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Evaluation of Sperm Motility and Viability in Honey-Included Egg Yolk Based Extenders

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Abstract: The proportion of honey, egg yolk and sodium citrate buffer suitable for the extension of West African Dwarf (WAD) buck (*Capra hircus* L.) spermatozoa at 5°C was studied. Semen was collected from four clinically healthy bucks certified free of any obvious andrological defects. Four diluents were prepared with egg yolk citrate as the control while other diluents consisted of increasing concentrations of honey and decreasing concentrations of egg yolk. Evaluations for motility and viability were done every two hours for six hours for each diluent. Results show that inclusion of honey in egg yolk based extender sustained sperm motility and liveability for up to 6 hours at 5°C. This effect was however found to be dependent on the ratio of honey to egg yolk in the extender. Diluent 2 (5ml honey + 15ml egg yolk + 80ml sodium citrate) gave the highest percentage motility at each of the hours tested. The live dead ratio observed at different hours gave high values for diluents 1, 2 and 3 with diluent 2 as the highest. Diluent 4 (20ml honey + 80ml sodium citrate) gave very low values except at 0 hour.The result of this study suggested that the addition of honey to egg yolk improves the motility and live dead ratio and thus viability of liquid goat semen. However, this effect is concentration dependent and higher concentrations may have negative effect on sperm viability.

Key words: Sperm motility · Viability · Honey · Egg yolk based extenders

INTRODUCTION

Artificial insemination (AI) has been postulated to have the potential of increasing productivity, enhancing biosecurity and aiding the genetic management of animals for either agricultural or conservation purposes [1]. However, AI is only economical if semen can be extended and used fresh or preserved to breed livestock with appreciable conception rate. A single buck's ejaculate can potentially inseminate 15-40 estrous does [2].

Extenders are certain ingredients added to ejaculated semen to sustain and protect the spermatozoa thereby preserving its fertility until they are used for insemination [3]. Egg yolk based extender has been the common and most extensively used extender but it is a good medium for the growth of microorganisms. Honey however contains high level of metabolizable energy in form of glucose and fructose and has antibacterial activity against microorganisms [4].

The use of honey to replace egg yolk in part or as a whole in egg yolk based extenders is the focus of this study since honey is a good source of glucose and fructose and also has antibacterial activity against some microorganisms which are resistant to the common antibiotics used in extenders [5].

MATERIALS AND METHODS

Four sexually matured West African Dwarf goats were used in this study. Their ages ranged between 2-3 years with an average weight of 12±1.5 kg.

They were dewormed with a subcutaneous injection of ivermectin (Ivomec®, Hoescht, Germany) at a dose rate of 1ml/50 kg body weight. They were also prophylactically treated with intramuscular injection of 5% oxytetracycline (Oxytetracyclina®, Invesa) at a dose rate of 5mg/kg body weight for 5 days.

They were kept in individual pens at the Small Ruminant unit of the Department of Veterinary Surgery and Reproduction, University of Ibadan. They were fed with concentrates and elephant grass (*Pennisetum purpureum*) and supplied with fresh clean water *ad libitum*.

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Freshly squeezed honey from the comb was obtained and used in this study.

Preparation of Extender: 2.9g of Sodium citrate salt ($Na_3C_6H_5O_7 2H_2O$) was weighed on an electronic laboratory scale and 100ml of fresh distilled water was warmed up to 40°C and added to the salt in a calibrated measuring cylinder. The solution was stirred gently with a glass rod and kept in a water bath maintained at 37°C.

Four types of semen extenders used in this study were prepared as follows;

- Diluent 1(Control): 20ml egg yolk + 80ml sodium citrate.
- Diluent 2: 5ml honey + 15ml egg yolk + 80ml sodium citrate.
- Diluent 3: 10ml honey + 10ml egg yolk + 80ml sodium citrate.
- Diluent 4: 20ml honey + 80ml sodium citrate.

All the four diluents were maintained at a temperature of 37°C.

Semen was collected from the four bucks using the electroejaculation method and pooled. Each buck had been judged satisfactory in a Breeding Soundness Examination prior to use.

