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Age and Seasonal Impacts on Bull Semen Quality: Current Findings and Future Directions

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ABSTRACT

This review explores the intricate interplay between age, season, and semen quality in bull reproduction, emphasizing the need for standardized assessment protocols, and advanced molecular technologies. Age-related changes in semen parameters such as volume, concentration, motility, and morphology are driven by physiological, and hormonal factors. Seasonal variations, influenced by environmental conditions, further affect reproductive success. Despite extensive research, inconsistencies in findings persist, highlighting the need for deeper investigation into the underlying mechanisms. Recent advancements underscore the significance of hormonal and nutritional influences on semen quality, advocating for targeted interventions. Key challenges in assessing semen quality include genetic variability, inconsistent methodologies, and a lack of longitudinal studies. Future research should focus on elucidating molecular pathways, integrating advanced technologies, and developing predictive models to optimize artificial insemination programs. Addressing these gaps requires a multidisciplinary approach to enhance reproductive performance and genetic progress in the cattle industry. This review offers valuable insights into the complexities of age and seasonal effects on bull semen quality, calling for standardized methods ,and innovative strategies to advance the field.

KEYWORDS: Bull semen quality, Age-related changes, Seasonal effects, Artificial insemination, Reproductive success, Genetic variability.

INTRODUCTION:

Bull semen quality is a crucial factor in the reproductive success of cattle breeding programs (Gafer *et al.*, 2015). It plays an important role in overall fertility and the success of artificial insemination programs, as well as in the production of the highest quality germplasm for future offspring. However, the quality of bull semen can be influenced by various factors such as age and seasonal effects (Kurniawan et al., 2020). Numerous studies have highlighted the influence of age on semen quality in bulls. For example, a study by Nirwana and Suparman reported that age has a significant impact on the semen quality of Bali bulls (Budiyanto *et al.*, 2021). Non-genetic factors such as the age of bulls, collection season, collection frequency, and bulls' category significantly affected the overall semen quality, sperm productivity, cryo-preservation capacity of ejaculates.(Mandal *et al.*, 2022)

The importance of bull semen in today's cattle breeding, especially in the era where artificial insemination (AI) is prevalent, cannot be overstated. Bull semen consists of sperm, and seminal plasma plays a critical role in determining

the fertility, conception rate, and overall productivity of the herd. In cattle breeding, the quality of the sperm is a crucial factor for reproductive success, and genetic progress. In cattle breeding, understanding age-related, and seasonal variations in semen parameters is crucial for optimizing reproductive success, and genetic improvement. Studies show that bulls aged 3 to 4 years often have the best semen quality ,and show differences in sperm motility, semen volume, etc. mass activity in different age groups. A study by Mridula Sharma *et al.,* (1984) found that the lowest sperm concentration was measured in the summer, suggesting that there may be age-related variations in semen quality in different seasons. However, this study did not provide specific age-related data. The correlation between age and semen volume suggests a lack of consensus or standardized understanding. The study by M. Sabs-Alsina *et al.,* (1984) focuses on the age-dependent effects of the temperature-humidity index on frozen sperm quality but does not provide details on the underlying mechanisms. While the section addresses environmental influences on semen quality, there may be gaps in understanding the specific contributions of individual environmental factors. Furthermore, the need for comprehensive longitudinal studies to track semen parameters in different age groups over time is suggested but not explicitly discussed. Furthermore, there is a lack of comprehensive studies that specifically examine how different genotypes affect semen quality under different environmental conditions. Identifying, and addressing these potential gaps through targeted research efforts could improve our understanding of the complex relationship between age, environmental factors, and bull semen quality in cattle breeding.

Future research should prioritize standardizing metrics, methods to consistently and uniformly assess age, season, and semen characteristics in bulls. This standardization is crucial to improving the comparability of results ,and obtaining more meaningful results in this area of study. The report highlights the complexity associated with the influence of age, and season on the semen characteristics of bulls. Although it offers valuable insights, it highlights the need for future research to standardize metrics, examine underlying mechanisms, examine interactions with other factors, and consider diverse populations. Adopting this comprehensive approach will contribute to a better understanding of bull reproductive health and improve cattle breeding practices.

MATERIALS AND METHODS:

Information sources: Database of Pub Med, web of science, Science Direct, and Google Scholar were searched to find articles related to the research interest.

