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Short Communications

Correlation between Sperm Parameters in West African Dwarf Goat Bucks during Storage

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ABSTRACT

The relationships among sperm parameters of West African Dwarf (WAD) goat semen were determined. The semen samples were extended in Tris-egg yolk extender and assessed for sperm motility, acrosome and membrane integrities, abnormality and malondialdehyde (MDA) concentrations from 0 to 240 h of storage at 5°C. All the parameters were found to have highly significant correlations (positive/negative) except between motility and MDA concentration as well as abnormality and MDA concentration. This study suggests that a significant correlation exists among the sperm parameters of WAD goat bucks and the semen stored at 5°C up to 48 h is suitable for artificial insemination beyond which deterioration to sperm viability occurred.

Keywords: Bucks, relationships, sperm quality, storage

INTRODUCTION

West African Dwarf (WAD) goats (*Capra hircus*) are found in the South-Western part of Nigeria. The demand for animal protein in the sub region is constantly high; hence, there are prospects for increasing the productivity of this breed of goat Artificial insemination (AI) to improve this breed presents a great potential to alleviate the problem of protein malnutrition in the region. Holt et al. (2007) stated that success of AI depends on the quality of its semen and its potential for fertilisation (as cited in Sharma et al., 2012).

The fertility potential of frozen or chilled semen is made on the basis of fertility rate in

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females mated, this notwithstanding, semen evaluation provides predictive information on expected performance of the male and insights into the fertilising capacity of the preserved spermatozoa (Januskauskas & Zilinskas, 2002). There is a link between fertility of semen in AI and its measurable parameters (Januskauskas & Zilinskas, 2002). Evaluation tests measuring the physiological and cytological parameters of spermatozoa *in-vitro* such as sperm viability, progressive motility, hypo-osmotic swelling tests, acrosome integrity and morphological abnormalities when carried out reduce the economic and time constraints in field conditions. Knowledge on the value of any one of these parameters could provide a fairly good and adequate prediction of the other. Ho (2006) reported that correlation of two variables is often used to predict the score of one on the other (as cited in Sharma et al., 2012). Adequate base-line information on the evaluation of semen for breeding purposes in WAD goats will be a valuable diagnostic tool to assess the fertility status of these bucks. There is little or no information in literature on the semen evaluation parameters as a valuable diagnostic tool to predict the quality of spermatozoa obtained from WAD goats. This study, therefore, investigated the correlation between sperm progressive motility, acrosome integrity, membrane integrity, percentage abnormality and malondialdehyde concentration of post-chilled spermatozoa obtained from WAD goat bucks.

MATERIALS AND METHODS

Animals and management

The WAD goat bucks used in this experiment were raised at the Goat unit of Teaching and Research Farm of Federal University of Agriculture Abeokuta, Nigeria. The University is located in south-western Nigeria with a prevailing tropical climate (mean annual rainfall of 1,037 mm, mean relative humidity of 82% and average temperature of 34.7 °C, latitude 7° 10' N and longitude 3° 2' E, and altitude 76 m above sea level). The bucks were aged between four and 5 and weighed 18 kg on average. The animals were managed intensively and fed (crude protein = 16 %, fat = 5%) at 300g/ body weight, supplemented with guinea grass (*Panicum maximum*) and fresh water *ad libitum*.

Semen collection, dilution and storage

Semen was collected from the bucks that had earlier responded well to semen collection using artificial vagina (AV). The semen samples collected from six bucks showing >80 % sperm motility were pooled to reduce individual differences. A Tris-egg yolk based extender [Tris [$OHCO_3$] amiozomethane (2.42 g/L), citric acid (1.36 g/L), glucose (1 g/L), penicillin (0.028 g/L), egg yolk (20% v/v) and pH adjusted to 7.03] was used for this study. The semen samples were diluted with the Tris-egg yolk based extender at room temperature. The diluted semen samples were drawn into vials, sealed and then cooled to 5°C, and maintained at

this temperature in a refrigerator for 240 hours. Following storage, evaluation of semen quality characteristics was carried out at interval of 24 hours.

Semen Evaluation

Subjective Microscopic Sperm Motility. Semen was warmed for 2 minutes in Clifton Water bath (Model: 74178 by Nickel Electro Ltd, Weston-S-Mare Somerset, England) at 37°C to determine its motility, using Celestron PentaView digital microscope (LCD-44348 by RoHS, China) at 400x magnification. Semen sample (5 µL) was placed directly on a warmed microscope slide overlaid with a cover slip. Each semen sample was examined for sperm motility rate from different slides. Ten microscopic fields were examined by three observers simultaneously to observe progressively motile spermatozoa that moved forward in basically a straight line. The mean (each from the three observers) of the 10 successive evaluations was recorded as the final motility score.

