Bull Selection Guideline for Genetic Improvement

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Dr Asrat Tera
Director General
Livestock Development Institute

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List of Abbreviations

AI	Artificial Insemination
BSE	Breeding Soundness Evaluation
BVD	Bovine Virus Diarrhea
CASA	Computer Assisted Semen Analysis
CFT	Compliment Fixation Test
EBV	Estimated Breeding Value
ELISA	Enzyme Linked Imuno Sorbent assay
GEBV	Genomic Estimated Breeding Value
HOST	Hypo Osmotic Swelling Test
IBR	Infectious Bovine Rhinotracheitis
LDI	Livestock Development Institute
MOT	Percent Total Motility
MOU	Memorandum of Understanding
MPPA	Most Probable Producing Ability
NAGII	National Animal Genetic Improvement Institute
NAIC	National Artificial Insemination Center
NCP	Non-Cytopathic
OIE	International Organization for Epizootics
PBV	Probable Breeding Value
PCR	Polymerase Chain Reaction
PI	Persistent Infection
PMOT	Progressive Motility
RBPT	Rose Bengal plate Test
TB	Tuberculosis

Scope

The scope of the guideline document for bull selection, titled "Bull Selection Guideline for Genetic Improvement," issued by the Livestock Development Institute (LDI) in Addis Ababa, Ethiopia, aims to standardize the bull selection process for artificial insemination (AI) and natural mating to enhance cattle genetic improvement in the region. It begins with a historical overview, highlighting the efforts in crossbreeding local and exotic cattle since the early 1950s through various governmental and non-governmental partnerships. The main sections of the document include:

Selection of Bull Dam Farm/Source: Criteria for choosing farms that contribute to breeding bulls, emphasizing the need for farms registered on the National Cattle Performance Recording database with organized data recording.

Selection Criteria for Candidate Breeding Bulls: Detailed processes and parameters for choosing breeding bulls, encompassing genetic selection strategies such as pedigree information, performance recording, estimated breeding values (EBVs), and genomic selection methods like genomic BLUP (GBLUP) and single-step GBLUP (ssGBLUP).

Breeding Soundness Evaluation (BSE): Extensive guidelines for assessing the physical and reproductive health of bulls, including detailed examinations of physical soundness, scrotal conformation, semen quality, and overall health status to ensure only the best candidates are selected for breeding.

The document also contains comprehensive appendices with tables and figures to support the evaluation processes, such as reference values for scrotal circumference and diagnostic tests for infectious diseases relevant to breeding bulls.

Overall, the guideline is designed to systematize and enhance the genetic evaluation and selection process of breeding bulls, ensuring high standards are maintained to achieve genetic progress in the cattle population of Ethiopia.

1. Background

Cattle genetic improvement initiatives in Ethiopia began in the early 1950s with crossbreeding programs aimed at enhancing productivity by integrating exotic and local breeds. Over the decades, governmental institutions such as the Ministry of Agriculture (MoA), Ethiopian Agricultural Research Organizations, and the National Artificial Insemination Center (NAIC) later restructured as the National Animal Genetic Improvement Institute (NAGII) and now the Livestock Development Institute (LDI) have spearheaded these efforts. Collaborating with nongovernmental partners, including the International Livestock Research Institute (ILRI) and the Swedish International Development Agency (SIDA), these programs utilized both natural mating and artificial insemination (AI) to develop crossbred cattle suited to local conditions.

Established in 1981, the LDI holds the national mandate to coordinate AI services, including training technicians, producing and importing semen, supplying liquid nitrogen, and managing animal identification and performance recording. The institute has historically partnered with regional agricultural bureaus to deliver AI services across rural, peri urban, and urban areas, ensuring broad accessibility to genetic improvement tools.

To support these efforts, the LDI utilizes exotic breeds such as Holstein Friesian and Jersey, known for their dairy productivity, alongside indigenous breeds like Borana, Begait, Fogera, Horro, Irob, and Sheko, which are valued for their adaptability and resilience. Indigenous bulls are typically sourced from their native environments to preserve genetic integrity, while exotic breeds are procured from government nucleus herds, private farms, or through international imports.

Selecting candidate bulls for AI centers and natural mating requires a comprehensive evaluation of phenotypic traits, genetic merit, breeding soundness (BSE), and health status. However, current practices often prioritize physical conformation, disease resistance, and basic breeding soundness evaluations, with limited emphasis on standardized genetic evaluation. This inconsistency underscores the absence of a unified national guideline to direct bull selection processes, leading to variable outcomes in genetic progress and productivity.

To address this gap, this guideline aims to establish a standardized, science-based framework for selecting bulls for AI and natural mating. By harmonizing evaluation criteria and procedures nationwide, it seeks to enhance the genetic quality of Ethiopia's cattle population, boost milk production, and improve reproductive efficiency, ultimately supporting sustainable livestock development and food security.

Objective

To establish a standardized protocol for selecting candidate bulls for artificial insemination and natural mating in Ethiopia, ensuring the consistent application of scientifically validated criteria. This objective aims to enhance genetic gains, improve milk yields, and strengthen the reproductive performance of the national cattle herd through rigorous phenotypic, genetic, health, breeding soundness evaluations and semen quality evaluation.

2. Selection of bull source farms

To improve the genetic quality of national herds, bull selection is primarily conducted from farms registered in the National Cattle Performance Recording Database managed by the Livestock Development Institute (LDI). These farms must follow structured data recording practices to qualify as sources for breeding bulls.

1. **Exotic Breeds**: Farms with predominantly purebred and crossbred populations of Holstein Friesian, Jersey, and other dairy or beef breeds are preferred for live bull procurement.

2. **Indigenous Breeds**: Local breeds such as Borana, Begait, Fogera, and Sheko are ideally selected from their regions of origin, where they thrive in their natural environments. This ensures the preservation of their unique genetic traits. Indigenous cattle may also be sourced from local markets within their native regions. While registration in the national database is not mandatory for these breeds, it is crucial for ensuring traceability and health standards. Additional selection of local breeds may be conducted from nucleus herds, typically maintained in government ranches, universities, and research institutes, where these breeds are actively preserved and studied.

Following the selection process, a Memorandum of Understanding (MoU) will be established between the LDI, regional Artificial Insemination (AI) centers, and the owners of the selected farms. This agreement will formalize collaboration and outline the responsibilities of each party in the supply of breeding bulls.

3. Selection Criteria for Candidate Breeding Bulls

Bulls born from the best-performing dams on each farm will be selected as candidates through a multi-stage selection process before being included in the institute's semen-producing bull program.

3.1. Genetic Selection

3.1.1 Pedigree Information and Recording

Pedigree records track the ancestry of individuals within a population, providing insights into genetic background and relationships. Therefore, pedigree information is critical for estimating the breeding value of a candidate bull. The candidate animal must have at least the following details:

- National identification number
- Birth date
- Breed
- Sex
- Dam and sire information

Performance records of the relatives including milk yield, body weight, dry date, calving date, AI service, and health and vaccination history, should also be recorded and entered into the national database.

3.1.2 Genotype Data

Whenever possible, genotype samples (hair, blood, or tissue) should be collected. Key considerations include:

- **Proper Handling**: Use sterile equipment and gloves to prevent contamination.
- **Labeling and Storage**: Clearly label samples with ID, type, and date. Store them appropriately to maintain DNA integrity.
- **Ethical Compliance**: Adhere to ethical standards and obtain necessary approvals for animal welfare.

