Journal of Bio-resource and Stress Management https://ojs.pphouse.org/index.php/IJBSM

Crossref

JBSM December 2024, 15(12): 01-06 **Article** AR5735a

Research Article *Natural Resource Management*

DOI: HTTPS://DOI.ORG/10.23910/1.2024.5735a

Effect of Age on Semen Quality and Freezability in Sahiwal Bulls

Anshul Gautam, Pawan Singh, Sriranga K. R. and Shruti Arya

Artificial Breeding Research Centre, ICAR-National Dairy Research Institute, Karnal, Haryana (132 001), India

Open Access

Corresponding anshulgautam789@gmail.com

0009-0002-5891-6743

ABSTRACT

The study was conducted at ICAR-National Dairy Research Institute, Karnal, Haryana, India during Feb–May, 2023 to find out the age window at which the bull produces quality semen. Fifteen Sahiwal bulls were selected and classified into three age groups (n=5 in each group) -(2–3.5), (>3.5–8) and (>8–12) years. Semen was collected twice a week with two ejaculates on each collection. Fresh and extended frozen semen was analysed at fortnightly interval. The data was subjected to statistical analysis by one-way Analysis of Variance (ANOVA) using SPSS software version 26.0 to draw scientific inferences. The results revealed that the mean value of semen volume (ml), sperm concentration (10^6 ml^{-1}), mass motility was high (p<0.05) in the >8-12 age group. Individual motility was high (p<0.05) in the >3.5–8 age group. Mean value of viability (%), HOST (%), Acrosome integrity (%) was lower in 2-3.5 age group for fresh as well as frozen semen. Post thaw motility was high (p<0.05) in >3.5-8 age group. In incubation test, the post thaw motility of the >3.5–8 age group was high (p<0.05) at 0, 90 and 120 min. Therefore, it can be concluded from the study that semen quality of Sahiwal bulls is influenced by age and it is better to use Sahiwal bulls only after 3.5 years of age and best semen quality is obtained from bulls of >3.5-8 years of age.

KEYWORDS: Age, bulls, fresh, frozen, semen quality

***Citation* (VANCOUVER):** Gautam et al., Effect of Age on Semen Quality and Freezability in Sahiwal Bulls. *International Journal of Bio-resource and Stress Management*, 2024; 15(12), 01-06. HTTPS://DOI.ORG/10.23910/1.2024.5735a.

Data Availability Statement: Legal restrictions are imposed on the public sharing of raw data. However, authors have full right to transfer or share the data in raw form upon request subject to either meeting the conditions of the original consents and the original research study. Further, access of data needs to meet whether the user complies with the ethical and legal obligations as data controllers to allow for secondary use of the data outside of the original study.

Conflict of interests: The authors have declared that no conflict of interest exists.

RECEIVED on 27th August 2024 RECEIVED in revised form on 20th November 2024 ACCEPTED in final form on 05th December 2024 PUBLISHED on 22nd December 2024

1. INTRODUCTION

With milk production of 230.58 mt and an annual growth rate of 3.83%, India is the world's greatest producer of milk (Anonymous, 2022–2023), however the productivity of our cows is relatively poor (3.36 kg day^{-1}). Artificial insemination with frozen semen is the most widely utilized method for boosting the genetic potential of dairy animals (Rugira et al., 2017) as a vast female population is being covered by a few number of genetically superior sires (Waldner et al., 2010). It is also more cost-effective than natural service for the management of sexually transmitted infections (Lemma and Shemsu, 2015). Precise seminal quality is crucial for the best use of genetically superior bulls for artificial insemination (Fuerst-Waltl et al., 2006; Christensen et al., 2011 and Ahmed et al., 2016). Sahiwal is the leading indigenous milch breed and it can be used to upgrade and selectively bred the nondescript bovine population in India.

