SPERMATOZOA QUALITY OF WEST AFRICAN DWARF GOAT SEMEN DILUTED WITH NORMAL SALINE AND STORED AT ROOM TEMPERATURE

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ABSTRACT
This study aimed at evaluating the ability of normal saline as diluent to sustain spermatozoa viability at 24-29℃ over a period of time. Semen was collected from 17 WAD bucks aged 1-3 years old with an average body weight of 12.15±1.51kg, pooled and divided into 5 portions. Four portions were diluted with normal saline at ratio 1:1 (T2), 1:2 (T3), 1:3 (T4) and 1:4 (T5), while undiluted portion (T1) served as control in a completely randomised design. Semen characteristics were determined 2 hours interval at 24-29℃ using standard procedures. Data were analysed using descriptive statistics and ANOVA. Results showed that at 2 hours, Spermatozoa motility in T2, T3, T4 and T5 were significantly higher than control (undiluted) and at 4 hours,T2 and T5 were significantly (P<0.05) higher than T1, T3 and T4. At 6 hours, spermatozoa motility in T2 was significantly (P<0.05) higher than T1, T3, T4 and T5. Acrosome integrity showed that at 8 hours, T1 was significantly lower than other treatments, except T4. Livability showed that at 6 hours, T2, T3, T5 were significantly higher than T1 and T4. At 8 hours, T2 was significantly higher than T1 and T5 though similar to T3 and T4. At 0, 2, 4, 6 and 8 hours, there was no significant differences among the treatments with respect to plasma membrane integrity. West African buck semen diluted with normal saline at ratio of 1:1 – 1:4 (Semen: diluent) at 24-29℃ maintained the highest viability of sperm cells compared to the undiluted semen. In order to ensure good motility and conception, semen diluted with normal saline should not be stored beyond four hours at room temperature.
Keywords: Caprine semen, Semen qualities, Normal saline, Semen diluent

INTRODUCTION

The use of Artificial insemination (AI) in goats can be enhanced with the improvement of diluents and method of storing semen. Increasing need for use of AI in WAD goats has necessitated extension of semen to make AI economically beneficial since semen can be extended to provide an appropriate volume of inseminate with sufficient number of sperm cells to give high fertility without wasting spermatozoa. So it is vital to develop reliable and on farm diluents for goat semen to facilitate the use of AI for goat reproduction in Nigeria since the ejaculates are small in volume with high concentration of spermatozoa (Mann *et al*., 1980) and to understand what factors affect semen quality between the time the semen is collected, suspended in semen extender and inseminated. The advantage of semen dilution includes a maximum use of good quality semen in short supply, reduction in ratio of males to females and valuable sires with low semen quantity can be used for many females. The study was designed to investigate the ability of normal saline diluent to sustain spermatozoa viability at 24 -29℃ room temperture and to determine the best dilution ratio for on-farm artificial insemination.

MATERIALS AND METHODS

The experiment was conducted at the Small Ruminant Unit of Teaching and Research Farm, University of Ibadan, while the analysis of semen was conducted at the Physiology Laboratory of Department of Animal Science of the same institution. Seventeen adult bucks aged 1-3 years old with an average body weight of 12.15±1.51kg were used for the study. Semen was collected using electro ejaculator according to Tingari *et al.*(1986). The semen was harvested into collection tubes in a warm flask and temperature was maintained at 37℃, diluted with normal saline at different ratios of 1:1 (T2), 1:2 (T3), 1:3 (T4) and 1:4 (T5), while unextended portion 1:0 (T1) served as control and were stored at room temperature(24 - 29℃) . The diluted semen was evaluated at zero hour and subsequently at two hours interval until sperm motility drop below 20%. Sperm motility, percentage livability and plasma membrane integrity were

assessed as described in Jimoh and Ewuola (2016). Data collected were subjected to descriptive statistics and one -way analysis of variance procedure of SAS (2011) and means were compared using Duncan's multiple range test of the same software.