Search strategy: The search was led by a list of pre-selected keywords. The search strategy was focused to review, and research articles; full text availability, and publication date was confined to 1955-2023. A meticulous systematic review methodology was employed, encompassing studies with distinct geographical locales, breeds, and methodologies. The focus was on discerning trends and discrepancies in results across varied contexts. The amalgamation of data from these diverse studies provides a comprehensive overview of the global landscape of factors influencing bovine semen quality. Keywords for search were confined to; Semen characteristics, Buffalo bulls, Age, Season. Seasonal effects, artificial insemination, Bubalus bubalis, dry season, rainy season, and semen cryopreservation.

It was observed that age played a crucial role in determining semen quality among bulls of various breeds, and found that older bulls tended to have lower sperm concentration, reduced motility, and increased defects in sperm morphology.. The influence of age on semen quality in bulls has also been observed by researchers (Budiyanto *et al.*, 2021). Other studies have confirmed that advanced age in bulls is associated with reduced semen quality. It is found that aging can lead to degeneration of testicular tissue, leading to a decrease in the volume and quality of semen produced by bulls (Pardede *et al*., 2020). Optimal semen quality in bulls aged 3 to 4 years (Anzar, 1984) the study by M. Sabs-Alsina

et al., shows that genetically elite young bulls can be identified at a very young age, but their semen quality can vary depending on the age at which puberty is reached. Better sperm motility in younger bulls is noticed (Bishop *et al.,* 1954). Age-dependent variations in semen volume and mass activity have been reported (Pant et al., 2003; Dutta and Deka, 1993; Younas, 1997). There is a gap in understanding and predicting variations in semen quality in genetically elite young bulls.

According to Budiyanto *et al.,* (2021), research suggests that older bulls may experience reduced semen volume, reduced sperm concentration and motility, as well as changes in testicular morphology and increased oxidative stress, all of which negatively impact sperm quality. Pardede *et al.,* (2020) note that the effects of aging on the semen quality of bulls are complex and may vary by breed. To mitigate these effects, Isnaini *et al.,* (2019) emphasize the importance of tailored management practices, including optimized nutrition ,and regular reproductive health assessments. Kurniawan *et al.,* (2020) highlight the importance of age on bull semen quality ,and emphasize the need for proactive strategies to maintain high-quality germplasm ,and ensure successful breeding programs. Furthermore, Budiyanto *et al.,* (2021) point out that age-related change, such as DNA damage, and seasonal fluctuations, highlight the importance of considering age in reproductive strategies, and management practices. In summary, age plays a critical role in various aspects of semen quality in bulls requires comprehensive understanding, and targeted interventions to achieve sustained reproductive success.

Buffalo bulls reach their maximum semen volume around nine years of age, after which it begins to decline (Younis, 1996; Jainudeen *et al.,* 1982). Age-related differences in sperm concentration were observed. Javed *et al.* (2000) reported significant differences in sperm concentrations, with concentrations being higher in younger bulls than in older ones (Javed *et al.,* 2000). Younis (1996) reported non-significant differences in sperm concentration between bulls of different age groups. Sperm concentration may vary with age. Gupta *et al.,* (1978) reported a sperm concentration of 1090 million/ml in buffalo bulls aged 7–9 years. Nordin *et al.,* (1990) reported concentrations of 5390 and 1130 million/ml in bulls of different age groups. Younger bulls tend to have better sperm motility than older ones. Bishop *et al.,(*1954) reported good motility in younger bulls. Higher motility percentages have been observed in bulls at 2–3 years of age and in certain breeds at 3 years of age (Khan, 1990; Gupta *et al.,* 1978). Nordin *et al.,*(1990) reported that sperm motility increased with age. Younas (1997) also reported significantly higher motility in young ,and adult bulls compared to old ones. Research has shown that older bulls tend to have lower semen volume, and concentration, as well as lower sperm motility, compared to younger bulls. These age-related declines may be due to changes in testicular morphology ,and function, such as decreases in the number of Leydig cells, seminiferous tubules and changes in Sertoli cell function. Additionally, aging bulls may be exposed to increased oxidative stress, which can negatively impact sperm quality, and function. Semen pH did not statistically differ between age groups in Nili Ravi buffalo bulls, as Younas (1997) reported. The pH values were 6.34 in young, 6.16 in adult and 6.27 in old bulls (Younas, 1997). However, a significant difference in semen pH was reported, with pH being lower in bulls aged 6 to 10 years (6.45) than in bulls older than 11 years (6.67) (Younas, 1997). Koonjaenak *et al.,* (2007) reported no statistically significant difference in pH between five Thai swamp buffalo bulls of different ages. Additionally, the effects of aging on semen quality of bulls may be influenced by breed-specific factors. Different breeds may experience different age-related declines in semen quality, highlighting the need for breed-specific management and reproductive strategies.

