

Effects of Type of Extender and Storage Conditions on the Motility of Goat Spermatozoa

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Abstract: The objective of this study was to test the effect of four different diluents on the motility of goat semen stored at room and refrigerator temperatures. To achieve this objective, four West African dwarf bucks aged 10-12 months were ejaculated weekly for five weeks using the electro-ejaculator method. The ejaculated semen were extended using four diluents namely sodium citrate (T_1), sodium bicarbonate plus glucose (T_2), sodium citrate plus glycine (T_3), and glucose plus glycine (T_4). Two storage conditions namely refrigerator temperature (5°C) and room temperature (25°C) were used. The semen was evaluated for colour, volume and motility before extending them with the diluents. Subsequently, motility was monitored for four days. The results indicated that storage days and type of extender were highly significant ($p < 0.01$) throughout the period. The highest motility was obtained during the first day of storage, thereafter, the motility declined drastically from day 2 to day 4. Sodium citrate preserved bucks semen better than the other extenders. This was followed by sodium bicarbonate plus glucose. Refrigerator temperature was significantly ($p < 0.05$, $p < 0.01$) better than room temperature in the preservation of bucks sperm motility. The semen stored in sodium citrate under refrigerator temperature maintained the highest spermatozoa motility throughout the period compared with the other storage conditions. It was concluded that goat semen can be preserved for 24 h in sodium citrate under refrigerator temperature.

Key words: Bucks, diluents, motility, Spermatozoa

INTRODUCTION

The West African Dwarf (WAD) goat is important as source of meat, milk, skin and fibre. WAD goats are known to have a remarkable ability to utilize roughages (Oyeyemi *et al.*, 2000). Management of the goat for reproductive soundness is one of the most important factors in goat breeding. Artificial insemination plays an important role in goat breeding especially in intensive system of production where it can be used as a tool to control reproduction and to improve the production of milk, hair and meat (Baillargeon, 2000). The success of an artificial insemination programme depends on the appropriate management of semen collection, storage and use (Etches, 1996). AI holds several genetic and economic advantages for goat production among which is utilization of proven sires (Bale, 2003). It also extends the usefulness of proven sires that for some physical reasons or ages are unable to copulate normally (Bale, 2003). Preservation of semen using suitable extenders is an important ingredient in the success of AI in goat reproduction. This is because it increases the volume of semen, thus permitting more insemination per ejaculate. Liquid goat semen destined for use within 12 h should be stored at 4°C (Lafalci *et al.*, 2002). Significant temperature changes are considered detrimental to semen

quality and under practical conditions, fluctuation in temperature may be easier to avoid at higher storage temperature than 4°C (Lafalci *et al.*, 2002). The viability and fertilizing ability of ejaculated semen are mainly judged by progressive motility (Etches, 1996) and morphologically normal spermatozoa (Eva-Marie *et al.*, 1989). Bucks semen presents some very specific challenges due to the presence of enzymes from bulbourethral gland secretion which interacts with skim milk constituents of some diluents (Pellicer-rubio *et al.*, 1997). Therefore, separation of seminal plasma from sperm cell is necessary for goat semen preservation (Pellicer-rubio *et al.*, 1998). However, study by Peterson *et al.* (2007) showed that the removal of the seminal plasma did not affect the quality of liquid stored or frozen thawed semen in Dutch dairy goat. The adoption of AI technique in animal production in Nigeria has been generally low. This has accounted for the little or no documented information on storage and AI technique for goat reproduction in Nigeria. It is important to develop reliable extenders for goat semen to facilitate the use of AI for goat reproduction in Nigeria. This will go a long way towards increasing goat production in the country. The specific objectives of this study includes:

- To determine the percentage motility of goat spermatozoa extended in four different diluents.

- To determine the effect of storage days and storage temperature on goats sperm motility.
- To determine the interactive effect of storage days, type of extender and storage temperature on the motility of goats spermatozoa.

