

Effect of Temperature Based Storage Conditions on Caudal Epididymal Spermatozoa of Yankasa Rams

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ABSTRACT

This study investigated the Effect of temperature-based storage Conditions (Ambient temperature and refrigeration) on Caudal Epididymal Spermatozoa of Yankasa Rams. Six pairs of ram testicles were collected from the Kano metropolitan abattoir. Left testes were transported in normal saline, and spermatozoa obtained were preserved at (18 - 22°C) ambient temperature (AT), while right testes were transported in an ice chest, and Spermatozoa obtained were preserved at (4 - 5°C) Refrigeration temperature (RT) for 24 hours. Results showed a consistent increase in sperm motility under refrigeration, with mean values rising from $78\% \pm 1.93$ to $87\% \pm 2.23$, indicating enhanced preservation of functional capacity at lower temperatures. In contrast, sperm concentration exhibited variable responses, with a slight decline from $2.06 \times 10^9/\text{mL} \pm 0.23$ to $1.93 \times 10^9/\text{mL} \pm 0.24$. The outcomes suggest that refrigeration benefits motility and will uniformly preserve sperm concentration. The study concluded that epididymides or testicles should be stored at (4 - 5°C) Refrigeration temperature for processing and evaluation.

Keywords: Temperature, Storage Conditions, Epididymal Spermatozoa, Yankasa Rams

DESCRIPTION OF PROBLEM

Spermatozoa obtained from the cauda epididymis of rams are valuable for genetic preservation, artificial breeding, and novel reproductive techniques, especially when natural mating is not feasible due to trauma, death, or geographical constraints (1). However, such spermatozoa's viability and fertility capacity primarily rely on post-recovery storage conditions, and temperature which serves as a crucial regulating parameter (2). Understanding the optimal temperature requirements for sperm quality maintenance is significant in breeds like the Yankasa ram, an influential Nigerian sheep breed prized for its adaptability and genetic capability (3). The cauda epididymis has been discovered to contain mature sperm with functional competence, and therefore, it can be a good source for sperm recovery after death (4). Improper storage temperatures pose a risk to key parameters of sperm like motility, viability, plasma membrane integrity, and acrosomal integrity (5). Although cold storage delays metabolic processes and reduces the pace of

deterioration, it may also induce cold shock in sperm cells in particular species (6).

Therefore, this study seeks to evaluate the effect of different temperature-based storage on the quality of the caudal epididymal spermatozoa of Yankasa rams.

MATERIALS AND METHODS

Experimental site

This research was conducted at the Kano state metropolitan abattoir and the department of Animal Science laboratory in Bayero University Kano. The area is characterized by tropical dry and wet climate which is suitable for livestock production (7).

Recovery of Spermatozoa

Six pairs of Yankasa ram testicles were collected from the Kano metropolitan abattoir. Left testes were transported in normal saline, and spermatozoa obtained were preserved at ambient temperature (AT) (18 - 22°C), while right testes were transported in an ice chest, and Spermatozoa

obtained were preserved at refrigeration temperature (RT) (4 - 5°C) for 24 hours.

The cauda epididymides were aseptically excised from the testis. Several longitudinal incisions (5–7) were made on the distal end of the cauda to expose the spermatozoa. Each cauda epididymidis was placed on a Petri dish for 25 minutes to allow the sperm swim out into the 2.5 ml of 2.9% sodium citrate buffer. An additional 1.5 ml of buffer was used to rinse spermatozoa off the incised part of cauda epididymidis. The total cauda epididymal sperm concentration (million/ml) was determined using the haemocytometer method, which involves counting sperm cells within the haemocytometer grid as described by (8).

Statistical Analysis

Data generated was analyzed using SAS statistical software suite version 9.1, values were

presented using descriptive statistics (Mean ± Standard deviation) and percentages in tables.

RESULTS AND DISCUSSION

The effect of the two temperature-based storage conditions on motility (%) of sperm harvested from caudal epididymides are presented in Table 1. An increase was observed in the sperm motility of the refrigerated semen. The average motility at room temperature was recorded as $78\% \pm 1.93$ while that of refrigerated temperature was recorded as $87\% \pm 2.23$. The highest motility was observed in Animal 4, which showed an improvement from 80% to 91%. This aligns with previous study by (9) hence, supporting the notion that cooler environments reduce metabolic activity, thereby minimizing cellular stress and maintaining motility for longer durations.