Studies on buffalo bulls have revealed notable differences in overall sperm abnormality depending on age ,and season (Kumi-Diaka *et al.,* 1980; Saeed *et al.,* 1987; Ahmad *et al.,* 1984; Javed, 1998; Koonjaenak *et al.,* 2007). The reported range of total sperm abnormalities in buffalo bulls is extensive, ranging from 9.93% to 35.90% (Anzar, 1984;

Younis, 1996). Age plays a significant role in the occurrence of head abnormalities in sperm, and shows an increasing trend with increasing age (Rao, 1971; Younas, 1997; Koonjaenak *et al.,* 2007). In addition, certain head abnormalities, such as pear-shaped heads, abnormal contours, and variable sizes are influenced by both the age and season of the bull.
Tail abnormalities in spermatozoa show a notable seasonal influence, with higher percentages observed in winter, and summer (Javed, 1998; Singh *et al.,* 1992). Bull age also contributes to the occurrence of tail abnormalities, with older bulls having a higher percentage (Koonjaenak *et al.,* 2007). Various tail abnormalities, including bent tails, curled tails, and tails wrapped around the head, are commonly reported in buffalo bulls (Javed, 1998; Younis, 1996).
In contrast, mid-piece abnormalities in buffalo bulls are relatively minor ,and show minimal variation across seasons, with a slightly increased incidence in older bulls (Javed, 1998; Younis, 1996). When exploring possible causes, it is suggested that abnormalities in the tail,and head of sperm are related to epididymal dysfunction and hormonal factors (Galloway, 1982; Gustafsson, 1965).

In a study by Gupta et al., in 1978, it was observed that age had a significant influence on the percentage of live ,and dead sperm. Bulls aged 3–4 years and over nine years of age had a higher percentage of dead sperm (17.81% and 19.97%, respectively) compared to bulls under three years of age (13.84%). Tomar *et al.,*(1985) also reported an increase in the percentage of dead sperm with increasing age . Nordin *et al.,*(1990), live sperm percentages were found to vary with age, ranging from 64.2% in 29-month-old bulls to 69.9% in bulls older than 65 months. Younas (1997) found that older bulls had a significantly higher percentage of dead sperm (14.57%) than young (13.96%) and adult (12.04%) bulls. Javed *et al.,* in 1997, different percentages of dead sperm were found in different age groups, with the highest proportion occurring in 8–9-year old bulls (21.16%). The total percentage of dead sperm in the study by Javed et al. in 1997 was 16.25%. Galloway and Norman (1980) proposed a threshold of 50% normal sperm as an indicator of normal reproductive function.

The influence of seasonal fluctuations on bull semen quality has been extensively studied. The environmental changes significantly affect production, and reproductive performance of animals. Among all climatic elements, temperature, and season are the most important parameters affecting animal fertility (Kunavongkrit *et al*., 2005). It has been consistently reported that semen quality tends to decline at certain times of the year, resulting in lower sperm concentration, motility, and viability. These changes in semen quality can be attributed to environmental factors such as temperature, photoperiod, and nutrient availability. For example, Sarastina *et al.,* conducted a study on dairy bulls, and found that semen quality was significantly lower in the summer months compared to other seasons (Collier *et al.,* 2017). Bhakat *et al.,* (2014) also discussed the influence of season on semen quality in Karan Fries bulls. Further studies by Brito L.F. *et al.,* (2002) and Nichi *et al.,* (2006) revealed seasonal variations in semen quality based on temperature, humidity, and photoperiod. Furthermore, Soderquist et al. (1997) highlighted seasonal variations in sperm morphology of Swedish dairy bulls.

