

REVIEW ARTICLE

Breeding Soundness Evaluation in Bulls: A Comprehensive Review

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Abstract

Breeding soundness evaluation (BSE) is a crucial process that assesses the reproductive potential of bulls intended for use as herd sires. This comprehensive method involves a thorough examination of the bull's physical health, reproductive organs, and semen quality, including overall examination, scrotal assessment, and semen evaluation. Among these components, precise semen evaluation is the most critical part of BSE. This method is reliable, cost-effective, and economically prudent for assessing the potential fertility of sire bulls. In this review, the information and application of BSE in bulls was summarized. Furthermore, knowledge gained from the reproductive biology of domestic animal species can be utilized through these BSE guidelines to study and develop assisted reproductive technologies, serving as tools for the ex-situ gene conservation of endangered wild animals such as argali sheep, yak, reindeer, and Przewalski horses—species that are vital members of Mongolia's wild fauna.

Key word

Breeding soundness evaluation, semen evaluation, morphology, morphometry, sperm collection method, chromatin status, Feulgen technique

Introduction

According to the United Nations Estimation report, the global population in 2023 is predicted to be 8.1 billion, and it is expected to grow to 9.7 billion people by 2050. This increase in population will result in a 70% increase in demand for global food production to meet the growing population's needs [1]. Animal breeding in developing countries is vital for efforts to increase production, as well as in addressing challenges related to climate change and promoting green sustainable development. As such, an increase in food production will require an improvement in livestock management, including better provender manufacturing, reducing gas emissions (carbon dioxide and methane etc.,) and waste, animal health, and fertility within herds [2].

Reproductive health in breeding male animals plays a key role in successfully breeding and maintaining healthy livestock by reducing the number of calves to achieve optimal reproductive outcomes. However, reproductive

health of the male animal can be affected by genetic, nutritional, infectious disease, and traumatic issue factors[3], [4]. For example, limited feed conditions might be one of the causes of low Body Condition Score (BCS) in cattle, resulting in abnormalities of reproductive function [5].

Breeding soundness evaluation (BSE) is an essential step in ensuring the reproductive success of all species of animals. It is an examination that evaluates the physical and reproductive soundness of livestock for breeding purposes [6]. There are several reasons to use this method: initially, it confirms that the animal is healthy and free from any physical abnormalities. Secondly, it's important to identify any reproductive issues in animals, as it can affect their ability to mate and reproduce. This is especially important for farmers and breeders since it can prevent economic loss [7], [8]. Ultimately, it can help to prevent or stop sexually transmitted diseases [9].

It is well known that breeding soundness exams are vital for successful reproduction in breeding animals. However, they do not replace routine health assessments that evaluate overall health.

Overall evaluation

Breeding soundness examination (BSE) is a method used to evaluate the reproductive health of animals, particularly bulls. This approach is reliable and efficient for screening bulls Routine health assessments may play a major role in detecting and treating diseases and conditions that may affect the animal's overall health and well-being.

regarding their fertility. Assessments should be performed on each bull within 30 to 60 days before each breeding season to allow adequate time for breeding [6].

Table 1

Suggested minimum scrotal circumference measurement (cm) for best practice guideline

Age of bull (months)	Minimum Scrotal Circumference recommendation
12	30
13	30
14	30
15-20	31
21-30	33
>30	34

Various criteria are evaluated, including breeding ability, overall body condition, reproductive system examination, and sperm production and quality evaluation. The fundamental components of semen quality assessment encompass macroscopic assessment, volume, concentration, sperm viability, and the percentage of normal sperm morphology and morphometry [6, 10, 11].

A physical examination analyzes the bull's overall appearance, including attitude, body condition, and fecal characteristics. Among these, body condition is a critical factor for evaluation. To detect female cows in heat, bulls must travel long distances; therefore, the health of their hooves and legs is crucial for their performance [80]. Additionally, the bull's eyes should be clear and free from injury or disease to effectively detect cows in heat. An early

Scrotal evaluation

The measurement of scrotal circumference (SC) has become widely utilized in the cattle industry to assess the reproductive capability of breeding bulls. SC is directly linked to sperm-producing tissue, spermatozoa normality, and the sexual maturity of female relatives [14, 15]. As one of the parameters for breeding soundness assessment, SC provides important indications of a bull's genetic merit. It is essential for

study evaluated the frequency of ocular tumors across different cattle breeds, revealing that Herefords had a higher incidence rate compared to the Red Poll breed [12]. Understanding breed differences can aid in the effective management of bull reproduction.

There are specific concerns regarding bulls that are overly thin or fat: thin bulls may lack the stamina to service several cows over large distances, while fat bulls may not mate to their full potential due to a lack of vigor. The Body Condition Score (BCS) method is a common practice for assessing fatness in beef cattle. This method typically involves a combination of visual inspection and palpation techniques, which are directly linked to semen quality [13]. Research indicates that bulls with a BCS of 5 or 6 tend to exhibit better sperm quality compared to those with scores below 5 or above 7 [13].

evaluating testicular volume and is strongly associated with sperm output [14]. To measure scrotal circumference, a self-tension tape (in centimeters) is used. The testes are drawn down into the lower part of the scrotum, and the tape is placed around the widest point to record the scrotal size measurement [16]. Bull BSE includes SC measurement indices based on age and breeding [17].

Table 1 provides an overview of scrotal measurements according to higher standard guidelines (Bull) [6].Larger SC measurements correlate with increased sperm output and improved fertility results, and they can depend on body weight [18], nutrition [19, 20], age [21], and breed [20]. Numerous studies have reported on the relationship between SC and semen quality across different bull breeds [22-24]. For example, research on crossbred Holstein Friesian bulls revealed a positive correlation between SC and semen volume. A similar study on Bali bulls reported a moderate relationship between SC and semen quality, with correlation coefficients of 0.63 for semen volume and 0.60 for sperm concentration [14] A study by Pérez-Osorio et al. (2016) investigated the association between scrotal circumference and physical characteristics of semen and sperm morphology in 191 Guzerat bulls, categorized by age into six groups ranging from 12 to 36 months [25]. They found a nearly perfect correlation (r = 0.94, p < 0.005) between SC and sperm motility by age group. Additionally, bulls with scrotal circumferences greater than 30 cm at 28 months of age produced the highest semen volumes under

Sperm Collection Methods

Currently, there are six primary methods for collecting semen from animals: artificial vagina (AV) [8, 31], internal artificial vagina (IAV) [9], electro-ejaculator (EE) [32], trans rectal massage (TM) [33], and post-mortem semen

Artificial Vagina (AV)

The artificial vagina (AV) is designed to replicate the vaginal orifice (labia minora) of female animals, facilitating artificial This method insemination (AI). offers genetic numerous benefits, including improvement, efficiency, and precise semen handling. Compared to other techniques, the AV method is more humane and closely resembles the natural breeding process, which can help prevent the transmission of diseases. Additionally, unlike natural methods, the AV approach does not require an expert palpatory. However, a disadvantage of natural breeding methods is the time needed to train bulls.