MATERIALS AND METHODS

The experiment was carried out in the physiological laboratory of the Department of Animal Science, Delta State University, Asaba campus. Four West African Dwarf (WAD) bucks aged 10-12 months were used for the experiment. The bucks were housed in the same pen in the goat unit of the teaching and research farm, Department of Animal Science, Delta State University, Asaba campus. The pen had a concrete floor covered with wood shavings as litter. The animals were allowed to graze from 7 am to 3 pm everyday on pastures consisting of carpet grass (*Axonopus compressors*), guinea grass (*Panicum maximum*) and elephant grass (*Pennisetum purpureum*). A corn based concentrate ration was provided in the evening as supplement. Water was provided *ad libitum*. Routine medication consisted of deworming using albendazole at a dosage of 1 mL / 5 kg body weight and vaccination against pneumo enteritis complex using tissue culture rinderpest vaccine. The materials used for the study includes sodium citrate, sodium bicarbonate, glucose, glycine, coverslips, buffer tablets, pH indicator paper, slides, eggs, penicillin, streptomycin, syringes and needles.

Preparation of buffer: Freshly boiled distilled water was added to a measured quantity of the ingredient in a 1000 mL volumetric flask and kept in a dark cupboard until the solution cooled. Some distilled water was added to make up 100 mL of the buffer. The ingredients used for preparation of the buffer were as follows:

- Trial 1: 2.90 g of sodium citrate.
 Trial 2: 2.10 g of sodium bicarbonate and 40.00 g of glucose.
 Trial 3: 10.00 g of sodium citrate and 5.00 g of glycine.
 Trial 4: 45.00 g of glucose and 6.00 g of glycine.

Preparation of diluents: A quantity of the buffer was measured into a cylinder. Egg yolk was measured and added to the buffer to make up 100 mL of the extender. Then 1 mL of antibiotic (penicillin and streptomycin) was added to it and the solution well shaken. The mixture was poured into centrifuge tubes for centrifugation. The supernatant obtained was decanted into universal bottle and the pH of the diluents adjusted to 7 using buffer tablet and measured with a pH meter. The diluents was kept in the refrigerator until required for use. The relative composition of the diluents are presented in Table 1.

Table 1: Relative composition of the various diluents

Trial	Buffer (mL)	Egg yolk (mL)	Antibiotic (mL)
1	90	10	1
2	75	25	1
3	70	30	1
4	90	10	1

Table 2: Initial semen characteristics of buck's semen

Parameters	Values
Colours	Milky white
Volumes (mL)	0.42±0.07
Motility (%)	92.00±0.86
Concentration (×10 ⁶)	2.05±0.21

Collection of semen: Semen was collected weekly by electro-ejaculator method (Oyeyemi *et al.*, 2000). Prior to semen collection, the buck prepuce was properly cleaned, the rectal probe was lubricated thoroughly with petroleum jelly and gently inserted into the rectum of the buck by a slow rotating movement with both electrodes down. The buck was held firmly in a standing position by two attendants. The electro-ejaculator was then plugged to a source of electricity and switched on. Voltage was applied to the animal by rotating the knob of the variable resistor, turning it back to zero, and repeating the process several times by gradually increasing the applied voltage. By regulating the voltage, the nerves responsible for ejaculation were stimulated and erection took place. This was followed by ejaculation. The ejaculate was collected into a graduated tube (bathed in warm water at 37°C) through a funnel held by an assistant. Semen collection was done once a week.

Pre-extension evaluation of semen: Immediately after semen collection, the volume of the semen was recorded with the aid of a graduated cylinder. The colour of the semen was evaluated by visual appraisal. For motility, a drop of semen was placed on a slide, covered with a cover slip and examined under a microscope. The concentration of the sperm cells were determined using a photometer as described by Peterson *et al.* (2007).

Semen extension: Buck semen was extended in sterilized tubes as described by Sule *et al.* (2007). The semen was extended by dispensing 0.1 mL semen into 0.5 mL extender (T1 to T4) previously kept at 37°C. The extended semen were stored at both ambient temperature and refrigerator (5°C) conditions. The extended semen were evaluated for motility every 24 h for 4 days.

Statistical analysis: Data collected were subjected to 4×4×2 factorial analysis in a completely randomized design (Steel and Torrie, 1980). Significant means were separated using Duncan multiple range test (Duncan, 1955).