The amount of semen in buffalo bulls can vary depending on the season. For example, Elwishy (1978) found that Iraqi buffalo bulls had higher semen volume in the fall. Reddy *et al.,* (1983) observed that Murrah and Surti buffalo bulls produced the most semen in the summer, followed by the rainy season. Nazir *et al.,*(1988) reported that the Nili Ravi breed had the highest semen volume in spring, followed by summer, winter, and autumn. Singh *et al.,* (1992) found no significant effect of the season on semen volume. Various factors, including genetics, reproductive health status, age, frequency of collection, pooled volume, nutrition, season, and management, can influence semen volume (Nazir, 1988; Soderquist *et al.,* 1992; Javed, 2000). Furthermore, Pant *et al.,*(2003) discovered a positive relationship between scrotal

circumference ,and semen volume. It is important to note that reported semen amounts may vary between studies due to different influencing factors.

Seasonal fluctuations can affect sperm concentration in buffalo bulls. Several studies have found that sperm concentration is higher in the summer compared to other seasons (Erb *et al.,* 1942; Heuer *et al.,* 1987; Mohan *et al.,* 1977). However, there are also reports indicating higher concentrations in certain months, such as October, and lower concentrations in other months, such as May (Dumitrescu *et al.,* 1988). Zafar *et al.,(*1988) reported the highest sperm concentration in April and October, while Shalash (1972) recorded lower concentrations in summer and higher concentrations in winter. The high ambient temperature increases the scrotal temperature and consequently a decline in the semen quality (Taylor and Bogart, 1988).Weirzbowsky *et al,,* (1980) found that the highest levels were observed during the breeding season (autumn) compared to other seasons. Javed *et al.,* (2000) observed a significantly higher sperm concentration in autumn than in winter, especially in older bulls. The semen quality of fresh samples was observed to be better during the winter season compared to the summer season, resulting in a satisfactory first AI conception rate for use in breeding programs (Parmar Kirankumar et al., 2022).

Various studies have reported fluctuations in the percentage of progressively motile sperm at different times of the year, suggesting that seasonal changes may influence sperm motility. Gill *et al.,(*1974) discovered that the Murrah breed had higher mobility in the winter than in the summer. Likewise, Mohan *et al.,* (1977) observed higher motility in winter, particularly in young bulls. Younas (1997) reported significantly higher motility in the autumn than in the summer in Nili Ravi bulls. However, there are also reports indicating higher motility in summer and no seasonal influence on sperm motility in certain breeds of buffalo.

The pH of Murrah buffalo semen was studied at different seasons of the year. Terezinha *et al.,* (1991) reported pH values of 6.82, 6.83, 6.92, and 6.93 in spring, summer, fall, and winter, respectively, with no significant differences between seasons. However, Younas (1997) discovered a significantly lower pH value in autumn (6.18) compared to summer (6.33). Javed et al. (2000) also observed a significantly lower pH value in autumn (6.05) compared to other seasons in bulls of different age groups. They also noted fluctuations in pH across different seasons (Javed *et al.,* 2000).

Sperm morphology can vary throughout the year, and certain seasons may be associated with an increased incidence of morphological abnormalities that may affect fertility. In a study by Javed (1998), higher sperm abnormalities were found in winter (30.12%) and summer (22–24%), while they were lowest in autumn (14.92%). Singh et al. (1992) also reported higher tail anomalies in the winter. Several studies have observed more frequent sperm abnormalities in the summer that are due to heat stress (Heuer et al., 1987). Igboeli *et al.,* (1987) reported variations in tail anomalies in different seasons, with the highest percentages occurring in the late wet season/early dry season and the lowest in the dry season. Koonjaenak *et al.,* (2007) reported variations in the overall proportion of tail defects across seasons, with the highest values occurring in the rainy season and the lowest in the summer. Javed (1998) suggested that the higher sperm abnormalities in winter could be due to improper handling of sperm. Younas (1997) reported greater head anomalies in autumn (and less in winter). Koonjaenak *et al.,* (2007) also observed differences in the overall proportion of tail defects across different seasons, with the highest values occurring in the rainy season, and the lowest in the summer. Heuer *et al.,* (1987) and Bhavsar *et al.,* (1990) reported higher sperm abnormalities during the hot season. Younas (1997) found greater fluctuations in sperm head in the secondary breeding season than in the main breeding season. Seasonal influence on head anomalies. Season can influence head anomalies in buffalo bulls, with differences observed between different seasons (Javed 1998). Igboeli *et al.,* (1987) reported variations in tail anomalies across seasons, with higher percentages in the late wet season, early dry season, and lower percentages in the dry season.