3.1.3 Estimated Breeding Value (EBV)

Estimated Breeding Value (EBV) represents an animal's genetic potential and is calculated using statistical models incorporating pedigree data, performance records, and/or genomic information. A positive EBV indicates superior performance relative to the population average, reflecting the genetic contribution to trait performance. For example, a positive EBV for milk yield is desirable, while a negative EBV is preferred for calving interval. Traits included in genetic evaluations should be selected based on their economic importance and data availability.

Genetic evaluation methods include:

- **Pedigree-based Best Linear Unbiased Prediction (BLUP)**
- **Genomic BLUP (GBLUP)**
- **Single-step GBLUP (ssGBLUP)**

Pedigree-based EBV Estimation:

This process involves three stages:

1. On-farm pedigree and performance data collection.
2. Data entry and validation.
3. Data analysis (EBVs and Indexes).

Genomic Selection Methods:

1. **Genomic BLUP (GBLUP)**: This method uses genomic relationships to estimate genetic merit. It involves genotyping young selection candidates and calculating Genomic Estimated Breeding Values (GEBVs) by summing the effects of relevant SNP alleles. A reference population of at least 1,000 individuals with reliable phenotypic and genotypic data is required.
2. **Single-step Genomic BLUP (ssGBLUP)**: This method integrates both genotyped and non-genotyped animals, using pedigree and genotype information simultaneously for more accurate evaluations.

By following these criteria and methods, the selection of candidate breeding bulls ensures the enhancement of genetic quality and productivity in national herds.

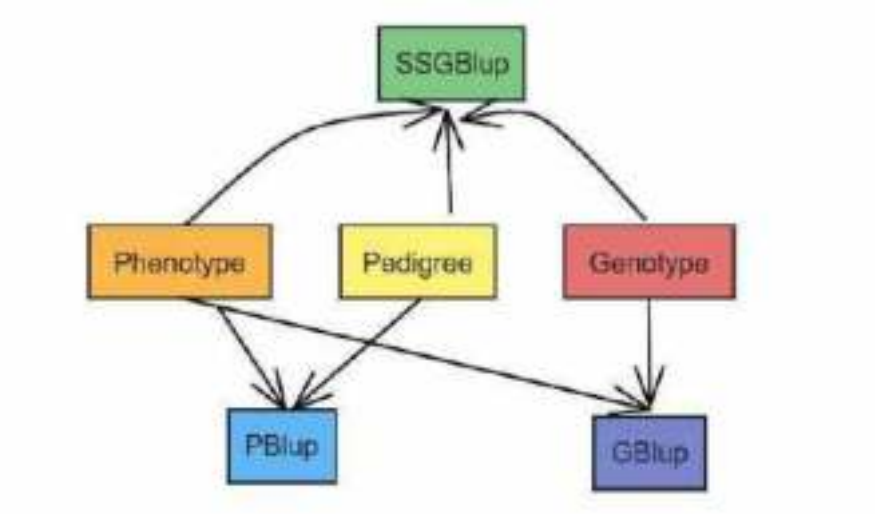


Figure 1. Schematic illustration of genetic evaluation methods

3.2. Breed Descriptions

To ensure the accuracy and consistency of bull selection, experts should establish standardized criteria by consulting resources such as the Ethiopian Biodiversity Institute, the Food and Agriculture Organization (FAO), and the Domestic Animal Genetic Resources Information System (DAGRIS). Additionally, published materials and documents from breed societies should be referenced, as there is currently no universally accepted standard for describing indigenous and commercial breeds. This collaborative approach will help maintain the integrity and effectiveness of breed improvement initiatives.

3.3. Breeding Soundness Evaluation (BSE)

The physical examination of a bull should encompass an assessment of its overall health, body condition, and structural conformation. Key aspects to evaluate should include:

- Whether the bull's vision is sufficient for it to navigate its environment effectively.
- Whether it possesses the structural integrity necessary for mounting and moving around in pastures or feeding areas.
- Whether it can maintain an appropriate body condition, avoiding being overly fat or thin.

Additionally, the examination should involve palpating the internal and external genitalia to identify any abnormal adhesions, inflammation, or abscesses that could affect breeding capabilities or facilitate the spread of diseases.

When selecting a breeding bull through physical examination, the following body parts and overall condition should be carefully evaluated:

Eyes

- The bull should exhibit a menace response (blinking) when its eyes are examined.
- The eyes should be well-set into the head to minimize exposure, with a strong forehead providing protection from sunlight (hooding).
- Bulging eyes should be avoided, as they are more prone to injury.
- Pigmentation around the eyes should be present, especially in white-faced breeds, to reduce the risk of eyelid cancer.
- A bull with impaired vision or blindness should not be selected for breeding.
- Conditions such as pinkeye or cancer eye should be noted, as they can hinder a bull's vision and reduce breeding effectiveness.
- A bull blind in one eye should be excluded from selection, as it poses a safety risk to handlers and cows during mating.
- Any abnormal eye conditions observed during evaluation should be examined by a veterinarian, and such bulls should be avoided.

Legs and Feet

- The front legs of the bull should appear straight when viewed from the front.
- On a structurally sound bull, a vertical line should be able to be drawn from the point of the shoulder to the middle of the claw.
- The condition of the claws should be examined, as it often reflects structural issues in the legs.
- Long or excessively short claws should be avoided, as they may indicate improper pastern angles, leading to uneven growth or wear.
- Overgrown, scissor-shaped, or severely curved claws should not be selected, as they affect mobility and performance.
- Mild curling of the claws is normal and acceptable.
- Bulls with normal front leg structure (as illustrated in Figure A of Figures 2 and 3) and correct pastern angles (Figure A) should be selected for breeding.
- Bulls with foot structures like those shown in Figure 4 (A) should be preferred for breeding purposes.

By following these guidelines, users should ensure that only bulls with optimal physical health and structural soundness are selected for breeding, thereby enhancing the overall quality and productivity of the herd.