The AV consists of various components, including a rigid casing, inner rubber liner, rubber bands, valve, director cone, semen vial, and warming bag [36]. The AV requires thermal

healthy conditions. While SC measurement is a crucial component of BSE, it should not be the sole factor in breeding decisions; other factors, such as semen quality and overall health, must also be considered for informed decision-making.

Furthermore, infrared thermography (IRT) is a valuable technique for studying scrotal and testicular function in both animal and human andrology. This non-invasive method is highly sensitive in measuring temperature differences across the skin surface, making it effective for monitoring scrotal surface temperature. Scrotal thermography is a simple, feasible, rapid, and cost-effective diagnostic method [26]. Initially utilized for assessing scrotal and testicular function in humans [27], IRT has also been adapted for use in bulls [28]. This technique is essential for determining scrotal surface temperature in bulls [29]. Research indicates a strong correlation between scrotal surface temperature and testicular temperature. Maintaining an appropriate temperature within the testes is crucial for normal spermatogenesis [30]. The ideal testicular temperature should be between 2°C and 6°C cooler than the normal body temperature to produce fertile sperm [20].

collection [34]. Among these, AV and IAV are regarded as methods that yield semen samples with a high sperm concentration, as they closely mimic natural breeding [35].

stimulation and mechanical to induce ejaculation. The pressure within the AV is crucial for obtaining ejaculates of optimum quality. For instance, the AV is filled with warm water (42 to 48°C), and temperature checks should be conducted at each semen collection [37]. Several studies have successfully employed the AV method in bulls [31, 38]. For example, when comparing the quality of bull semen collected via AV and EE methods, the AV method yielded a lower volume (2.8 ml vs. 6.3 ml) but a higher concentration (625 vs. 299 million sperm/l) [38]. However, this approach necessitates the use of previously trained bulls and a mount cow, bull, or a so-called phantom. It is important to note that bulls are powerful animals, and even seemingly docile individuals can become aggressive.

Consequently, this method may not be suitable for bulls that are not accustomed to human

Electro-Ejaculator (EE)

Electro-ejaculators (EE) are designed to stimulate the pelvic splanchnic nerves with low electromotive force and amperage, resulting in ejaculation. An EE set comprises several components, including a carrying case, rectal probe, control devices, battery charger, power cord, probe cord, semen collection handle, collection cone, and collection vial [37]. In the field of animal reproduction, EE is a remarkable method for collecting semen from both domestic and wild animals. This technique is safe, easy to employ, and requires minimal facilities.

Sarsaifi et al. (2013) assessed the response of Bali bulls to different semen collection techniques (EE, RM, and RM+EE) and their effects on sperm cell characteristics [33]. The study found that Bali bulls responded better to

Internal Artificial Vagina (IAV)

The internal artificial vagina (IAV) method is simple, easy to use, repeatable, and cost-effective. It allows for semen collection from bulls without the critical skills typically required by a technician [9]. This method mimics natural mating, facilitating simultaneous semen collection and evaluation of libido and mating ability. The IAV consists of a flexible plastic tube and a wire frame supporting a 7.5 cm diameter rubber tubing that can be placed into a cow's vagina [42].

In terms of instrument and labor costs, the IAV

Rectal Massage (RM)

The rectal massage (RM) method requires two personnel: one to perform the massage and another to collect the semen [37]. Initially, the bull is placed in a chute, and feces must be cleared from the rectum before semen can be collected by massaging the internal reproductive structures (vesicular glands, ampullae, pelvic urethra). This technique has been successfully employed in several studies [7, 33]. A combination of massaging both the seminal vesicular glands and the ampullae has proven effective in collecting spermatozoa from various bull breeds [7, 33].

The RM method has its pros and cons. Advantages include inexpensive devices and reduced pain compared to the EE method.

handling [7].

EE and the combination of RM+EE than to RM alone. Although the EE method produced better semen characteristics than RM [33], the quality of semen collected was not as high as that obtained from the AV method [39]. It is worth noting that while EE may seem less humane due to the associated pain, understanding the pain experience in animals remains complex. Research has indicated that nerve stimulation for semen release in humans is often associated with discomfort [40]. Moreover, complications may arise from natural animal behaviors, such as lying down, struggling, or becoming recumbent. Palmer et al. (2005) recommended that operators apply electrical stimulation as gently as possible [41]. One advantage of the EE method is that bulls do not require prior training [40].

may be more expensive than the EE method due to the need for additional mount cows. Potential limitations of the IAV method include disease transmission and animal welfare issues [9]. Nevertheless, it offers several advantages, such as being a cost-effective and more humane semen collection technique compared to EE. Additionally, the IAV method allows for semen collection while simultaneously assessing the bull's sexual drive and serving ability, enhancing the selection process for healthy breeding bulls [9].

Disadvantages include the necessity for an expert palpator, the requirement of libido for mating, potential contamination of samples, and variable semen volume and concentration [44]. Nonetheless, the primary consideration is obtaining a representative semen sample with accurate semen quality measurements.

A study by Sarsaifi et al. (2013) reported that the success rate for semen collection using EE was 100%, whereas the RM method achieved an approximately 80% success rate [33]. In another study comparing EE and RM methods for semen collection in beef bulls, the RM method required more time, and the viability of sperm and live sperm in the samples was lower; however, no differences in sperm morphology

were noted [7].

Post-Mortem Semen Collection

Post-mortem semen collection involves several attempts to harvest semen samples from wild animal species after culling or castration, primarily from the epididymis. This method was applied in the cryopreservation of epididymal spermatozoa from white-tailed deer (Odocoileus virginianus), with semen collection and processing completed within 1 to 5 hours post-mortem for two Virginian deer [44]. In another study, spermatozoa were collected from the epididymis of two different deer species: red deer (Cervus elephus hippelaphus) and fallow deer (Dama dama). The sampling time for red deer ranged from 1.5 to 15 hours, whereas for fallow deer, it ranged

from 3 to 11 hours [34].

Other researchers have successfully applied this approach to other species, including reindeer (Rangifer tarandus), wild sheep or argali (Ovis Ammon), and yaks (Bos grunniens) [45-47]. The characteristics of sperm cells collected, such as concentration, motility percentage, and intact acrosome status, were satisfactory [34]. Numerous studies have focused on bull semen collection, highlighting both advantages and disadvantages associated with the process. The existing literature suggests that while there are benefits to semen collection in bulls, further research is warranted to fully understand the implications (see Table 2).