TABLE 1: MEAN ESTIMATES OF SPERM MOTILITY AT AMBIENT (18 - 22°C) AND (4 - 5°C) REFRIGERATED TEMPERATURES

Animal ID	Spermatozoa Motility (%)	
	Ambient storage (AT)	Refrigerated storage (RT)
1	70	81
2	77	89
3	81	80
4	80	91
5	75	90
6	83	93
Mean±SD	78±1.93	87±2.23

Table 2 outlines sperm concentration ($\times 10^9/\text{mL}$) under two conditions of storage. For animal ID 5, refrigeration had an increase from (1.8 to 2.9), whereas Animal ID 4 and 6 experienced extreme decline. Average concentration at room temperature was $2.06 \times 10^9/\text{mL} \pm 0.23$, Mean concentration at refrigerated temperature was recorded as $1.93 \times 10^9/\text{mL} \pm 0.24$. This result corroborates the findings of (10) in West African dwarf rams.

CONCLUSION AND APPLICATION

Caudal Epididymal Spermatozoa of Yankasa Rams can be preserved at an atmospheric temperature in normal saline if spermatozoa are to be used immediately. However, refrigeration temperature is best for storage of epididymides in the laboratory for future use.

REFERENCES

- 1 Mercedes, Á., Tamayo, C. J., Martinez, R. C., López, U. E., Gomes, A. S., Anel, L., Martínez, P. F. and De Paz. (2012). Specificity of the extender used for freezing ram sperm depends of the spermatozoa source (ejaculate, electroejaculate or epididymis). *Animal reproduction science*. 132: 145-54. doi: 10.1016/j.anireprosci.2012.05.006.
- 2 Rizkallah, N., Chambers, C. G., De Graaf, S. P. and Rickard, J. P. (2022). Factors Affecting the Survival of Ram Spermatozoa during Liquid Storage and Options for Improvement. *Animals* (Basel). 12(3):244. doi: 10.3390/ani12030244.
- 3 Osinowo, O. A., Bale, J. O. and Eduvie, L. O. (1982). Semen quality of Yankasa rams. *Tropical Animal Health Production*. 14(3):189 - 194. doi: 10.1007/BF02242155.
- 4 Abu, A.H., Kisani, A.I. and Ahemen, T. (2016). Evaluation of sperm recovered after slaughter from cauda epididymides of red Sokoto bucks. *Veterinary World*. 9(12):1440-1444. doi: 10.14202/vetworld.
- 5 Jurado, C. A, Soria-Meneses P. J., Arenas, M. M., Alonso, M. C., Rodríguez, R. V., Soler, A. J., Garde, J. J. and Del Rocío, F. M. (2023). Minimizing sperm oxidative stress using nanotechnology for breeding programs in rams. *Journal of Animal Science and Biotechnology*. 14(1):106 - 110. doi: 10.1186/s40104-023-00907-3.
- 6 Khan, I. M., Cao, Z., Liu, H., Khan, A., Rahman, S. U., Khan, M. Z., Sathanawongs, A. and Zhang, Y. (2021). Impact of Cryopreservation on Spermatozoa Freeze-Thawed Traits and Relevance OMICS to Assess Sperm Cryo-Tolerance in Farm Animals. *Frontiers in Veterinary Science*. doi: 10.3389/fvets.2021.609180.
- 7 Mohammed, H. B., Muhammad, I. R., Aji, Z. A., Abdullahi, A. H., Hassan, U.I. and Baba, M. (2018). Wafer As Dry Season Supplement for Livestock Formulated with Different Sources of Binders and Levels of Inclusion in the Semi -Arid Region. In proceedings of 43rd Annual Conference of the Nigerian Society for Animal Production. 746 – 748
- 8 Chaveiro, A., Cerqueira, C., Silva, J., Franco, J., Moreira da Silva, F. (2015). Evaluation of frozen thawed cauda epididymal sperms and in vitro fertilizing potential of bovine sperm collected from the cauda epididymal. *Iran Journal of Veterinary Research*. 16 (2):188-193.

Table 2: Mean estimates of sperm concentration at ambient (18 - 22°C) and (4 - 5°C) refrigerated temperatures

Animal ID	Spermatozoa Concentration (x10 ⁹ /mL)	
	Ambient storage (AT)	Refrigerated storage (RT)
1	2.1	2.1
2	1.9	1.8
3	2.1	1.9
4	3.1	1.8
5	1.8	2.9
6	1.4	1.1
Mean±SD	2.06±0.23	1.93±0.24

- 9** Bamanga, U. M., Abba, A., Mustapha, A. R., Maina, V. A., Bukar, M. M., Waziri, M. A. and Amin, J. D. 2022. Characteristics Of Uda Ram Semen in Maiduguri, Nigeria. *Journal of Innovative Research in Life Sciences*. 4(1): 8 - 13.
- 10** Ososanya, T., Adeniji, S. A., and Aree, O. S. (2017). Growth and Semen Characteristics of West African Dwarf Rams Fed Ammonium Sulphate Supplemented Diets. *Journal of Animal Production Research*. 29, 70-82.