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Effects of cold storage time on the quality of epididymal sperm recovered from White Fulani bulls after slaughter

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Abstract

Sperm recovery from the cauda-epididymis can be very useful after the unexpected death of an animal with superior genetic traits. This study evaluated the effects of storage time at 4℃ on the quality of cauda-epididymal spermatozoa recovered from White Fulani bulls after slaughter. Testes along with their epididymides of 20 White Fulani bulls were recovered after slaughter and stored at 4℃ in the refrigerator for 0 – 3, 6, 12, 24 and 48 hours, after which the quality of the cauda-epididymal spermatozoa was analyzed following standard procedures. Each group consisted of four pairs of testes-epididymides. Results showed that sperm motility (%) decreased significantly ($p < 0.05$) after 24 hours and 48 hours of storage compared with 0 – 3 hours group, which served as the control. Sperm viability (%) did not differ significantly ($p > 0.05$) between the 0 – 3, 6, 12 and 24 hour groups. However, there was a significant decrease ($p < 0.05$) in sperm viability (%) after 48 hours of storage when compared with the 0 – 3 hour group. Acrosome integrity (%) significantly ($p < 0.05$) declined after 48 hours of storage when compared with the 0 – 3 hour group, but no significant difference ($p > 0.05$) was detected between 0 – 3 hour group and the 6, 12 and 24-hour groups in acrosome integrity. The percentage of sperm with abnormal morphology increased significantly ($p < 0.05$) after 24 hours and 48 hours of storage when compared with the 0 – 3 hour group, however there was no significant difference ($p > 0.05$) between 0 – 3 hour group and the 6 and 12 hour groups. No significant difference $p > 0.05$ was observed in the sperm concentration between the groups. The results of this study showed that viable epididymal spermatozoa with intact acrosomes can be recovered from the cauda-epididymis of slaughtered White Fulani bulls stored at 4℃ for up to 24 hours.

Keywords: Epididymal spermatozoa; Cold storage time; White Fulani bulls; Sperm evaluation; Assisted reproduction technologies; Sperm recovery.

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Introduction

The White Fulani breed of cattle is originally found in Northern and Southern Nigeria, North-Eastern part of Cameroon, South of Chad and Western Sudan (where they are called Fellata or Red Fulani). In Nigeria, this breed accounts for about half of the total national herd and are mainly kept for meat and milk production and for research purposes (Santoze and Gicheha, 2019). Reproduction in cattle can be achieved either naturally through mating, or artificially through assisted reproductive technologies (ART) such as; artificial insemination (AI), *in vitro* fertilization (IVF) and intra-cytoplasmic sperm injection (ICSI). In recent times, researchers have shown an increased interest in the use of epididymal sperm for ART. Epididymal sperm collection is very necessary when natural mating or the use of ejaculated semen is not possible, which could be due to difficulty in handling intractable animals, or in cases of sudden death of an animal (Foote, 2000; Kaabi *et al.*, 2003; Edeneil *et al*., 2015).

In cases where an animal dies suddenly or experiences severe injuries from accidents, it is possible to recover epididymal spermatozoa for use in ART. This helps preserve and propagate the genetic material of the animal. However, the duration between recovery and use, along with storage conditions, can impact sperm quality. Therefore, it is crucial to develop storage techniques for epididymal spermatozoa to preserve genetic material of valuable or endangered animals, and to expand their gene pool using ART. Currently, there are limited research reports on how cold storage time affects the quality of caudal epididymal spermatozoa of White Fulani bulls.

The goal of spermatozoa evaluation is to measure the ability of the sperm cells to accomplish fertilization (Eilts, 2005). The following parameters are the most commonly evaluated while assessing the quality of a semen sample: sperm motility, sperm concentration, sperm viability, acrosome integrity. The present study determined the effects of storage time at 4°C on the quality of epididymal spermatozoa recovered from White Fulani bulls after slaughter. This was to be achieved by evaluating the effect of storage time at 4°C on the sperm motility, viability, concentration and acrosomal integrity of epididymal sperm recovered from White Fulani bulls after slaughter.

