

A Bull Breeding Soundness Evaluation System with Greater Emphasis on Molecular Genetics

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Abstract

Bull is known to be half of the herd. As bull contribute large number of progenies to the next generation as compared to females so determining the potential fertility of the bull is much more important than determining the fertility of any individual cow. It becomes critical to study and analyse the breeding potential of a bull before breeding season for getting best out of progeny. Breeding soundness evaluation (BSE) is a practical method to predict bull's potential fertility. Evaluation should be conducted on every bull at least 30–60 days before each breeding season to allow enough time for replacement of deferred or unsatisfactory bulls. Parameters have been shown to be the most reliable and repeatable in nature and consist of (A) History; (B) Physical examination; (C) Measurement of scrotal size; and (D) Semen evaluation for sperm motility (movement) and morphology (structure and shape). Along with all these the accessory sex glands such as vesicular glands, ampullae, and prostate should be looked for evidence of inflammation, adhesions, fibrosis or enlargements. New techniques such as Ultrasonography, Pelvic measurement, Trichomoniasis testing, Virgin Heifer test can also be used for BSE. Molecular genetics also plays an important role as, with new advancements it helps us to investigate and identify major genes which are responsible for various diseases in animals. Genes affecting bull fertility can also be identified. Molecular tools such as RFLP, RAPD, Mini-satellites, AFLP's can also be used. BSE every year will help farmer in identifying infertile and subfertile and gain maximum from dairy farming.

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INTRODUCTION

Breeding soundness refers to bull's ability to conceive a cow successfully. Genetic progress in a farm can be accelerated if female animals are bred with high value bulls. As bull contribute large number of progenies to the next generation as compared to females so determining the potential fertility of the bull is much more important than determining the fertility of any individual cow. It becomes critical to study and analyse the breeding potential of a bull before breeding season for getting best out of progeny.

WHAT IS SOUNDNESS EVALUATION?

Breeding soundness evaluation (BSE) is a practical method to predict bull's potential fertility based on various measurements which shows a high correlation with fertility and are highly heritable. Soundness in terms of traits such as increased growth rate, monthly body

weight, and early maturation can be used in bull evaluation. This evaluation should be conducted on every bull at least 30–60 days before each breeding season to allow enough time for replacement of deferred or unsatisfactory bulls [1].

BASIS OF BULL SOUNDNESS EVALUATION?

A complete BSE is normally conducted by a veterinarian. There are different approaches to evaluate a breeding bull, out of which four parameters have been shown to be the most reliable and repeatable in nature and consists of (A) History; (B) Physical examination; (C) Measurement of scrotal size; and (D) Semen evaluation for sperm motility (movement) and morphology (structure and shape).

History

Bulls of elite sire lines or dams line usually follow their ancestry, which make it important

to consider reproductive performance records of its ancestors (sire, grand sires, dam), lateral and collateral relatives (half sibs, full sibs, cousins). Bulls with poor history are usually found to be unsatisfactory in later stages of soundness evaluation.

Physical Examination of Bull

The physical examination should consist of a general evaluation of health, well-being, and body condition of bull. A bull should be true to its breed characters. A bull's ability to impregnate female can be hindered due to disabilities in feet, legs, eyes, testicles, penis, or internal reproductive structures, which render bull as unsatisfactory in soundness evaluation. It is divided in two parts: (1) external examination and (2) internal examination.

External Examination

Conformational and Structural Soundness: All four legs and all joints should be clean and free from any swelling or evidence of old injuries. Posture and gait of bull should be thoroughly checked by moving it on hard surface as well as loose in open area. Limb problems such as sore hocks, camped out hind legs, bow legs, sickle hocks, post legs, puffy hocks should be discriminated against since they are heritable and lead to lameness of bull which hampers his willingness or ability to travel, breed and can also affect sperm production if the bull spends a lot of time lying down.

General Health and Eyes: Bull should have masculine body with good body conformation score. It should be little fat before breeding season as to compensate weight loss in breeding season. Bull should be free from visual defects such as pink eye, scars, squamous cell carcinoma etc.

Condition of Accessory Sex Organs

Penis: The penis and prepuce are generally examined during electro-ejaculation for a semen sample. The penis and sheath should be examined for any sores, lacerations, abscesses, scar tissue, hair rings, warts, or adhesions. A perfectly erect penis should extend from the sheath in a straight line with the body of the bull. Persistent penile frenulum (tied back penis) is commonly found in young bulls during this part of the examination, but it can be easily corrected.

Penis or prepuce should be checked for old

lacerations and adhesions may prevent the penis from being fully extended or cause pain during breeding which can lead to lose in their desire to breed cows and become ineffective.