Table 2

The pros and cons of semen collection method

Semen collection	Advantages	Disadvantages
Methods		
Artificial vagina	 Mimics natural breeding More humane To prevent disease transmission No need specialist palpatory High concentration Inexpensive 	more time to train bulllow semen volume
Internal artificial vagina	 Mimics natural mating Evaluation of libido and mating ability simultaneously Expensive 	ExpensiveDisease transmissionAnimal welfare problem
Electro-ejaculator	 Safe to employ Easy, and require minimal facilities High quality semen High semen volume Not needed to be trained Time-consuming 	Not seems more humane
Transrectal massage	 Inexpensive Time-consuming Only two people required to collect semen No need to be trained No expensive equipment is required No pain 	 Skilled palpators are needed Need for libido, mating or breeding Potential contamination of samples Variable semen volume and concentration
Post mortem	 To save the genetic diversity of endangered species High quality sperm cell No need to train Cost-effective 	- Culling or castration - Time limitation - Time consuming

Sperm Analysis

Semen is a body fluid composed of a suspension of spermatozoa in seminal fluid. A comprehensive assessment of semen requires consideration of several factors, including volume, color, consistency, density, smell, and

3.1 Volume

Semen volume can be influenced by various factors such as the season, breed, size, age of the animal, and collection methods [48, 49]. A study by Abah et al. (2023) reviewed approximately 100 papers and found that the

3.2 Macroscopic Evaluation

The color of bull semen typically ranges from creamy white to yellow-gray or even greenish. A normal ejaculate is usually creamy white and should have a uniform, opaque appearance, indicative of high sperm concentration. Translucent semen may suggest a lower sperm count [6]. Semen should be free of contaminants, such as blood, urine, pus, or hair. Pathological colors and their implications include:

• **Pink or red:** Indicates blood, often due to penis abrasion or urinary stones.

3.3 Concentration

Sperm concentration is a critical measure for determining fertility levels, expressed as the number of sperm cells per milliliter of ejaculate. Various methods can be employed to determine sperm concentration, including:

 Hemacytometer: A traditional method involving a specially designed microscope slide for manual counting.

3.4 Motility and Viability

Sperm motility evaluation estimates the percentage of active spermatozoa, providing insight into viability and quality. Light microscopic analysis is commonly used for assessing motility in both raw and extended semen [10, 12]. However, measuring motility in raw semen can be challenging due to high sperm concentrations, necessitating dilution with a quality extender [11, 34]. Standard practice involves incubating sperm samples at 37°C for 2 minutes before measurement.

the presence of foreign materials or blood. Proper documentation of these observations is crucial for accurate analysis and diagnosis, typically conducted through visual assessment [31].

average ejaculate volume from bulls ranges between 2 ml and 10 ml [49]. Notably, the volume of semen tends to increase with age, with bulls around five years old generally producing higher-quality semen [48, 49].

- **Green:** Suggests the presence of pus.
- Yellow: Indicates potential contamination with urine.
- Watery white: Suggests a low spermatozoa count [31, 50].

The smell of normal semen is similar to cow's milk [31]. Kocks et al. (2014) established a standard density value for whole bull semen at 1.053 g/ml, serving as a benchmark for routine bovine artificial insemination practices [51].

- Spectrophotometer and Colorimeter: More precise and rapid techniques that require calibration for accurate results.
- Computer-Aided Sperm Analyzer (CASA): Automated methods like flow cytometry provide accurate and efficient assessments.

In young and mature bulls, normal sperm concentration ranges from 2×10^8 sperm/ml to 1.8×10^9 sperm/ml, respectively [6].

Viability is determined by placing a drop of semen on a clean slide and observing it under a phase-contrast microscope at 37°C [11, 47]. Sperm motility is typically classified as follows:

- **Very good:** 80-100% motile sperm
- **Good:** 60-80% motile sperm
- **Fair:** 40-60% motile sperm
- **Poor:** 20-40% motile sperm
- **Very poor:** <20% motile sperm [6, 31].

3.5 Morphology

Sperm morphology refers to the physical shape of spermatozoa, with normal sperm featuring a head, neck, and tail. Abnormalities can occur, and while light microscopy is commonly used for initial assessments, electron microscopy (EM) provides higher resolution images for detecting subtle defects [52]. Phase-contrast microscopy at 1000× magnification or higher is typically used for detailed morphological

3.6 Morphometry

Sperm morphometry involves measuring the physical dimensions of sperm cells to assess their quality and fertilization potential. Various microscopy techniques, including light and electron microscopy, are utilized alongside specific morphometric analysis software [55]. While electron microscopy is costly and time-consuming, light microscopy combined with

3.7 Chromatin Status

Sperm chromatin, a complex of DNA and protein, undergoes significant changes during spermatogenesis, with histones being replaced by protamines. This condensation is crucial for sperm function, and any abnormalities can lead to reduced fertility and poor embryo quality [62, 63]. Techniques for assessing chromatin status include:

• Fluorescent microscopy and flow cytometry: Common methods for

Importance of Sperm Morphology and Morphometry

Semen samples typically contain abnormal sperm cells, and there is a strong correlation between morphological abnormalities and fertility in livestock. Consequently, a reliable method for assessing male animal subfertility and sterility is essential, particularly in morphometric evaluations. **Computer-assisted sperm image analysis** has emerged as a promising approach to reduce subjectivity and

Staining Techniques

A significant challenge in assessing sperm morphology and morphometry is the lack of standardization in staining techniques. The precision of sperm morphology assessments is contingent upon the staining method employed, as different chemical compositions of reagents can lead to varying effects on the stained assessments [10, 11, 47, 53]. Abnormalities may include:

- **Head defects:** Knobbed acrosome, pear-shaped heads, and detached heads.
- **Tail defects:** Distal midpiece, coiling, or double bends.
- **Cytoplasmic droplets:** Indicating immaturity during spermatogenesis [10, 11, 34, 52, 54].

analysis software provides a more practical solution for routine assessments [34, 47, 55]. Computer-assisted sperm morphometry analysis (ASMA) has been adopted across various species, including bulls [57, 58]. Flow cytometry has also emerged as an essential tool for rapid, precise analysis of sperm cells, enabling the assessment of multiple parameters simultaneously [54, 59].

- evaluating chromatin condensation.
- Staining techniques: silver nitrate, acridine orange, aniline blue, and chromomycin A3 are employed to assess DNA integrity and chromatin structure.

For instance, silver nitrate can detect chromatin proteins, while acridine orange distinguishes between normal and damaged DNA, emitting different fluorescence colors based on the DNA structure [10, 63]

variability in evaluating sperm morphometry, particularly regarding the percentage of normal spermatozoa within a sample. However, the effectiveness of this method hinges on proper sample preparation and staining techniques, with accuracy influenced by the staining properties of the spermatozoa (e.g., Giemsa, India ink, William's Karras, nigrosin-eosin, eosin-aniline blue) [10, 52].

cells.For instance, Banaszewska et al. (2015) evaluated four different staining techniques (SpermBlue®, Papanicolau, eosin + gentian violet complex, and silver nitrate) on spermatozoa from Holstein-Friesians. Their findings revealed that while SpermBlue and eosin + gentian violet complex yielded similar

sperm head dimensions, significant differences were observed across all staining techniques. Eosin + gentian violet complex has been commonly utilized to assess the morphology of Black-and-White breed bulls [68, 69].