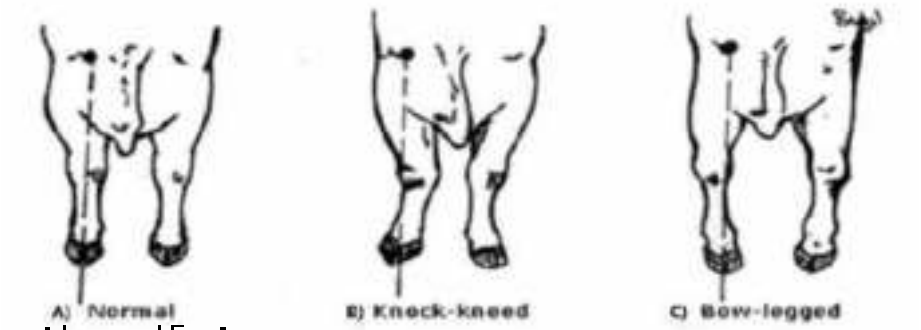


Figure 2. Front leg and Feet

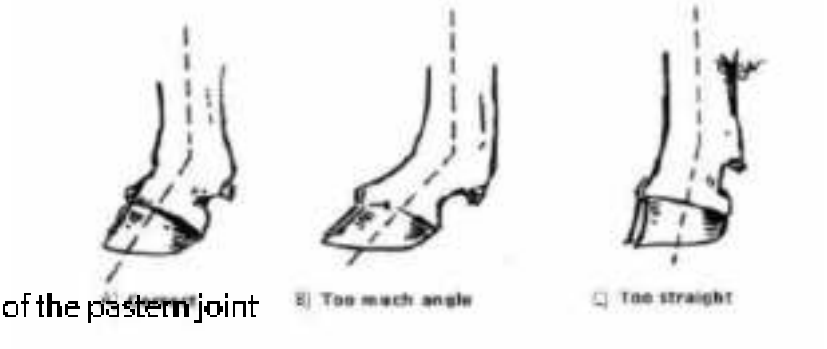


Figure 3. Angle of the pastern joint

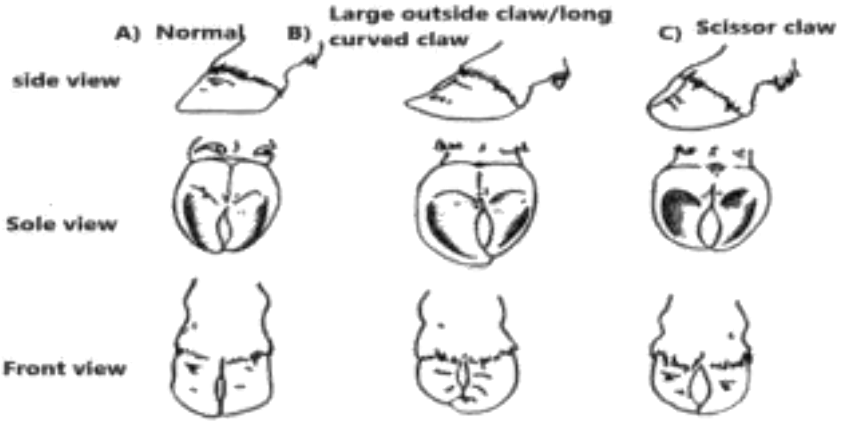


Figure 4. Feet Structure

The structure of the hind legs should be evaluated in a manner similar to that of the front legs. Rear leg conformation should be given high priority, as the hind legs are the primary load-bearing structures during mounting and breeding and are essential for proper mobility. The hind legs should have well-defined angles at the hip, stifle, hock, and pastern joints, as illustrated in Figure 5 (A). Deviations from these correct angles, as shown in Figure 5 (B & C), should be avoided, as they can lead to excessive wear and tear on the joints, resulting in early breakdown. Hind leg problems are one of the most common reasons for bulls breaking down, making this evaluation critically important.

When viewed from the rear and side, the bull depicted in Figure 5 (A) and Figure 6 (A) demonstrates the correct structure of the hind legs and feet. Bulls with such conformation should be preferred for selection to ensure optimal mobility, breeding performance, and longevity.

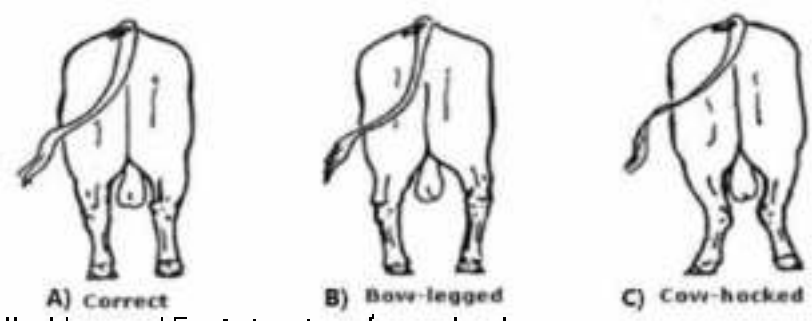


Figure 5. Hind leg and Feet structure (rear view)

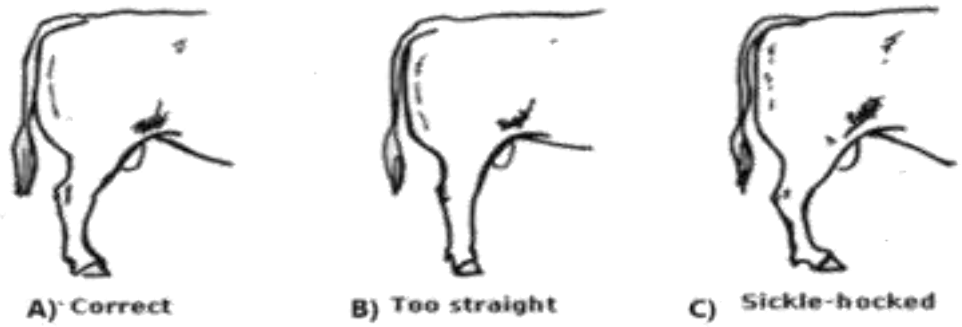


Figure 6. Hind leg and Feet structure (side view)

Walk

The bull's walk should be observed carefully to assess its gait and movement. A free-moving gait should be looked for, with the hind feet stepping into the footprints of the front feet, as illustrated in Figure 7. Overstepping (where the hind feet land ahead of the front footprints) or understepping (where the hind feet land behind the front footprints) should be noted, as these are indications of structural problems. Additionally, uneven footprints from the claws should be checked, as they may also signal underlying issues.

When the bull walks, the hind feet stepping into the footprints of the front feet should be considered the correct walk style. Bulls demonstrating this gait should be preferred for selection for breeding purposes.

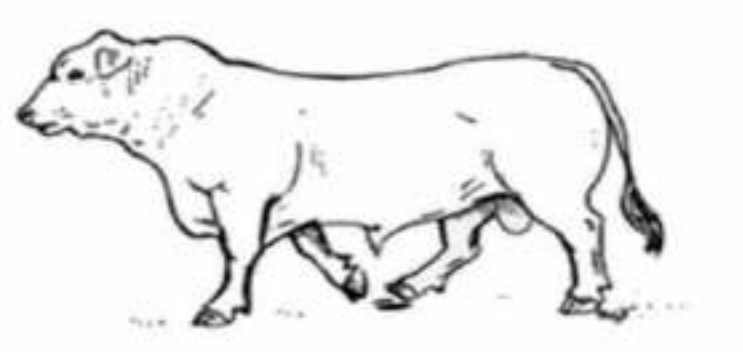


Figure 7. Proper Walk of the bull

Top line/ the back:

The top line of the bull should be evaluated as the total length of the animal, measured from the front of the poll to the back of the rump. The top line should be divided into three measurements: neck length, back length, and rump length, as illustrated in Figure 8. These three measurements together make up the total top line length.

Ideally, 2/3 of the top line should be composed of the rump length and back length. The total top line or true top line should be calculated using the formula:

Total Top Line = 2/3 Top Line × 1.5

For example, if a bull measures 70 inches in total length and its body length (back – rump) is 48 inches, the neck length should be calculated as follows:

- Neck Length = Total Length – Body Length
- Neck Length = 70 inches – 48 inches = 22 inches

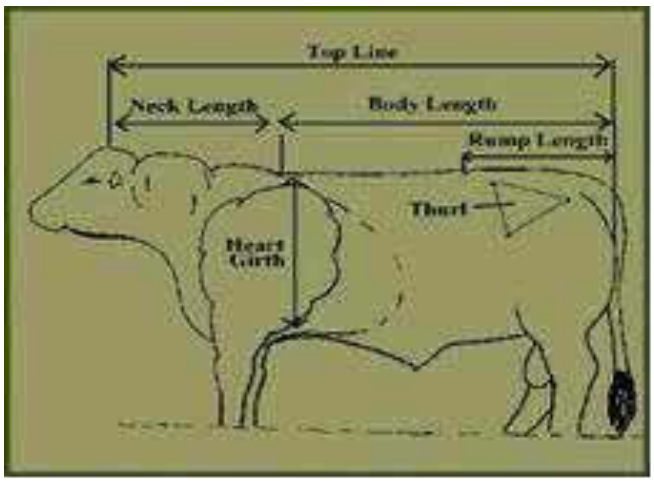


Figure 8. Body length description

Heart Girth

The heart should be measured as the total distance around the animal's chest, just behind the shoulder blade. At 12 months of age, the heart girth should be equal to or larger than the total top line.