Materials and Methods

The research was carried out at the Physiology Laboratory of the College of Veterinary Medicine, Michael Okpara University of Agriculture, Umudike, Nigeria. The University is situated in the southeast geopolitical zone of Nigeria and located at latitude 5°40' and 6°4' North and longitude 7°10' and 7°34. The area has a warm mean temperature range of 21.9˚C to 31.5˚C. High temperatures are experienced between late January and April in this area. The climate is purely tropical with wet and dry seasons. The rainy season goes from March to October while the dry season is from November to February. The mean daily rainfall and relative humidity are 146.5 mm and 80%, respectively (Amaechi *et al*., 2022).

Sample collection and Experimental design: Twenty (20) scrotal sacs were excised from apparently healthy sexually matured White Fulani bulls immediately after slaughter at the abattoir and placed in a thermos flask containing ice packs. The study was done between August and December, 2022. Age of the bulls was determined using their dentition. Samples collected were randomly assigned to five storage groups each containing 4 scrotal sacs. The control group (Group I) contained samples that were processed and analyzed within the first three hours after slaughter. Groups II, III, IV and V consisted of 4 samples each, which were processed and analyzed after storage at 4°C for 6, 12, 24 and 48 hours, respectively.

Cauda epididymal sperm collection from the scrotal sac: Sperm cells were collected from the cauda epididymides at room temperature by the incision method. Incisions were made on the lower end of the epididymides to enable sperm cells swim out into pre-warmed (37°C) 5 ml of 2.9% sodium citrate buffer in a petri dish.

Determination of spermatozoan progressive motility: Spermatozoan progressive motility of the various storage groups including control (0 – 3 hours) were evaluated by placing a drop (10 µl) of sperm sample on a pre-warmed, grease-free-slide (Zemjanis, 1970). A cover slip was put over the drop and examined under the high-power (40×) magnification of a microscope, and the percentage of progressive motility was determined (Zemjanis, 1970).

Determination of sperm concentration: The sperm concentration of each sample was enumerated using the improved Neubauer haemocytometer after appropriate dilution in 0.05% formol saline (Rekwot *et al*., 1987). A 1:100 dilution of the sperm sample in 0.05% formol saline was made. A specific amount (20µl) of the sperm sample was placed on both sides of the haemocytometer. The number of sperm cells in five of the large central squares (made up of 16 smaller squares) were counted on both chambers and their average calculated. Sperm concentration per ml was then calculated using the formula: Number of spermatozoa × Multiplication factor × 50,000.

Determination of sperm viability: Viability of sperm cells was determined by placing a drop (10 µl) of sperm sample on a clean, grease-free slide and mixing it with a single drop of Eosin-Nigrosin stain (Blom, 1977; Hoseinzadeh-Sani *et al.*, 2013). The spermatozoa were allowed to interact with the stain for at least 2 minutes and then a smear was made. The smear was air-dried and examined under oil immersion objective (100× magnification). Spermatozoa that stained either partially or completely were considered as dead and the spermatozoa that appeared colourless were considered as live. Two hundred spermatozoa were randomly examined in different fields and the percentage of live sperm cells was determined. The mean results were expressed as percentage of viable spermatozoa (Hoseinzadeh-Sani *et al.*, 2013).

Determination of acrosome integrity: Acrosome status was determined by adding a small (10 µl) drop of the sample on a clean slide and making a smear. The smear was air-dried and kept in Hancock's fixative for 15 – 20 minutes in a coupling jar. After the prescribed time interval, the slides were washed under slow running tap water for another 15 – 20 minutes and finally rinsed with distilled water. The slides were kept in a coupling jar containing Giemsa working solution overnight. The next day, the stained slides were rinsed with slow running tap water air-dried, and observed under oil immersion objective (×100 magnification). Two hundred spermatozoa were randomly examined and the percentage of spermatozoa with intact acrosome was determined and the mean results were expressed as percentage of spermatozoa with intact acrosomes (Watson, 1975; Mir *et al.*, 2012).

Determination of spermatozoa morphological abnormality: Spermatozoa morphological abnormality was determined by viewing the Giemsa stained slides under the microscope. Spermatozoa (n = 200) were examined and the percentage of spermatozoa with abnormal morphology was determined. The mean results were expressed as percentage of spermatozoa with abnormal morphology (Chima *et al*., 2017).

