Buck Semen Preservation at Ambient Temperature

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ABSTRACT

A study was conducted at the teaching and research farm of Abubakar Tafawa Balewa University, Nigeria to investigate buck semen storage at ambient temperature. Semen samples were collected from 3 Red Sokoto bucks (average age 20 months) and pooled for the study . Motility under ambient temperature conditions of storage were assessed using the diluents, skimmed milk (SM),skimmed milk with 0.9g glucose (SMG), Cornell University Extender (CUE),Glucose-Citrate Extender (GCE). The average motility for the first three days of storage declined from 85% for the respective diluents to 44.33%, 49.00%,49.67% and 53.35% . There were no significant ($P>0.05$) differences between these values but CUE and GCE which had synthetic buffers tended to store better. Using GCE under three conditions of storage (aerobic vs anaerobic oil vs anaerobic jar) motility declined with time with mean values of 41.5%,43.0% and 68.67% for the three conditions respectively. Significant differences ($P<0.05$) were observed in the overall mean values of these conditions of storage being 48.27% ,49.75% and 53.42% in that order. The best storage condition for ambient temperature preservation of semen motility is under anaerobic condition.

Key words: Buck semen, Preservation, Ambient Temperature, Nigeria.

RÉSUMÉ

Une étude sur la recherche des techniques de conservation à la température ambiante des semences du bouc à été conduite à la ferme d'application et recherche de l'université Abubakar Tafawa Balewa du Nigeria. Les échantillons de semences ont été collecté sur trois boucs du race Red Sokoto (age moyenne de 20 mois) et mis en commun pour l'étude. La mobilité dans les conditions de conservation la température ambiante a été évaluée utilisant les diluants : Lait écrémé (SM), le lait écrémé avec 0.9 g (SMG) de glucose, 'Cornell University Extender'(CUE) et 'Glucose-Citrate Extender'(GCE). La mobilité moyenne pour les trois premiers jours de conservation était de 44.33, 49.00 ,49.67 et 53.35% respectivement. Aucune différence significative ($P>0.05$) à été observé entre les valeurs mais le CUE et GCE qui ont eu un tampon synthétique ont tendance à mieux conserver. En utilisant le GCE dans trois conditions de conservation (Aerobique ≠ Anaerobique sous l'huile ≠ Anaerobique dans un bocal) la mobilité baisse avec le temps avec des moyennes de 41.50, 43.0 et 68.67% pour les trois conditions respectivement. Les différences significatives ($P<0.05$) ont été observée entre ces moyenne dans ces conditions de conservation. La meilleur condition de conservation des semences à la température ambiante est dans les conditions anaerobique.

Mot clés : Semence du bouc, Conservation, Température ambiante, Nigeria.

INTRODUCTION

The ability of the male animal to synthesise proteins in the testicles is of commercial and clinical importance. More so in any enterprise concerned with breeding of livestock the male animals represents half of the reproductive potential of the herd or flock. (Maclaren,1988). Sperm concentration, motility, morphology and volume are believed to be very relevant to the chance of oocyte penetration (Den Daas, 1992). The motility of spermatozoa provides a simple means of evaluating the physiological status of a sample of semen. Although the motile character of a sperm cell is itself not an accurate predictor of its potential fertilising capacity, sperm motility however continues to be a useful tool in evaluating the viability of spermatozoa (Hafez,1980).

The in vitro preservation of semen motility is aimed at prolonging the fertilising capacity of spermatozoa. This could be achieved by reducing or arresting their motility and metabolic reactions (Evans and Maxwell, 1987) and also by providing a ready source of nutrients and a rapid means of removing waste products (Butswat et al.,1992). Short-term storage with diluents which provide nutrients for the spermatozoa and buffer against changes in pH as well as storage under reduced oxygen environment might provide a simple means for storage of buck semen. Storage at room temperature in an atmosphere of carbon dioxide has been tested for ram semen based on a method developed for the storage of bull semen(Evans and Maxwell, 1987). However there appear to be no reports on the practicability of this method for storage of goat semen.

The experiments herein reported were conducted to test the effect of diluent of storage and conditions of storage on the motility of buck semen.

MATERIALS AND METHODS

Semen collection and diluents

Semen was collected twice daily from 3 red sokoto bucks (average age 20 months) by artificial vagina method for 34 weeks, every collection day was followed by a rest period of 3 days before the next collection. After collection, semen samples were pooled and divided into four equal volumes for the first experiment and into three for the second experiment. These volumes corresponded to the number of diluents in the respective experiments. Only semen with good sperm motility (at least 70%) were pooled and used in the experiments. A total of 70 semen collections were retained for both experiments with 40 collections for the first experiment and 30 for the second experiment.

