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Review Paper

A Review on Traditional and Recent Approaches for Predication of Breeding Soundness Evolution in Bull

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ABSTRACT

The breeding soundness examination (BSE) predicts about bull's potential to get cows pregnant. To identify a bull's potential fertility, breeding-soundness examinations are frequently used in the beef industry. Bull BSE is a simple, cheap, and essential tool for a cow-calf operation. BSE enhances risk management, strategic bull use, herd fertility, and economics. It is carried out to determine a bull's libido, and reproductive status, and to test for different genital diseases. Based on the BSE bulls are given one of three classifications: "satisfactory," "unsatisfactory," or "classification postponed". Accurate Semen evaluation is an important factor of the BSE. Competent physical/reproductive exams and appropriate semen evaluations can contribute greatly to the fertility and economics of individual herds as well as understanding of factors which affect fertility

Keywords: Bull, Breeding Soundness, Fertility, Semen Quality

Bull is more than half of the herd is a proverb that highlights the importance of choosing the best bull from a population. Regardless of the reproductive strategy employed—natural, regulated mating, or artificial insemination - bulls have a significant impact on the reproductive efficiency of herds. Thus, a lack of bull fertility or a loss in bull fertility results in a decrease in productive parameters and a potential decline in farm profitability, especially in extensive livestock production systems. A cow herd's reproductive management includes a wide range of elements. The majority of the focus is on female reproduction. When a cow didn't get pregnant in the past, it was supposed that she was at fault.

Breeding soundness evaluations (BSE) are a crucial financial aspect of herd management (Williams, 1927; McCosker *et al.* 1989; Chenoweth, 1980; Farin *et al.* 1989; Wiltbank, 1983). Physical soundness, sufficient semen quality, and serving capability all contribute to breeding soundness (Barth, 1994). The term "breeding soundness" describes a bull's capacity to impregnate cows. Despite the fact that 20–40% of bulls may have decreased fertility, very few are completely sterile (Kastelic *et al.* 2000).

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Bulls with sub-fertility have delayed pregnancy, extended calving intervals, lower calf weaning weights, and higher rates of female culling. Breeding Soundness is influenced by a range of factors, including age, nutrition (physical condition), seasons, management, breed (genetic group), and their interactions (Roberts *et al.* 2010; Menon *et al.* 2011; Lemma and Shemsu, 2015). BBSE contains biometric measurements, namely scrotal circumference (SC) and semen analysis (sperm concentration, motility, and morphology), but behaviour (libido) is not taken into account, despite the fact that this characteristic may be used to predict prospective bull fertility (Lessard *et al.* 2011). Breeding soundness evaluation (BSE) is a combination of numerous physical, biochemical, and microscopic tests to predict the reproductive potential of bulls. Therefore, a breeding bull's appraisal properly may increase a dairy farm's total profitability and reproductive efficiency.

In 1983, the Society for Theriogenology (SFT) released the first list of guidelines for interpreting the BSE system (Ball *et al.* 1983). Bulls were divided into three categories based on a numerical score system: satisfactory potential breeder, questionable potential breeder, and unsatisfactory potential breeder. Later in 1992, new standards for assessing and classifying bulls for breeding soundness were developed based on the most recent scientific findings.

Classification of Breeding Potential

Satisfactory: Bulls that equal or exceed the minimal requirements for scrotal circumference, sperm motility, and sperm morphology and that don't exhibit any genetic, infectious, or other problems or faults that can affect breeding or fertility.

Unsatisfactory: Bulls that are below one or more thresholds and which are highly unlikely to ever improve their status. Bulls that exhibit genetic problems or irrecoverable physical issues (such as infectious diseases) that would impair reproduction or fertility.

Deferred: Bulls that do not fall under the above categories and that could benefit from a retest. This includes bulls that are substandard but can improve. (Usually, a re-examination test date is suggested.

The young bulls found unsatisfactory at the time of evaluation may turn into satisfactory breeders if re-tested after 3 months. This is because the process of spermatogenesis requires around 60 days, plus an additional 15 days for travel from the reproductive tract and maturation. It should be remembered that the BSE is an observation made on a particular day and that it may alter over time due to physical defects or injury to the bull.