Statistical analysis: Data collected were subjected to one-way analysis of variance (ANOVA) and expressed as mean ± standard error of the mean (SEM) using GraphPad Prism version 9.20 for Windows. The differences

between means were analyzed by Tukey's multiple comparisons test. The level of significance was set at 5%.

Results

The mean percentage sperm motility of the storage time groups are presented in Figure 1. Sperm motility decreased significantly ($p < 0.05$) after 24 hours and 48 hours of storage compared to the control group (0 – 3 hours). However, no significant difference ($p > 0.05$) was observed between the control group and Groups II (6 hours) and III (12 hours).

Figure 2 shows the result for mean (SEM) sperm concentration of each storage group. No significant difference ($p > 0.05$) was observed in the mean sperm concentration of the control group and all the storage groups.

Sperm viability decreased significantly ($p < 0.05$) after 48 hours of storage compared to the control group. However, no significant difference was observed between the control group and the 6, 12 and 24-hour groups (Figure 3).

The results for acrosome integrity was similar to that obtained for sperm viability. No significant ($p > 0.05$) difference was observed between the control group and the 6, 12, and 24-hour groups (Figure 4). However, after 48 hours of storage, a significant ($p < 0.05$ decline was observed in acrosome integrity compared to that of the control (Figure 4).

The percentage of morphologically abnormal sperms in each storage group is presented in Figure 5. There was a significant ($p < 0.05$) increase in the percentage of morphologically abnormal sperm cells after 24 and 48 hours of storage compared to the control. However, no significant ($p > 0.05$) difference was observed between the control group and the 6-hour and 12-hour storage groups as regards the percentage of morphologically abnormal sperm.

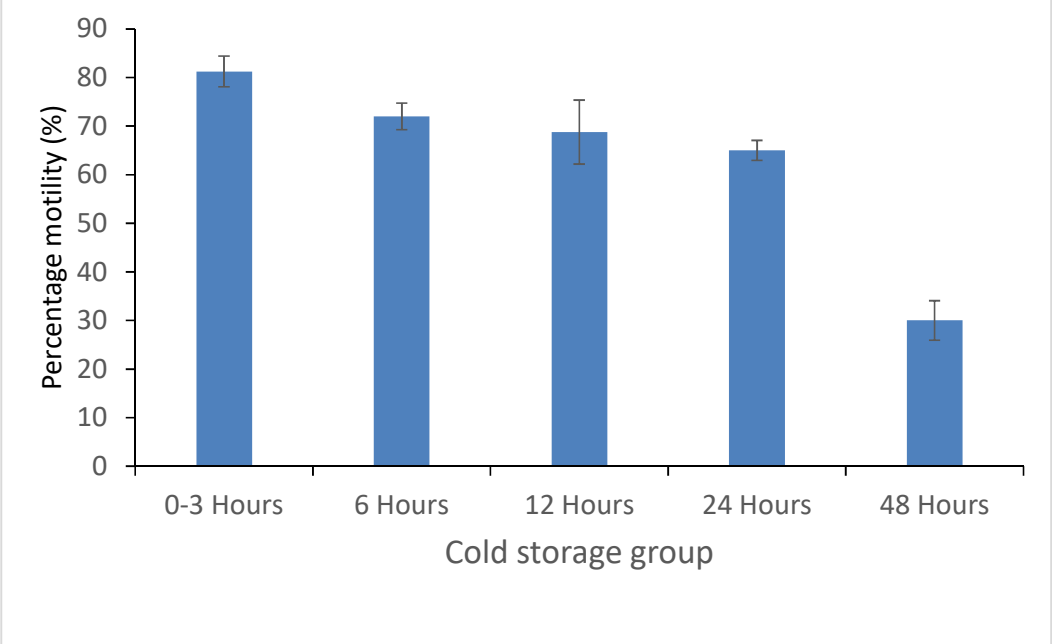

Figure 1. Effect of cold storage (4°C) on the mean (SEM) percentage motility of cauda-epididymal spermatozoa of White Fulani bulls recovered after slaughter.