Both genetic and non-genetic factors (Hirwa et al., 2017; Tohura et al., 2018) such as age (Meena et al., 2023), testicular thermoregulation (Rizzoto and Kastelic, 2019), season of collection (Bhutta et al., 2020), season of birth (Dangar et al., 2021) testicular size (Susilawati et al., 2020) affects the quality of semen produced by bulls. The age of a bull at semen collection is known to affect the characteristics of the semen (Mahmood et al., 2014; Argiris et al., 2018; Murphy et al., 2018; Rai and Dorji, 2021; Al-Asadi et al., 2021; Tyagi et al., 2023). Management strategies to reduce age at first semen donation are emphasized now -a- days. (Naha et al., 2015; Singh et al., 2020). Nonetheless, the wide range in the age at which puberty begins within and within breeds accounts for the majority of the diversity in the reproductive performance of young bulls (Barth and Waldner, 2002). Also, the percentage of normal sperms and major sperm defects are significantly affected by the age of bulls (Vilakazi and Webb, 2004). Age effects the scrotal circumference and testicular thermoregulation which in turn effects sperm quantity and quality (Sivaselvam et al., 2022; Wahyudi et al., 2022). It also effects testosterone production and libido (Dasrul et al., 2020) ultimately affecting the ejaculatory performance of bull (Singh et al., 2015). The undeveloped size of the testis and the compromised thermoregulatory system may be the cause of the younger bulls' poor semen qualities and high aberrant spermatozoa levels. Moreover, DNA is more prone to fragmentation (Carreira et al., 2017) due to a lack of protamination (Westfalewicz et al., 2021). Semen quality deteriorate with age as a result of disintegration of bodily tissues, notably testicular tissues, alterations in seminiferous tubule degeneration and more production of reactive oxygen species (Rafiq, 2022). Around the scrotum, a bull may begin to accumulate fat as it ages, thereby decreasing the scrotal ability to radiate heat (Brito et al., 2002). Cryopreservation is known to effect the semen functionality (Sonar et al., 2016; Peris et al., 2020) which also varies among different age groups (Kipper et al., 2016). Bulls of different age reacts differently to environmental stress (Vince et al., 2018).

Therefore, a bull produces its best quality semen at a certain age window. Given the foregoing explanation, it is necessary to address this holistically with Sahiwal bulls as, the effect of age on a Sahiwal bull's reproductive performance has not been investigated. For this reason, the current study was designed to look into how age affects the quality and freezability of the semen produced by Sahiwal bulls.

2. MATERIALS AND METHODS

The study was conducted at Artificial Breeding Research Centre (ABRC) of ICAR-National Dairy Research Institute (NDRI), Karnal, Haryana, India for the period of February, 2023–May, 2023. To study the effect of age on fresh and frozen semen quality, fifteen Sahiwal bulls were used. The age of bulls ranged from 2–12 years and were divided into three age categories: 2 to 3.5 years (G1), >3.5 to 8 years (G2), and 8 to 12 years (G3). The semen was collected twice a week using bull specific Artificial Vagina (IMV Technologies, France) method, with standard semen collection procedure. For semen collection, a Sahiwal bull was used as a dummy. Semen was collected in the morning hours beginning at 7:30 AM. The semen quality was assessed at 15 days interval. Immediately after collection, the ejaculate was brought in the laboratory and kept in water bath at 32 ^{o}C for assessing volume, mass activity, individual motility, sperm concentration, viability % (Campbell et al., 1953), hypo-osmotic swelling test (HOST) (Correa and Zavos, 1994), acrosome reaction (Watson, 1975) and subsequent experimentation after freezing.The data obtained in the study were subjected to statistical analysis by one way Analysis of Variance (ANOVA) using SPSS software version 26 and means were compared using Duncan's multiple range test to draw scientific inference.

3. RESULTS AND DISCUSSION

The details regarding the effect of age on fresh and extended frozen semen with respect to ejaculate volume, concentration, mass motility, Individual motility, live %, HOST % and acrosome integrity % is presented in Table 1.