Table 3: Mean Squares and degree of freedom obtained from the analysis of variance for bucks sperm motility from week 1 to 5

Source of variation	Mean square					
	DF	Week 1	Week 2	Week 3	Week 4	Week 5
Storage days (SD)	3	3178.47**	2457.49**	3367.36**	3401.44**	4553.81**
Type of extender (TE)	3	420.17**	463.75**	426.07**	616.55**	336.70**
Storage temperature (ST)	1	299.45*	365.24**	601.53**	668.50**	609.04*
SD × TE interaction	9	43.01 ^{NS}	45.35 ^{NS}	41.65 ^{NS}	59.61*	16.50 ^{NS}
ST × SD interaction	3	53.75 ^{NS}	32.13 ^{NS}	108.00 ^{NS}	113.95*	174.34 ^{NS}
ST × TE interaction	3	213.32*	150.07*	202.14*	358.03*	203.01*
Residual	9	30.70	35.05	51.81	19.58	58.11

*: p<0.05; **: p<0.01; NS = Not Significant

Table 4: Effects of days of storage on the motility of bucks spermatozoa (%)

Weeks of collection	Storage days			
	1	2	3	4
1	70.00±2.67 ^C	17.50±9.01 ^b	5.00±5.00 ^a	5.00±5.00 ^a
2	65.00±3.27 ^d	17.50±7.96 ^C	10.00±6.55 ^b	5.00±5.00 ^a
3	72.50±3.13 ^d	16.25±9.62 ^C	10.00±6.55 ^b	5.00±5.00 ^a
4	72.00±4.12 ^C	15.00±9.62 ^b	12.50±8.40 ^b	5.00±5.00 ^a
5	70.00±3.78 ^d	15.00±9.82 ^C	10.00±6.55 ^b	0.00±0.00 ^a

For each row values, a<b<c<d (p<0.05)

Table 5: Effect of type of extender on bucks sperm motility (%)

Week of collection	Type of extender			
	T1	T2	T3	T4
1	40.00±12.10 ^c	25.00±11.02 ^b	15.00±9.82 ^a	17.50±11.46 ^a
2	40.00±11.34 ^c	27.50±9.21 ^b	15.00±9.82 ^a	15.00±9.82 ^a
3	38.75±11.72 ^c	32.50±13.06 ^b	15.00±9.82 ^a	17.50±11.46 ^a
4	45.00±13.50 ^c	27.50±11.14 ^b	15.00±9.82 ^a	17.50±11.45 ^a
5	43.33±14.98 ^b	45.33±14.98 ^b	20.00±12.65 ^a	20.00±12.65 ^a

Mean values on the same row with different superscript letters are significantly different (p<0.01)

RESULTS

Table 2 presents the initial semen colour, volume, motility and concentration of WAD buck used for the experiment. The semen was generally milky white in colour, with mean volume, concentration and motility of 0.42 mL, 2.5×10⁶ and 92.00%, respectively. The summary of the analysis of variance for bucks sperm motility subjected to different storage conditions for 5 days is presented in Table 3. The effect of storage days and type of extender were highly significant (p<0.01) throughout the period of the trials. Storage temperature was highly significant (p<0.01) in weeks 2, 3 and 4 and significant (p<0.05) in weeks 1 and 5. The interaction between storage temperature and type of extender were significant (p<0.05) throughout the period while storage days by type of extender and storage temperature by storage days interactions were not significant (p>0.05). It will be observed from Table 4 that the highest motility was attained during the first 24 h of storage, thereafter, motility declined drastically from day 2 to day 4. Table 5 shows that sodium citrate (T1) was the best extender used, followed by sodium bicarbonate plus glucose (T2),

Table 6: Mean±S.E for sperm motility of bucks stored at room and refrigerator temperatures (%)

Week of collection	Room temperature	Refrigerator temperature
1	22.14±8.04 ^a	29.38±8.07 ^b
2	18.75±7.18 ^a	30.00±7.30 ^b
3	18.75±8.11 ^a	33.13±8.10 ^b
4	18.75±8.16 ^a	33.75±8.10 ^b
5	23.33±10.10 ^a	40.00±9.12 ^b

For each row values, a<b (p<0.05)

sodium citrate plus glycine (T3) and glucose plus glycine (T4) in that order. Table 6 shows that refrigerator temperature (5°C) preserved sperm motility better than room temperature (25°C) throughout the period of the trials. Table 7 presents the effect of storage temperature and type of extender on the motility of buck spermatozoa. It will be observed that semen extended in sodium citrate (T1) and under refrigerator temperature gave the highest motility throughout the five weeks period compared with other storage conditions. This was followed by sodium bicarbonate plus glucose under refrigerator temperature and sodium citrate under room temperature. The poorest motility values were obtained in sodium citrate plus glycine (T3) and glucose plus glycine (T4) under refrigerator and room temperatures, respectively.

