

Epididymal Spermatozoa Recovery from Post-Mortem Cattle Preservative Effects of Skimmed Milk, Coconut Water and Egg Albumin on Caudal Epididymis Sperm

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Abstract:- with the objective of comparing the preservative effects of skimmed milk, coconut water and egg albumin for epididymal spermatozoa recovered from cattle after death a floating technique under refrigerated conditions (5°C) was employed. For the recovery of spermatozoa, thirty-two testis-epididymis complexes were collected after slaughter of the bulls, dissected and divided into 3 groups for treatments. Motility, vigor and spermatid pathologies were daily analyzed. In all treatments was possible to recover viable spermatozoa with the values of motility and vigor of $25.44 \pm 7.82\%$ and $21.22 \pm 6.62\%$ Vs $12.20 \pm 7.87\%$ and $2.22 \pm 0.58\%$ Vs $1.91 \pm 0.49\%$ and $0.92 \pm 0.61\%$ for skimmed milk, egg albumin and coconut water respectively. Skimmed milk and egg-albumin showed better results, however, there were no significant differences between the three preservatives ($P > 0.05$). The results of this research show that it is possible to preserve recovered bulls' spermatozoa viable after death of the donor for a maximum period of 6 days using skimmed milk and egg white and 3 days for coconut water.

Keywords:- Recovery Spermatozoa; Floating Method; Motility; Viability; Bulls.

I. INTRODUCTION

The Artificial Vagina (AV) and Electroejaculation (EE) are two conventional methods frequently used for semen collection's purposes from bulls (Alvarez *et al.*, 2020). However, there are situations that can prevent the use of these methods, such as unexpected death or erectile dysfunction (Prieto *et al.*, 2014; Kang *et al.*, 2018; Pleshkov *et al.*, 2022). In these cases, the loss of genetic material from these animals is inevitable. The spermatozoa recovery is an alternative method, which has been used in addition to AV and EE, allowing the conservation of germplasm from dead sires and its use through Artificial Insemination for livestock genetic improvement (Cunha *et al.*, 2019; Dziekońska *et al.*, 2020). Furthermore, this method has also been successfully used in several species, both domestic and wild, when by any reason they have to be esterilized or killed and thus represents a very useful resource for the creation of germplasm banks for futher use. Several techniques have been used to recover spermatozoa from the tail of epididymis namely cutting,

floating, mincing, and flushing (Kang *et al.*, 2018; Raseona *et al.*, 2018).

In Mozambique, most of livestock smallholder farmers are unable to carry out the collection and conservation of semen from their males. However, due to indiscriminate livestock slaughter for human consumption, particularly from stolen stock, bulls and bucks of high genetic merit and up are being slaughtered before their potential has been fully used. As a result, there is great waste of genetic material, contributing for farmers and country's economic loss due to unexpected death of males, particularly bulls. In this sense, the aim of this study is to evaluate the effect of skimmed milk, coconut water and egg-albumin on recovered epididymal spermatozoa.

II. MATERIAL AND METHODS

A. Study location

The study was carried out in the facilities of the Animal Genetic Resources and Assisted Reproduction Techniques Center of the Directorate of Animal Science located in Matola (CRGTRA – DCA / IIAM) and in the Reproduction laboratory of the Faculty of Veterinary Medicine in Maputo, Mozambique.

B. Collection and sampling

The harvest of Epididymis Testicles Complexes (ETC) was carried out at the Municipal Slaughterhouse in Maputo, Mozambique. The ETC were transported to the laboratory in an isothermal box with recyclable ice to keep the temperature low and avoid compromising the samples. Transport from the slaughterhouse to the laboratory took approximately half an hour. 32 ETC from bulls of different breeds, reared in different production systems, aged between 2 -8 years old, weighing between 250-350 kg were used.

C. Spermatozoa recovery method

Caudal epididymis spermatozoa were recovered by the floating technique in a Petri dish with a diameter of 90x15mm. After dissection, the tail of the epididymis was sliced and let to rest for 15 minutes in a preservative medium composed by 20 ml of each skimmed milk, coconut water or egg-albumin to allow the spermatozoa to migrate into the medium and then be recovered.