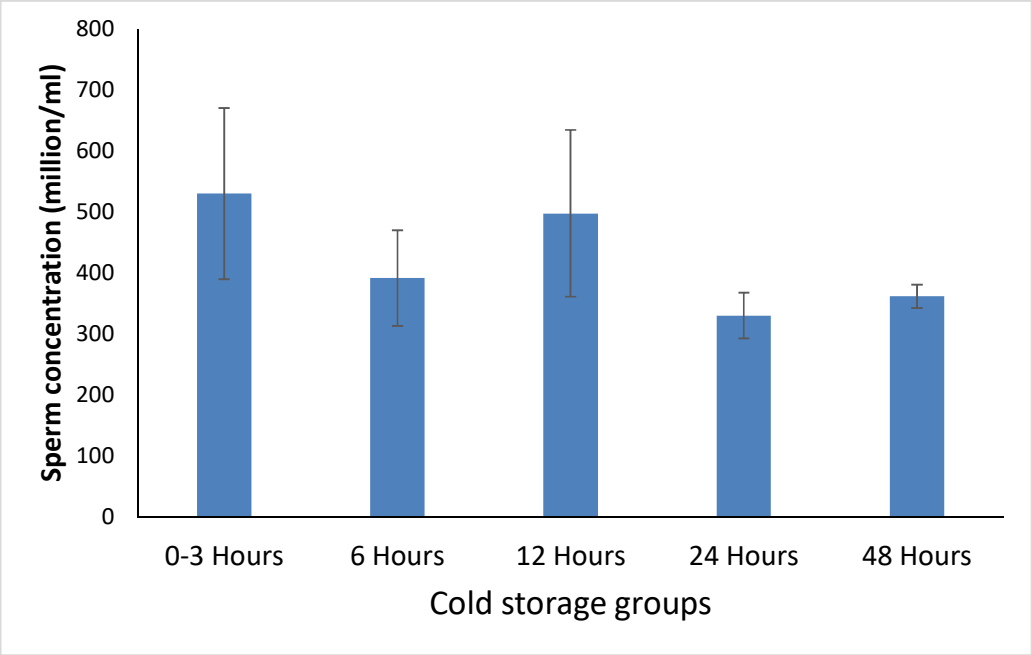

Figure 2. Effect of cold storage (4°C) on mean (SEM) sperm concentration of cauda-epididymal spermatozoa of White Fulani bulls recovered after slaughter.

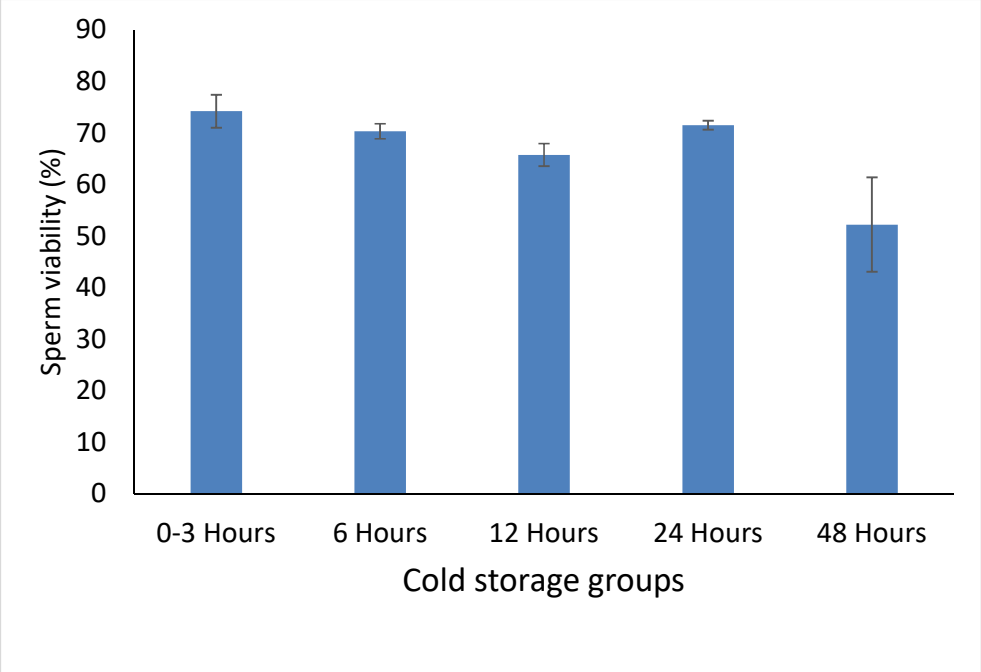

Figure 3. Effects of cold storage (4°C) on mean (SEM) sperm viability of White Fulani bulls recovered after slaughter.