Guidelines for Measuring Heart Girth Using a Tape:

1. *Position the bull so that its front legs are evenly placed on the ground.*
2. *Place the tape measure just behind the shoulder blade.*
3. *Wrap the tape around the bull's chest, down the fore-ribs, and under the body behind the elbow.*
4. *Pull the tape snugly enough to keep the hair flat but not so tight that it indents the flesh.*

Figure 9. Heart girth measurement

Rump angle: The rump angle should be evaluated to ensure proper structural conformation. The pin bones should be slightly lower than the hip bones, as illustrated in Figure 9 (A). A pin bone higher than the hip bones is often, but not always, undesirable. Bulls with an extreme slope to the rump may also exhibit undesirable hock alignment or hind leg movement issues, which can affect mobility and breeding performance.

A) Pins higher than hooks B) slightly slope from hips to pin. C) extreme slope from hips to pin

Figure 10. Rump angle

Shoulders: The shoulders and front leg structure of the bull should be assessed as shown in Figure 10. The shoulders should have a natural slope, with an angle of 45 60 degrees considered acceptable. The shoulder should blend smoothly against the rib cage. Bulls with shoulders that are wide at the point of the shoulder (base of the neck) or wide between the shoulder blades (when viewed from above) may produce heavily shouldered calves, increasing the risk of calving difficulties (Figure 11).

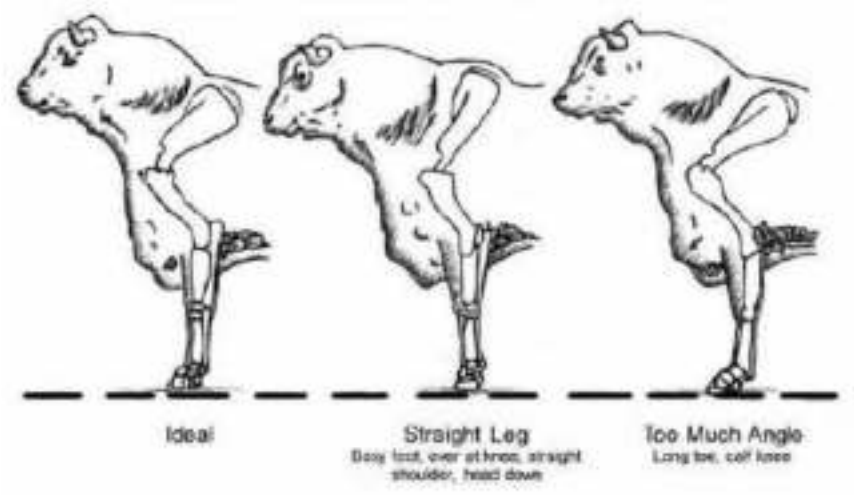


Figure 11. Front leg and shoulder structure of the bull

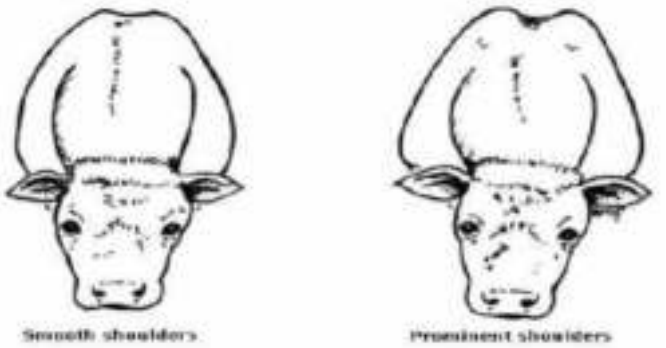


Figure 12. Shoulder structure from view

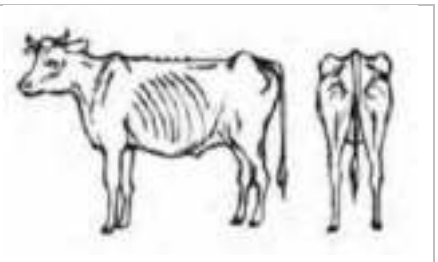
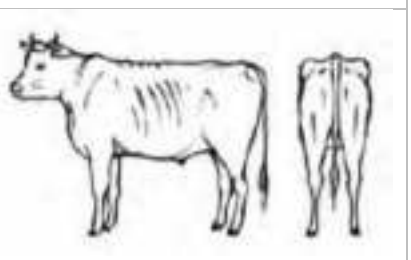
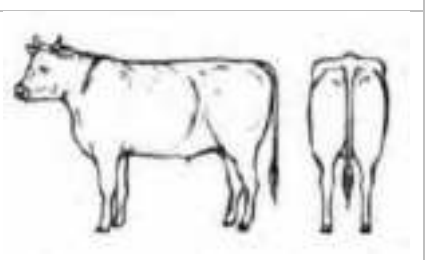
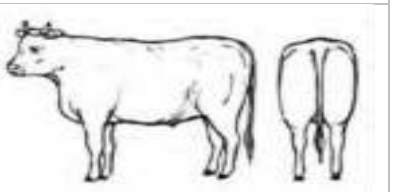
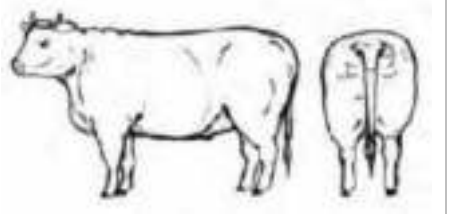
Body Condition Score: Body Condition Scoring (BCS) is a practical tool for managing the nutrition and fertility of breeding stock. Scoring is subjective, so all staff should undergo training, and the same person should routinely perform the scoring to minimize variation between scorers. BCS is assessed on a five point scale, where:

1 = Very thin

5 = Obese

Bulls with a BCS of 2 or less should be deemed unsatisfactory, as they are unlikely to perform adequately during an intensive breeding period. Maintaining bulls within an optimal BCS range (typically 3-4) is essential for ensuring their health, fertility, and breeding performance.

Body condition score key:

Score 1 Backbone prominent Hips and shoulder bones prominent Ribs clearly visible Tail-head area recessed skeletal body outline	
Score 2 Backbone visible Hips and shoulder bones visible Ribs visible faintly Tail-head area slightly recessed Body outline bony	
Score 3 Hip bones visible faintly Ribs generally not visible Tail-head area not recessed Body outline almost smooth	
Score 4 Hip bones not visible Ribs well covered Tail-head area slightly lumpy Body outline round	
Score 5 Hip bones showing fat deposit Ribs very well covered Tail-head area very lumpy Body outline bulging due to fat	

Temperament: It should be understood that temperament, whether docile or aggressive, is a heritable trait. While some behavioral traits may not be evident at an early age, certain bulls may display aggressive behavior as they mature. Therefore, bulls that pose a risk to attendants, handlers, or experts due to aggressive behavior should be identified and culled from breeding service.

Examination of Scrotum: the scrotum of a bull can be described as follows.

Straight-sided scrotum: This shape is usually due to a fat-pad at the base of the scrotum which could interfere with testicular thermoregulation. The testicles in a straight-sided scrotum are frequently only moderately sized (Figure a).

Normal scrotum: This should be selected for breeding purposes and large sized testicles are most frequently found in a normal-shaped scrotum (Figure b).

Pointy scrotum/Wedge-shaped scrotums: Testicles in a pointy scrotum are held too close to the body and are most often undersized (Figure c).