Table 7: Effect of storage temperature and type of extender on goats sperm motility (%)

Week of collection	Room temperature				Refrigerator temperature			
	T1	T2	T3	T4	T1	T2	T3	T4
1	22.50 ^b (9.31)	22.50 ^b (6.52)	15.00 ^a (5.60)	17.50 ^a (7.60)	55.00 ^d (9.57)	35.00 ^c (6.58)	15.00 ^a (5.60)	17.50 ^a (7.50)
2	22.50 ^b (8.93)	20.00 ^{ab} (4.14)	15.00 ^a (5.00)	17.50 ^a (7.50)	55.00 ^d (9.57)	35.00 ^c (6.58)	15.00 ^a (5.60)	17.50 ^a (7.60)
3	22.50 ^b (9.31)	22.50 ^b (10.00)	15.00 ^a (5.00)	17.50 ^a (7.50) ^a	55.00 ^d (9.57)	45.00 ^c (7.80)	15.00 ^a (5.60)	17.50 ^a (7.60)
4	25.00 ^b (9.31)	17.50 ^a (10.50)	15.00 ^a (7.40)	17.50 ^a (5.00)	65.00 ^d (10.41)	37.00 ^c (9.36)	15.00 ^a (5.60)	17.50 ^a (7.60)
5	22.50 ^b (9.31)	22.50 ^b (10.00)	15.00 ^a (5.00)	17.50 ^a (7.50)	55.00 ^d (9.57)	45.00 ^c (7.80)	15.00 ^a (5.60)	17.50 ^a (7.60)

Mean values along the same row with different superscript letters are significantly different (p<0.05); Standard errors are in parentheses

DISCUSSION

From the experiment, sodium citrate (T1) exhibited the highest ability to sustain the viability of sperm motility followed by sodium bicarbonate and glucose (T2). Sodium citrate plus glycine (T3) and glucose plus glycine (T4) exhibited the least capabilities to sustain the viability of sperm motility under storage. This could be attributed to the ability of these extenders to supply the nutrients needed for the metabolic maintenance of the sperm cells, control the pH of the sperm cells to the pH of freshly ejaculated semen, afford protection from cold shock and inhibit microbial growth. The usefulness of sodium citrate as a good semen extender was discussed by Adeyemo *et al.* (2007) who reported that sodium citrate performed excellently well in maintaining the viability of stored fish semen. It was observed from Table 4 that the highest sperm motility was obtained during the first 24 hours of storage throughout the trial period. After that motility declined drastically from day 2 to day 4. This implies that diluted liquid goat semen stored in refrigerator condition should not be kept beyond 24 h before insemination in order to obtain a good conception rate. This result is in line with the observation of Peterson *et al.* (2007) that the percentage of motile spermatozoa in buck semen stored in liquid form for 72 h, progressively declined overtime irrespective of whether storage occurred at 4 or 18°C. The drastic decline in sperm motility of stored liquid semen could be attributed to gradual depletion of nutrients such as potassium, sodium and plasma protein required for high metabolic demands of sperm transport through the female genital tract. From Table 6, it was observed that refrigerator temperature (5°C) had significantly (p<0.01) higher motility values compared to room temperature (25°C) throughout the period of the trials. This was because refrigerator temperature help to stop metabolic process of stored liquid semen which resulted in the utilization of nutrients such as fructose by the sperm cells (Aboagle and Terada, 2003). This observation was in line with the reports of Bayemi *et al.* (2010) that refrigerator temperature

recorded the highest viability of stored bull semen with a general motility of 44.50% over the whole experimental period. From Table 7, it was observed that the semen extended in sodium citrate under refrigerator temperature gave the highest sperm motility throughout the trial period. This could be attributed to the cooling effect of refrigerator condition that help to prevent damage of sperm cells by temperature shock and to the fact that sodium citrate based extender maintained the viability of the sperm cells better than others (Lafalci *et al.*, 2002).

CONCLUSION AND RECOMMENDATION

It was concluded that liquid goat semen stored in sodium citrate under refrigerator condition maintained the highest viability of the sperm cells compared to other storage conditions.

It was also concluded that diluted liquid goat semen stored at refrigerator temperature should not be kept beyond 24 h before insemination in order to obtain a good conception rate. Based on the results obtained in this study, it was recommended that sodium citrate should be used for effective storage of bucks semen in order to ensure viability and good conception rate.

It was also recommended that refrigerator temperature should be used as a storage medium.

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