Ejaculate volume (ml) differed significantly among the groups and was found to be higher ($^{*}p<0.05$) in G3 group as compared to G1 and G2 group, whereas it did not differ between G1 and G2. However, Bhakat et al. (2011) and Meena et al. (2023) in their study found that ejaculate

Table 1: Semen quality parameters in Sahiwal bulls of different age groups

Age groups (years)	Fresh semen							Frozen semen		
	Volume (ml) Mean± SE	Conc. (106 ml^{-1}) Mean± SE	Mass motility (0-5 score) Mean ±SE	Individual motility (%) Mean± SE	Viability (%) Mean± SE	Host (%) Mean± SE	Acrosome integrity (%) Mean± SE	Viability (%) Mean± SE	HOST (%) Mean± SE	Acrosome integrity (%) Mean± SE
2-3.5	3.74[a] ± 0.43	1289.43[a] ± 112.91	2.69[a] ± 0.18	71.81[a] ± 1.84	72.13[a] ± 3.18	54.50[a]± 2.87	70.38[a] ± 3.03	45.85[a] ± 2.97	34.05[a] ± 1.99	60.07[a] ± 2.84
>3.5-8	4.06[a] ± 0.25	1339.43[a] ± 93.46	3.03[ab] ± 0.08	77.00[b] ± 1.42	78.05[b] ± 1.38	64.17[b]± 1.51	81.48[b] ± 1.26	59.08[b] ± 2.06	39.60[b] ± 1.63	68.23[b] ± 1.51
8-12	5.47[b] ± 0.49	1759.36[b] ± 95.07	3.26[b] ± 0.10	75.16[ab] ± 1.54	81.63[b] ± 1.47	64.25[b] ± 1.64	77.55[b] ± 1.43	64.56[b] ± 1.91	43.48[b] ± 2.03	68.53[b] ± 1.80

volume significantly increased up to an age, after which it began to decline. Abdullah (2016) in Sahiwal bulls and Javed et al. (2000) in Nili Ravi bulls did not find any significant differences among the age groups. As 90% of semen is seminal plasma, which is released by accessory sex glands, the increase in ejaculate volume may be primarily caused by an increase in the size and secretions of accessory sex glands, as well as an increase in testicular size and, to some extent, spermatozoa generation.

The sperm concentration (10^6 ml^{-1}) differed significantly among age groups. It was high ($^*p$<0.05) in G3 group as compared to G1 and G2 groups. It did not differ between G1 and G2 groups. Opposite results to the present findings were found by Javed et al. (2000) where highest sperm concentration was found in the younger age group (<5 years old). Meena et al., 2023 found that sperm concentrations decreases from young to adult group but it again increases for older groups (72 months). Ahmad et al. (2003), Bhakat et al. (2011) and Rehmann et al. (2016) found insignificant differences among the age groups. The difference between the present study and findings of Javed et al. (2000) may be due to the fact that the younger group formed in the present study had lower age (2–3.5 years) than the latter and the study conducted by Bhakat et al. (2011) and Rehmann et al. (2016) involved groups with less difference in age and that's why they did not find any difference among the age groups in sperm concentration.

Mass motility (0–5 scale) was significantly high ($^*p$<0.05) in G3 group followed by G2 and G1 group. Similarly, Younis et al. (1998) in Nili-Ravi bulls and Bhakat et al. (2011) in Sahiwal bulls found significantly higher mass activity in adult bulls as compared to young bulls. Contrary to the findings of the present study, Ahmad et al. (2003) and Abdullah (2016) found no significant difference between the age groups in Sahiwal bulls.

The motility (%) of individual sperm was significantly ($^*p$<0.05) high in G2 group followed by G3 and G1. The results are in agreement with Ahmad et al. (2003) who also found significantly higher motility in adult age group as compared to other age groups. On the contrary, Carreira et al. (2017) found high motility in younger age as compared to the other two groups. Rehmann et al. (2016) in Sahiwal bulls and Javed et al. (2000) in Nili-Ravi bulls found no significant differences between the age groups.

The viability (%) for fresh semen was significantly ($^*p$<0.05) lower in G1 group as compared to G2 and G3 groups while no significant difference was found between the G2 and G3 groups. For extended frozen semen, it was significantly ($^*p$<0.05) lower in G1 group as compared to G2 and G3 groups. No significant difference was found between the G2 and G3 groups. Rehmann et al. (2016) and Abdullah (2016) found no significant difference between the age groups for viability in Sahiwal bulls. Low viability in the young age group in our study indicates that their sperm are more susceptible to management and environmental factors and also to cryopreservation techniques.