Studies of the effects of age, and season on bull semen quality have revealed complex patterns. Maulana *et al.,* (2022) found deterioration in semen quality in summer, while Collier *et al.,* (2017) observed seasonal influences near the equator and questioned the consistency of these effects. Anderson's study in Kenya reported seasonal effects on sperm volume and motility (Collier *et al.,* 2017). The conflicting results highlight the need for further research to understand the underlying mechanisms and interactions between age and season (Maulana *et al.,* 2022). Despite discrepancies, ongoing studies emphasize the importance of age and season on bull semen quality and require tailored management practices for reproductive success (Luz *et al.,* 2018). Future studies should include larger sample sizes, different locations, standardized protocols, and hormonal analyzes to deepen our understanding of these complex relationships.

The effect of season on live/dead sperm ratio was studied by Dumitrescu et al. examined. (1988) and Singh *et al.,* (1992). Dumitrescu et al. reported higher percentages of live sperm in July (93.60%) and lower percentages in May (90.2%) and October (87.3%). Singh et al. also found that the live rate is higher in summer compared to winter. Various researchers (Ahmad *et al.,* 1984; Bamabe *et al.,* 1992; Cook *et al.,* 1994; Younas, 1997) recorded a significantly higher percentage of dead sperm in buffalo bulls in summer. Kapoor (1973) recorded different percentages of live sperm in different seasons, and Gupta *et al.,* (1978) reported differences in the percentage of live sperm between seasons. Javed *et al.,* (1997) reported a significantly lower percentage of dead sperm in autumn (13.0%) compared to dry summers (19.58%) ,and wet summers (18.10%). The higher percentage of dead sperm in summer has been attributed to possible impairments in spermatogenesis due to environmental and nutritional stress (Ahmad *et al.,* 1984; Younas, 1997).

Potential Mechanisms behind Age and Seasonal Influences:

Javed *et al.,* (2000) investigated the interplay between age, and seasonal effects on bull semen parameters. They found that motility varied with age, and season, with higher motility observed in certain age groups during fall compared to summer, and winter. Younger bulls showed no significant seasonal effect. Additionally, age, and season influenced other semen parameters such as mass activity, progressive motility, sperm concentration, semen pH, percentage of live or dead sperm, and proportion of abnormal sperm. However, discrepancies exist in the reported results across studies (Bishop *et al.,* 1954).

Possible mechanisms underlying age, and seasonal influences on semen quality in bulls include:

1. Physiological Changes: Older bulls undergo post-pubertal physiological developments such as increased body mass, and testicular growth, resulting in improved semen production, and quality.

2. Hormonal Regulation: Age-related hormonal changes, particularly increased testosterone levels after puberty, contribute to improved semen quality in adult bulls. Seasonal fluctuations in hormone levels due to changes in photoperiod also affect reproductive physiology.

3. Heat Stress: High ambient temperatures in summer lead to heat stress, disrupting endocrine regulation and impairing spermatogenesis, resulting in reduced sperm production and quality.

4. Direct effect on the testes: Elevated temperatures directly affect the testes, and accessory reproductive glands, affecting sperm development, and semen quality.

5. Photoperiod modulation: Fluctuations in day length across seasons affect the pituitary-gonadal axis, affecting reproductive hormone regulation, and therefore semen quality.

6. Nutritional status: Seasonal fluctuations in food supply forage quality influence bulls' diet and affect energy balance, metabolism ,and testicular function, which also affects semen quality. Understanding these potential mechanisms is crucial for developing effective reproductive management strategies, and improving bull fertility.

Further research is needed to elucidate the specific physiological and molecular processes underlying age and seasonal effects on semen quality in bulls. However, the exact mechanisms ,and interactions between age, season and genetic factors are not yet fully understood. Future research should aim to elucidate these mechanisms.

