International Journal of Veterinary Sciences and Animal Husbandry

ISSN: 2456-2912
VET 2023; 8(6): 100-102

www.veterinarypaper.com
Received: 13-06-2023
Accepted: 22-07-2023

Ruthrakumar R
M.V.Sc., Scholar, Department of Veterinary Gynaecology and Obstetrics, Veterinary College and Research Institute, Namakkal, TANUVAS, Tamil Nadu, India

Selvaraju M
Dean, Veterinary College and Research Institute, Namakkal, TANUVAS, Tamil Nadu, India

Manokaran S
Associate Professor and Head, Department of Veterinary Gynaecology and Obstetrics, Veterinary College and Research Institute, Salem, TANUVAS, Tamil Nadu, India

Yogeswari R
Assistant Professor, Department of Clinics, Veterinary College and Research Institute, Namakkal, TANUVAS, Tamil Nadu, India

Corresponding Author:
Selvaraju M
Dean, Veterinary College and Research Institute, Namakkal, TANUVAS, Tamil Nadu, India

Biochemical evaluation of fresh Kangayam Bull Semen

Ruthrakumar R, Selvaraju M, Manokaran S and Yogeswari R

DOI: https://doi.org/10.22271/veterinary.2023.v8.i6b.818

Abstract
Evaluation of the semen is necessary to assess the bull fertility. For the accuracy in the result of semen evaluation strict quality control measures should be applied. The current necessity is the rapid proliferation of native cow breeds, particularly Kangayam cows, via artificial insemination using high-grade semen. For the investigation, 5-7-year-old Kangayam bull Nos. NK 16, NK 17 and NK 30 were used. The semen was collected twice in a week and two ejaculates in each time were collected. They were subsequently transported immediately to the laboratory for processing semen and maintained in a water bath at 34° C. After the primary screening of semen through microscopical analysis the semen samples were subjected to biochemical analysis. From the biochemical analysis of bull semen out of 24 samples, 20 samples showed a pH of 6.7 and 4 samples with 6.8. The mean (± SE) methylene blue dye reduction time (MBRT) of fresh semen samples was 4.20±0.01 minutes. The mean fructolytic index of fresh semen samples was 1.02±0.37. This study shows the significance in evaluating the biochemical characteristic of the fresh Kangayam bull semen.

Keywords: Kangayam, Bull, MBRT, Fructolysis, Semen, evaluation

1. Introduction
In cattle, it is very important to determine fertility in male animals which is used for breeding purposes and artificial insemination (Elamurugan *et al.*, 2020) [4]. Semen should be analysed to assess the fertility in bulls by measuring the number of sperms having the capacity to fertilize oocyte both in vivo and *in vitro* and it provides information about normal functioning of the testis for sperm production and seminal fluid (Aitken *et al.*, 1982) [2]. *In vitro* quality assessment of the fresh semen has become mandate in the bull semen station to ensure the post thaw quality of the semen in the field for successful pregnancies (Abril-Parreno *et al.*, 2023) [1]. Therefore, in this investigation, the ejaculates were assessed for biochemical parameters. The objective of this investigation is to determine the importance of the biochemical evaluation for the utilization of the semen for further processing.

2. Materials and Methods
2.1. Source of Experimental Animals: Kangayam Bull Nos. NK 16 (Ram), NK 17 (Lakshman) and NK 30 (Barathan) aged 5-7 years maintained at Frozen Semen Bank, Department of Veterinary Gynaecology and Obstetrics, Veterinary College and Research Institute, Namakkal were utilized for this study. The semen was collected on Tuesday and Friday of every week during the trial period from the bull nos. NK 16, 17 and 30. The collected ejaculate was immediately sent to a water bath at 34 °C in the semen processing laboratory.

2.2. Biochemical Evaluation of Fresh Semen
2.2.1. pH: To determine the pH of the semen sample, semen sample was placed on the pH meter (AD 1020, pH Bench Meters, Adwa Instruments). The pH was displayed on the primary LCD after immersing the pH electrode tip into the semen sample.

2.2.2. MBRT: The metabolic activity of spermatozoa was assessed by using MBRT. In a clean, sterile test tube, 0.2 ml of fresh semen was taken and mixed with 0.8 ml of Tris buffer

and 0.1 ml of methylene blue solution. Liquid paraffin was placed 1 centimetre above the mixture and placed in a water bath maintained at a temperature of 46.5 °C. The time taken for changing blue color to colourless solution were noted.