Testes: When evaluating the testes, size, shape, form and consistency should be checked. Normally testes are always symmetrical and equal in size. Condition such as cryptorchidism, Testicular hypoplasia (underdevelopment), high-flanker should be avoided as these defects are heritable in nature. Testes should not be close to the body wall as it may lead to testicular degeneration resulting in impaired fertility.

Scrotum: It should be pendulous and well suspended. The outer skin of the scrotum should be examined for indications of earlier damage such as scratches, punctures, lacerations, frostbite and infections. Any soft enlargement of the scrotal contents palpated can be suspected for scrotal hernias especially evident at its base and can be verified by rectal palpation of abdominal viscera passing through the internal inguinal ring.

Internal Examination

The accessory sex glands such as vesicular glands, ampullae, and prostate should be looked for evidence of inflammation, adhesions, or fibrosis or enlargements.

The tail of epididymis is a good indication of sperm production; it should be palpated rectally as it stores sperm.

Vesicular glands are a lobulated pair of organs that lie in the pelvis cavity. The most common pathological condition of the accessory sex glands is vesicular adenitis.

The ampullae are located between the vesicular glands which has a normal circumference slightly greater than that of a standard pencil. Ampullitis usually occurs in conjunction with inflammation of other reproductive organs.

Scrotal Circumference

Scrotal circumference (SC) is measured using a flexible tape by forcing the testes to the bottom of the scrotum. It gives a relatively accurate estimate of the semen producing ability of a young bull. The scrotum should have a distinct neck; testes should be freely movable, similar in size, firm and resilient, with normal

epididymides and spermatic cords.

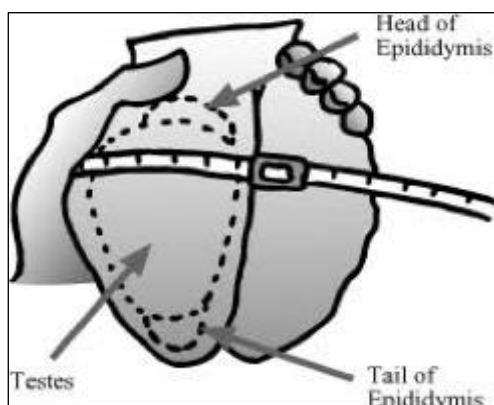


Fig. 1: Measuring Scrotal Circumference.

Scrotal circumference is a valuable indicator of semen production. In young bulls, SC, actual testicular size and daily sperm production are all highly related. Bulls with large testicles produce more semen and sire sons with bigger testicles. Bulls with larger testicles generally reach puberty at an earlier age and sire earlier maturing heifers (Figure 1).

Table 1: Recommended Scrotal Circumference as per Journal Society of Theriogenology (1983) (Chenoweth et al;1992) [4].

Age	Minimum SC (cm)	Good SC (cm)
<15 months	30	>34
15–18 months	31	>36
18–21 months	32	>37
21–24 months	33	>38
>24 months	34	>39

Scrotal circumference is highly correlated with paired testes weight, which is correlated with daily sperm production and semen quality. Bulls with large SC have half-sib heifers and daughters with earlier puberty and greater fertility [2] (Table 1).

Evaluation of Semen Quality

Semen is collected from bulls by a variety of methods such as AV method, Massage method, but electro-ejaculation is the most common under normal field conditions. Various parameters such as volume, colour, and consistency should be checked as per species.

Semen Motility: Motility is best determined by

observing a drop of collected semen and also a drop of diluted semen, using a good microscope. It is defined as the rate of progressive forward movement of sperm towards the ovum after ejaculation has occurred. It depends upon time, temperature, concentration, contamination and the method of evaluation; all affect the motility score. Requirements for motility are that 30% of sperm must have forward, progressive activity (Table 2).

Table 2: Grading of progressive motility of sperm in bulls (Ball et. al 1983) [5].

Grade	Progressive Motility	Points
Good	50–69%	12
Few	30–49%	10
Poor	<30%	3

Manual for BSE of bulls Journal Society of Theriogenology (1983)

Semen Morphology: Semen morphology means the study of the shape of sperm in the ejaculate and is measured as the percentage of normal and abnormal sperm. It depicts true picture of spermatogenesis. The evaluation of viability of sperm was evaluated under a light microscope (1000X) following Eosin Nigrosin staining. Sperm with an unstained head (white arrows) were regarded as the live sperm.

