

NOTE ON SOME RAMS SEMEN CHARACTERISTICS STORED IN SOYMILK EXTENDER WITH VARYING WATER LEVELS.

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Summary

Six Yankasa rams aged 12-48 months were used for this study. The animals were kept under semi-intensive management system. Semen collection was done early in the mornings by electroejaculation before the animals were taken out for grazing. A day was left free in-between each ejaculation day. The semen were evaluated, extended and preserved in soymilk extenders with different water levels (90, 60 and 30 percent water levels), for four days at refrigeration temperature (5°C) before they were re-evaluated for sperm motility and live spermatozoa.

The mean rams semen characteristics, viz: sperm concentration, percent sperm motility, percent live-spermatozoa and percent sperm abnormalities were $2.28 \pm 4.75 \times 10^9/\text{ml}$, $77.50 \pm 1.23\%$, $85.24 \pm 1.24\%$, $6.94 \pm 0.69\%$, respectively at 100 percent water level. The means initial and final percent sperm motility when the soymilk water level was reduced to 90, 60 and 30 percent were $84 \pm 3.76\%$ and $60.00 \pm 2.08\%$, 74.33 ± 3.53 and $43.67 \pm 4.37\%$, $52.00 \pm 6.43\%$ and 32.00 ± 4.58 , respectively. The initial and final percent live spermatozoa were $86.33 \pm 3.76\%$ and $41.67 \pm 2.03\%$; $76.00 \pm 3.51\%$ and $50.00 \pm 5.03\%$; 54.00 ± 8.33 and 33.67 ± 5.36 for the 90, 60 and 30 percent water levels, respectively. There were no significant differences in the initial and final percent motility and live spermatozoa in the different soymilk extenders water levels, however, the soymilk extender with 60 percent water level compared favourably with whole milk extender and give clear sperm visibility.

Introduction

Artificial insemination (A.I) and embryo transfer (ET) are two technologies that have been evolved by scientist's to enhance reproduction in farm animals. Aside from reproductive efficiency, genetic improvements in farm animal species are secured by the application of these technologies. The use of these technologies necessarily involves storage of the reproductive cells (Sperm and Ova) in diluents or media to maintain their viability at temperatures varying from room to liquid nitrogen temperatures before the cells are used for fertilisation.

In the case of A.I., many extenders have been developed as a result of the discovery that sperm in undiluted semen lived for only short periods of time and that cooling of undiluted semen very rapidly to 5°C caused the death of many sperm (Garden and Fuquay, 1980). Therefore, apart from increasing the ejaculate volume, the extender would have to protect the sperm during cooling as well as extend the life of the sperm.

Established extenders include skim milk - glycerol, Lactose-egg yolk glycerol, egg-yolk citric acid, Tris (hydroxymethyl) aminoethane citric acid, egg yolk - glycerol, Illini Variable Temperature (IVT) extender, Cornell University Extender (CUE), Tris- coconut milk, coconut milk citric acid, etc (Sekoni, 1990; Keskinetepe, *et al*, 1998). Sperm survival has been found to vary in these extenders.

According to Keskinetepe *et al* (1998), skim milk-glycerol extender gives higher fertilisation rates than egg yolk- glycerol citrate extender. Some of these extenders are of higher cost than others as they are not readily available locally. Soymilk is readily available and relatively cheaper than the known regular extenders but has not been tested as a semen extender. The purpose of this work was to evaluate the quality of spermatozoa in soymilk extender with varying water levels.

Materials and methods

Animals and semen collection: A total of six healthy Yankasa rams in the flock of sheep kept at the research farm of the Federal University of Technology, Akure were used for this study. The rams were between 12-84 months of age. They were managed semi-intensively as they were taken out for grazing at 008 hours and returned to the pen at 1400 hours. In the pens, water was made available liberally.

Semen collection was done in the mornings at 007 hours every other day allowing a day of rest for a period of 60 days which represent one spermatogenic cycle (Osinowo *et al*, 1988). Semen collection was by electroejaculation (Adegbile, 1993), and the semen was collected into clean, graduated test-tubes and immediately placed in a flask containing warm water at 37°C. The semen samples were immediately analysed for the following characters- sperm volume and colour, sperm concentration, progressive motility,

live-dead ratio and sperm abnormalities and classified as very good, good or bad (Theriotogenology, 1976). The samples adjudged to be very good or good were left in the water bath for extension while the bad ones were discarded.