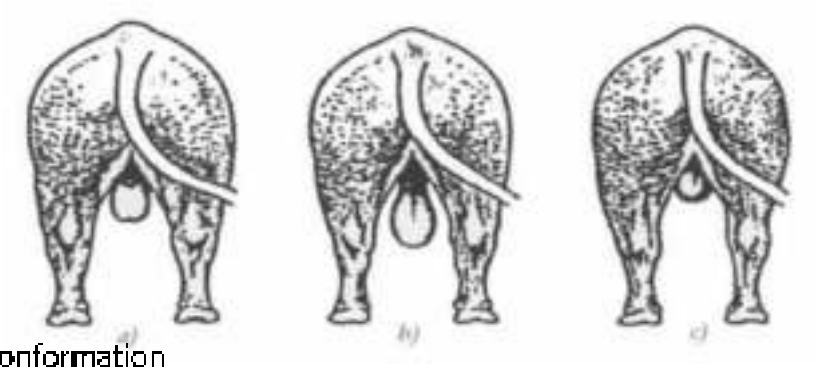


Figure 13. Scrotal conformation

Scrotal circumference (SC): The scrotal circumference indicates the combined testicle size and is one of the easiest ways to estimate of a normal tone having a greater capacity to produce semen and inseminate a larger number of cows. In Table 1 are the minimum recommended scrotal circumference categorized per age group that a bull must have to pass a BSE.

Table 1. Reference for Scrotal Circumference

Age (month)	Bos Taurus (cm)	Bos indicus (cm)
<= 15	30	26
>15 <=18	31	28
>18 <= 21	32	30
>21 <=24	33	31
>24	34	32

Technique for SC Measurement: The measurement is taken with scrotal tape. The testes should be cradled in the scrotum by one hand held at the neck of the scrotum with thumb and forefinger held either side of the neck of the scrotum (Figure 13). The thumb should not be pushed in between the spermatic cords as this can push the testes apart thus increasing the SC falsely. The tape is placed around the widest part of

the scrotum and pulled snugly until the skin is indented. The procedure should be repeatable and accurate within 0.5cm with different operators using the same tape.

Figure 14. Technique for SC Measurement

Examination of testicles: Examination of the testes and associated structures is to check for any abnormalities or unevenness. This will identify a range of diseases which may permanently or temporarily alter the function of the testes. The testes should be of no genetic abnormalities (e.g. hypoplasia), adequate size, normally formed and no swelling or hardening, no excessive fat deposition in the neck of the scrotum, well-developed epididymis and no cryptorchidism.

Examination of the prepuce and penis: The penis should be felt to ensure that it can move freely within the prepuce (sheath) and that there are no swellings or growths on either the prepuce or penis (Figure 14). It is not possible to examine the penis fully in the standing conscious bull; thus, it is important to check the penis when is extruded during the testing of mounting ability (or when using an electro-ejaculator to collect semen).

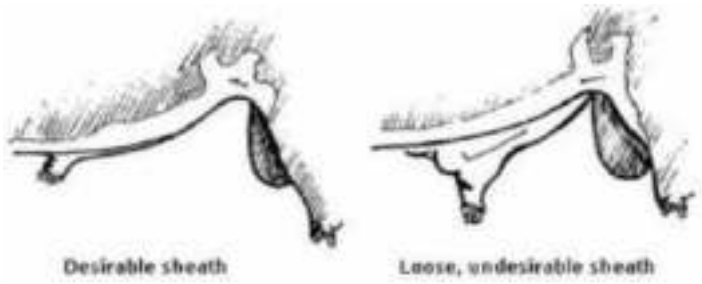


Figure 15. Sheath Conformation

Accessory sex glands: Accessory sex glands (seminal vesicle, Prostate and bulbourethral) can be assessed for their development upon rectal palpation, and bulls with accessory sex glands of well-developed shall be pass to the next level of breeding soundness evaluation. Moreover, for tough appreciate of the development of accessory sex glands, the evaluation shall be carried out using ultrasound instead of rectal palpation.

3.4. Disease control

Health monitoring and disease surveillance of candidate and semen-producing bulls before isolation, during isolation/quarantine, and throughout their residency at the AI

center are critical to safeguarding both the health of the seminal donors and the herds that utilize their semen.

3.4.1.Pre-entry to Isolation

Candidate bulls that are intended to enter a semen production center shall be healthy and free of infectious or contagious diseases and shall not originate from a herd under quarantine. Subsequent to the pre- entry testing, the bulls should not be used for natural service and should be isolated from the herd. The following pre-entry examination and diagnostic tests shall be conducted.

Physical Examination: Conduct a physical examination by a veterinarian within 30 days prior to entry to ensure the bull shows no clinical symptoms of infectious or contagious diseases.

Bovine Tuberculosis: Conduct a Single Intradermal Comparative Cervical Test (SICCT) within 60 days prior to entry and ensure the result is negative. If the SICCT result is not negative, perform a confirmatory Comparative Cervical Test (CCT) within 10 days of the initial SICCT injection. If 10 days have passed, wait 60 days before administering the CCT; and ensure the result to be negative.

Bovine Brucellosis: Conduct a Rose Bengal Plate Test (RBPT) within 30 days prior to entry. If the result is not negative, perform a confirmatory brucellosis test, such as a Complement Fixation Test (CFT), within 30–60 days of the initial test and ensure the result to be negative.

Bovine Viral Diarrhea Virus (BVDV): Conduct Competitive Enzyme-Linked Immunosorbent Assays (c-ELISA) within 30 days prior to entry to detect antibodies against BVDV in serum samples, ensuring a negative result. Use Antigen Capture ELISA (AC-ELISA) as the preferred confirmatory test to detect Bovine Viral Diarrhea virus antigens and identify persistently infected (PI) candidate bulls. Hence, LDI's test of preference shall be Antigen Capture ELISA test to test BVDV for candidate semen producing bulls.

Infectious Bovine Rhinotracheitis (IBR): Conduct serological tests to detect antibodies against the IBR virus in candidate bulls within 30 days prior to entry; and ensure a negative result for it.

Bovine Campylobacteriosis (Vibriosis): Accurate diagnosis for campylobacteriosis is essential for candidate breeding bulls. It is possible to use direct microscopy test or gold standard test (culture and isolation test) to identify the bacteria.

- Use aseptically collected Preputial wash sample
- At field condition, use direct microscopy tests. But it has low sensitivity
    - Use staining techniques (e.g., Gram staining) to identify campylobacter organisms in preputial smears.
- Use gold standard test: Culture the sample on specialized media (e.g., Skirrow's medium) under microaerophilic conditions and isolate the bacteria

NB:

- The bacterium is sensitive to environmental conditions, reducing test sensitivity. So, take percussion for it.
- Use multiple diagnostic methods, when possible, to improve accuracy
- Testing should be repeated periodically
- Timely diagnose and remove infected animals to control the disease.
- ***Bovine Venereal Trichomoniasis Test:*** Accurate and timely diagnosis for Bovine Venereal Trichomoniasis is essential for candidate breeding bulls. It is possible to use direct microscopy test or gold standard test (culture and isolation test) to identify the protozoa.
- Collect preputial wash sample aseptically
- Transport the sample to the laboratory promptly to ensure protozoan viability
- At field condition, use direct fresh sample microscopy tests for motile protozoa. But it has low sensitivity as the motility of *T. foetus* may decrease over time
- Use gold standard test: Culture the sample on specialized media (e.g., Diamond's medium), and isolate identify the protozoa
- Regularly test the breeding bulls and remove positive animals.

NB:

- Ensure samples are fresh and kept in optimal conditions (e.g., warm and anaerobic) during transport to the laboratory.
- Protozoa may die during sample transport, leading to false negatives.
- Culturing the sample requires several days (up to 7 days) to confirm results.
- Negative test results typically require confirmation through repeated testing (at least three negative tests at weekly intervals).
- Bulls are the primary carriers, so routine testing of breeding bulls is essential.