The HOST % for fresh semen was significantly ($^*p$<0.05) lower in G1 group as compared to G2 and G3 group. No significant difference was found between the G2 and G3 age groups. HOST % of extended frozen semen was significantly ($^*p$<0.05) lower in G1 group as compared to G2 and G3 groups. No significant difference was found between the G2 and G3 groups. The results are in agreement with Rehmann et al. (2016) and Rafiq et al. (2022) who found significantly lower HOST values for lower age groups. Bhave et al. (2020) in their study found that there was a gradual improvement in HOST reactive sperms up to a certain age after which it begins to decline.

The acrosome integrity (%) for fresh semen was significantly (*p<0.05) lower in G1 group as compared to G2 and G3 groups. No significant difference was found between the G2 and G3 age groups. For extended frozen semen, it was significantly (*p<0.05) lower in G1 group as compared to G2 and G3 groups. No significant difference was found between the G2 and G3 groups. These finding are similar to Rafiq et al. (2022) in Murrah bulls, Gupta et al. (1984) in Surti bulls and Sekharam and Rao (1986) in Murrah bulls. Ahmed et al. (2018) in Nili Ravi buffalo bulls and Abdullah (2016) in Sahiwal bulls found no significant difference in acrosome integrity between the different age groups.

Post thaw motility (%) was significantly (p<0.05) high for G2 group followed by G3 and G1. Mandal et al. (2021) found significant increase in the post thaw motility as the age increased. Ahmad et al. (2003) in Sahiwal bulls and Bhave et al. (2020) in Gir bulls did not find any significant difference in post thaw motility among the age groups.

3.1. Post thaw incubation test

The details regarding the effect of age on post thaw motility at 0, 30, 60, 90,120 min is presented in Table 2.

The sperm motility (%) was significantly (p<0.05) high in G2 age group as compared to G1 and for G3 group at 0, 90 and 120 min. At 30 and 60 min no significant difference was observed among the groups.

No published literature is available for the effect of age on incubation test of sperms in animals.

Table 2: Post thaw motility % in Sahiwal bulls of different age groups

Age group (Years)	Motility (%) Mean±SE				
	0	30	60	90	120
G1 (2-3.5)	52.25[a] ±1.86	51.75 ±1.85	51.00 ±2.05	46.50[a] ± 2.10	43.02a± 2.93
G2(>3.5-8)	57.00[b] ± 0.93	56.16 ±0.79	55.16 ±0.70	53.33[b] ±0.86	52.33b± 1.18
G3(8-12)	53.83[ab] ± 1.84	53.33 ±1.87	51.66 ±2.14	47.66[a] ±2.29	45.00a± 2.87

4. CONCLUSION

Age had significant influence on the semen quality parameters. Not much difference was observed in the semen quality of >3.5–8 years and 8–12 age group, though it was slightly better in the middle age group. The sperm of 2–3.5 years age group bulls was found to be more susceptible for cryopreservation techniques. Therefore, it is better to use semen for AI from bulls only after 3.5 years of age and it can be used up to 12 years of age without much deterioration in quality.

5. REFERENCES

Abdullah, M., 2016. Andrological evaluation of Sahiwal males for breeding soundness. PhD (Doctoral dissertation), NDRI (Deemed University), Karnal, Haryana.

Ahmad, M., Asmat, M.T., Rehman, N.U., Khan, M.Z., 2003. Semen characteristics of Sahiwal bulls in relation to age and season. Pakistan Veterinary Journal 23(4), 202–206.

Ahmed, H., Andrabi, S.M.H., Jahan, S., 2016. Semen quality parameters as fertility predictors of water buffalo bull spermatozoa during low-breeding season. Theriogenology 86(6), 1516–1522.

Ahmed, S., Khan, M.I.U.R., Ahmad, M., Iqbal, S., 2018. Effect of age on lipid peroxidation of fresh and frozen-thawed semen of Nili-Ravi buffalo bulls. Italian Journal of Animal Science 17(3), 730–735.

Al-Asadi, F.A., Kassim, W.Y., Hassan, A.F., 2021. Effect of season and age on semen characteristics and physiological changes in the testis of Jonobi Bull Breed in Iraq. Annals of the Romanian Society for Cell Biology 25(4), 2535–2542.