Timing

BSE should be performed on all bulls before breeding; however, they are most frequently done on young bulls before they are sold or bred for the first time. In principle, the BSE should happen as soon as possible after breeding. It is common to conduct the initial BBSE within 4-6 weeks after sale or introduction to the breeding herd, however, ample time should be provided for treatments, retesting, or replacement before breeding.

Evaluation of bull fertility

The major elements of the BBSE are:

1. Identification and history

Before performing the BSE, the bull's past medical history should be examined. Age, breed, identification number, vaccinations administered and the site of administration, fever, retained testicles, hernias, and the prevalence of chronic debilitating illnesses are among the basic details. All these elements greatly impact the sperm production ability and quality of semen of a particular bull.

2. Structural soundness and physical exam

The physical examination covers the eyes, teeth, legs, and overall body confirmation. Any physical defect may lead to lower fertility when mating naturally. The bull should be freed from ringworm, warts, and other skin conditions. Vision plays a crucial role in identifying potentially receptive females in natural service likewise as during mounting on the dummy. the attention should be healthy, clean, and unblemished. The teeth should be investigated for age together with wearing and missing. Additionally, it aids in ensuring proper meal chewing, which helps breeding bulls maintain their regular weight. Successfully copulate or

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ejaculate in the artificial vagina, he must be able to physically support a significant amount of his weight on his back legs. The bull shouldn't have cracked or rotted hooves, swelling knee and stifle joints, scars around the coronary band, or foot rot. Numerous conformational flaws, such as cow hock, sickle hock, post-leggedness, crooked legs, or splayed toes, might be detrimental or harmful to the feet during the mating season.

3. Reproductive system analysis

It involves examining the following —

(a) External reproductive organs- Testis, penis, prepuce and scrotal circumference

(b) Internal accessary glands - Seminal vesicles, prostate and ampulla for detection of any abnormalities

(a) External reproductive examination

Examining the external reproductive organs involves both manual palpation and visual inspection.

Testis: The capacity to produce sperm and the onset of early puberty in females are closely associated with the size of the testis. Bull testicles should be well spaced, symmetrical, and easily moveable inside the scrotum. Any asymmetry might be a sign of hydrocele, hernia, or testicular atrophy. The size and consistency of the testes should also be checked. Testes should have the consistency of a flexed bicep muscle.

Prepuce: The prepuce should first be visually evaluated for signs of prolapse. When examining the external orifice, it is important to pay close attention to any precipitated crystals on the hairs since they may indicate the presence of urinary calculi, which can put bulls at risk for urethral blockage and even rupture, making a bull unfit for breeding. In addition to the visual examination, the entire external preputial sheath should be thoroughly palpated to check for any lacerations, adhesions, stenosis, or enlargements of the preputial sheath. Phimosis (inability to extend the penis from the prepuce) and paraphimosis (inability to retract the penis into the prepuce) are symptoms of preputial disorders (Bruner *et al.* 1992).

Penis: The penis should be examined as soon as possible after a natural mating before the penile retracts into the prepuce or both before and after semen collection using an artificial vagina. Since artificial deviations may develop during electro-ejaculation and/or manual protrusion. The extended penis should be evaluated for the presence of fibropapillomas, hair rings, and penile deviations. Bulls intended for breeding shouldn't have secondary illnesses like phimosis or paraphimosis (Hopkins, 1997).

Testicular consistency: Tonometers are frequently used to estimate the consistency of the testicles and provide information on the parenchyma's consistency. Some studies relate the tonometer reading to the ability to produce high-quality sperm. It has been proposed that the tonometer gives a quick, quantitative way to judge a dairy bull's potential fertility and semen quality. However, there is no agreement that it can be used as a measure to forecast bull fertility. It is necessary to conduct more research on the tonometer's usefulness in assessing young bulls' reproductive potential.

Scrotum and its contents: The scrotum (and its contents) should be examined visually and palpated. This visual examination of the scrotum's size and shape should be performed on a relaxed bull in a warm environment since the scrotum will be at its maximum pendulous under these situations (Johnson, 1997). The presence of a clear, fat-free scrotal neck is necessary for adequate thermoregulation. Straight-sided and wedge-shaped scrotums, as well as typical scrota with fat deposits in the scrotal neck, are linked to decreased testicular thermoregulation, which can lead to abnormal sperm production. Scrotal, testicular, and epididymal palpation should be done following the visual examination. The testicular cords should be examined for the presence of fat, abscesses, varicoceles, or viscera in case of a scrotal hernia, as well as the thickness of the scrotal wall and the fat content of the scrotal neck (Warner, 2004).