NB:

In addition to its negative results for the above listed disease tests, verify that the candidate bull has been vaccinated within the past six months for common diseases, including Anthrax, Blackleg, Pasteurellosis, Lumpy Skin Disease (LSD), and Foot-and-Mouth Disease (FMD), before selection for semen production.

3.4.2. Isolation/Quarantine

Each bull shall successfully complete the isolation/quarantine protocol before being permitted to enter the facilities occupied by resident semen producing bulls and before any semen from that bull is released for use.

Each candidate bull shall be held in isolation/quarantine throughout the period of time necessary to conduct the tests listed below.

Bovine Tuberculosis: Conduct a Single Intradermal Comparative Cervical Test (SICCT) at least 60 days after the date of pre-entry test and no sooner than 21 days after entry into the isolation facility; and ensure a negative result. If the SICCT result is not negative, perform a confirmatory Comparative Cervical Test (CCT) with a negative result within 10 days of the initial SICCT injection. If 10 days have passed, wait 60 days before administering the CCT.

Bovine Brucellosis: Conduct a Rose Bengal Plate Test (RBPT) no sooner than 30 days after the pre-entry test and no sooner than 21 days after entry into the isolation facility; and ensure a negative result. If the RBPT result is not negative, perform a confirmatory brucellosis test, such as a Complement Fixation Test (CFT), within 30–60 days of the initial test and ensure the result to be negative.

Bovine Viral Diarrhea Virus (BVDV): Conduct Competitive Enzyme-Linked Immunosorbent Assays (c-ELISA) to detect antibodies against BVDV in serum samples no sooner than 30 days after the pre-entry test for brucellosis and no sooner than 21 days after entering the isolation facility and ensure a negative result. Use Antigen Capture ELISA (AC-ELISA) as the preferred test to detect Bovine Viral Diarrhea virus antigens, and to identify persistently infected (PI) candidate bulls, and confirm negative results from c-ELISA. Prioritize AC-ELISA as the test of choice to screen BVDV in candidate semen-producing bulls.

Any bull that has active or persistent infection for BVDV is not eligible for semen collection and is not permitted to remain in the isolation facility or resident herd.

Infectious Bovine Rhinotracheitis (IBR): Conduct serological tests to detect antibodies against the IBR virus in candidate bulls no sooner than 30 days after the pre-entry test and no sooner than 21 days after entering the isolation facility; and ensure a negative result.

Bovine Campylobacteriosis (Vibriosis): Accurate diagnosis is essential for controlling the disease in breeding herds. It is possible to use direct microscopy test or gold standard test (culture and isolation test) to identify the bacteria.

- Use aseptically collected Preputial wash sample
- Use direct microscopy tests. But it has low sensitivity
  - Use staining techniques (e.g., Gram staining) to identify Campylobacter organisms in preputial smears.
- Use gold standard test: Culture the sample on specialized media (e.g., Skirrow's medium) under microaerophilic conditions and isolate and identify the bacteria.

NB:

- The bacterium is sensitive to environmental conditions, reducing test sensitivity. So take percussion for it.
- Use multiple diagnostic methods when possible to improve accuracy
- Testing should be repeated periodically
- Timely diagnose and remove infected animals to control the disease.

Bovine Venereal Trichomoniasis Test: Accurate and timely diagnosis is crucial for controlling Bovine Venereal Trichomoniasis in breeding herds. It is possible to use direct microscopy test or gold standard test (culture and isolation test) to identify the protozoa.

- Collect aseptically preputial wash sample
- Transport the sample to the laboratory promptly to ensure protozoan viability
- Use direct fresh sample microscopy tests for motile protozoa. But it has low sensitivity, as the motility of *T. foetus* may decrease over time
- Use gold standard test: Culture the sample on specialized media (e.g., Diamond's medium), and isolate/identify the protozoa
- Regularly test the breeding bulls and remove positive animals.

NB:

- Ensure samples are fresh and kept at optimal conditions (e.g., warm and anaerobic) during transport to the laboratory.
- Protozoa may die during sample transport, leading to false negatives.
- Culturing the sample requires several days (up to 7 days) to confirm results.
- Negative test results typically require confirmation through repeated testing (at least three negative tests at weekly intervals).
- Bulls are the primary carriers, so routine testing of breeding bulls is essential.

3.4.3. Resident Herd

Once a bull has completed the isolation testing outlined above with satisfactory test results, it may enter the resident herd where it shall continue to be tested in accordance with the below listed test procedures.

Bovine Tuberculosis: Conduct a Single Intradermal Comparative Cervical Test (SICCT) at least 60 days after the date of isolation test and no sooner than 21 days after entry into the resident facility; and ensure a negative result. If the SICCT result is not negative, perform a confirmatory Comparative Cervical Test (CCT) with a negative result within 10 days of the initial SICCT injection. If 10 days have passed, wait 60 days before administering the CCT.

- *Conduct bovine tuberculosis (TB) testing in a semen production center at regular intervals, ideally annually.*
- *If biosecurity measures are not strict, test bulls every six months.*
- *Pause semen collection and distribution immediately for any bull that tests positive for bovine TB.*
- *Remove TB-positive bulls from the semen production program immediately.*

Bovine Brucellosis: Conduct a Rose Bengal Plate Test (RBPT) no sooner than 30 days after the pre-entry test and no sooner than 21 days after entry into the resident facility; and ensure a negative result. If the RBPT result is not negative, perform a confirmatory brucellosis test, such as a Complement Fixation Test (CFT), within 30–60 days of the initial test and ensure the result to be negative.

- *Conduct Bovine brucellosis testing in a semen production center at regular intervals, ideally every six months.*
- *If biosecurity measures are not strict, test bulls more frequently.*
- *Pause semen collection and distribution immediately for any bull that tests positive for bovine brucellosis.*
- *Remove Bovine brucellosis-positive bulls from the semen production program immediately.*

Bovine Viral Diarrhea (BVD): testing BVD in a semen production center should be conducted regularly to ensure the health of breeding bulls and the safety of collected semen.

- *Conduct Bovine Viral Diarrhea (BVD) testing in the semen production center biannually.*
- *Increase testing frequency if biosecurity measures are not strict.*
- *Permanently cull any bulls identified as persistently infected (PI) with BVD.*

***Infectious Bovine Rhinotracheitis* (IBR):** testing IBR in a semen production center should be conducted regularly to ensure the health of breeding bulls and the safety of collected semen.

- *Conduct IBR testing in the semen production center biannually.*
- *Increase testing frequency if biosecurity measures are not strict.*
- *Permanently cull any bulls identified as positive with IBR*

Bovine Campylobacteriosis (Vibriosis): Accurate diagnosis is essential for controlling the disease in breeding herds. It is possible to use a direct microscopy test or gold standard test (culture and isolation test) to identify the bacteria

- Use aseptically collected Preputial wash sample
- Use direct microscopy tests. But it has low sensitivity
    - Use staining techniques (e.g., Gram staining) to identify Campylobacter organisms in preputial smears.
- Use gold standard test: Culture the sample on specialized media (e.g., Skirrow's medium) under microaerophilic conditions and isolate and identify the bacteria

NB:

- The bacterium is sensitive to environmental conditions, reducing test sensitivity. So, take percussion for it.
- Use multiple diagnostic methods, when possible, to improve accuracy
- Testing should be repeated periodically
- Timely diagnose and remove infected animals to control the disease.