In the first experiment four diluents being Skimmed milk (SM) , Skimmed milk with 0.9g glucose per 100ml (SMG), Cornell University Extender (CUE) and Glucose Citrate Extender (GCE) were used. The composition of these diluents (in this case without egg yolk) were as described by Foote and Bratton (1960) for CUE and Evans and Maxwell (1987) for the other three diluents as shown in Table 1. In the second experiment only Glucose Citrate Extender (which was the best for the first trial) was used.

Semen storage

This involved dilution of semen with each diluent inside Mccartny bottles at a dilution rate of 1: 4 (v/v semen : diluent). The bottles where then tightly capped and stored in dark room at ambient temperature. This was done for the first experiment. For the second experi-

Table 1: Composition of the diluents used for the preservation of buck semen at ambient temperature.

Diluent component	TYPE OF DILUENT			
	Skimmed Milk	Skimmed Milk + 0,9g Glucose	Cornell University Extender	Glucose Citrate Extender
Milk powder(g)	90	90	-	-
Glucose(g)	-	9	3.0	8.0
Sodium citrate(g)	-	-	14.5	23.7
Sodium bicarbonate(g)	-	-	2.1	-
Potassium chloride(g)	-	-	0.4	-
Glycine(g)	-	-	9.37	-
Sulphanilamide(g)	-	-	3.0	-
Citric acid(g)	-	-	0.87	-
Penicillin(Sodium salt) (IU/ml)	1000	1000	1000	1000
Streptomycin sulphate(mg/ml)	1	1	1	1
Distilled water(ml)	1000	1000	1000	1000

Table 2 : Motility of buck semen using different diluents with time for buck semen preserved under aerobic conditions at ambient temperature.

Diluent	Storage period(Days)			Mean
	0	1	2	
SM	85	33	15	44.33
SMG	85	47	15	49.00
CUE	85	54	10	49.67
GCE	85	56	19	53.35
Mean	85.00	47.50	14.75	

SM= Skimmed Milk CUE=Cornell Univ. Extender
SMG=SM+0.9g Glucose GCE=Glucose-Citrate Extender

ment the above condition of storage was compared to two other conditions of storage which were, storage under oil and storage in the anaerobic jar. For oil storage paraffin oil was used to make a sufficient film over diluted semen in Mccartny bottles. The mixture was capped and stored as above. Anaerobic storage involved placing diluted semen in bottles in the anaerobic jar without the cap.

Experiments.

In the first experiment, the effect of type of diluent on the motility of semen diluted 1:4(v/v semen: diluent) with each of the four diluents were studied simultaneously at ambient temperature. In the second experiment the effect of storage condition on the motility of semen in GCE at ambient temperature was studied. Forty trials with three replicates per diluent were done for the first experiment. The same procedure was followed for the thirty trials of the second experiment. Average values for these trials constituted raw data for statistical analysis.

Data Analysis

Analysis of variance and Duncan's multiple range test (Steel and Torrie, 1980) were used for statistical analysis of values of motility following angular transformation (from percentages to degrees) of the data. The effect of diluent of storage on the motility of buck semen were compared in the first experiment while the effect of storage conditions were compared in the second experiment.

RESULTS

Results obtained from the first experiment are summarised in Table 2. The average motility of spermatozoa for the first three days of storage for the diluents were 44.33, 49.00,49.67 and 53.33% respectively. Table 3 shows results for the analysis of variance for the data following angular transformation. There were no significant differences between the diluents of storage. Significant differences ($P<0.05$) were however observed for the periods of storage between day 0 (67.21) and day 2 (22.48).

The results obtained from the second experiment which are summarised in Table 4 shows an average motility for the first three days of storage for aerobic storage condition, anaerobic storage under oil and jar to be 41.50, 43.00, 68.67% respectively.

Table 5 shows that there is a significant ($P<0.05$) difference between the conditions of storage and the periods of storage. Overall mean values for the storage conditions were 48.27, 49.75 and 53.42% respectively while for the storage periods (0 vs 1 vs 2), the values were 65.65, 53.02 and 32.77% respectively. Corresponding coefficients of variability for these values are shown under the respective parameters.

Table 3: Effect of type of diluent and storage time on the motility of buck semen (in degrees) preserved under aerobic conditions at ambient temperature.