Scrotal circumference measurement: In breeding soundness examination to assess a bull's ability to produce semen, testicular dimensions, particularly the scrotal circumference (SC), have been widely employed. The association between testicular biometry and characteristics related to male reproduction and semen quality, such as the quantity and quality of normal sperm, sperm concentration, sperm motility, and daily sperm production, is extensively researched (Kumaresan). Numerous

studies have shown a positive correlation between a bull's scrotal circumference and the probability of conception and/or pregnancy (Kastelic and Thundathil, 2008; Waldner *et al.* 2010; Ahmad *et al.* 2011).

Scrotal circumference tapes can be used to measure it. Bulls' ability to produce sperm is closely correlated with their scrotal circumference (SC), testicular volume (TV), testicular weight (TW), and testicular shape (Bailey *et al.* 1996). A larger-than-average SC measurement has typically been related to higher sperm production and testicular mass, whereas a smaller-than-average SC, frequently associated to smaller testicles, has been linked to infertility (Chacon *et al.* 1999). Bulls with smaller than average SC and longer testicles may have a larger TV or TM than bulls with higher SC and shorter testicle in compared to bulls with higher SC and shorter testis, bulls with lower than average SC and longer testis may have a larger TV or TM. Therefore, combining SC with TL and TW would result in a fairly accurate representation of TV and TM. SC is highly heritable and gives an indirect indication of testicular weight and size, which are closely related to the production of sperm (Sylla *et al.* 2007). Scrotal circumference measurement is important when examining yearling bulls since it may help identify whether the animal is pubertal (Brinks, 1994).

Table 1: Minimum bull scrotal circumference (in cm) in relation to the bull's age

Age (months)	Minimum scrotal circumference (cm)
> 12 - 15	30
> 15-18	31
> 18-21	32
> 21-24	33
> 24	34

(b) Internal reproductive examination

The internal accessary glands are investigated per-rectum after proper removal of dung. It should not have any anomalies when the prostate gland, which feels like a band across the urethra, is palpated. The ampulla is found on the distal portion of the ductus deferential, and the chance of any disease affecting this gland occurring is abnormal. On either side of the pelvic urethra, two seminal vesicles feel like a bunch of grapes when palpated per rectally. Vesiculitis is probably the most prevalent illness in bulls and is characterized by any asymmetry and pain observed on palpation. A large opening or the presence of an intestinal loop through the inguinal ring, which should also be palpated, may cause future problems with reproduction.

Semen collection and evaluation: Bull semen can be collected using an artificial vagina (AV), an electro-ejaculator, or by massaging the internal reproductive organs (vesicular glands, ampullae, and pelvic urethrae). Each technique has benefits and drawbacks (Beggs, 2013). The most important factor, regardless of the method employed, is obtaining a representative semen sample; in this regard, the AV approach has benefits over the others since it more reliably provides a full ejaculate. The volume of ejaculation produced and the morphology and motility of the sperm cells are factors that affect the quality of the sperm. It's important to remember that poor diet, extreme weather conditions, and disease can all affect the quality of the semen. The quality of semen from a single bull can change with time.

Motility is estimated by examining the semen on a clean, warm slide, and it is decreased by high temperatures and environmental pollutants. Mass motion can be seen at low power, but increasing motility has to be evaluated at medium power (400 X). Concentrated samples are diluted with warm, fresh saline before applying a cover slip. According to http://www.therio.org, the minimum percentage of progressively motile sperm for a Satisfactory Potential Breeder is 30%. Under oil immersion, the morphology of the sperm should be assessed. Semen is diluted in eosin-nigrosin (smear) or 10% neutral buffered formalin (wet mount) and at least 100 sperm cells (up to 300 if there are multiple abnormalities) are analysed. There are several techniques for classifying sperm; compensable and uncompensable abnormalities are reasonable approaches. By increasing the dosage, compensable anomalies can be eliminated (Saacke *et al.* 2000). For example, it is unlikely that an ovum would be fertilised by sperm with knobbed acrosomes or bent tails. Nuclear vacuoles, on the other hand, are considered as uncompensable. The probability of the damaged sperm fertilizing an ovum is about equal to its proportion in the ejaculate, regardless

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of the quantity of normal sperm present. According to http://www.therio.org (Barth, 2007), fertility will generally be reduced if there are more than 30% of morphologically defective sperm or more than 20% head deformities.