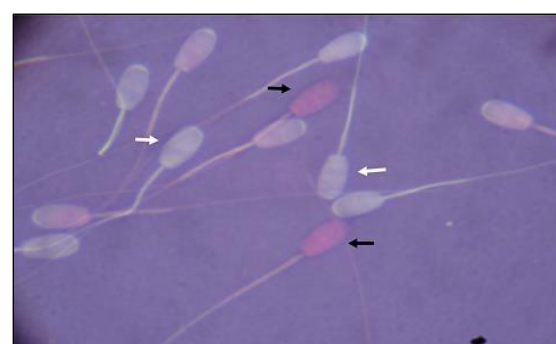


Fig. 2: Sperm Having Red Head (Black Arrows) were Deemed as Dead Sperm.

Abnormalities are classified as either primary or secondary based on whether the defect occurred in the testis or after the sperm left the testis. When primary defects are greater than 18–20% the fertility is reduced. Secondary defects are not generally as serious and do not affect fertility unless a large number are present (Figure 2).

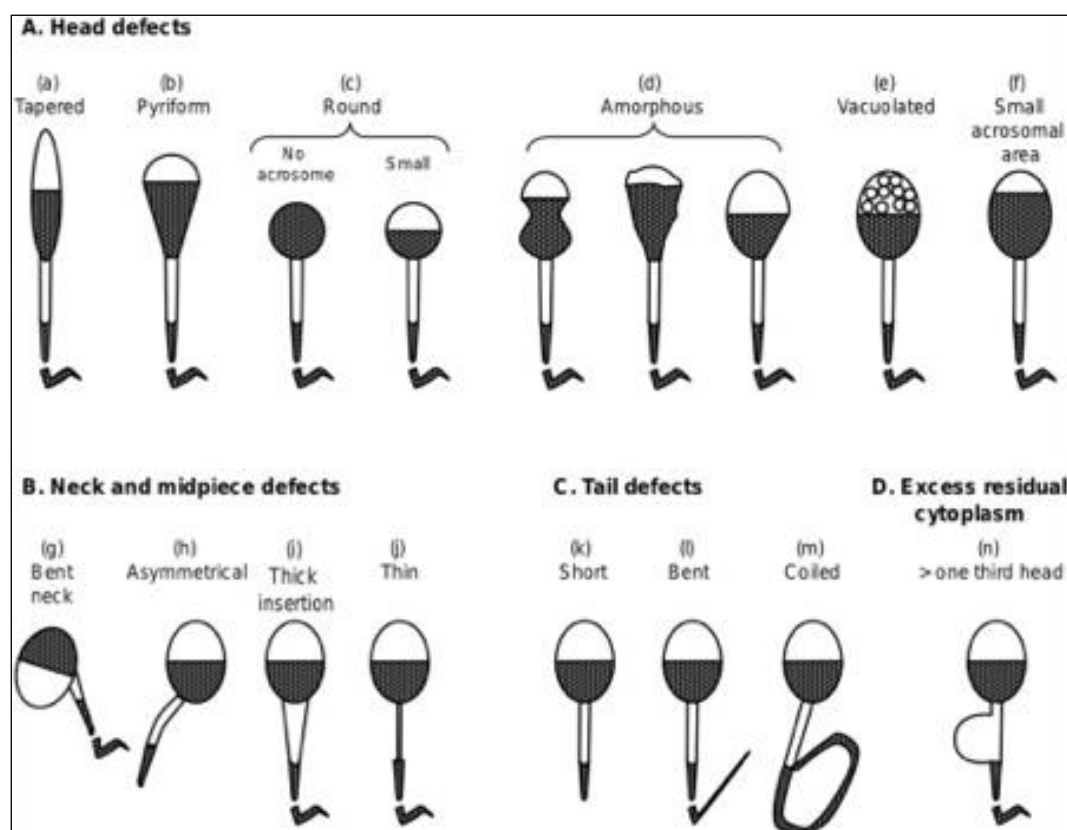


Fig. 3: Common sperm abnormalities.

Most common defects are broken neck, absence of tail, diadem effect, dag defect, knobbed sperm, etc. as shown in Figure 3. The abnormal sperm should not exceed 18–20% in normal semen sample.

Libido/Mating Ability: Libido is defined as the "willingness and eagerness" of a bull to attempt to mount and service, while mating ability refers to the ability and competence of the bull in fulfilling this aspiration [3] or is the ability of the sire to locate, mount and serve cows that are in standing heat.

No practical way is known to estimate a bull's potential mating ability except to observe the bull servicing cows in standing heat. Semen production, scrotal size, or hormone levels do not relate well to the bull's mating performance. Assessment of libido and mating ability is important to help detect physical abnormalities that would prevent a bull with good semen from impregnating cows. It has been demonstrated that there is a significant increase in the ability to impregnate heifers by bulls that are graded high and medium for libido as compared to bulls with low libido.

NEW TECHNIQUES TO BE USED IN BREEDING SOUNDNESS EVALUATION

Ultrasonography

It is a non-invasive, accurate method to evaluate internal structures such as testicular parenchyma, mediastinum testes width etc. which can be time effective and reliable to judge the fertility of bull.