The semen was examined macroscopically for color, volume and consistency. A drop of semen was also examined for pre dilution assessment of mass activity, live dead ratio and motility by conventional methods [6, 7].

Semen extension was done by adding 0.4ml of semen to 20ml of each of the diluents and kept at 37°C. Initial evaluation of motility and live/dead ratio were done and the extenders were kept in a refrigerator at 5°C for evaluation at every 2 hours for 6 hours i.e. at 2 hours, 4 hours and 6 hours post extension.

The experiments were repeated for each of the diluents after three days for accuracy.

RESULTS

The average volume of the pooled ejaculate was 2.5ml. It was creamy in color and homogenous without flakes or clumps. Average gross and individual motility were assessed as +++ and 95% respectively. Average sperm concentration was found to be 2.5 x 10^9 spermatozoa/ml.

Table 1: Motility Count

	Motility (Mean±SD%)			
Percentage	0 hour	2hours	4hours	6 hours
Diluent 1	92.5±2.50	82.5±2.50	80.0±0.00	77.50±2.50
Diluent 2	95.0±0.00	90.0±0.00	87.5±2.50	85.38±0.00
Diluent 3	22.5±2.50	10±0.00	-	-
Diluent 4	-	-	-	-

Table 2: Percentage Liveability

	Liveability (Mean±SD%)			
Percentage	0 hour	2hours	4hours	6 hours
Diluent 1	95.0±0.00	90.0±0.00	82.5±2.50	80.0±0.00
Diluent 2	98.0±0.00	95.0±0.00	90.0±0.00	87.5±0.00
Diluent 3	22.5±2.50	10±0.00	-	-
Diluent 4	-	-	-	-

DISCUSSION

The mean semen volume from each buck recorded in this study was 0.42±0.007ml which agrees with 0.41±0.04ml recorded by Akusu *et al.* [8] but lower than the report of 0.7ml average ejaculate of WAD buck by Okere *et al.* [9].

The average percentage live/dead ratio for prediluted semen in this study was 94.8±5.07% which agrees with the report of 93.3±1.7% by Oyeyemi and Akusu, [7] for WAD bucks.

In this study, the inclusion of honey in egg yolk based extender was found to sustain sperm motility and liveability for up to 6 hours at 5°C. This effect was however found to be dependent on the ratio of honey to egg yolk in the extender. The percentage motility was high for all the diluents and this was found to be decreasing over time. Diluent 2 gave the highest percentage motility at each of the hours tested. The inclusion of honey as an additional source of glucose in an egg yolk buffer diluting medium used in this study supports the report of Smith *et al.* [10] that the addition of small amounts of glucose to an egg yolk buffer increases and prolongs active motility of the spermatozoa. This could be the reason why 5ml honey + 15ml egg yolk yielded the maximum percentage motility count and high values for live/dead ratio even up to 6 hours post dilution when stored at 5°C.

Diluents 3 and 4 did not sustain the motility of the goat semen compared to when 5mls of honey was added to 15mls of egg yolk suggesting that the effects of honey on sperm motility might be dependent on concentration.

Although the exact mechanism for this is unknown, similar findings have been reported in rats when 1250µg/ml of hyaluronan was reported to produce a negative effect on sperm motility compared to 750µg/ml [11].

The live dead ratio observed at different hours gave high values for diluents 1, 2 and 3 with diluent 2 as the highest. Diluent 4 gave very low values except at 0 hour.

The findings in this study also supported the work of Parandekar [12] and Moore *et al.* [13] that 10-20% egg yolk in an extender was optimum for successful storage of buck semen at refrigeration temperature of 3-6°C. It also agrees with Parkinson [14] that high concentration of egg yolk is toxic to buck spermatozoa because of lysolecithin and so should be maintained within a narrow limit.

In conclusion, the results of this study suggested that the addition of honey to egg yolk improves the motility and live dead ratio and thus viability of liquid goat semen. However, this effect is concentration dependent and higher concentrations may have negative effect on sperm viability.

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