Table 2: Minimum Recommended Motility is: 30% or Fair.

Mass Activity (Gross)	Rating	Individual
Rapid Swirling	Very Good	≥ 70%
Slower Swirling	Good	50 - 69%
Generalized Oscillation	Fair	30 - 49%
Sporadic Oscillation	Poor	< 30%

Source: *Hopkins, Spitzer (1997).*

Sperm Plasma Membrane Viability: It is important to evaluate the viability (integrity) of the sperm plasma membrane since it is essential for fertilization. Non-viable sperm (pink to red sperm heads) are permeated by eosin, while viable sperm are white. Various fluorescent probe combinations that are specific for viable or non-viable sperm are also used; the most common of them is Syber-14 with propidium iodide (Gillan *et al.* 2005). In "viable" sperm (sperm with an intact plasma membrane), syber-14 penetrates, creating the DNA bright green (Gillan *et al.* 2005), whereas propidium iodide penetrates, creating the DNA red (dead and non-viable sperm). Fluorescent microscopy or flow cytometry is used to determine the percentage of living and dead sperm (green and red, respectively).

Brito *et al.* (2003) investigated the association between cleavage and a variety of techniques for determining the vitality of sperm membranes, including eosin nigrosin, Trypan blue, fluorescent probes, and sperm response to hypoosmotic fluid exposure (IVF). However, these stains evaluate the physical integrity of sperm membrane, and the hypoosmotic swelling test (HOST; Jeyendran *et al.*, 1984) evaluates its functional integrity. The success of in vitro fertilization could only be predicted using this method of plasmalemma evaluation, which was the only one that helped conventional sperm quality testing (based on cleavage rate; Brito *et al.* 2003).

Externalization of phosphatidyl serine of cryopreserved bovine semen is evaluated using an Annexin-V binding test utilizing fluorescence-activated cell sorting (FACS) (Anzar *et al.* 2002; Januskauskas *et al.* 2003). This test identifies translocation of membrane phosphatidyl serine, which initiates loss of membrane integrity during cryopreservation-induced, apoptosis-like cell degradation. But other investigations found different patterns in its relationship to the fertility of frozen-thawed bull semen (Rodriguez-Martinez, 2003).

Sperm–Oviduct Interaction: The ability to predict fertility is also provided by in vitro sperm-oviduct binding tests. The interaction of sperm with in vitro oviduct epithelial explants, as a screening test for fertility, must be cautiously interpreted because the endosalpinx undergoes cyclic morphological and histological changes and therefore the sperm cell membrane is modified during sperm transport through the uterus and oviduct (Hunter *et al.* 1987; 1991; Hunter, 2008).

Sperm Capacitation: Sperm undergo capacitation before fertilization (Yanagimachi, 1994). The ability of sperm to perform an acrosome reaction can be used to measure similar cryopreservation-induced alterations (premature capacitation; Cormier *et al.* 1997), which decrease the fertility of frozen semen (Watson, 2000). (Thundathil *et al.* 1999; Januskauskas *et al.* 2000). Modifications in the activity of sperm proteins involved with capacitation may accompany premature induction of capacitation. Sperm Na+ K+ ATPase (sperm membrane protein) was inhibited, which caused capacitation and reduced motility (Thundathil *et al.* 2006). Moreover, cryopreservation processes inhibited the activity of Na^+/K^+ ATPase (Zhao and Buhr, 1996). Therefore, this protein may regulate the premature capacitation of sperm.