Day of storage	TYPE OF DILUENT				Overall Mean
	SM (10)*	SMG ((10)	CUE (10)	GCE (10)	
0					
1	35±1.56	43.66±1.47	47.0±1.48	48.25±1.44	41.27 ^{ab}
CV(%)	13.5	13	12.4	11	
2	22.52±0.3	22.79±0.45	18.33±1.67	26.3±1.67	22.48 ^{bc}
CV(%)	4	6	9	7	
Overall Mean	41.59^{a}	41.55^{a}	44.21^{a}	47.26^{a}	

Means(± S.E) on the same row or column bearing different superscripts differ significantly ($P<0.05$).
*number in parenthesis represents the number of semen collections used per diluent.

Table 4: Motility of buck semen under different storage conditions for buck semen preserved at ambient temperature.

Storage Condition	STORAGE PERIOD (DAYS				Mean
	0	1	2	3	
Aerobic	83	60	23	0	41.50
Anaerobic	83	63	26	0	43.00
Anaerobic jar	83	68	40	15	68.67
Mean	83.00	63.67	29.67	5.00	

DISCUSSION

The average spermatozoa motility values for Table 2 are lower than values reported elsewhere (Butswat et al.,1992) on ram semen with Cornell University Extender for the first 3 days of storage at ambient temperature using continuous and discontinuous flow dialysis techniques. The higher values were probably because of the possibility of elimination of waste products produced by the spermatozoa during that experiment. Significant differences observed between day 0 and day 2 (Table3) may be related to a depletion of the energy reserves of the diluents by the sperm cells as well as an increasing accumulation of waste products resulting from sperm metabolism within these diluents. However, energy reserves and waste products of the respective diluents on day 1 did not significantly affect sperm motility.

Results for the first experiment(Table 2 and 3) also showed that buck semen was better preserved in diluents containing synthetic buffers than those with natural buffers (SM and SMG). This is the case with CUE which had a synthetic buffer (a mixture of citric acid, sodium citrate and sodium bicarbonate) and GCE with just sodium citrate as buffering agent. A slight improvement in the storage capacity of GCE as opposed to CUE may be attributed to the high level of energy (glucose 8.0g/litre) contained in GCE than CUE (3.0 g glucose/litre). A similar trend is shown between SM and SMG where SMG has an additional input of 9g glucose/litre as opposed to SM without any additional energy substrate.

Results for anaerobic storage (second experiment) in Table 4 are comparable to those reported by Butswat et al., (1992). The values for anaerobic storage on day 1 also corroborates with the findings of Azawi et al., (1990) who reported an individual motility percentage for bovine semen after 24 hours storage to be 65.35±3.16 under Iraqi environment.

This study clearly indicates that buck semen motility can best be preserved under anaerobic conditions especially in the anaerobic jar. This may be because of the ageing of sperm cells due to metabolic activity owing to the continued availability of oxygen for respiratory processes during aerobic storage. Faster accumulation of waste products which might result in auto-intoxication may be associated with aerobic storage. A similar observation has been made (Azawi et al.,1990). In the study, significant ($P<0.01$) enzyme activity was observed after 120 hours of storage for bovine semen under the same condition of storage. It was also observed that the enzyme activities released to the extracellular fluid were negatively correlated with sperm motility and positively correlated with aged acrosomes. Breenswa, (1972) also concluded that semen with low fertility showed a significantly high level of aspatate amino transferase (AST).

It could therefore be concluded here that anaerobic storage of buck semen may be of particular importance for short-term storage in developing countries where steady supply of electricity cannot be guaranteed. It is not only simple it is also cheap.

Table 5: Effect of storage condition and time on the motility of buck semen (in degrees) preserved at ambient temperature.

Day of Storage	STORAGE CONDITIONS			Overall mean
	Aerobic (10)*	Anaerobic oil (10)	Anaerobic jar (10)	
0	65.65±1.69	65.65±1.69	65.65±1.69	65.65^{a}
CV(%)	8	8	8	
1	50.77±1.25	52.74±0.68	55.56±0.79	53.02^{b}
CV(%)	7.4	3.9	4.3	
2	28.40±1.9	30.86±3.42	39.04±1.35	32.77^{c}
CV(%)	14.46	12.2	10.7	
Overall mean	48.27^{a}	49.75^{b}	53.42^{c}	

Means(±SE) on the same row or column with different superscripts differ significantly($P<0.05$).
*number in parenthesis represents the number of semen collections and trials used per diluent per storage condition.

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