D. Spermatozoa viability

The evaluation of motility and vigor consisted on the deposition of a drop of semen in sodium chloride solution (0.9%) for dilution, with subsequent removal of a sample and depositing it on a pre-heated blade (37°C). This was inferred using a contrast microscope, under a 10 and 40x objective, and the result was issued as a subjective percentage, according to the criterion adapted from Derivaux (1976), and Baracaldo (2007). Vigor was rated using a scale from 0 to 5.

Morphology parameters were analyzed through observation, under the microscope, of fixed smears with methanol and stained with Giemsa, adapted from the technique described by Diaz and Arancibia (1970). Percentages of abnormalities were obtained from 100 sperms counted.

E. Statistical analysis

Samples were chosen and randomly divided into 3 treatments, skimmed milk, egg albumin and coconut water. For the statistical analysis of the data, the means and standard deviations for the sperm parameters were obtained, using the analysis of variance (ANOVA), through the statistical program (SPSS), and using the Tukey test $P < 0.05$ to assess the significance of the differences of means between the three tested preservatives.

III. RESULTS

Conservation tests showed effect up to 6 days (148 hours), with skimmed milk showing the best result in preserving sperm motility and vigor, followed by egg albumin and finally coconut water. There were no significant differences between three preservatives under refrigerated conditions of 5°C ($p > 0.05$). The results of motility and vigor are shown below in figure 1 and 2 respectively.

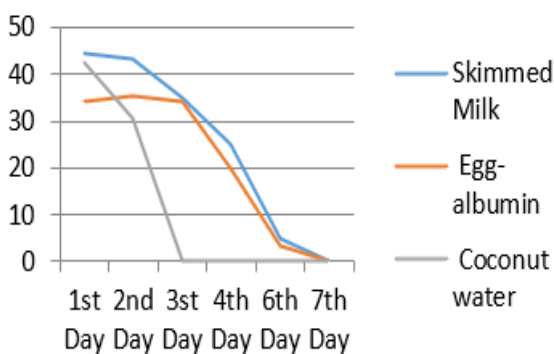


Fig. 1. Percentages of spermatozoa motility

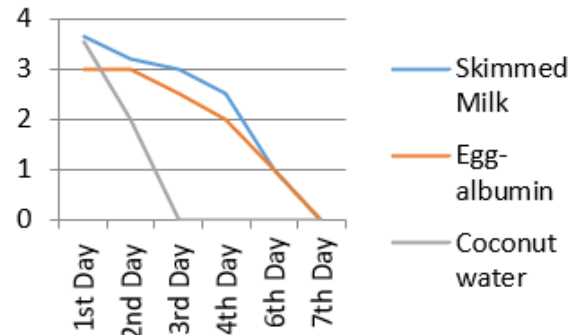


FIG. 2. VALUES OF SPERMATOZOA VIGOR

Skimmed milk had a greater preservative effect compared to egg albumin and coconut water, with a maximum motility preservative effect of about 44.33% on the first day and a minimum of 5% on the sixth day (148 hours) after the death of the bulls and sperm recovery. On the other hand, it was observed that egg albumin similarly presented viable spermatozoa for the same period as milk, however with relatively lower motility percentages and having presented a maximum preservative effect of 34.33% on the first day and a minimum of 3.30% on the sixth day. Coconut water had a variation in sperm motility of 42.66% to 30.54% between the first and second day, showing total sperm inactivity from the third day after sperm recovery. With regard to sperm vigor, an almost similar trend to motility was observed over the period, with a preservative effect greater than 3 in the first day after recovery for the three treatments.

Turning to abnormalities, defects in the tail were predominant, showing a percentage variation of 12, 19 and 29% in samples preserved in milk, egg albumin and coconut water, respectively, as illustrated below (Table 1). Abnormalities in the head were more significant in milk samples with 32% and the least in coconut water with 8%. Intermediate part defects range from 6% to 19%.