Sperm–Zona Pellucida Interaction: Widespread access to abattoir-derived bovine ovaries facilitates using homologous systems for investigating sperm–oocyte interactions. Sperm interact with the zona pellucida after capacitation (ZP). In this interaction, zona glycoprotein (ZP3) is the ligand and galactosyl transferase (Larson and Miller, 2000), p47 (Ensslin *et al.* 1998), sp56 (Cheng *et al.* 1994), and zonadhesin (Hardy and Garbers 1995) seem to be sperm receptors (Yanagimachi, 1994). The inner acrosomal membrane interacts with a second glycoprotein, ZP2, causing an acrosome reaction that facilitates the secondary binding of sperm to the zona matrix after penetration (Yanagimachi,

1994; Aitken, 2006). A hemizona assay (Fazeli *et al.* 1997) compares the ability of control sperm (known fertility) and test sperm (unknown fertility) to bind using both halves of a ZP. Non-return rates and zona binding and hemizona binding tests were significantly correlated. In vivo fertility has been predicted using in vitro zona penetration assays (Puglisi *et al.* 2004). However, the value of this test is limited by the fact that sperm penetration varies with the sperm-oocyte ratio, incubation time, and heparin content.

Sperm Oolemma Fusion and Sperm DNA Decondensation: The effects of accessory gland fluid from low-fertility vs. high-fertility bulls were demonstrated by Henault *et al.* (1995) using a homologous zona-free oocyte penetration assay. Furthermore, competitive penetration of zona-free bovine oocytes by fluorochrome-labeled bull sperm was associated with in vivo fertility (Henault and Killian, 1995) and assessed the capacity of sperm to undergo chromatin decondensation and pronucleus formation. All sperm have an equal chance of fusing with the egg membrane, going through DNA decondensation, and becoming pronuclei in the absence of a ZP (Thundathil *et al.* 2001).

The ability of sperm to produce normal pronuclei is significantly influenced by the integrity of the sperm chromatin. Based on resistance to acid denaturation, the sperm cell structure assay (SCSA) uses flow cytometry to determine chromatin integrity. Acridine orange, which binds to double-stranded DNA (intact) or single-stranded DNA (denatured) and generates green or red fluorescence, respectively, is used to stain sperm at low pH levels. Chromatin denaturation is measured by the ratio of red to (red + - green) fluorescence, which has significantly correlated with fertility (Ballachey *et al.* 1987, 1988; Januskauskas *et al.* 2001; Waterhouse *et al.* 2006). In brief, IVF-based tests evaluate the sperm's ability to undergo DNA decondensation and pronuclear formation during fertilization, whereas flow cytometry-based approaches provide a quantitative measurement of the structural integrity of sperm chromatin based on a large number of sperm.

Association Between *In Vitro* Fertilization and Fertility: Bull field fertility may be predicted using pronuclear formation, according to Marquant-Le Guienne *et al*. (1990). According to Zhang *et al.* (1997), blastocyst production varied between test dates, however, fertilization based on cleavage rate was highly correlated with non-return rates. Moreover, fertility estimations were more accurate when based on many laboratories assays vs a single assay (Truelson *et al.* 1996). Considering this, a seven-variable model (post-thaw total motility, post-thaw sperm with a linear motile pattern, sperm concentration, sperm concentration after swim-up, sperm ZP-binding, cleavage rate of total oocytes, and blastocyst rate of total oocytes) explained 84.6 percent of the variation in non-return rates (Zhang *et al.* 1999).

(d) Others

Endocrine profile and fertility of bulls: It is commanly believed that testosterone levels are related to libido or sexual drive in male animals. Since testosterone's influence on the spermatogenic process is well known, it is predicted that testosterone levels will positively correlate with the quality of the sperm. Conversely, several researchers have suggested that the quantity of testosterone in the blood is unrelated to libido and semen quality (Foote *et al.* 1976; Sekasiddhi *et al.* 1997), even though certain studies have shown a positive correlation between testosterone and semen quality (Parkinson, 1985).