Recent Advances in Semen Quality Research:

Recent research has highlighted the complicated relationship between age, season and semen parameters in bulls (Syarifuddin *et al.,* 2017). Age has a significant impact on semen quality, with older bulls generally having higher semen volume and better sperm quality than younger ones (Murphy *et al.,* 2018). This improvement is attributed to physiological changes after puberty and during sexual maturation that lead to increased semen production. However, studies on seasonal effects provide conflicting results, with some pointing to higher semen volume and sperm production in summer, while others find no significant differences. To deepen our understanding, future research should address the underlying mechanisms, interactions between age, season and semen quality. This may include research into hormonal changes, epigenetic changes and nutritional interventions. By elucidating molecular signaling pathways, targeted interventions could be developed to improve semen quality in older bulls, and counteract seasonal fluctuations. Additionally, it is important to consider the effects of diet. Research focuses on identifying specific nutritional components or supplements to improve semen quality and mitigate age-related and seasonal effects.

Challenges in Assessing Semen Quality

Despite advances in semen quality research, challenges remain in accurately assessing, and predicting semen quality in bulls. The variability between individual bulls caused by genetic factors makes evaluation difficult (Indriastuti *et al.,* 2020). Furthermore, inconsistent seed assessment methods hinder comparability between studies, and guideline development. External factors such as handling and storage conditions make the assessment even more difficult. Long-term studies tracking changes in semen quality over time, and seasons are lacking, making it difficult to understand age and seasonal effects. To address these challenges, future research should establish standardized evaluation protocols and comprehensive databases to track semen quality over time. By incorporating advanced technologies such as transcriptomics, proteomics and metabolomics, molecular mechanisms underlying age ,and seasonal effects can be uncovered, enabling targeted interventions. By overcoming these hurdles, researchers can make significant progress in improving semen quality prediction and ultimately improving reproductive success in the cattle industry.

Future Perspectives on Bull Semen Quality Improvement

Future research to improve semen quality in bulls should prioritize several key areas. Understanding the physiological and molecular mechanisms that determine age and seasonal effects is critical to developing targeted strategies to improve reproductive success in the cattle industry. Efforts should focus on elucidating hormonal changes, epigenetic changes, and nutritional influences. By exploring molecular signaling pathways, novel interventions could be identified to improve semen quality in older bulls ,and counteract seasonal fluctuations. Studying the effects of nutrition, particularly in older bulls, is essential and requires research into specific dietary components or supplements.

Standardized assessment protocols ,and comprehensive databases are needed to overcome challenges in assessing, and predicting semen quality. The integration of advanced technologies such as transcriptomics, proteomics and metabolomics can deepen the understanding of the underlying mechanisms. By addressing these challenges, researchers can make significant progress in improving prediction of semen quality and increasing reproductive success in bulls, benefiting the cattle industry as a whole

Conclusion: Bridging the Gap in Bull Semen Quality Research

The influence of age, and seasonal effects on bull semen quality highlights the need for further research to understand underlying mechanisms, and develop targeted interventions (Murphy et al., 2018). Standardized assessment protocols, and advanced technologies such as transcriptomics, proteomics and metabolomics offer promising opportunities to gain insights into these effects, identify biomarkers and signaling pathways related to semen quality. Future perspectives should focus on developing predictive models that integrate age, season, and genetic information to optimize artificial insemination programs in the cattle industry. To close the research gap, a multidisciplinary approach is required to comprehensively understand molecular mechanisms, and improve reproductive performance in bulls. While the influence of age ,and seasonal variations on semen quality is recognized, the ongoing debate highlights the need for further study and consensus in the field of reproductive biology.

Comprehensive Review of Studies on Factors Influencing Semen Quality in Bulls: Investigations, Limitations, Conclusion and Results (Table-1)

SI.No	Reference	Investigation	Limitations	Conclusion and Results
1	Bhakat *et al.*, (2014)	Seasonal effect on semen quality in bulls.	Limited to specific crossbred bulls, single location.	Season significantly impacts semen quality in Karan Fries bulls.
2	Bredderman & Foote (1969)	Examined sperm volume and motility relation.	Older study, focused on physical characteristics.	Larger sperm volume linked to reduced motility and fertility.
3	Brito *et al.*, (2002)	Investigated environmental, age, and genotype.	Limited to Bos indicus and taurus bulls in Brazil.	Environmental factors, age, and genotype affect semen production.
4	Chacon *et al.*, (1999)	Evaluated breeding soundness in managed bulls.	Specific to Costa Rica, extensive management.	Demonstrated acceptable breeding soundness in extensively managed bulls.
5	Chandler *et al.*, (1985)	Explored environmental and genetic sources.	Limited to Holstein bulls, older study.	Both genetics and environment influence seminal quality.
6	Diarra *et al.*,	Studied genetic and	Focused on young Holstein	Genetic and