Table I. Percentages of abnormalities

Treatment	Type of abnormalities and location (%)			Total of abnormalities (%)
	H	I.P	T	
Skimmed Milk	32	6	62	50
Coconut water	8	13	79	50
Egg-albumin	14	19	67	47

H: Head, I.P: Intermediate Peace and T: Tail

IV. DISCUSSION

The results found in this study can be explained according to Dziekońska *et al.* (2020) who mention that the tail of epididymis provide an environment which allow to store gametes in physiological situations and, thus, viable spermatozoa from this portion can be recovered after the death of the males. In addition, Martinez-Pastor *et al.* (2006), O'Hara *et al.* (2010), Monteiro *et al.* (2013) and Nichi *et al.* (2017) consider that refrigerating epididymis at 5°C, before sperm recovery, increases the sperm viability period by slowing down the metabolic and degeneration rate.

Skimmed milk showed higher rates of preservative effects in comparison to the remaining two. This may be regarded to its protein fraction and abundance of carbohydrates (lactose) that can be used as source of energy. Besides that, milk contains casein that enhances the kinetic activity of sperm (Martins, 2018). Some preservative effects of skimmed milk in bovine semen was also studied by Thacker and Aimqist (1958) who found that it is possible to extend sperms lifetime up to 16 days using skimmed milk.

Egg albumin showed an ability to preserve the spermatozoa viability relatively lower than skimmed milk however higher than coconut water. Albumin is a viscose media and this may have led to much energy expenditure during the sperm migration from epididymis to the respective media resulting in reduced effects agreeing with Ratnawati *et al.* (2020) who refereed that viscosity of diluent can influence the capability of spermatozoa to penetrate albumin. However, due to scarcity of references, the reasons behind the effect of albumin are unknown.

Coconut water has largely been used as a natural preservative of semen especially in goats and sheep. It is a sterile solution and contains salts and some important nutrients such as proteins, growth factors, sugars, vitamins, neutral fats. In addition to cell division inducers and supplier of various electrolytes, coconut water gives to the sperm cells density and pH compatible for their conservation (Teixeira *et al.* 1994). However, at the present study it experienced the least preservative effects, which, according to previous studies might have been influenced by the spermatozoa recovery technique. Hori *et al.* (2015) demonstrated that some techniques could influence the spermatozoa survival because of demerits of blood contamination from blood vessels in the surface of caudal epididymis and mixing of tissues. This can induce increase in acrosome reaction and decrease in motility and viability (Martinez-Pastor *et al.* 2006). Moreover, fresh coconut water has shown satisfactory results when used for the conservation of sperm from species such as dogs (Cardoso *et al.* 2003) sheep (Machado, 1991) goats (Brasileiro *et al.*, 2019) and swine (Toniolli *et al.*, 2010), suggesting a species preference to sperm preservative medium therefore the probable negative effects of coconut water in recovered bull spermatozoa.

The occurrence of abnormalities in spermatozoa can be an indicate of pathological process in the reproductive tract of bulls or an indication of decreasing semen fertility

(Dziekońska *et al.*, 2020). Based on the location, this can be divided into primary (abnormalities in the spermatozoa's head), secondary (abnormalities in the spermatozoa's mid piece) and tertiary (abnormalities in the spermatozoa's tail) (Corbet *et al.*, 2012). In this study, the majority of abnormalities were found in the tail. This can be a result of the refrigerating process which, according to Souza *et al.* (2017) abnormalities in this portion increases significantly after cooling as well as the total pathologies. Similar results of the morphological evaluation of the spermatozoa recovered from the epididymis were found by Costa *et al.* (2011) in which this problem was attributed to the immaturity of the material recovered directly from the epididymis meaning that possibly the spermatozoa were recovered from young bovine males .

In conclusion, the results of this study demonstrated that floating is a very viable technique for the recovery of epididymal spermatozoa in cattle after death and that skimmed milk, egg albumin and coconut water have natural preservative effects that may be helpful to reduce the loss of germplasm in Mozambique.

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