Anonymous, 2022–23. Department of Animal Husbandary, Dairying and Fisheries, Ministry of Agriculture and Farmers Welfare, Govt of India. Available at: https://dahd.nic.in/document/annual_report. Accessed on 15th June, 2024.

Argiris, A., Ondho, Y.S., Santoso, S.I., Kurnianto, E., 2018. Effect of age and bulls on fresh semen quality and frozen semen production of Holstein bulls in Indonesia. In IOP Conference Series: Earth and Environmental Science 119, 012033.

Barth, A.D., Waldner, C.L., 2002. Factors affecting breeding soundness classification of beef bulls examined at the Western College of Veterinary Medicine. The Canadian Veterinary Journal 43(4), 274.

Bhakat, M., Mohanty, T.K., Raina, V.S., Gupta, A.K., Khan, H.M., Mahapatra, R.K., Sarkar, M., 2011. Effect of age and season on semen quality parameters in Sahiwal bulls. Tropical Animal Health and Production 43(6), 1161–1168.

Bhave, K.G., Jawahar, K., Kumarasamy, P., Sivakumar, T., Joseph, C., Shirsath, T., Deshmukh, P., Venkataramanan, R., 2020. Genetic and non-genetic factors affecting semen production and quality characteristics of Gir cattle breed under semi-arid climate. Veterinary World 13(8), 1714–1718.

Bhutta, M.F., Tariq, M., Tunio, M.T., Sufyan, A., Rauf, H.A., Javed, M., Imran, S., 2020. Season induced changes in seminal characteristics of Sahiwal breeding bulls. Advances in Animal and Veterinary Sciences 8(6), 601–607.

Brito, L.F.C., Silva, A.E.D.F., Rodrigues, L.H., Vieira, F.V., Deragon, L.A.G., Kastelic, J.P., 2002. Effects of environmental factors, age and genotype on sperm production and semen quality in *Bos indicus* and *Bos taurus* AI bulls in Brazil. Animal Reproduction Science 70(3–4), 181–190.

Campbell, R.C., Hancock, J.L., Rothschild, L., 1953. Counting live and dead bull spermatozoa. Journal of Experimental Biology 30(1), 44–49.

Carreira, J.T., Trevizan, J.T., Carvalho, I.R., Kipper, B., Rodrigues, L.H., Silva, C., Perri, S.H.V., Drevet, J.R., Koivisto, M.B., 2017. Does sperm quality and DNA integrity differ in cryopreserved semen samples from young, adult, and aged Nellore bulls? Basic and Clinical Andrology 27(1), 1–8.

Christensen, P., Labouriau, R., Birck, A., Boe-Hansen, G.B., Pedersen, J., Borchersen, S., 2011. Relationship among seminal quality measures and field fertility of young dairy bulls using low-dose inseminations. Journal of Dairy Science 94(4), 1744–1754.

Correa, J.R., Zavos, P.M., 1994. The hypo-osmotic swelling test: its employment as an assay to evaluate the functional integrity of the frozen-thawed bovine sperm membrane. Theriogenology 42, 351–360.

Dangar, N.S., Brahmkshtr, B.P., Deshmukh, N., Modi, L.C., Khasatiya, C.T., Ramani, U.V., Pandya, G.M., 2021. Effect of season and year of birth on pubertal age, body weight and scrotal circumference of Kankrej bulls at first semen collection. The Indian Journal of Veterinary Sciences and Biotechnology 17(1), 35–38.

Dasrul, D., Wahyuni, S., Sugito, S., Hamzah, A., Zaini, Z., Haris, A., Gholib, G., 2020. Correlation between testosterone concentrations with scrotal circumference, and semen characteristics in Aceh bulls. In E3S Web of Conferences 151, 01015.

Fuerst-Waltl, B., Schwarzenbacher, H., Perner, C., Solkner, J., 2006. Effects of age and environmental factors on semen production and semen quality of Austrian Simmental bulls. Animal Reproduction Science 95(1–2), 27–37.