The **eosin** + **gentian violet complex** is particularly valuable for sperm quality assessment, but it complicates the observation of acrosome and midpiece structures in bulls. Moreover, this technique, alongside trypan blue staining, can effectively distinguish between live and dead sperm cells [68, 70]. Other notable staining methods include Giemsa and eosin-nigrosine approaches, as well as the **Rapiddiff** (**Diff-Quik**) method, which was previously applied to human spermatozoa morphology assessment [10, 70, 71].

• **Eosin-Nigrosin Stain Method**: This twostep technique differentiates live from dead sperm cells based on membrane

Image Analysis Options

Various image analysis software, such as **Sperm Sizer**, enable precise measurements of sperm components (head, midpiece, tail) by allowing users to select key points for analysis.

permeability. Eosin penetrates the membranes of dead cells, coloring them pink, while live cells remain colorless. The procedure involves mixing eosin and nigrosine with a semen sample, smearing it on a slide, and evaluating 200 spermatozoa [73, 74].

- Papanicolau Staining Technique: This method is labor-intensive, requiring over 20 processing stages, including air-drying slides, fixation in ethanol, and staining. The complexity and use of numerous chemicals can hinder its application [68].
- SpermBlue® Staining Technique: A straightforward two-stage process involving fixation and staining. The slides are air-dried, fixed in SpermBlue fixative, and stained, taking only 12-15 minutes for completion [68].

This software facilitates the visualization of measurements as digital photos, which can be saved for further analysis [75].

Importance of Chromatin Status and DNA Fragmentation

DNA fragmentation is a critical biomarker in sperm cells, with approximately 15% of sperm in standard semen samples exhibiting fragmented DNA. While some degree of fragmentation is considered normal, an increase is often associated with age. Notably, DNA fragmentation correlates with decreased

Analysis Techniques

Three primary categories of tests exist for evaluating sperm quality:

- 1. Sperm Chromatin Structural Probes.
- 2. Direct Evaluation Assays for Sperm DNA Fragmentation.
- 3. Sperm Nuclear Matrix Tests.

These tests, commonly employed in male fertility diagnostics, include methodologies such as the Sperm Chromatin Structure Assay (SCSA), TUNEL Assay, In Situ Nick Translation (ISNT), and Comet Assay [78]. These assays assess sperm fertility by measuring DNA damage, with TUNEL and

The Feulgen Technique

The Feulgen staining procedure is rapid and cost-effective, aiming to mitigate the effects of

fertilization rates, reduced pregnancy rates, and diminished embryo quality, emphasizing that sperm with fragmented DNA should be avoided in in vivo fertilization [76, 77]. Importantly, DNA fragmentation is independent of sperm morphology and viability, necessitating specific staining for assessment.

SCSA being particularly favored for their reliability and sensitivity [76, 85].

- TUNEL Assay: A fluorescence-based technique used to detect DNA damage in individual sperm cells.
- SCSA: A flow-cytometry-based analysis assessing sperm chromatin susceptibility to acid-induced denaturation.
- Comet Assay: An electrophoresis method distinguishing intact DNA from damaged DNA but requires specialized software for image analysis [64].

staining on sperm morphometry outcomes. It labels chromatin to reduce variations caused by

staining and membrane integrity. The procedure involves thawing frozen sperm, preparing

smears, and employing a series of staining steps to achieve the desired results [10].

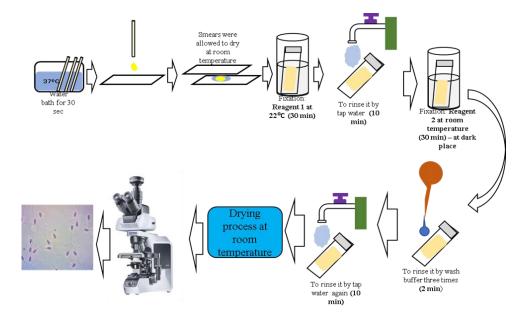


Figure 1. Scheme of the Feulgen staining procedure

Practical Importance of Breeding Soundness Evaluation in Mongolian Animal Husbandry

Animal husbandry is a cornerstone of Mongolia's economy, contributing over 10% to the Gross Domestic Product (GDP) and employing approximately one-third of the country's labor force. In light of food safety concerns, with more than 20% of food products imported, enhancing domestic animal

production is crucial. Therefore, breeding soundness evaluation (BSE) plays a pivotal role in improving livestock fertility and productivity, particularly in the harsh and variable climate of Mongolia, which presents unique challenges for animal husbandry.

1. Significance of Animal Husbandry in Mongolia

The Mongolian agricultural sector is deeply intertwined with the nation's cultural and economic fabric. Livestock species, such as yaks, goats, sheep, and horses, not only serve as vital food sources but also support traditional nomadic lifestyles. With extreme weather

change, effective management practices, including breeding soundness evaluations, become essential for maintaining healthy and productive livestock populations.

conditions and the rising threat of climate

2. Challenges in Animal Reproduction

Given Mongolia's unique geographical landscape, including mountains and steppes, conducting breeding soundness evaluations presents several challenges. Factors such as:

- Logistics: Difficulties in transportation can hinder access to remote herds, complicating the sampling process. For example, significant distances must be traveled on horseback to reach herders.
- Specialized Training: There is a scarcity of qualified specialists in the field, making it difficult to train local

veterinarians or herders in effective breeding soundness evaluation techniques.

• **Infrastructure**: The lack of adequate facilities and testing equipment limits the ability to conduct thorough evaluations.

These challenges highlight the necessity for practical, standardized protocols to improve the efficiency and accuracy of breeding soundness evaluations.

3. Benefits of Breeding Soundness Evaluation (BSE)

Breeding soundness evaluation is a relatively simple, cost-effective method for assessing sperm quality in livestock. Key benefits include:

- Field Applicability: BSE can be performed in field conditions with minimal resources. Typically, only two people and basic equipment (microscopes, slides, and mobile CASA) are required, making it accessible for rural veterinarians and herders.
- **Rapid Assessment**: This evaluation method is reliable and efficient, allowing

Conclusion

The practical importance of breeding soundness evaluation in Mongolian animal husbandry cannot be overstated. Given the critical role of livestock in the economy and the unique challenges posed by climate and geography, implementing effective BSE practices is essential for ensuring sustainable and

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References

- [1] FAO (2009) 'How to Feed the World in 2050', Insights from an expert meeting at FAO, 2050 (1), pp.1-35. Available at: http://www.fao.org/wsfs/forum2050/wsfs-forum/en/
- [2] Chatellier, V. (2021) Review: International trade in animal products and the place of the European Union: main trends over the last 20 years, Animal. The Author(s), 15, p. 100289. https://doi.org/10.1016/j.animal.2021.100
- [3] Yoo, H. S. (2010) 'Infectious causes of reproductive disorders in cattle', Journal of Reproduction and Development, 56 (SUPPL.). https://doi.org/10.1262/jrd.1056S53
- [4] Pal, R. P., Mani, V., Mir,S.H., Singh, R.K., & Sharma,R. (2017) Importance of Trace Minerals in the Ratio of Breeding Bull - A Review, International Journal of Current

- for quick decision-making regarding animal breeding. By identifying sub-fertile or infertile males, farmers can make informed breeding decisions that enhance herd fertility and productivity.
- Long-term Genetic Improvement: Regular BSE enables herders to select for superior breeding stock, ultimately improving the genetic quality of livestock over time. This is especially important in the context of conserving genetic diversity among native breeds.

productive animal husbandry. By addressing logistical, training, and infrastructural challenges, BSE can significantly enhance reproductive performance and contribute to the overall resilience of Mongolia's agricultural sector.