Bovine Venereal Trichomoniasis Test: Accurate and timely diagnosis is crucial for controlling Bovine Venereal Trichomoniasis in breeding herds. It is possible to use direct microscopy test or gold standard test (culture and isolation test) to identify the protozoa.

- Collect aseptically preputial wash sample
- Transport the sample to the laboratory promptly to ensure protozoan viability
- Use direct fresh sample microscopy tests for motile protozoa. But it has low sensitivity as the motility of *T. foetus* may decrease over time
- Use gold standard test: Culture the sample on specialized media (e.g., Diamond's medium), and isolate/identify the protozoa
- Regularly test the breeding bulls and remove positive animals.

NB:

- Ensure samples are fresh and kept in optimal conditions (e.g., warm and anaerobic) during transport to the laboratory.
- Protozoa may die during sample transport, leading to false negatives.
- Culturing the sample requires several days (up to 7 days) to confirm results.
- Negative test results typically require confirmation through repeated testing (at least three negative tests at weekly intervals).
- Bulls are the primary carriers, so routine testing of breeding bulls is essential.

NB:

- *The semen station must remove bulls (within 48 hours) which are positive for IBR, BVD, Brucellosis and TB.*
- *Bulls found positive for Campylobacteriosis and Trichomoniasis shall be isolated and treated.*
- *In addition to its negative results for the above listed disease tests, vaccinate bulls annually as per the recommended schedule for common diseases, including Anthrax, Blackleg, Pasteurellosis, Lumpy Skin Disease (LSD), and Foot-and-Mouth Disease (FMD).*

- *The semen station shall also cull those bulls which provide enough doses of semen, whichever is achieved earlier. In addition, the bulls with poor libido, poor semen quality, incurable lameness, etc. shall also be culled.*

3.4.4. Measures for Semen collected from infected bull

If a semen producing bull gets positive for either of the diseases, follow the steps below to properly discard its semen:

- Immediately identify and segregate its semen.
- Label it clearly as "BIOHAZARD" to avoid accidental use or distribution.
- Keep the semen in a designated storage area until disposal is carried out.
- Use separate cryogenic containers to prevent cross-contamination
- Test each batch of the semen for the disease suspected
- Dispose of all batches starting from the one that tested positive by thawing to deactivate any viruses, bacteria, or protozoa.
- Dispose of the treated semen and any related materials in accordance with regulations for biohazardous waste.
- Clean and disinfect all equipment, containers, and workspaces that came into contact with the contaminated semen.
- Investigate the source of contamination and enhance biosecurity protocols to prevent recurrence.
- Retest the bull to determine its infection status.

3.5. Libido and serving ability

- Recognize libido as highly heritable in cattle and associated with good reproductive performance.
- Include libido and serving ability assessments when selecting bulls for breeding purposes, as they are key predictors of fertility potential.
- Consider factors such as age, sexual experience, locomotion or back problems, and social dominance, which can affect libido and serving ability, potentially reducing test reliability.
- Evaluate libido on a 0–3 scale:
  0: Shy or no desire to approach the teaser.
  1: Dull or very reluctant to approach the teaser.
  2: Active or willingly approaches the teaser.
  3: Aggressive or move towards the teaser in an uncontrolled manner.
- Assess mating ability on a 0–3 scale based on the number of attempts required for a successful ejaculate:
  0: Poor – more than 4 attempts.

1: Fair – 3–4 attempts.
2: Good – 2–3 attempts.
3: Excellent – successful on the first attempt.

- Disqualify bulls scoring 0 or 1 in libido and/or mating behavior.

3.6. Quality Semen Production Ability

The most representative method to evaluate semen fertilizing capability is through vivo fertility results. However, this is very sophisticated and time taking to practice. For this reason, to predict fertility potential of the bull, semen qualities must be assessed.

Macroscopic parameters: Assess semen quality parameters such as color, volume, and consistency to diagnose accessory gland functionality, potential sperm concentration, and estimated doses (Dhurvey et al., 2012).

Color of bull semen: Ensure the bull semen has a milky to creamy appearance and is free from contaminants such as pus, blood, feces, urine, or debris. And Identify deviations in semen color as follows (Barszcz, 2012):

- Pink/red suggests the presence of blood (which can be the result of penis abrasion, fistulas of cavernous bodies, urinary stones). Green suggests the presence of pus, yellow suggests the presence of urine, and watery white suggests lower quantity of spermatozoa.

Volume of semen: Measure bull ejaculate volume, which typically ranges from 5–8 ml but can vary between 1–15 ml.

- Use ejaculate volume, sperm concentration, and motile sperm proportion to determine the correct semen straw dosage during processing. Nevertheless, small ejaculate volume is not problematic unless it is accompanied by low sperm concentration and reduced motility (David, 2003).
- Account for genetic and non-genetic factors affecting semen volume, such as age, breed, ejaculation frequency, nutrition, season, bull preparation, collection errors, and urogenital diseases.

PH: Measure the pH of bull semen, which should typically range from 6.2 to 6.8 (Barszcz, 2012). However, the pH can increase above 7 in cases of incomplete ejaculation, overuse of the bull, yearling bulls, or pathological conditions affecting the testis, epididymis, ampulla, or seminal vesicles (Sori, 2004). On the other hand, dense semen samples with excellent motility are often associated with lower pH values (Barszcz, 2012). Therefore, it is important to consider these factors during semen evaluation.

Microscopic parameters: Assess microscopic parameters of semen, including sperm motility, morphology, and viability. According to Graham (2001), the correlation between fertility and these parameters differs, with sperm motility ranging from 0.15 to 0.84, sperm morphology from 0.06 to 0.86, and sperm viability from 0.33 to 0.66.

Mass activity: Assess this parameter promptly during semen analysis, and as it is the most susceptible to change during semen analysis if not assessed promptly (Dhurvey et al., 2012).

- Ensure the recommended value to be ≥3 on a 0–4 scale:
  0: No mass activity.
  1: Weak activity without wave formation.
  2: Small, slow-moving waves.
  3: Vigorous movement with moderately rapid waves and eddies.
  4: Dense, very rapid waves and eddies.

Individual Motility: Ensure bull sperm motility to be above 70% in fresh and above 40–50% in frozen semen to avoid reduced conception rates or poor fertility outcomes (David, 2003; Karolina et al., 2012). This is one of the most widely used parameters to evaluate the quality of semen intended for artificial insemination.

Viability test: Ensure the minimum acceptable standard for live spermatozoa in bull semen to be 70% before freezing, when collected using an artificial vagina (David, 2003).

- Assess sperm viability using vital stains like Eosin-Nigrosin to verify the accuracy of individual motility estimates.
  - Note that the percentage of dead sperm should be lower than the percentage of immotile spermatozoa.
  - A higher percentage of dead sperm may indicate underlying issues such as diseases, advanced age, or dysfunction of the accessory glands or testes. Therefore, while evaluating the viability of sperm considering these factors is necessary.

Sperm Morphology: Ensure that the proportion of morphologically abnormal spermatozoa does not exceed 15% in fresh and 20% in frozen semen for fertile bulls (Barszcz, 2012; Karolina et al., 2012).

- Consider factors such as genetics, health conditions, and the bull's age, as young and old bulls are more prone to producing sperm with structural defects. Therefore, while evaluating morphology of sperm considering these factors is necessary.

Concentration: An average of 1.2 X 10^9 sperm cells/ml (ranging: 0.3 to 2.5 X 10^9 sperm cells/ ml) is expected in a normal bull ejaculate.

- Note that concentration measurements alone cannot differentiate between live and dead sperm cells (Only it provides approximate counts).
- Combine concentration evaluation with additional tests, such as live-to-dead ratio and motility percentage, to ensure the required population of progressively motile live sperm cells per dose is achieved (Dhurvey *et al.*, 2012).