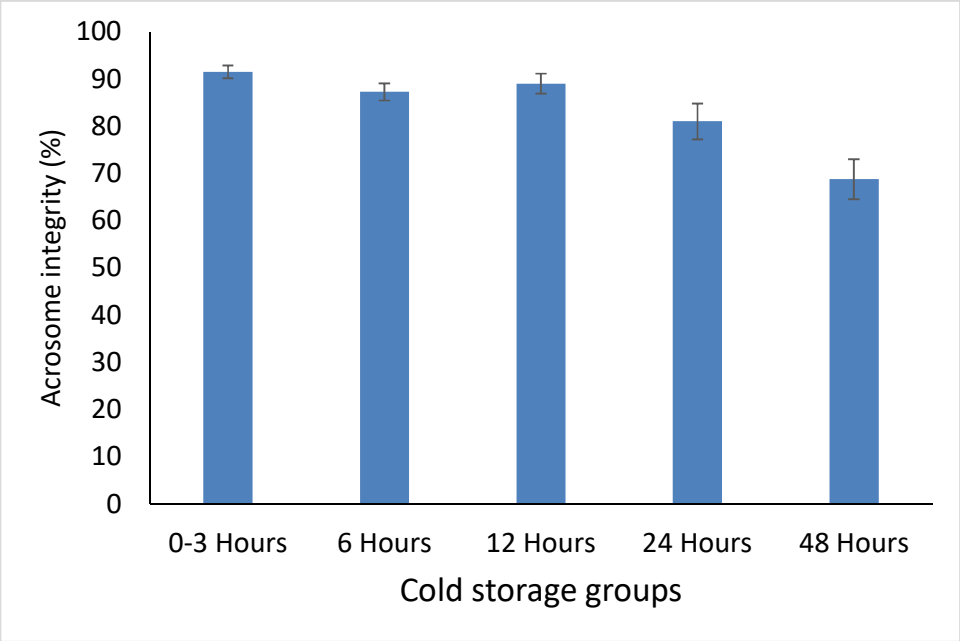

Figure 4. Effect of cold storage (4°C) on mean (SEM) acrosome intergrity of epididymal spermatozoa of White Fulani bulls recovered after slaughter.

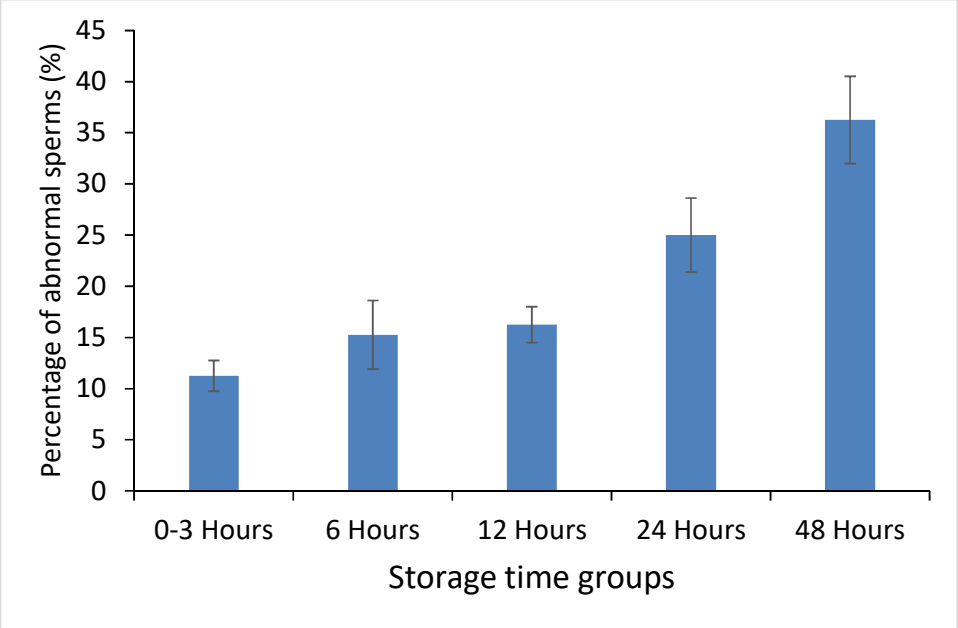

Figure 5. Effect of cold storage (4°C) on the percentage of morphologically abnormal epididymal sperm of White Fulani bulls recovered after slaughter.