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- Microbiology and Applied Sciences, 6(11), pp. 218-224. https://doi.org/10.20546/ijcmas.2017.611.
- [5] Azis, I. U., Agus, A., Astuti, A., Yusiati, L. M., & Anas, M.A. (2023) 'Effect of Mineral Premix Supplementation on Intake and Digestibility of Repeat Breeder Cows', IOP Conference Series: Earth and Environmental Science, 1183(1). https://doi.org/10.1088/1755-1315/1183/1/012017
- [6] Watts, R. (1997) Assisted reproductive technology, Collegian (Royal College of Nursing, Australia), 4(1), p. 12. https://doi.org/10.1016/S1322-7696(08)60200-0
- [7] Palmer, C. W. (2005) Welfare aspects of theriogenology: Investigating alternatives to electroejaculation of bulls, Theriogenology, 64(3), pp. 469-479.

- https://doi.org/10.1016/j.theriogenology.2005. 05.032
- [8] Palmer, C. W. (2016) Management and Breeding Soundness of Mature Bulls, Veterinary Clinics of North America - Food Animal Practice. Elsevier Inc, 32(2), pp. 479-495.
 - https://doi.org/10.1016/j.cvfa.2016.01.014
- [9] Barth, A. D., Arteaga. A.A., Brito, L.F.C., & Palmer, W.C. (2004) Use of internal artificial vaginas for breeding soundness evaluation in range bulls: An alternative for electroejaculation allowing observation of sex drive and mating ability, Animal Reproduction Science, 84(3-4), pp. 315-325. https://doi.org/10.1016/j.anireprosci.2004.0 2.010
- [10] Nagy, S. Johannisson, A., Wahlsten, T., Ijäs, R., Andersson, M., & Rodriguez-Martinez, M. (2013) Sperm chromatin structure and sperm morphology: Their association with fertility in AI-dairy Ayrshire sires, Theriogenology. Elsevier Inc, 79(8), pp. 1153-1161. https://doi.org/10.1016/j.theriogenology.20 13.02.011
- [11] Nagy, S., Kovacs, B., & Johannisson, A. (2023) Evaluation of domestic animal sperm head morphology via flow cytometric DNA labelling and pulse shape analysis using bull and stallion spermatozoa as model species, Reproduction in Domestic Animals, 58(11), pp. 1569-1575. https://doi.org/10.1111/rda.14472
- [12] Bailey CM., Hanks DR., Hanks MA., (1990) Circumocular pigmentation and incidence of ocular squamous cell tumors in Bos taurus and Bos indicus X Bos taurus cattle. J Am Vet Med Assoc. 1990 May 15;196(10):1605-8. https://doi.org/10.2460/javma.1990.196.10. 1605
- [13] Renáta Toušová., Luděk Stádník., Jan Beran, and Jaromír Ducháček. (2011). Effect of bulls' breed, age and body condition score on quantitative and qualitative traits of their semen. Acta Universitatis Agriculturae et Silviculturae Mendelianae Brunensis Vol 59 (6). Pp. 37-44. https://doi.org/10.11118/actaun2011590600
- [14] Saputra, D., Ihsan, M., & Isnaini, N. (2017) Korelasi Antara Lingkar Skrotum Dengan Volume Semen, Konsentrasi Dan Motilitas

- Spermatozoa Pejantan Sapi Bali, TERNAK TROPIKA Journal of Tropical Animal Production, 10(2), pp. 59-68. https://doi.org/10.21776/ub.jtapro.2017.018
- [15] Penitente-Filho, J. M. Silva, E.F.F., Guimarães, S.F., Waddington, B., Costa, E.P.D., León, V.G., Siqueira, J.B., Okano,D.S., Maitan,P.P., & Guimarães, J.D. (2018) Relationship of testicular biometry with semen variables in breeding soundness evaluation of Nellore bulls, Animal Reproduction Science. Elsevier, 196 (May), pp. 168-175. https://doi.org/10.1016/j.anireprosci.2018.07.010
- [16] Alexander, J. H. (2008) Bull breeding soundness evaluation: A practitioner's perspective, Theriogenology, 70(3), pp. 469-472.
 - $\frac{\text{https://doi.org/10.1016/j.theriogenology.20}}{08.05.030}$
- [17] Susilawati, T., Sholikah, N., Wahjuningsih, S.I., Herwiyanti, E., & Yekti, APA. (2020) Relationship of scrotal circumference with spermatozoa production in various breed of Indonesian local bulls, American Journal of Animal and Veterinary Sciences, 15(2), pp. 102-107. https://doi.org/10.3844/ajavsp.2020.102.10
- [18] Hopkins, F. M., & Spitzer, J. C. (1997) The new Society for Theriogenology breeding soundness evaluation system., The Veterinary clinics of North America. Food animal practice. Elsevier Masson SAS, 13(2), pp. 283-293. https://doi.org/10.1016/S0749-0720(15)30341-8
- [19] Barth, A. D., Brito, L. F. C. & Kastelic, J. P. (2008) The effect of nutrition on sexual development of bulls, 70, pp. 485-494. https://doi.org/10.1016/j.theriogenology.20 08.05.031
- [20] Kastelic, J. P. (2014) Understanding and evaluating bovine testes, Theriogenology. Elsevier Inc, 81(1), pp. 18-23. https://doi.org/10.1016/j.theriogenology.20 13.09.001
- [21] Chanbacher, D.B., & Amann, R. P. (1983) Physiology of male reproduction, Journal of Animal Science, Volume 57 (2), pp. 380-403.