Pelvic Measurement

Another evolving technology is pelvic evaluation. It has been observed in various research reports that there is high genetic correlation between male and female pelvic area which implies that selection for pelvic size in bulls should result in increased pelvic size of daughter offspring. It will be helpful in decreasing the incidences of dystocia in herd.

Trichomoniasis Testing

Trichomoniasis—a venereal disease—is responsible for early abortion within the first four months of pregnancy in pregnant cow. It is mandatory in herd to check for trichomoniasis. Mucus sample is collected from the deepest

portion of the sheath and cultured this material to allow the trichomoniasis organisms to grow. The culture is observed for 3–7 days for any live trichomonads. Bulls testing positive should be culled from the herd.

Virgin Heifer Test

Bull semen is possibly tested for presence of *Campylobacter fetus* venerealis which is causative agent for vibriosis, and act as phantom against reproductive management of cow. In this, 2–3 virgin heifers were inseminated by bull and vaginal mucous and uterine biopsy were collected after 3–4 days of insemination and tested for *Campylobacter fetus* venerealis.

Role of Molecular Genetics

The new advancements in molecular genetics helped us to investigate and identify major genes which are responsible for various diseases in animals. Some diseases were found to be heritable in nature and can pass on this malfunctioning to next generation. Some of the genetic diseases along with responsible genes are given in Table 3.

Table 3: Common genetic diseases in Bovine (Shuster et al., 1992; Dennis al.1989; Schwenger et al., 1993 and Marron et al., 2004) [7-10].

Diseases	Genes responsible
Bovine leukocyte adhesion Deficiency (BLAD)	Integrin beta 2 (ITGB2)
Citrullinemia	Argininosuccinate Synthetase
DUMPS	Uridine monophosphate Synthetase
Factor XI deficiency	UDP-Nacetylglucosamine

Table 4: Genes affecting fertility in Bovines (Bichile et al.2014) [3].

Gene symbol	Protein encoded	Site of Expression	Pathology
TSPY	Testis-specific protein Y	Testis	Gonadoblastoma
DAZ	Deleted in azoospermia	Testis	Oligospermia, Azoospermia
BPY2	Basic protein Y	Testis	Infertility
CDY	Chromodomain Y	Testis	Spermatogenetic failure, Azoospermia
HSFY	Heat shock transcription factor Y	Testis	Azoospermia
VCY	Variable charge Y	Testis	Infertility

GENES AFFECTING BULL FERTILITY

With advancements in molecular genetics now we have additional criteria to select bulls on the basis of expression level of various genes which directly influence the fertility of bull (Table 4).

MOLECULAR TOOLS

- Restricted fragment length polymorphism (RFLP).
- Random amplified length polymorphism (RAPD).
- Mini-satellites.
- Microsatellite.
- Single nucleotide polymorphism.
- Amplified fragment length polymorphisms (AFLPs).

Microsatellites

Single random repeats (STRs) are stretch of DNA a few nucleotides long, about 2–6 base pairs repeated several times in tandem repeats. It is highly polymorphic and present throughout genome; it has high mutation rate. It is a PCR-based technique, allows high throughput analysis. It is codominant in nature. Microsatellites have been proven to be useful markers for a variety of purposes such as identification of animals, evaluation of genetic resources, parentage determination, disease research, determination of genetic variation within and among breeds, because of their high abundance in the genome, extremely high degree of polymorphism and easy detection.

ADVANTAGES OF BREEDING SOUNDNESS EVALUATION

- It increases the transparency in sale and purchase of bulls. A well certified bull fetches more price in the market.
- It gives farmer the greatest assurance that the bull will be reproductively sound during the breeding season in the herd.
- It eliminates the chance of infertile bulls to mate with healthy cow, which saves a lot of time and money from farmer prospective.
- Bulls with low fertility are culled from the herd.
- It helps in improving genetic base for fertility within breed.
- It can be used as an aid to selection of elite bull in herd which can be further progeny tested.

CONCLUSION

Bull has been always a centre of attraction when there is requirement for genetic improvement in the herd. BSE has been a standard for determining if a bull is a good potential breeder and a satisfactory method of identifying subfertile bulls which requires a thorough understanding of spermatogenesis and sperm maturation process. For selecting bulls we should take into account all important aspects, starting from its physical condition, reproductive fitness, and absence of disease to the level of molecular genetics which can ultimately result in improved genetic advancement of breeds and sustainable development of livestock. BSE every year will help farmer in identifying infertile and subfertile bulls and ultimately have higher pregnancy rates in your herd and definitely improve socioeconomic condition of farmer.

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