	(1997)	environmental factors.	bulls, older study.	environmental factors impact young Holstein bulls' semen.
7	Drevius & Eriksson (1966)	Investigated osmotic swelling of spermatozoa.	Limited to sperm osmotic swelling, older study.	Provided insights into sperm osmotic swelling.
8	Drevius (1972)	Explored sperm permeability to various agents.	Limited to bull sperm, older study.	Provided insights into sperm membrane permeability.
9	Edel M. Murphy *et al.*,(2018)	Study on semen production and sperm motility in Holstein Friesian bulls. Investigated effects of bull age, ejaculate number, and season.	Focuses on bulls <1 year and older, missing detailed age-related nuances. Limited to Holstein Friesian bulls, reducing generalizability to other breeds.	Young bulls had poorer semen production and motility. Second ejaculates from young bulls had similar post-thaw motility.
10	Fiaz *et al.,* (2010)	Evaluated semen quality in subtropical environment.	Limited to specific breeds, subtropical region.	Environmental factors impact semen quality in subtropical bulls.
11	Fields *et al.,* (1979)	Investigated age, season, and breed effects.	Limited to young beef bulls, older study.	Age, season, and breed affect testicular volume and semen traits.
12	Gadea *et al.,* (2004)	Examined glutathione content in boar sperm.	Specific to boar sperm, related to cryopreservation.	Glutathione affects boar sperm cryopreservation.
13	Gajendra Solanki *et al.*,(2023)	Study on seasonal impact on Gir bull semen quality. Evaluation of HSP70 and HSP90 expression and fertility correlation.	Age-related effects were not addressed in the study on HSP expression and semen functionality.	Season affects physico-morphological parameters and HSP70 expression in semen.HSP70 expression correlates positively with semen quality and fertility.
14	Godfrey *et al.,* (1990)	Studied season and location on semen quality.	Specific to Brahman and Hereford bulls, extensive locations.	Seasons and locations influence semen quality in these bulls.
15	Goswami *et al.,*	Explored the effect of	Limited to zebu-taurus bulls	Hormones and semen

	(1991)	meteorological factors.	in India.	traits are influenced by meteorological factors.
16	Graffer *et al.*, (1988)	Investigated semen production in Norway.	Limited to semen production in Norway, older study.	Provided insights into semen production in Norwegian AI bulls.
17	Hancock (1959)	Explored the morphological .characteristics.	Older study, limited to morphology.	Morphology of spermatozoa is related to fertility.
18	Hankiewiez *et al.*, (1964)	Studied enzyme activity in spermatozoa.	Specific to enzymology, older study.	Enzyme activity related to sperm function and fertility.
19	Hirwa Claire *et al.*, (2017)	Study on bovine semen quality influenced by breed, season, age.	Older bulls have lower semen quality. Summer season negatively affects semen quality.	Friesian bulls had superior semen volume compared to Jersey and In ambo. - Short rainy season showed best semen quality characteristics.
20	Ibrahim *et al.*, (1983)	Analyzed seasonal and bacterial effects on semen.	Limited to Hungarian Simmental bulls, older study.	Season and bacterial contamination affect semen quality.
21	Igboeli & Rakha (1971)	Investigated seasonal changes in ejaculate.	Limited to Angoni bulls, older study.	Seasonal changes in ejaculate characteristics in Angoni bulls.
22	Ramajayan *et al.*,(2022)	The effect of non-genetic factors on semen production traits of Murrah buffalo bulls maintained in an organized semen station was analyzed using least-squares analyses under General Linear Model (GLM).	Findings may be specific to the study's organized semen station conditions. Lacks insights into biological mechanisms driving observed traits.	Poor semen quality, old age, and poor libido caused disposals. Early selection and training under preferable conditions increase semen production.
23	Jeyendran *et al.*, (1984)	Developed assay for assessing sperm membrane.	Limited to human sperm, specific to membrane.	Developed assay for assessing human sperm membrane integrity.
24	Kumi-Diaka *et al.*,	Explored seasonal and age-	Limited to tropical	Seasonal and age-related