- https://doi.org/10.2527/animalsci1983.57 Supplement 2380x
- [22] Rashid, M. M., Hoque, M.A., Hoque,S.K., & Bhuiyan,H.F.K.A. (2015) Effect of Semen Collection Frequency and Scrotal Circumference on Semen Quality Parameters in Brahman x Local Crossbred Bulls, Advances in Animal and Veterinary Sciences, 3(12), pp. 677-684. https://doi.org/10.14737/journal.aavs/2015/3.12.677.684
- [23] Gopinathan, A., Sivaselvam, S.N., Karthickeyan, S.M.K., & Kulasekar, K. (2016) Effect of body weight and scrotal circumference on semen production traits in crossbred Holstein Friesian bulls, Indian Journal of Animal Reproduction, 39(1), pp. 24-27. https://doi.org/10.21887/tijovsab.v12i1.11
- [24] Indriastuti, R., Ulum, M.F., Arifiantini, R.I., Memili, E., & Purwantara, B. (2020) Relationship among Body Weight, Scrotal Circumference and Sperm Quantity of Bali Bulls in Baturiti Artificial Insemination Center, IOP Conference Series: Earth and Environmental Science, 478(1). https://doi.org/10.1088/1755-1315/478/1/012004
- [25] Osorio, J.P., Álvarez, J.A.C., León, V.E.G., & Arroyo, R.G.O. (2016) 'Relação entre o perímetro escrotal e parâmetros da qualidade do sêmen em machos da raça Guzerá, da puberdade até os 36 meses de idade', Ciencia y Agricultura, 13(2), pp. 29-38. https://doi.org/10.19053/01228420.v13.n2

.2016.5549

- [26] Kulis, T., Kolaric, D., Karlovic. K., Knezevic, M., Antonini, S., & Kastelan, Z. (2012) Scrotal infrared digital thermography in assessment of varicocele - pilot study to assess diagnostic criteria, Andrologia, 44(SUPPL.1), pp. 780-785. https://doi.org/10.1111/j.1439-0272.2011.01265.x
- [27] Gold.R.H., Richard.M.E., Barry. S., Dowdy.A., & Young. R.T. (1977) Scrotal Thermography, Journal of Diagnostic Radiology, Jan. https://doi.org/10.1148/122.1.129
- [28] Purohit. R.C., Hudson.RS., Riddell MG., Carson RL., Wolfe DF., & Walker DF (1985) Thermograppy of the bovine

- scrotum. American Journal of Veterinary Research, 01 Nov 1985, 46(11):2388-2392 PMID: 4073651
- [29] Ahirwar. M.K., Mukund. A.K., Ramesha. K.P., Pushpadass. A.H., Jeykumar.S., Revanasiddu. D., Kour. J.R., Nath.S., Nagaleekar. K.A., & Nazar. S. (2017) Influence of season, age and management on scrotal thermal profile in Murrah bulls using scrotal infrared digital thermography. https://doi.org/10.1007/s00484-017-1415-00
- [30] Barth.A.D., & Ominski.K.H., (2000) The relationship between scrotal circumference at weaning and at one year of age in beef bulls. Can Vet J. Jul; 41(7): 541-546.
- [31] Barszcz, K., Wiesetek, D., Wąsowicz, M., & Kupczyńska, M. (2011) Bull Semen Collection and Analysis for Artificial Insemination, Journal of Agricultural Science, 4(3), pp. 1-10. https://doi.org/10.5539/jas.v4n3p1
- [32] Barker, C.A.V. (1958) The collection of semen from bulls, rams and bucks by electro-ejaculator, Canadian Journal of Comparative Medicine, 22(1), pp. 4-8.
- [33] Sarsaifi, K., Rosnina, Y., Ariff, MO., Wahid, H., Hani,H., Yimer, N., Vejayan, J., Naing, S.W., & Abas, Mo. (2013) Effect of semen collection methods on the quality of pre- and post-thawed bali cattle (bos javanicus) spermatozoa, Reproduction in Domestic Animals, 48(6), pp. 1006-1012. https://doi.org/10.1111/rda.12206
- [34] Zomborszky, Z., Nagy, S., Nánássy, L., & Bodó, Szabari, M., Sz. (2005)**Experiences** in deer sperm cryopreservation under practical conditions - A pilot study, Animal Reproduction Science, 90(1-2), pp. 185https://doi.org/10.1016/j.anireprosci.2005. 01.014
- [35] Ott RS. Examination of bucks for breeding soundness. Vet Med Small Anim Clin. 1978 Dec;73(12):1561-3. PMID: 252347.
- [36] Baracaldo, M. I., Barth, A. D. & and Bertrand, W. (2007) Steps for Freezing Bovine Semen: From Semen Collection to the Liquid Nitrogen Tank, IVIS Reviews in Veterinary Medicine IVIS Ed, pp. 1-11. Available at: www.ivis.org

- [37] Mulu, M., Adane, M., & Moges, N. (2018) Review on process, advantages and disadvantage of artificial insemination in cattle, ~ 8 ~ International Journal of Veterinary Sciences and Animal Husbandry, 3(6), pp. 8-13.
- [38] Austin, J. W., Hupp, E. W., & Murphree, R. L. (1961) Comparison of Quality of Bull Semen Collected in the Artificial Vagina and by Electroejaculation, Journal of Dairy Science, 44(12), pp. 2292-2297. https://doi.org/10.3168/jds.S0022-0302(61)90057-1
- [39] Jiménez-Rabadán, P., Ramón, M., García-Álvarez, O., Maroto-Morales.A., Del Olmo, E., Pérez-Guzmán., Bisbal. A., Fernández-Santos. M. R., Garde, J.J., & Soler, A.J. (2012) Effect of semen collection method (artificial vagina vs. electroeiaculation). extender centrifugation on post-thaw sperm quality of Blanca-Celtibérica buck ejaculates, Animal Reproduction Science. Elsevier B.V., 132(1-2), 88-95. pp. https://doi.org/10.1016/j.anireprosci.2012. 04.005
- [40] Dana A. Ohl. (1993). Electroejaculation. Urologic Clinics of North America. Volume 20, Issue 1, February, Pages 181-188. https://doi.org/10.1016/S0094-0143(21)00474-2
- [41] Palmer, C. W., Brito, L.F.C., Arteaga, A.A., Söderquist, L., Persson, Y., & Barth, A.D. (2005) 'Comparison of electroejaculation and transrectal massage for semen collection in range and yearling feedlot beef bulls', Animal Reproduction Science, 87(1-2), pp. 25-31. https://doi.org/10.1016/j.anireprosci.2004. 09.004
- [42] Cruz, F. B., Lohn, L., Marinho, L.S.R., Mezzalira, J.C., Neto, G.S., Martins, L.T., Vieira, A.D., Barth, A.D., & Mezzalira, A. (2011) Internal artificial vagina (IAV) to assess breeding behavior of young Bos taurus and Bos indicus bulls, Animal Reproduction Science. Elsevier B.V., 126(3-4), pp. 157-161. https://doi.org/10.1016/j.anireprosci.2011. 06.003
- [43] Srivastava, N. & Pande, M. (2017) Protocols in semen biology (comparing assays), Protocols in Semen Biology (Comparing Assays), pp. 1-288.