Acrosome Integrity. Percentage of spermatozoa with intact acrosome was determined by adding 50 µL of each semen sample to a 500 µL formalin citrate solution (96 mL of 2.9% sodium citrate with 4 mL of 37 % formaldehyde) and mixed carefully. A small drop of the mixture was placed on a glass slide and a total of 200 spermatozoa were counted in different microscopic fields for each sample. Normal apical ridge of spermatozoa was assessed as intact acrosome using Celestron PentaView LCD digital microscope (400x magnification).

Sperm Membrane Integrity. Hypo-osmotic swelling test (HOST) assay was carried out by incubating chilled semen (10 µL) in 100 µL of a 100mOsm hypo-osmotic solution (9 g fructose plus 4.9 g tri-sodium citrate dihydrate mixed with 1000 mL of distilled water) at 37°C for 30 min. Thereafter, 0.1 mL of the mixture was dispersed on a warmed slide overlaid with a with a cover slip and observed under Celestron PentaView LCD digital microscope (400x magnification). A total of 200 spermatozoa were counted on each slide. Spermatozoon that swelled in response to the test is indicative of having an intact plasma membrane and was counted.

Sperm Abnormality. Sperm abnormality was carried out using eosin-nigrosine stain. Semen sample (3 *u*L) was placed on a microscopic slide and 2 *u*L of eosin-nigrosin was dropped on it and dried. The percentage of morphologically abnormal spermatozoa with defects in the head, midpiece and tail were observed under Celestron PentaView LCD digital microscope (400x magnification).

Malondialdehyde concentrations. Malondialdehyde concentration in the stored semen was measured in a thiobarbituric acid reactive substances (TBARS) according to Pipan et al. (2014, pp. 132-133). This was carried out by incubating sperm suspension

(0.1 mL) in 0.1 mL of 150 mM Tris-HCl (pH 7.1) for 20 min at 37°C. Subsequently, 1 mL of 10 % trichloroacetic acid (TCA) and 2 mL of 0.375 % thiobarbituric acid was added and incubated in boiling water for 30 minutes. Thereafter, it was centrifuged for 15 minutes at 3000 g. The absorbance was read using UV spectrophotometer (SW7504 model by Surgifriend Medicals, England) at 532 nm. The concentration of malondialdehyde was calculated as follows: Malondialdehyde (nmol/mL) = AT – AB/1.56 × 10^5; where AT = the absorbance of the semen sample, AB = absorbance of the blank, 1.56 × 10^5 is the molar absorptivity of malondialdehyde.

Statistical analysis. Data were subjected to analysis of variance using general linear model of SAS package. Duncan Multiple Range was used to separate means while Pearson's correlation coefficients were used to determine the relationship between the semen quality characteristics.

RESULTS

The means of sperm parameters for the different hours of cold storage are presented in Table 1. The results of this study showed that period of storage had significant effect on motility, acrosome integrity, membrane integrity and percentage abnormality. The percentage motility, acrosome integrity and membrane integrity decreased with increased storage periods while the percentage of sperm abnormality and malondialdehyde concentration increased in tandem with increased storage periods. The relationships between the various sperm parameters along with their regression equations are shown in tables 2 and 3. All the parameters had significant correlations (positive/ negative) except correlation between motility and malondialdehyde concentration as well as percentage abnormality and malondialdehyde concentration. Motility showed significant positive correlation

Table 1
Sperm parameters evaluated for the different hours of storage at 5°C (Mean ± SD)