Testicular cytology: Testicular functions can be

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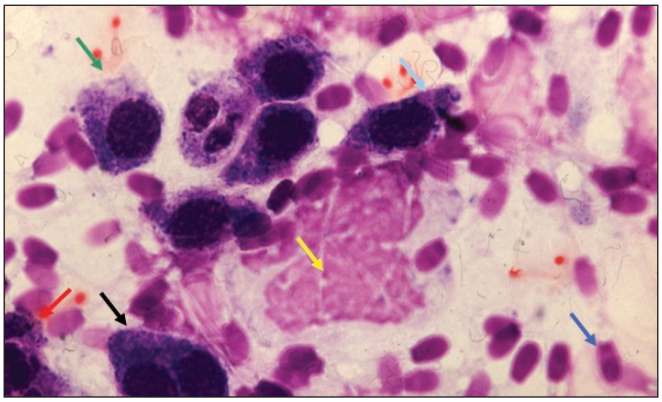

The spermatogenic cells identifiable are:

Spermatogonia ( ); Spermatocytes ( ); Spermatids ( ); Mature spermatozoa ( ); Sertoli cells ( ); Some amorphous material ( ) is also seen.

Fig. 1: Testicular FNA cytology showing normal testicular cells proportions from a healthy six-year-old Angus bull. (Magnification x400, Speedy-Diff stain)

Testicular cytology indices

Spermatic index = Total no. of spermatozoa per 100 spermatogenic cells; Sertoli cell index = Total no. of sertoli cell per 100 spermatogenic cells; Sperm- Sertoi cell ratio = Ratio of total no. of spermatozoa to sertoli cells; Spermatogram = Proportion of particular developmental forms of spermatogenic cells per 100 spermatogenic cells; Mitotic index = Number of spermatogenic cells undergoing division per 100 spermatogenic cells; Spermatogenic: Sertoli cell ratio = Ratio of total no. of spermatogenic cells to total no. of Sertoli cells.

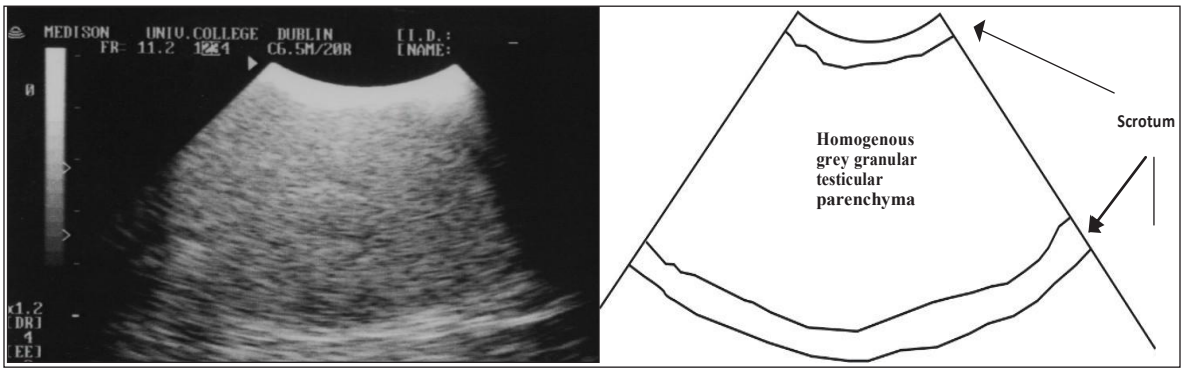

Fig. 2: A three-year-old Angus bull with normal testicular ultrasonography displaying homogeneous granular parenchyma. A portable B-mode real-time ultrasound scanner (SonoAce 600V: Edinburgh) with a 7.5 MHz linear array transducer was used to obtain the ultrasonograph

measured by evaluating various cell indices of spermatogenic cells, Sertoli cells, and Leydig cells. Various techniques, including the open method, split needle biopsy, needle punch biopsy, and fine needle aspiration cytology (FNAC), are used to assess the testicular cytogram. Testicular FNAC is regarded as a simple, rapid, and less invasive method to evaluate spermatogenesis as compared to other methods.

To determine the relationship between several cytological indices and the reproductive status of the bulls, the percutaneous needle aspiration biopsy (PNAB) technique was used. It was shown that in bulls with high fertility, the number of Sertoli cells, Sertoli cell index, and the spermatogenic: Sertoli cell ratios were significantly higher than those of bulls with low fertility (Rajak, 2012).

Trans-scrotal ultrasonography: Trans-scrotal scanning allows accurate scrotal circumference measurement and the visualization of tissue interfaces within the scrotum (Love, 1992). Additionally, trans scrotal scanning allows the assessment of testicular lesions that are both palpable and not palpable (Ahmad and Noakes, 1995). The method is practical and non-intrusive, making it ideal used on farms.