- https://doi.org/10.1007/978-981-10-5200-2 https://doi.org/10.1007/978-981-10-5200-2
- [44] Jacobson, H. A., Bearden, H. J., & Whitehouse, D. B. (2016) Trials whitetailed artificial, 53(1), pp. 224-227. https://doi.org/10.2307/3801338 https://doi.org/10.2307/3801338
- [45] Kovács A, Tumennasan Kh, Demberel Sh, Nagy Sz, Kútvölgyi G, Oláh J, Jávor A. (2007). Argáli spermiumok mélyhűtése: Cryopreservation of argali spermatozoa. Fővárosi Állat- és Növénykert (2007) 90 p. pp. 80-81., 2p.
- [46] Shimazaki, M., Sambuu, R., Yoko, S., Kim,D.L.T., Fuminori, T., Masayasu,T., & Takeshiga,O. (2015) Effects of orvus es paste on the motility and viability of yak (Bos Grunniens) epididymal and ejaculated spermatozoa after freezing and thawing, Cryo-Letters, 36(4), pp. 264-269
- [47] Ariuntungalag.J., Gabriella, Kútvölgyi., & Szabolcs, T. Nagy. (2021). Chromatin condensation status of post mortem collected Przewalski's horse (Equus ferus) spermatozoa. XXVII. Ifjúsági Tudományos Fórum. Hungarian Conference proceedings (Book) Scientific. pp. 33-35. ISBN: 9786156338044
- [48] Bhakat, M., Mohanty, K.T., Raina, S.V., Gupta, K.A., Khan, M.H., Mahapatra, K.R., & Sarkar, M. (2011) Effect of age and season on semen quality parameters in Sahiwal bulls, Tropical Animal Health and Production, 43(6), pp. 1161-1168.

 https://doi.org/10.1007/s11250-011-9817-1
- [49] Abah, K.O., Fontbonne, A., Partyka, A., & Nizanski, W. (2023) Effect of male age on semen quality in domestic animals: potential for advanced functional and translational research?, Veterinary Research Communications. Springer Netherlands, 47(3), pp. 1125-1137. https://doi.org/10.1007/s11259-023-10159-1
- [50] Foote, R. H. (2002) The history of artificial insemination: Selected notes and notables the online version of this article, along with updated information and services, is located on the World Wide Web at: The history of artificial insemination: Selected

- notes and notab, Journal of Animal Science, 80, pp. 1-10. https://doi.org/10.2527/animalsci2002.80E-Suppl 21a
- [51] Kocks, J. E. M. & Broekhuijse, M. L. W. J. (2014) The practical use of density of
- [52] Brito, L. F. C. (2007) Evaluation of Stallion Sperm Morphology, Clinical Techniques in Equine Practice, 6(4), pp. 249-264.

https://doi.org/10.1053/j.ctep.2007.09.004

[53] Nagy, S., Balázs, K., Pál, L., Havasi, M., Bercsényi, M., & Husvéth, F. (2016) Effects of high ambient temperature on fish sperm plasma membrane integrity and mitochondrial activity - A flow cytometric study, Acta Biologica Hungarica, 67(2), pp. 125-132.

https://doi.org/10.1556/018.67.2016.2.1

- [54] Hossain, M.S., Hohannisson, A., Wallgren, M., Nagy, S., Siquira, P.A., & Rodriguez-Martinez, H. (2011) Flow cytometry for the assessment of animal sperm integrity and functionality: State of the art, Asian Journal of Andrology, 13(3), pp. 406-419. https://doi.org/10.1038/aia.2011.15
- [55] Yániz, J. L., Soler., & C. Santolaria, P. (2015) Computer assisted sperm morphometry in mammals: A review, Animal Reproduction Science. Elsevier B.V., 156, pp. 1-12. https://doi.org/10.1016/j.anireprosci.2015.0 3.002
- [56] Tanga, B. M., Qamar, A.Y., Raza, S., Bang, S., Fang, X., Yoon, K., & Cho, J (2021) Semen evaluation: Methodological advancements in sperm quality-specific fertility assessment A review, Animal Bioscience, 34(8), pp. 1253-1270. https://doi.org/10.5713/ab.21.0072
- [57] Sailer, B. L., Tost, L. K., & Evenson, D. P. (1996) Bull sperm head morphometry related to abnormal chromatin structure and fertility, Cytometry, 24(2), pp. 167-173. <a href="https://doi.org/10.1002/(SICI)1097-0320(19960601)24:2<167::AID-CYTO9>3.0.CO;2-G">https://doi.org/10.1002/(SICI)1097-0320(19960601)24:2<167::AID-CYTO9>3.0.CO;2-G
- [58] Ostermeier, G. C., Sargeant, G.A., Yandell, B.S., & Evenson, D.B. (2001) Relationship of bull fertility to sperm nuclear shape, Journal of Andrology, 22(4), pp. 595-603. https://doi.org/10.1002/j.1939-4640.2001.tb02219.x

- whole bull semen to determine the volume of an ejaculate at AI laboratories, Animal Reproduction Science. Elsevier B.V., 146(3-4), pp. 98-102. https://doi.org/10.1016/j.anireprosci.2014.0 2.015
- [59] Martínez-Pastor, F., Mata-Campuzano, M., Álvarez-Rodríguez, M., Álvarez, M., Anel, L., & De Paz, P. (2010) Probes and techniques for sperm evaluation by flow cytometry, Reproduction in Domestic Animals, 45(SUPPL. 2), pp. 67-78. https://doi.org/10.1111/j.1439-0531.2010.01622.x
- [60] Benaron, D. A., Gray, W.J., Gledhill, L.B., Lake, S., Wyrobek, J.A., & Young, T. I. (1982) Quantification of mammalian sperm morphology by slit-scan flow cytometry, Cytometry, 2(5), pp. 344-349. https://doi.org/10.1002/cyto.990020512
- [61] Ramdzan, Y. M., Poling, S., Chia, S.P.Z., Ng,I.H.W., Ormsby,A.R., Croft, N.P., Purcell, A.W., Bogoyevitch, M.A., Ng, D.C.H., Gleeson,P.A., & Hatters, D.M. (2012) Tracking protein aggregation and mislocalization in cells with flow cytometry, Nature Methods, 9(5), pp. 467-470. https://doi.org/10.1038/nmeth.1930
- [62] Ward, W. S. (2009) Function of sperm chromatin structural elements in fertilization and development, Molecular Human Reproduction, 16(1), pp. 30-36. https://doi.org/10.1093/molehr/gap080
- [63] Andraszek, K., Banaszewska, D., Czubaszek, M., Wójcik, E., & Szoztek., M. (2014) 'Comparison of different chromatin staining techniques for bull sperm', 57, pp. 1-15. https://doi.org/10.7482/0003-9438-57-013
- [64] González-marín, C., Gosálvez, J., & Roy, R. (2012) Types, Causes, Detection and Repair of DNA Fragmentation in Animal and Human Sperm Cells, pp. 14026-14052. https://doi.org/10.3390/ijms131114026
- [65] Ben-yehuda, K., Mirsky, K.S., Levi, M., Barnea, I., Meshulach, I., Kontente, S., Benvaish, D., Cur-Cycowicz, R., Nygate, N. Y., & Shaked. T. N. (2021) Simultaneous Morphology, Motility, and Fragmentation Analysis of Live Individual Sperm Cells for Male Fertility Evaluation. Advanced Intelligent System, (April), pp. 1-31. https://doi.org/10.1101/2021.05.11.443542