Gupta, R.C., Sharma, A.K., Khurana, N.K., 1984. Testosterone levels and libido in buffalo bull 10thInt. Cong. Animal Reproduction Artificial Insemination, University Illinois, Urban Champaign, Chicago, USA, 282.

Hirwa, C.D., Kugonza, D.R., Amahoro, E., Ingabire, C., Niyiragira, V., Myambi, C., Manzi, M., Murekezi, T., Nyabinwa, P., Nshimiyimana, A.M., Kanyandekwe, C., 2017. Influence of breed, season and age on quality bovine semen used for artificial insemination. International Journal of Livestock Production 8(6), 72–78.

Javed, M.T., Khan, A., Kausar, R., 2000. Effect of age and season on some semen parameters of Nili-Ravi buffalo (*Bubalus bubalis*) bulls. Veterinarski Arhiv 70(2), 83–94.

Kipper, B.H., Trevizan, J.T., Carreira, J.T., Carvalho, I.R., Mingoti, G.Z., Beletti, M.E., Perri, S.H.V., Franciscato, D.A., Pierucci, J.C., Burkhardt de Koivisto, M., 2016. Sperm morphometry and chromatin condensation in Nelore bulls of different ages and their effects on in vitro fertilization. Theriogenology 87, 154–160.

Lemma, A., Shemsu, T., 2015. Effect of age and breed on semen quality and breeding soundness evaluation of pre-service young bulls. Journal of Reproduction and Infertility 6(2), 35–40.

Mahmood, S.A., Ijaz, A., Ahmad, N., Rehman, H., Zaneb, H., Farooq, U., Karaniwala, S.P.U., 2014. A study on relationships among age, body weight, orchidometry and semen quality parameters in adult Cholistani breeding bulls. The Journal of Animal and Plant Sciences 24(2), 380–384.

Mandal, D.K., Kumar, M., Tyagi, S., 2021. Impact of management factors on seminal attributes and frozen semen doses production in holstein friesian crossbred dairy bulls. Applied Veterinary Research 2(1), 2023004. https://doi.org/10.31893/avr.2023004.

Meena, S.K., Saini, S., Gahan, M.K., Swami, P., Gurjar, D., Chandra, V., 2023. Effect of non-genetic factors on seminal traits of indigenous and crossbred bulls under semi-arid climatic conditions. International Journal of Bio-resource and Stress Management 14(2), 298–305.

Murphy, E.M., Kelly, A.K., O'Meara, C., Eivers, B., Lonergan, P., Fair, S., 2018. Influence of bull age, ejaculate number, and season of collection on semen production and sperm motility parameters in Holstein Friesian bulls in a commercial artificial insemination centre. Journal of Animal Science 96(6), 2408–2418.

Naha, B.C., Chakravarty, A.K., Mir, M.A., Jamuna, V., Singh, A.P., Maher, D., 2015. Identifying factors affecting age at first semen freezing and age at first semen use in Sahiwal bulls. Veterinary World 8(7), 928–931.

Peris-Frau, P., Soler, A.J., Iniesta-Cuerda, M., Martín-Maestro, A., Sánchez-Ajofrín, I., Medina-Chávez, D.A., Fernández-Santos, M.R., García-Álvarez, O., Maroto-Morales, A., Montoro, V., 2020. Sperm cryodamage in ruminants: understanding the molecular changes induced by the cryopreservation process to optimize sperm quality. International Journal of Molecular Sciences 21(8), 2781.

Rafiq, M., 2022. Effect of age on lipid composition and semen quality in Murrah buffalo bulls. M.V.Sc Thesis, NDRI Deemed University, Karnal, Haryana.

Rai, D., Dorji, D., 2021. Assessment of non-genetic factors affecting the quality of bovine semen production under bhutanese environment. Bhutan Journal of Animal Science 5(1), 72–81.

Rehman, H., Alhidary, I.A., Khan, R.U., Qureshi, M.S., Sadique, U., Khan, H., Yaqoob, S.H., 2016. Relationship of age, breed and libido with semen traits of cattle bulls. Pakistan Journal of Zoology 48(6), 1793–1798.

Rizzoto, G., Kastelic, J.P., 2019. A new paradigm regarding testicular thermoregulation in ruminants? Theriogenology 147, 166–175.