	(1981)	related semen changes.	environment, older study.	changes in semen quality and morphology.
25	Majic *et al.,* (2012)	Investigated age and environmental effects.	Limited to Simmental bulls, specific environment.	Age and environmental factors impact semen quality and oxidative parameters.
26	Mathevon *et al.,* (1998)	Explored environmental, management, and genetics.	Limited to Holstein bulls, older study.	Environmental, management, and genetic factors affect semen production.
27	Meyerhoeffer *et al.,* (1976)	Analyzed effect of elevated temperature.	Specific to elevated temperature, older study.	Elevated ambient temperature affects bull physiology.
28	Mishra *et al.,* (2013)	Studied the effect of ambient temperature.	Limited to ambient temperature, specific breeds.	Ambient temperature affects sperm membrane integrity.
29	Moghdum *et al.,* (2012)	Examined seasonal variation in semen traits.	Limited to Iranian crossbred rams, older study.	Seasonal variation in semen quantity and quality in crossbred rams.
30	Nagarcenkar (1982)	Discussed breeding for dairy production.	Limited to general breeding principles.	Emphasized the importance of breeding for dairy production.
31	Nichi *et al.,* (2006)	Studied seasonal variation in semen quality.	Limited to Bos indicus and taurus bulls, tropical conditions.	Seasonal variation in semen quality under tropical conditions.
32	Nurul Isnaini *et al.,*(2021)	Studied seasonal effects on semen quality of Pasundan bulls.	Scarcity of information on seasonal effects.	Semen quality of Pasundan bulls reduced in dry season. Sunshine duration increase associated with reduced semen quality.
33	Parkinson *et al.,*(1987)	Observed seasonal variations in semen quality.	Correlated with environmental temperature.	Seasonal variations in semen quality correlated with environmental temperature.
34	Parmar Kirankumar(2022)	Seasonal variation in Jaffarabadi buffalo bulls'	Age data is not provided in the study.	Winter semen quality

		semen quality and conception rate.		better than summer for breeding program. - First AI conception rate satisfactory in winter compared to summer.
35	Pushp Raj Shivahre *et al.,* (2017)	Study on Murrah buffalo bulls' semen production characteristics at organized station. Investigated influence of season and period of birth on semen traits.	Focuses solely on Murrah buffalo bulls, limiting generalizability to other breeds or species. Does not account for factors like nutrition, health, or genetic variations, which could impact results.	Season and period of birth did not significantly affect semen characteristics. Introducing training at an early age can reduce age at donation.
36	Rekwot *et al.,* (1987)	Explored seasonal influence on semen traits.	Limited to ejaculate characteristics, specific location.	Seasonal influence on ejaculate characteristics in Nigerian bulls.
37	Richthoff *et al.,* (2003)	Investigated the impact of cigarette smoking.	Limited to lifestyle factors, specific to smokers.	Cigarette smoking affects reproductive characteristics in young males.
38	Roussel *et al.,* (1963)	Explored artificial light, temp, and humidity	Limited to specific environmental factors	Artificial light, temperature, and humidity impact physiological response in dairy bulls
39	Saacke (1970)	Emphasized the importance of sperm morphology	Limited to sperm morphology, older study	Sperm morphology is crucial for fertility assessment
40	Salah *et al.,* (1992)	Studied seasonal variation in semen traits	Specific to semi-arid environment, Holstein bulls	Seasonal variation in semen quantity and quality in semi-arid conditions
41	Schwab *et al.,* (1987)	Explored factors affecting semen production	Limited to semen production factors	Factors affecting semen production in artificial insemination bulls
42	Sekoni *et al.,*(1988)	Assessed seasonal variation in sperm quality	Limited to sperm morphology, older study	Seasonal variation in sperm morphology in Swedish dairy AI bulls

43	Siswanto Imam *et al.*,(2017)	Study on fresh and frozen semen quality of Holstein bulls. Investigated age and bulls' impact on semen production in Indonesia.	Seasonal effects on bulls in frozen semen production were not addressed in the paper.	Age and bulls significantly affect fresh and frozen semen quality.Bulls produce optimal frozen semen at 3-9 years old.

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