- [66] Barroso, G., Mercan, R., Ozgur, K., Morshedi, M., Kolm, P., Coetzee, K., Kruger, T., & Oehninger, S. (1999) Intraand inter-laboratory variability in the assessment of sperm morphology by strict criteria: Impact of semen preparation, staining techniques and manual versus computerized analysis, Human Reproduction, 14(8), pp. 2036-2040. https://doi.org/10.1093/humrep/14.8.2036
- [67] Hirano, Y., Shibahara, H., Obara, H., Suzuki, T., Takamizawa, S., Yamaguchi, C., Tsunoda, H., & Sato, I. (2001) Relationships between sperm motility characteristics assessed by the computer-aided sperm analysis (CASA) and fertilization rates in vitro, Journal of Assisted Reproduction and Genetics, 18(4), pp. 213-218. https://doi.org/10.1023/A:1009420432234
- [68] [68] Banaszewska, D., Andraszek, K., Czubaszek, M., & Biesiada-Drzazga, B. (2015) 'The effect of selected staining techniques on bull sperm morphometry', Animan Reproduction Science. Elsevier B.V., 159, pp. 17-24. https://doi.org/10.1016/j.anireprosci.2015.0 6.019
- [69] Kondracki, S., Banaszewska, D., Wysokińska, A., & Chomicz, J. (2006) Sperm morphology of cattle and domestic pigs., Reproductive biology, 6 Suppl 2, pp. 99-104
- [70] Nagy, S., Házas, G., Bali Papp, A., Iváncsics, J., Szász, F., Szász Jr, F., Kovács, A., & Foote,R.H. (1999) Evaluation of sperm tail membrane integrity by light microscopy, Theriogenology, 52(7), pp. 1153-1159. https://doi.org/10.1016/S0093-691X(99)00207-1
- [71] Freneau, G. E., Chenoweth, P.J., Ellis, R., & Rupp, G. (2010) Sperm morphology of beef bulls evaluated by two different methods, Animal Reproduction Science, 118(2-4), pp. 176-181. https://doi.org/10.1016/j.anireprosci.2009.0 8.015
- [72] Kruger, T. F., Ackerman, S.B., Simmons, K.F., Swanson, R.J., Brugo, S.S., & Acosta, A.A. (1987) A quick, reliable staining technique for human sperm morphology, Systems Biology in Reproductive Medicine, 18(3), pp. 275-277. https://doi.org/10.3109/0148501870898849

- [73] Abhishek Kumar., J.K. Prasad., N. Srivastava, and S.K. Ghosh. (2019). Strategies to Minimize Various Stress-Related Freeze-Thaw Damages During Conventional Cryopreservation of Mammalian Spermatozoa. Bio preservation and Bio banking Vol. 17, No. 6. https://doi.org/10.1089/bio.2019.0037
- [74] Cao, X. W., Lin, K., Li, CY., & Yuan, CW. (2011) A review of WHO Laboratory Manual for the Examination and Processing of Human Semen (5th edition), National journal of andrology, 17(12), pp. 1059-1063
- [75] McDiarmid, C. S., Li, R., Kahrl, F.A., Rowe, M., & Griffith, C.S. (2021) Sperm Sizer: a program to semi-automate the measurement of sperm length, Behavioral Ecology and Sociobiology. Behavioral Ecology and Sociobiology, 75(5). https://doi.org/10.1007/s00265-021-03013-4
- [76] Berger, R. E., Muller, H. C., & Singh, P.N. (2003) Effects of age on DNA double-strand breaks and apoptosis in human sperm, 80(6). https://doi.org/10.1016/j.fertnstert.2003.04.002
- [77] Cohen-bacrie, P., Belloc, S., Menezo, R.J.Y., Sci, D., Clement, P., Hamidi, J., & Benkhalifa.M. (2009) Correlation between DNA damage and sperm parameters: a prospective study of 1, 633 patients. 91(5), pp. 0-4. https://doi.org/10.1016/j.fertnstert.2008.01. 086
- [78] Erenpreiss, J., Spano, M., Erenpreisa, J., Bungum, M., & Giwercman, A. (2006) Sperm chromatin structure and male fertility: Biological and clinical aspects, Asian Journal of Andrology, 8(1), pp. 11-29. https://doi.org/10.1111/j.1745-7262.2006.00112.x
- [79] Nagy, S. (2014) Male reproduction in the polluted environment review, 18, pp. 6-10
- [80] Bayasgalan, B., R. Mijiddorj., P.Gomboluudev., D. Oyunbaatar., M. Bayasgalan., A.Tas., T.Narantuya., & M.Lkhagvasuren. (2009) Climate change and sustainable livelihood of rural people in Mongolia. G. O'Brien, P. O'Keefe, & Dr. Tellam (Eds.), The adaptation continuum: Groundwork for the future, pp. 193-213.
- [81] Takahashi, Y., M.Dooloikhuu., Ito.A., & Murata.K.(2019) How to improve the performance of agriculture in Mongolia by

- ICT, Applied Studies in Agribusiness and Commerce, 13(3-4), pp. 59-64. https://doi.org/10.19041/APSTRACT/2019/3-4/7
- [82] Baldan, T., P.Myagmardulam., T. Ankhbayar., D.Jambatsadmid., & B. Khosbayar. (2022) Research results on the use of frozen yak sperm, Grand Altai Research & Education, 1(1(17)), pp. 58-66. https://doi.org/10.25712/ASTU.2410-485X.2022.01.008
- [83] Lueders, I. & Allen, W. R. T. (2020) Managed wildlife breeding-an undervalued conservation tool, Theriogenology. Elsevier Ltd, 150, pp. 48-54.

- https://doi.org/10.1016/j.theriogenology.2 020.01.058
- [84] Wildt, D., Pukazhenthi, B., Brown.J., Monfort, S., Howard, J., & Roth,T., (1995) Spermatology for understanding, managing and conserving rare species, Reproduction, Fertility and Development, 7(4), pp. 811-824. https://doi.org/10.1071/RD9950811
- [85] Özbek, M., Hitit, M., Kaya, A., Jousan.F.D., & Memili, E. (2021) Sperm Functional Genome Associated With Bull Fertility, Frontiers in Veterinary Science, 8(June), pp. 1-17. https://doi.org/10.3389/fvets.2021.610888