Hypo osmotic swelling test /HOST/: Use the Hypo-Osmotic Swelling Test (HOST) as a basic method to evaluate the structural and functional membrane integrity and activity of spermatozoa.

- Ensure the HOST reactive percentage for frozen bull semen is greater than 40% for better quality standards.

Acrosome Integrity: Confirm that the acrosome is structurally and biochemically intact, as it contains enzymes essential for penetrating the ovum and achieving fertilization.

- Ensure that the percentage of acrosome integrity in frozen bull semen is greater than 65%.

Advanced technique of semen quality evaluation: The Computer Assisted semen analysis system can assess various parameters such as total motility (MOT), progressive motility (PMOT), curvilinear velocity (VCL), average path velocity (VAP), straight line velocity (VSL), amplitude of lateral head displacement (ALHD), linearity (LIN), straightness (STR), beat cross frequency (BCF), and mean angular displacement (MAD). The minimum acceptable values for these indices in bovine semen are as follows.

Table 2. Sperm kinematic indices for approval of bovine semen

kinematic index/indices	Minimum value for approval
VCL of <10μm/s	Static
VCL of 10- 25μm/s	Slow
VCL of 25 - 50μm/s	Medium
VCL of >50μm/s	Rapid
VCL of 25 – 50μm/s and STR > 70%	medium progressive motile sperm
VCL of >50 μm/s and STR > 70%	rapidly progressive motile sperm
Progressivity	STR >70%
Other functional kinematic parameters (VAP, SLV, STR, LIN) can be calculated indirectly using the individual spermatozoon's VCL trajectories through the ISAS software program.	

4. Selection Criteria Form

Bulls to be selected as candidates for semen production and/or those who are in production should be sound for breeding purposes and should pass and attain at least the minimal expected threshold criteria. Hence, while candidate bull selection is carried out and while evaluating semen producing bulls, the following points should be considered and assessed for their fitness/soundness (For the standards set in bull selection guidelines).

Owner Name		Date	Case No.
Address		Bull ID	Breed
Tel. No. (Mobile)		Birth Date	Age (Month)
Name and address of the Farm			

SN	Criteria to be considered	
1.	**General And Reproductive Physical Examinations**	
1.1.	***General Physical Examinations***	
	Breed characteristics	The bull should consistently exhibit the defining traits of its breed. Fit ---- Suspicious ---- Unfit ----
	Physical conformation	The bull's physical conformation should exhibit normal. Fit ---- Suspicious ---- Unfit ----
	Body condition	The bull should maintain a moderate body condition score (BCS – 3.5) or slightly higher, with careful consideration given to its age, breed, and size during body condition evaluation. Fit ---- Suspicious ---- Unfit ----
	Temperament	The bull to be selected for semen production should be calm in its character Fit ---- Suspicious ---- Unfit ----
1.2.	**Reproductive Structural and Physical Examinations**	
	Scrotum	Scrotum of the bull should be pendulous with a distinct neck at point of attachment and it should have no wrinkling Fit ---- Suspicious ---- Unfit ----
	Scrotal Circumference	Scrotal circumference for a yearling candidate bull should be at least 30 cm, Measured value ---- Fit ---- Suspicious ---- Unfit ----
	Testicles	Testicles of the bull should be palpated entirely for their size, consistency and mobility in the scrotum and should be appropriate for such parameters and be symmetrical and uniform in size and consistency. Testicular biometrics (length, width, thickness, volume and weight) should have also been taken into consideration. Fit ---- Suspicious ---- Unfit ----

Owner Name		Date	Case No.
Address		Bull ID	Breed
Tel. No. (Mobile)		Birth Date	Age (Month)
Name and address of the Farm			

SN	Criteria to be considered	
	Epididymis	It should be palpated to its entire length to record its form and consistency. Fit——— Suspicious———— Unfit————
	Copulatory Organs	Visualized inspection and/or palpation have to be taken for their form, consistency and abnormalities (if any). Fit——— Suspicious———— Unfit————
	Accessory sex glands	Palpation has to be taken for their form, consistency and abnormalities (if any). Fit——— Suspicious———— Unfit————
	Secondary Sex Character	Here the masculine developmental status, libido (sexual desire) and mating behavior (ability to complete the service) of the bull to be selected have to be considered Fit——— Suspicious———— Unfit————
	Semen quality	
	Color	The bull's semen has to be milky to white creamy Observed color——— Fit——— Suspicious——— Unfit———
	Volume	The bull's semen volume has to be 5-8ml in most cases Measured Value———— Fit——— Suspicious——— Unfit———
	Concentration	The bull's semen concentration has to be 0.3 to 2.5 X 10^9 sperm cells / ml. Measured Value——— Fit——— Suspicious——— Unfit———
	Mass activity	The bull's spermatozoa mass activity shall be >= 3 Measured Value———— Fit——— Suspicious——— Unfit————
	Individual sperm motility	The bull's spermatozoa individual motility for fresh and frozen semen shall be >= 70% and >=40% respectively Measured Value———— Fit——— Suspicious——— Unfit———
	Morphology	The bull's spermatozoa morphological abnormality for frozen semen shall be <=20% Measured Value——— Fit——— Suspicious——— Unfit————
1.3.	*Viability*	The bull's spermatozoa viability shall be 70% before freezing

Owner Name		Date	Case No.
Address		Bull ID	Breed
Tel. No. (Mobile)		Birth Date	Age (Month)
Name and address of the Farm			

SN	Criteria to be considered	
		Measured Value----- Fit-----· Suspicious-----· Unfit-----·--
	HOST reactivity	The bull's spermatozoa HOST reactivity shall be >=40% for frozen semen Measured Value----- Fit-----· Suspicious-----·-- Unfit-----·--
	Acrosome integrity	The bull's spermatozoa acrosome integrity shall be >=65% for frozen semen Measured Value----- Fit-----· Suspicious-----· Unfit-----·--
1.4.	Pedigree and Performance Recording	An individual's ancestor's trait (milk production) will be considered and bull(s) with relative best EBV has/have to be selected. Measured Value----- Fit-----· Suspicious----- Unfit-----·--
1.5.	Genetic Evaluation	As much as possible and conditions allow to take genomic evaluation. bull(s) in the herd or population with best genetic merit for a specific intended trait (milk production) has/have to be assessed and selected
1.6.	Disease	The bull to be selected as candidate and/or in production should be free of any diseases in particular for those the diseases transmitted by semen (like IBR, BVD. Tuberculosis, Brucellosis, Campylobacteriosis and Trichomoniasis). *PRE-ENTRY TO ISOLATION/QUARANTINE TESTING:* 1. ☐ Bulls intended to enter AI Center shall be: • Healthy • Free of infectious or contagious disease • Not under quarantine • Should not be used for natural service after physical exam and testing • Should be isolated from other cattle after physical exam and testing 2. ☐ Tests and exam must be performed, and results must be known within 30 days before bull may enter the AI center isolation facility *ISOLATION TESTING* 1. ☐ Sampling may not commence until Pre-Entry test results are known and before the bull has entered the AI center isolation facility.

Owner Name		Date	Case No.
Address		Bull ID	Breed
Tel. No. (Mobile)		Birth Date	Age (Month)
Name and address of the Farm			

SN	Criteria to be considered	
		N.B: • Testing for Bovine Trichomoniasis and Campylobacteriosis shall be completed for seven weeks (at least weekly intervals) • Testing for Bovine brucellosis, BVD and IBR on week 6 (Day 35) after entry • Testing for Bovine tuberculosis on Week 9 (Day 63) after entry

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