Duration (h)	MOT (%)	ACI (%)	MI (%)	ABN (%)	MDA (µmol/L)
0	88.00 ± 6.00^{a}	93.58 ± 0.87^{a}	90.54 ± 1.26^{a}	1.02 ± 0.63^{g}	0.00^{h}
24	69.33 ± 8.08^{b}	92.37 ± 0.12^{a}	84.37 ± 4.37^{b}	2.48 ± 1.40^{f}	0.13 ± 0.11^{g}
48	58.00 ± 6.92^{c}	87.25 ± 1.25^{b}	76.37 ± 5.37^{c}	3.36 ± 1.15^{e}	0.22 ± 0.16^{fg}
72	49.33 ± 8.08^{d}	80.12 ± 4.12^{bc}	71.37 ± 3.96^{cd}	4.10 ± 1.10^{de}	0.28 ± 0.18^{f}
96	41.33 +21.93^{e}	76.50 ± 3.50^{c}	66.25 ± 5.25^{d}	4.63 ± 1.25^{d}	0.31 ± 0.16^{e}
120	36.00 ±31.17^{f}	69.50 ± 5.50^{d}	58.75 ± 6.75^{e}	5.36 ± 1.73^{c}	0.32 ± 0.16^{e}
144	34.66 ± 30.02^{fg}	64.50 ± 7.00^{e}	54.50 ± 5.00^{ef}	5.44 ± 1.69^{c}	0.34 ± 0.17^{de}
168	28.00 ± 24.24^{g}	58.75 ± 6.75^{f}	49.50 ± 5.50^{f}	5.96 ± 1.78^{bc}	0.35 ± 0.18^{d}
192	26.66 ± 23.09^{h}	49.37 ± 9.37^{g}	41.75 ± 6.49^{g}	6.49 ± 1.51^{b}	0.56 ± 0.36^{c}
216	26.67 ± 23.09^{h}	42.75 ± 10.75^{h}	39.87 ± 6.38^{g}	6.86 ± 1.73^{b}	0.62 ± 0.36^{b}
240	10.66 ± 9.23^{i}	36.75 ± 8.75^{i}	33.00 ± 5.50^{h}	8.13 ± 1.29^{a}	0.78 ± 0.53^{a}

^{a,b,c,} ….i Mean values with different superscripts within columns are significantly different at $p<0.05$.
MOT: Motility, ACI: Acrosome Integrity, MI: Membrane Integrity, ABN: Abnormality, MDA: Malondialdehyde

Table 2
Correlation between the evaluated sperm parameters

	MOT (%)	ACI (%)	MI (%)	ABN (%)	MDA (μmol/L)
MOT (%)	1.00000	0.47332**	0.49986**	-0.81133***	-0.03150^{NS}
AI (%)		1.00000	0.98193***	-0.60388***	-0.80019***
MI (%)			1.00000	-0.62657***	-0.75699***
ABN (%)				1.00000	0.12981^{NS}
MDA (μmol/L)					1.00000

** (P<0.01), *** (P<0.001), ^{NS} (not significant), MOT: Motility, ACI: Acrosome Integrity, MI: Membrane Integrity, ABN: Abnormality, MDA: Malondialdehyde

Table 3
Inter-relationship between semen evaluation parameters in WAD bucks

S/No	Relationship between parameters	Correlation coefficient	Regression estimate	Regression equation
1	MOT with ACI	0.47332**	0.3198	y = - 11.758 + 0.796x
2	MOT with MI	0.49986**	0.3865	y = -12.787 +0.914x
3	MOT with ABN	-0.81133***	0.7373	y = 91.816 - 10.049x
4	MOT with MDA	-0.03150^{NS}	0.0215	y = 49.851 – 20.245x
5	ACI with MI	0.98193***	0.9577	y = 5.822 + 1.032x
6	ACI with ABN	-0.60388***	0.4503	y = 96.910 – 5.839x
7	ACI with MDA	-0.8001***	0.6798	y = 87.661 – 54.059x
8	MI with ABN	-0.62657***	0.5153	y = 89.479 – 5.903x
9	MI with MDA	-0.75699***	0.6206	y = 78.154 – 49.13x
10	ABN with MDA	0.12981^{NS}	0.0628	y = 4.069 + 2.313x

** (P<0.01), *** (P<0.001), ^{NS} (Not significant), MOT: Motility, ACI: Acrosome Integrity, MI: Membrane Integrity, ABN: Abnormality, MDA: Malondialdehyde

with acrosome integrity (r=0.47332) and membrane integrity (r=0.49986). Acrosome integrity also showed significant positive correlation with membrane integrity (r=0.98193). However, a negative correlation (P<0.001) was recorded between percentage abnormalities with the following sperm parameters: motility (r=-0.81133), acrosome integrity (r=-0.60388), membrane integrity (r=-0.62657) as well as MDA with acrosome integrity (r=-0.80019) and membrane integrity (r=-0.75699).