2.2.3. Fructolytic Index: The fructose concentrations in all the three groups were determined colorimetrically (Lu *et al.*, 2007) [5]. The neat semen was centrifuged at 3000 g for 15 minutes. Fructose concentrations in seminal plasma after 0, 2 or 4 hours of storage at room temperature (25 °C) were detected. In brief, 2.9 mL of distilled water was combined with a 0.1 mL fresh seminal plasma sample. After that, 0.5 mL each of 0.15 M barium hydroxide and 0.175 M zinc sulphate were added, thoroughly mixed and left to stand for 5 min at room temperature (28 °C). The mixture was centrifuged at 3000 g for 15 minutes after incubation. 1 mL of the supernatant was mixed with 3 mL of 10 M hydrochloric acid (Himedia), 1 mL of 8.47 mM resorcinol, 1 mL of the supernatant and incubated at 90 °C for 10 min. To serve as a standard, standard fructose solution (0.28 mmol/L) was substituted for the supernatant and distilled water was substituted for the blanks. The tubes were put on ice as soon as the incubation process was finished. By drawing a standard curve using optical density readings taken at 490 nm against a blank in a UV spectrophotometer (Libra S32, Biochrom, Cambridge, UK), the fructose concentration (mmol/L) in semen was calculated. The content of fructose in seminal plasma was calculated as mmol/L: absorbance value of test tube/ absorbance value of standard tube × 11.12.

3. Results and Discussion

3.1. pH: A combination of secretions from the testes, epididymis, and accessory sex glands constitutes seminal plasma. HCO_3^-/CO_2, inorganic ions, organic acids, carbohydrates, lipids, steroids, polyamines, amino acids, nitrogenous bases and proteins in seminal plasma are the key factors that contribute for the higher buffering capacity than other body fluids (Zhou *et al*., 2015) [15]. One of the major factors influencing viability, motility and metabolic rate of sperms is pH (Liu *et al*., 2016) [6]. Twenty of the twenty four samples (83 per cent) had pH of 6.7, while four (17 per cent) had pH of 6.8 (Table. 1). Elamurugan (2021) [3] reported that two samples of Kangayam bull semen had a pH of 6.7 and six samples had a pH of 6.8. Similar values were obtained by Patel and Siddiquee (2013) [8], Ratnawati *et al*. (2018) [12] and Prihantoko *et al*. (2020) [10] from their study.

3.2. MBRT: The biochemical activity of the spermatozoa is an indirect indicator of their potential for motility and fertility and assessed mainly by MBRT and resazurin reduction tests (Nidhi *et al*., 2023) [7]. The mean (± SE) methylene blue dye reduction time (MBRT) of fresh semen samples in this experiment was 4.20±0.01 (Table. 1) minutes which was in contrast with Elamurugan (2021) [3] who reported that the time required for discoloration in Kangayam bull semen was 4.01±0.03 minutes. In accordance with our findings, Saini *et al*. (2017) [13] found that the mean MBRT (min) in Murrah bull was 4.38±0.20. However, Sharma *et al*. (2017) [14] reported a higher MBRT value of 8.27±0.86 minutes in Murrah bulls. The variation results of the present study might be due to the difference in the concentration, viability, motility and plasma membrane integrity between the bulls in their respective study as reported by Rajashri *et al*. (2017) [11].

3.3. Fructolytic index

The appropriate function of the seminal vesicle is critical for fertility maintenance. Seminal plasma is an essential indicator of seminal vesicular function. Fructose is the primary glucose source and is required for sperm movement (Lu *et al.*, 2007) [5]. Hence, it is highly important to measure the concentration of fructose in freshly ejaculated semen. Perusal of literature revealed there were no studies on fructolytic index in Kangayam bull semen. In our study (Table. 1), the mean fructose concentration (± SE) in seminal plasma standing for 0 and 2 hours after centrifugation of semen were 322.75±0.57 and 315.20±0.63, respectively with the fructolytic index of 1.02±0.37. Similar results were reported by Patel *et al.* (2014) [9] in HF bulls (1.09±0.06), Kankrej × HF bulls (1.17±0.09) and Kankrej × Jersey bulls (0.98±0.06). The present study showed that there was no obvious difference of fructose concentration in seminal plasma between 0 and 2 hours of incubation.