Proteomic biomarkers in sperm and seminal plasma

The systematic analysis and documenting of the proteins in biological samples is known as proteomics. By doing a systematic analysis of protein expressions, it provides fascinating new insight into the mechanism behind cellular function. It may be viewed as a mass screening approach to molecular biology, with the end goal of elucidating the relationships and functional roles of the proteins involved by first characterizing the individual proteins of interest. Several proteins found in seminal plasma and sperm are essential for many processes, including motility, capacitation and acrosome reaction, immune modulation in uterus, creation of tubal sperm reservoir, zona penetration, sperm-egg fusion, and embryo development.

Earlier, Killian *et al*. (1993) used the 2D-PAGE technique to find four proteins linked with fertility in the seminal plasma of Holstein bulls. They discovered that two proteins (26 kDa, pI 6.2, and 55 kDa, pI 4.5) occurred more frequently and densely in bulls with higher fertility, whereas two other proteins (16 kDa, pI 4.1, and 16 kDa, pI 6.7) were more prominent in bulls with lower fertility. They also developed a multi-regression equation to describe how the presence of the proteins associated with fertility and bull fertility relate to one another. Later these proteins were discovered to be osteopontin33 (55 kDa) and Lipocaine type prostaglandin D synthase (26 kDa), which overexpressed in high fertile bulls (Gerena *et al.*, 1998). According to studies (Bellin *et al.* 1998; McCauley *et al.* 1999), bovine seminal plasma protein (BSP) 30 kDa and phospholipase A237 are more common in high fertility bulls, however, spermadhesin Z13 levels were adversely connected to fertility (Moura *et al.* 2006). Moreover, a comparative proteomic study of spermatozoa revealed a relationship between the expression levels of several proteins and an animal's fertility status (Peddinti *et al.*, 2008; Park *et al*, 2012; Soggiu *et al.*, 2013).

Animal proteomics studies use several methods, including SDS-PAGE, two-dimensional electrophoresis (2D), and difference gel electrophoresis (DIGE). Mass spectrometry techniques like MALDI TOF and LC MS/MS are being developed to determine the proteins that are differentially expressed (Wright *et al.* 2012). Usually, methods like western blotting are used to validate the results of the experiments (Park *et al.* 2012). The fertility associated with proteomic profiling of bull spermatozoa was initiated by pioneer work of Peddinti *et al.* in 2008. They identified 125 potential biomarkers of fertility using differential detergent fractionation multidimensional protein identification technology (DDF-Mud PIT). They reported that proteins involved in energy metabolism, cell communication, spermatogenesis, and cell motility were abundantly expressed in the spermatozoa from high fertile bulls. Later, D'Amours *et al.* (2010) used the 2D-PAGE technique to compare the proteome profiles of high, medium, and low fertile HF bulls and validated the results using western blotting.

Theriogenology Lab (NDRI) conducted the study with technical support from the All-India Institute of Medical Sciences (AIIMS), New Delhi, and found many possible chemicals in the seminal plasma and sperm of crossbred bulls that may serve as a panel of reproductive biomarkers (Aslam *et al.* 2014). The difference in expression between the two groups ranged from 1.5 to 5.5 folds. Protection of Telomeres-1 Protein (POT1) was greatly overexpressed (2.9 fold) in the high fertile group and Prostaglandin E2 receptor EP3 (PTGER3) was extremely abundant (5.5 fold) in the low fertile group.

CONCLUSION

A breeding soundness examination will identify bulls that are grossly aberrant. Moreover, a comprehensive approach, including evaluating sperm function and fertility at the molecular, cellular, and whole-animal levels, is needed to estimate fertility of bulls that are producing normal sperm. This implies that using these techniques together enhances breeding soundness examination in bulls by differentiating highly fertile bulls from low fertility bulls.

According to the above conclusion, the following recommendations are suggested:

- Breeding soundness evaluation of bulls should get special attention to reduce or eliminate subfertile and sterile animals.

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- Regular libido evaluation and testing for infertility disorders should be included in BSE since they will have an adverse effect on the quality of the semen.
- Bull should be always screened before their use since results are most valid at the time of examination only.
- Educating farmers about the importance of breeding soundness examination to increase their cows' conception rates.

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