Rugira, K.D., Elyse, A., Claire, I., Vincent, N., Celestin, M., Maximillian, M., Daphrose, G., 2017. Influence of breed, season and age on quality bovine semen used for artificial insemination. International Journal of Livestock Production 8(6), 72–78.

Sekharam, S., Rao, A.R., 1986. Biometry of testes and semen characteristics of Murrah buffalo bulls. The Indian Veterinary Journal 63, 228–232.

Singh, S., Bhakat, M., Mohanty, T.K., Chakravarty, A.K., Gupta, A.K., Singh, P., Kumar, R., 2015. Relationship between sexual behaviour and seminal attributes of young Sahiwal bulls. Indian Journal of Dairy Science 68(3), 266–270.

Singh, S., Mohanty, T., Sinha, M.B.R., Rahim, A., Kumar, P.P., Dewry, R., Abdullah, M., 2020. Management interventions in indigenous bull to reduce age at first semen donation. Indian Journal of Animal Production and Management 35, 3–4.

Sivaselvam, S., Karthickeyan, S., Venkataramanan, R., Gopinathan, A., Kulasekar, K., 2022. Effect of body weight and scrotal circumference on semen production traits in murrah buffalo bulls. Indian Journal of Animal Sciences 92(1), 59–61.

Sonar, B.P., Tiwari, R.P., Poyam, M.R., Mishra, G.K., Pandey, A.K., Nair, A.K., Sahasrabudhe, S.A., 2016. Characteristics and freezability of Gir bull semen. Indian Journal of Animal Sciences 86(3), 264–272.

Susilawati, T., Sholikah, N.U., Wahjuningsih, S., Herwiyanti, E., Yekti, A.P.A., 2020. Relationship of scrotal circumference with spermatozoa production in various breed of Indonesian local bulls. American Journal of Veterinary and Animal Sciences 15(2), 102–107.

Tohura, S., Parvin, A., Siddique, A.B., Assaduzzaman, M., Zohara, B.F., Islam, M.F., 2018. Factors affecting the semen quality of breeding bulls. The Bangladesh Veterinarian 35(1–2), 32–39.

Tyagi, S., Raja, T.V., Sirohi, A.S., Chand, N., Kumar, S., Pande, M., Mahajan, S., 2023. Effect of age, season and sire on semen quality traits in Frieswal breeding bulls. The Indian Journal of Animal Sciences 93(10), 979–984.

Vilakazi, D.M., Webb, E.C., 2004. Effect of age and season on sperm morphology of friesland bulls at an artificial insemination centre in South Africa. South African Journal of Animal Science 34(1), 62–69.

Vince, S., Zaja, I.Z., Samardzija, M., Balic, I.M., Vilic, M., Duricic, D., Milinkovic-Tur, S., 2018. Age-related differences of semen quality, seminal plasma, and spermatozoa antioxidative and oxidative stress variables in bulls during cold and warm periods of the year. Animal 12(3), 559–568.

Wahyudi, I., Qalfin, M., Susanti, R., Widiatningrum, T., 2022. Relationship between scrotal circumference and quality of semen production in bulls: a meta-analysis review. Jurnal Sain Peternakan Indonesia 17(3), 159–169.

Waldner, C.L., Kennedy, R.I., Palmer, C.W., 2010. A description of the findings from bull breeding soundness evaluations and their association with pregnancy outcomes in a study of western Canadian beef herds. Theriogenology 74(5), 871–883.

Watson, P.F., 1975. Use of a Giemsa stain to detect changes in acrosomes of frozen ram spermatozoa. Veterinary Record 97(1), 12–5.

Westfalewicz, B., Słowinska, M., Judycka, S., Ciereszko, A., Dietrich, M.A., 2021. Comparative proteomic analysis of young and adult bull (*Bos taurus*) cryopreserved semen. Animals 11(7), 2013.

Younis, M., Samad, H.A., Ahmad, N., Ahmad, I., 1998. Studies on semen quality of young, adult and old buffalo bulls during low and peak breeding seasons. Pakistan Veterinary Journal 18(3), 134–141.