Preparation of soymilk extenders: Soymilk extender was prepared by first soaking a measured quantity (0.5kg) of Soya beans in water for about twelve hours after which the coat/husk was removed. The beans was ground into a smooth paste, about 500ml distilled water was added and mixed thoroughly to remove lumps. The slushy was first drained through a sieve and later through a folded clean white cloth. As much filtrate as possible was squeezed out, this was boiled for about 15 to 20 minutes, stirred, cooled and stored in the refrigerator. (Thio, 1978).

Equal amount (25ml) of the soymilk were placed in three 25ml beaker and evaporated in the oven at 30°C to three water levels (90, 60 and 30 percents). Eighty-seven percent each of these evaporated (condensed) soymilk were then mixed with 10% egg yolk and 3% buffer containing 0.5g glucose and made up to 50ml with distilled water prior to use. These were then labelled 90-water extender, 60-water extender and 30-water extender.

Semen extension: The three soymilk extender water level (excluding glycerol) and the semen were placed in the water bath to equilibrate the temperatures. Four millilitres of each extender was then divided into two equal parts of 2ml each and glycerol was added to one part at twice the desired level. The semen and extenders (excluding glycerol) were then placed along with the water bath in the refrigerator and allowed to cool to about 5°C by leaving it for about two hours, depending on the volume, after which one millilitre of the semen was mixed with the extender (2:1).

Later, the part containing glycerol was gradually added to the mixture. This was done by dividing the extender plus glycerol into four parts and gradually adding one part to the mixture at fifteen minutes interval. A dilution rate of 1 to 4 was used i.e. one millilitre of semen to four millilitres of extender. After processing, the extended semen was packed in labelled bijou bottles and stored in the refrigerator for four days (Hearden and Fuquay, 1980) before it was re-evaluated for motility and live spermatozoa.

Data analysis:- All parametric data were subjected to a one-way analysis of variance (ANOVA) using MINITAB (V.10.1, Minitab Inc., USA) statistical package

Result and discussion

The mean initial sperm motility for the three water levels (90, 60, and 30 percent) of soymilk extender were 84.67 ± 3.76 , 74.33 ± 3.53 and 52.00 ± 6.43 percent, while the mean final motility were 40.00 ± 2.08 , 43.67 ± 4.37 and 32.00 ± 4.58 percent respectively for the 90, 60 and 30 percent soymilk extender water levels. As the mean initial live spermatozoa for the three water levels of the soymilk extender were 86.33 ± 3.76 , 76.00 ± 3.51 and 54.00 ± 8.33 percent, the mean final live spermatozoa were 41.67 ± 2.03 , 45.00 ± 5.03 and 33.67 ± 5.36 percent respectively for the 90, 60 and 30 percent soymilk extender water levels (Table 1).

The 30 percent soymilk extender water level had the least initial final percent motility and live-spermatozoa respectively. Although there was no significant differences in the treatments, the 60 and 90 percent soymilk-extender water level performed fairly favourably better than the 30 percent water level with the 60 percent soymilk extender water level having slightly higher initial and final motility and live-spermatozoa. Sperm cells were more clearly observed under the microscope in semen preserved in soymilk extender compared to other extenders tested in this study as in an earlier study (Okukpe, 1999).

As could be seen in Table 1 the percent final motility and live-spermatozoa improved appreciably, though not significantly when the soymilk water content was reduced as against the poor performance by the initial soymilk extender (Okukpe, 1999). Therefore the low performance of the soymilk extenders could be due to the percentage of water contained in it among other factors such as antinutritional factors and method of preparation of soymilk. This supports the work of Moss *et al* (1979) and Sekoni (1990) that water is spermicidal. It could be suggested that the higher the concentration of water in soymilk, the greater the lethal effect on spermatozoa, though very low water level in soymilk could also be detrimental to spermatozoa characteristics.

Therefore, it would be necessary to carry out further studies to ascertain the best method of preparation for reduced antinutritional factors and the right water content of soymilk required for optimum performance of soymilk as an extender component. In addition, following successful testing of soymilk extender for semen preservation, a fertility trial of semen stored in soymilk extender versus other tested extenders would be imperative.

Table 1: Effect of soy-milk extender with variable water content on sperm motility and live ability

Percent water level	Mean motility		Mean live-spermatozoa	
	Initial	Final	Initial	Final
90	84.67 ± 3.76 ^a	40.00 ± 2.08	86.33 ± 3.76 ^a	41.67 ± 2.03
60	74.33 ± 3.53 ^a	43.67 ± 4.37	76.00 ± 3.51 ^a	45.00 ± 5.03
30	52.00 ± 6.43 ^b	32.00 ± 4.58	54.00 ± 8.33 ^b	33.67 ± 5.36

^{a,b} Means with different superscripts along same column differ significantly ($P < 0.05$).

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