DISCUSSION

The assessed viability parameters (motility, acrosome integrity and membrane integrity) were found to deteriorate with time while sperm abnormality and malondialdehyde concentrations increased with time. The survival of ejaculated sperm in seminal plasma alone is limited to few hours (Kheradmand et al., 2006, p. 40). The present study therefore indicated the importance of storage period for the refrigeration of buck sperm. Storage of semen at low temperature

decreases the functional integrity of spermatozoa (Rasul et al., 2001).

Contrary to the positive correlation between sperm motility and membrane integrity observed in this study, Ollero et al. (1998) reported that many sperm cells that exhibit damaged membranes are motile after thawing. Ollero et al. (1998) attributed the inclusion of non-viable sperm cells into the population of motile sperm cells as a possible reason. Similar to other small ruminants, the plasma membrane of buck sperm is rich in polyunsaturated fatty acids, and this makes it susceptible to peroxidative damage (Jones & Mann, 1976). Lipid peroxidation is known to induce membrane damage (Aitken, 1995), and this is evidenced by the positive correlation between sperm motility and membrane integrity observed in this study contrary to negative correlation with sperm motility earlier reported in (Kasimanickam et al., 2006).

The significant correlation among the parameters assessed in this study is consistent with Jeyendran et al. (1984). The results obtained in this study could probably be due to the dependence of sperm motility on compounds transported across membrane of spermatozoa (Jeyendran et al., 1984,). For this reason and in line with Kordan and Strze_ zek (1997), plasma membrane damage due to death or anisosmotic conditions causes a rapid leakage of intracellular adenosine triphosphate (ATP), which is required to maintain sperm motility. The content of ATP has been reported to be highly correlated with progressive sperm motility of fresh and cryopreserved bull semen (Januskauskas & Rodriguez-Martinez, 1995). Moreover, as the production of mitochondrial ATP is a membrane-dependent process, any negative change to the cell membrane could reduce mitochondrial ATP production that could possibly lead to mitochondrial membrane potential (Januskauskas & Rodriguez-Martinez, 1995). In the absence of sufficient energy, spermatozoa are not progressively motile. Therefore, the correlation between the membrane integrity and sperm motility reflected this observation.

When the relationship between motility and percentage of sperm with intact acrosome was evaluated in semen from yearling Hereford or Angus bulls, Bemdtson et al. (1981) reported that post-thawed motility and acrosome integrity constituted distinct features of sperm integrity that varied independently of each other. The results obtained in this study were in agreement with previous work on cattle (Lodhi et al., 2008) and human (Jeyendran et al., 1984), semen. The correlation that was recorded among motility, acrosome integrity and membrane integrity was in line with Brito et al. (2003) and this was expected since they are all related to plasma membrane integrity and possibly because the sperm plasma membrane is a continuous structure covering the head, mid-piece and tail (Karp, 2009). Membrane integrity and the stability of its semi-permeable features are required for viable spermatozoon and this is because if the plasmalemma is intact but functionally unstable, the spermatozoon is not capable of interacting with its environment and thus,

is unable to fertilise (Rodriguez-Martinez, 2007).

Furthermore, the correlation between the membrane integrity and motility may be attributed to the fact that motility is a function of intracellular ATP content (Januskauskas & Rodriguez-Martinez, 1995). Higher correlation between motility and membrane integrity was probably due to the fact that both are determinants of the integrity of the tail membrane. Similarly, a correlation has earlier been established between acrosome intact sperms with percentage of motile sperms (Kirk et al., 2005). Negative correlations of these parameters with morphological abnormalities at various periods of storage have also been reported (Vyas et al., 1992). The significant relationship of the morphological abnormalities established with acrosome integrity in this study could be attributed to the principle of classification of the abnormalities. The relationship with acrosome integrity may be ascribed to the association of acrosome abnormalities with head abnormalities.

Kobayashi et al. (1991) demonstrated that malondialdehyde concentration in spermatozoa was significantly related to the number of non-motile spermatozoa. In the present study, the negative correlation between the malondialdehyde activity and membrane integrity was consistent with the finding of Kobayashi et al. (1991). Increased malondialdehyde activity could represent the pathologic lipid peroxidation of sperm membrane and inhibition of sperm motility.

CONCLUSION

The results indicated positive correlations between motility, acrosome integrity and membrane integrity, while abnormality was negatively correlated with motility, acrosome integrity and membrane integrity. The MDA concentration was negatively correlated with acrosome integrity. The parameters provide fair estimate of sperm quality of WAD goat bucks and the semen stored at 5°C up to 48 hours is suitable for artificial insemination beyond which deterioration of sperm viability is witnessed.

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