) . This experiment established that paternal miRNAs that are transferred to the oocyte at the time of fertilization are indeed crucial to proper embryo development.

Proteomics and spermatozoa longevity

As estrous synchronization and fixed time artificial insemination (FTAI) implementation increased, it became apparent that certain bulls consistently performed well in a FTAI setting (indicating improved sperm longevity) while other bulls had poorer conception rates in a FTAI setting compared to when they were used in either a natural service or heat detection scenario. This observation stimulated inquiry into what contributes to sperm longevity and sperm transport.

The plasma membrane of spermatozoa is coated with a plethora of glycoproteins (Magargee et al., 1988; Mahmoud and Parrish, 1996; Geussova et al., 1997) , while the epididymis contains several enzymatic proteins that are thought to be involved in spermatozoa protection (Girouard et al., 2011) or motility (Frenette et al., 2003; Frenette et al., 2004; Frenette et al., 2005) . To better understand how these different proteins may contribute to longevity and/or transport, proteins from epididymal fluid and sperm and protein from ejaculated fluid were analyzed. Proteins found in the epididymal fluid likely affect motility and mitochondrial activity while proteins found on sperm located in the epididymis are enzyme inhibitors or catalytic subunits of proteasomes. Meanwhile, the proteins found in ejaculated fluid had little interaction with one another but could be involved in functions of the extracellular region. Conversely, proteins found on ejaculated sperm are likely involved in enzymatic activities.

Conclusion

A basic BSE is the start to identifying infertile bulls and will likely always be the starting point for bull fertility as it can be conducted chute side in a pasture. However, to separate sub-fertile from fertile bulls, work is being done to advance technologies that will investigate the role of surface proteins in sperm transport and longevity as well as the role of microRNAs in early embryo development and survival.

References

Albertson, D. G. 1984. Formation of the first cleavage spindle in nematode embryos. Dev. Biol. 101(1):61-72. doi: 10.1016/0012-1606(84)90117-9

Alves, M. B. R., R. P. de Arruda, T. H. C. De Bem, S. A. Florez-Rodriguez, M. F. Sa Filho, C. Belleannee, F. V. Meirelles, J. C. da Silveira, F. Perecin, and E. C. C. Celeghini. 2019. Sperm-borne miR-216b modulates cell proliferation during early embryo development via K-RAS. Sci Rep 9(1):10358. doi: 10.1038/s41598-019-46775-8

Barth, A. D. 2000. Bull breeding soundness evaluation. 2nd ed. Western Canadian Association of Bovine Practitioners.

Boerke, A., S. J. Dieleman, and B. M. Gadella. 2007. A possible role for sperm RNA in early embryo development. Theriogenology 68 Suppl 1:S147-155. doi: 10.1016/j.theriogenology.2007.05.058

Chenoweth, P. J., and F. J. McPherson. 2016. Bull breeding soundness, semen evaluation and cattle productivity. Anim. Reprod. Sci. 169:32-36. doi: 10.1016/j.anireprosci.2016.03.001

Chenoweth, P. J., J. C. Spitzer, and F. M. Hopkins. 1992. A new bull breeding soundness evaluation form. In: Proc. Ann. Mtng. Soc. for Theriogenology, San Antonio, TX. p 63-71.

Frenette, G., C. Legare, F. Saez, and R. Sullivan. 2005. Macrophage migration inhibitory factor in the human epididymis and semen. Mol. Hum. Reprod. 11(8):575-582. doi: 10.1093/molehr/gah197

Frenette, G., C. Lessard, E. Madore, M. A. Fortier, and R. Sullivan. 2003. Aldose reductase and macrophage migration inhibitory factor are associated with epididymosomes and spermatozoa in the bovine epididymis. Biol. Reprod. 69(5):1586-1592. doi: 10.1095/biolreprod.103.019216

Frenette, G., C. Lessard, and R. Sullivan. 2004. Polyol pathway along the bovine epididymis. Mol. Reprod. Dev. 69(4):448-456. doi: 10.1002/mrd.20170

Geussova, G., J. Peknicova, J. Capkova, P. Kalab, J. Moos, V. V. Philimonenko, and P. Hozak. 1997. Monoclonal antibodies to canine intra-acrosomal sperm proteins recognizing acrosomal status during capacitation and acrosome reaction. Andrologia 29(5):261-268. doi: 10.1111/j.1439-0272.1997.tb00480.x

Girouard, J., G. Frenette, and R. Sullivan. 2011. Comparative proteome and lipid profiles of bovine epididymosomes collected in the intraluminal compartment of the caput and cauda epididymidis. Int J Androl 34(5 Pt 2):e475-486. doi: 10.1111/j.1365-2605.2011.01203.x

Koziol, J. H., and C. L. Armstrong. 2018. Manual for Breeding Soundness Examination of Bulls. Second ed. Society for Theriogenology.

Krawetz, S. A. 2005. Paternal contribution: new insights and future challenges. Nat Rev Genet 6(8):633-642. doi: 10.1038/nrg1654

Lalancette, C., D. Miller, Y. Li, and S. A. Krawetz. 2008. Paternal contributions: new functional insights for spermatozoal RNA. J Cell Biochem 104(5):1570-1579. doi: 10.1002/jcb.21756

Lunstra, D. D., J. J. Ford, and S. E. Echternkamp. 1978. Puberty in beef bulls: hormone concentrations, growth, testicular development, sperm production and sexual aggressiveness in bulls of different breeds. J. Anim. Sci. 46(4):1054-1062. doi: 10.2527/jas1978.4641054x

Magargee, S. F., E. Kunze, and R. H. Hammerstedt. 1988. Changes in lectin-binding features of ram sperm surfaces associated with epididymal maturation and ejaculation. Biol. Reprod. 38(3):667-685. doi: 10.1095/biolreprod38.3.667

Mahmoud, A. I., and J. J. Parrish. 1996. Oviduct fluid and heparin induce similar surface changes in bovine sperm during capacitation: a flow cytometric study using lectins. Mol. Reprod. Dev. 43(4):554-560. doi: 10.1002/(SICI)1098-2795(199604)43:4<554::AID-MRD19>3.0.CO;2-Z

NAHMS. 2017. Beef 2017 Report 1: Beef Cow-calf Management Practices in the United States. In: U. APHIS (ed.).

O'Brien, J., H. Hayder, Y. Zayed, and C. Peng. 2018. Overview of MicroRNA Biogenesis, Mechanisms of Actions, and Circulation. Front Endocrinol (Lausanne) 9:402. doi: 10.3389/fendo.2018.00402

Saunders, C. M., K. Swann, and F. A. Lai. 2007. PLCzeta, a sperm-specific PLC and its potential role in fertilization. Biochem Soc Symp (74):23-36. doi: 10.1042/BSS0740023

Trenkle, A., and R. L. Willham. 1977. Beef production efficiency. Science 198(4321):1009-1015. doi: 10.1126/science.198.4321.1009

Yuan, S., A. Schuster, C. Tang, T. Yu, N. Ortogero, J. Bao, H. Zheng, and W. Yan. 2016. Sperm-borne miRNAs and endo-siRNAs are important for fertilization and preimplantation embryonic development. Development 143(4):635-647. doi: 10.1242/dev.131755