4. Conclusion

Present study concluded that out of 24 samples, 20 samples showed a pH of 6.7 and 4 samples with 6.8. The mean methylene blue dye reduction time (MBRT) was 4.20±0.01 minutes. The mean fructolytic index of 1.02±0.37. Thus Kangayam bull which is familiar for jallikattu possess very good biochemical properties and that can be used for genetic propagation.

5. References

1. Abril-Parreño L, Carthy TR, Keogh K, Štiavnická M, 'Meara CO, Lonergan P, *et al*. Genome-wide association study reveals candidate markers related to field fertility and semen quality traits in Holstein-Friesian bulls. Animals. 2023;17(6):100841.
2. Aitken RJ, Best FS, Richardson DW, Djahanbakhch O, Templeton A, Lees MM. An analysis of semen quality and sperm function in cases of oligozoospermia. Fertil Steril. 1982;38:705-11.
3. Elamurugan K. Sperm viability and fertility rate of Kangayam bull semen extended with different diluents. M.V.Sc., Thesis submitted to Tamil Nadu Veterinary and Animal Sciences University, Chennai, Tamil Nadu, 2021.
4. Elamurugan K, Ezakial Napoleon R, Selvaraju M, Murali N, Palanisamy M, *et al*. Macro Seminogram in Neat Semen of a Kangayam Bull – An Ingenious Study. Int. J. Curr. Microbiol. App. Sci. 2020;9(10):1809-1814.
5. Lu JC, Chen F, Xu HR, Huang YF, Lu NQ. Standardization and quality control for determination of fructose in seminal plasma. J Androl. 2007;28(2):207-213.
6. Liu CH, Dong HB, Ma DL, Li YW, Han D, Luo MJ, *et al*. Effect of pH during liquid storage of goat semen on sperm viability and freezing potential. Anim. Reprod. Sci. 2016;164:47-56.
7. Nidhi S, Palod J, Singh D, Singh S, Study of Certain Biochemical Attributes of Pantja Buck Semen. Indian J Animal Prod. Manag. 2023;37(2):103-106.
8. Patel BR, Siddique G. Physical and morphological characteristics of Kankrej bull semen. Vet. World. 2013;6(7):405- 408.
9. Patel KV, Chauhan MK, Mehta VM, Dugwekar YG. Reaction time, seminal fructose, fructolytic index and blood testosterone profile in Holstein-Friesian and crossbred bulls. Indian J Anim. Sci. 2014;71(2):109-111.

10. Prihantoko K, Yuliastuti DF, Haniarti H, Kusumawati A, Widayati DT, Budiyanto A, *et al*. The Effect of Genistein on the Plasma Membrane Integrity of frozen Ongole Grade Bull Semen Based on Skim Milk – Soy Lecithin Extender, K. IOP Conf. Series: Earth Environ. Sci. 2020;6(2):465.
11. Rajashri M, Ramchandra Reddy K, Aruna Kumari G, Nalini Kumari N, Kesharwani S. Correlation between hypo-osmotic swelling test (host) and other seminal characteristics of Deccani Ram semen. J Exp. Biol. Agri. Sci. 2017;5(2):195-200.
12. Dian R, Nurul I, Trinil S. Character motility of liquid semen on Ongole crossbreed (po), Bali and Madura bulls with different diluents at cold storage, Asian J Microbiol. Biotechnol. Environ. Sci. 2018;20(1):21-28
13. Saini J, Dhande PL, Gaikwad SA, Shankhapal VD, Shelar RR. Physico-morphological characters of semen of Murrah buffalo bull. Indian J Anim. Reprod. 2017;38(1):54-55.
14. Sharma M, Yaqoob B, Singh A, Sharma N, Rawat S. Effect of temperature humidity index on semen quality of bovine bull. Int. J Curr. Microbiol. Appl. Sci. 2017;6(12):1822-1830.
15. Zhou J, Chen LI, Li J, Li H, Hong Z, Xie M, *et al*. The semen pH affects sperm motility and capacitation. *Plos one*. 2015;10(7):e0132974.