Discussion

The recovery and cold storage of cauda epididymal spermatozoa at 4°C has been reported to be an effective way to preserve spermatozoa for use in the short term in other breeds of cattle (Strand *et al*., 2016) and in different species (Soler *et al.,* 2003; H*ori et al.,* 2005; Abu *et al* 2016; Mir *et al.,* 2012). Refrigeration slows down metabolic functions, hence allowing the spermatozoa to survive for longer periods than keeping at room temperature (Hishunuma *et al*., 2003).

In the present study, epididymal spermatozoa were still very motile after 24 hours of storage at 4°C, however sperm motility decreased severely after 48 hours of storage. This finding is in agreement with the findings of Strand *et al*. (2016) in Danish Holstein bulls. In contrast to the present finding, sperm motility has been reported to not significantly change after 48 hours of cold storage in dogs (Chima *et al*., 2017; Hori *et al*., 2005), goats (Abu *et al*., 2016), ram (Mir *et al*., 2012), rats (Kishikawa *et al*., 1999), African buffalo (Kilian *et al*., 2000) and camel (El-Harairy *et al*., 2016).

Cold storage had no significant effect on White Fulani bull's epididymal sperm concentration. This agrees with the reports of Chima *et al*. (2017) and Hori *et al*. (2005) in dogs.

Acrosome integrity decreased significantly after 48 hours compared to control, however, the percentage of spermatozoa with intact acrosomes remained high. Malcotti *et al*. (2012) made similar reports in Hereford bulls, where they observed that cold storage did not have much effects on acrosome integrity. Findings in other species also show that acrosome integrity did not significantly decrease after 48 hours of storage (Mir *et al*., 2012; Abu *et al*., 2016; Chima *et al*., 2017).

The percentage of spermatozoa with morphological abnormality was observed to have increased with storage time in the current study. This finding concurs with the findings of Strand *et al*. (2016) in Danish Holstein bulls, Hori *et al*. (2005) in dogs, and Hoseinzadeh-Sani *et al*. (2013) in goats.

The overall decline in epididymal sperm quality observed after prolonged cold storage might be attributed to the accumulation of toxic products of metabolism, especially reactive oxygen species (ROS) (Fernández-Santos *et al*., 2009). Epididymal spermatozoa may be more susceptible to oxidative stress than ejaculated spermatozoa, since they are not exposed to seminal plasma which contains the complex secretions of the accessory sex glands which are regarded as the major source of antioxidant protection for spermatozoa (El-Harairy *et al.,* 2016). Furthermore, sudden changes in osmotic pressure of the epididymal fluid, also known as osmotic shock, during the process of cold storage and thawing, may have an irreversible deleterious effect on the sperm's motility and metabolic activity, and can inflict damages to the acrosome and plasma membranes (Khan and Ijaz, 2008).

Conclusion: Viable epididymal spermatozoa with intact acrosome were recovered from the cauda epididymis of slaughtered White Fulani bulls after 48 hours of storage at 4°C. However, their motility declined severely after 24 hours of storage at the same temperature. This implies that, in conditions of sudden death of a White Fulani bull, its epididymal spermatozoa can be recovered and stored at refrigeration temperatures for up to 24 hours without having a significant decline in their quality and hence can be used to facilitate reproduction via assisted reproductive technologies such as artificial insemination, intracytoplasmic sperm injection and cryopreservation.

Recommendations: We recommend that when cryopreservation facilities are distant from the collection site, the testis along with the epididymis of White Fulani Bulls can be shipped at a temperature of 4°C for over 24 hours without having a deleterious effect on the epididymal spermatozoa. Further studies are needed to evaluate the fertilizing

capabilities of cold stored epididymal sperm of White Fulani Bulls.

Conflict of interest

The authors declare no conflict of interest.

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