RESULTS AND DISCUSSION

Progressive spermatozoa motility of WAD goat semen diluted with normal saline and stored at room temperature is presented in Figure 1. At zero hour, spermatozoa motility in T5 was significantly ($P<0.05$) higher than T2 and T1, but similar to T3 and T4. At 2 hours, spermatozoa motility in T2, T3, T4 and T5 were significantly ($P<0.05$) higher than T1 which served as the control (undiluted). At 4 hours, T2 and T5 were significantly ($P<0.05$) higher than T1, T3 and T4. At 6 hours, spermatozoa motility in T2 was significantly ($P<0.05$) higher than T1, T3, T4 and T5 and at 8 hours, T1 was significantly ($P<0.05$) lower than T2, T3, T4 and T5. Motility is a very important parameter to consider when carrying out on-farm artificial insemination. At collection time, motility of spermatozoa across the treatments was vigorous and progressive. Treatments diluted with normal saline at ratios 1:1 – 1:4 recorded higher motility than the control (undiluted), this could be attributed to dilution. Dilution of semen creates more surface area for sperm cells to move more than when it was not diluted. At 2 hours, treatments diluted with normal saline at ratios 1:1 – 1:4 were significantly higher than the control. This could also be attributed to the ability of the diluent to serve as buffer needed for the metabolic maintenance of the sperm cells and modulate the medium for spermatozoa survival (Peterson *et al.*, 2007; Ude and Oghenesode, 2011). For the success of artificial insemination, motility should not be less than 50%. Treatments diluted with normal saline at 4 hours recorded sperm motility above 50% unlike the control. At 6 hours, treatment diluted with normal saline at ratio 1:1 had significantly higher motility than other treatments. At this period, dilution level of 1:1 still sustained motility of the sperm cells at 60% which is good for the success of any artificial insemination. John *et al.* (2003) rated spermatozoa motility between 50 and 70% as good motility. In the undiluted treatment, motility was 35% at 6 hours, which is contrary to the findings of Ajala *et al*. (2012) who observed the motility of undiluted goat semen to be 0% at 6th hour. At 8 hours, motility of goat semen in the control was significantly lower than T2, T3, T4 and T5. Motility was 5%. From the results, normal saline exhibited good ability to sustain the viability of spermatozoa up to dilution ratio of 1:4. At room temperature (24 –29℃), motility of WAD goat semen diluted with normal saline declined after 4 hours except for T2. This implies that diluted goat semen stored at room temperature condition should not be stored beyond 4 hours for on-farm artificial insemination in order to obtain a good conception rate. This result is in line with the observation of Peterson *et al.* (2007) and Ude and Oghenesode (2011) who observed that the percentage of motile spermatozoa in buck semen stored at room temperature condition progressively declined over time. The decline in spermatozoa motility could be attributed to gradual depletion of nutrients such as sodium and plasma protein required for high metabolic demands. However, best motility was achieved in dilution ratio of 1:1 at 6 hours of storage at room temperature.

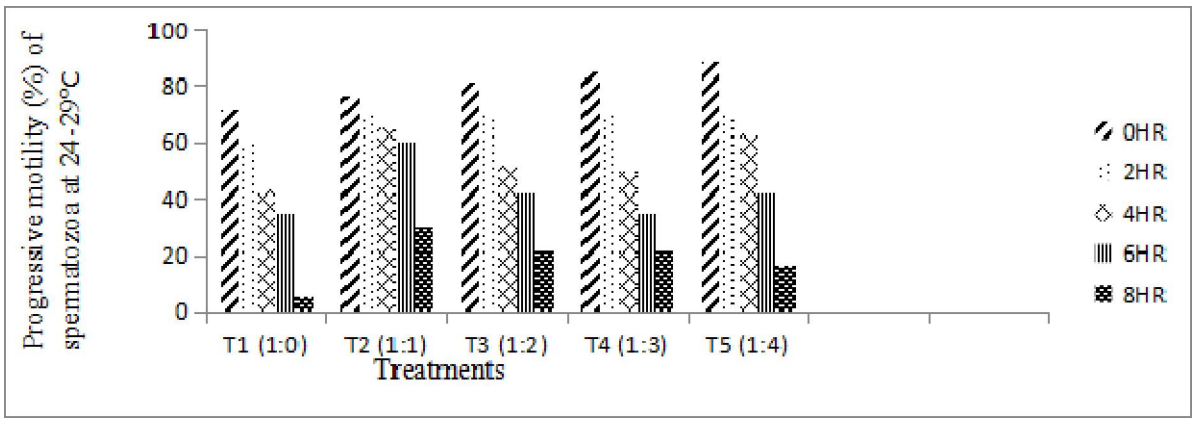

Figure 1: Progressive Motility (%) of West African Dwarf goats (WAD) semen extended with Normal saline stored at room temperature (24 -29℃)

At 0, 2, 4 and 6 hours, there were no significant (P>0.05) differences among the treatments with respect to acrosome integrity (Figure 2). At 8 hours, T1 was significantly (P<0.05) lower thanT2, T3 and T5 but similar to T4. Acrosome integrity was intact across the treatments from time of collection up to 8 hours. However, acrosome integrity declined with increase in storage time at room temperature. This result support the findings of Waberski *et al.* (2011) who reported that storage of semen for longer period causes ultra-structural, biochemical and functional damage to the spermatozoa resulting in a reduction of motility, viability, fertility and impaired acrosome integrity and transport. The changes were attributed to certain sperm abnormalities or exposure to external environmental conditions and adjustments to the sudden changes in the environment resulted in the reduction in sperm quality. Also, reduction in acrosome integrity will result in induced acrosome reaction of greater portion of spermatozoa before reaching the site of fertilization (Waberski *et al.*, 2011) and (El-Mulla *et al.,* 1996).

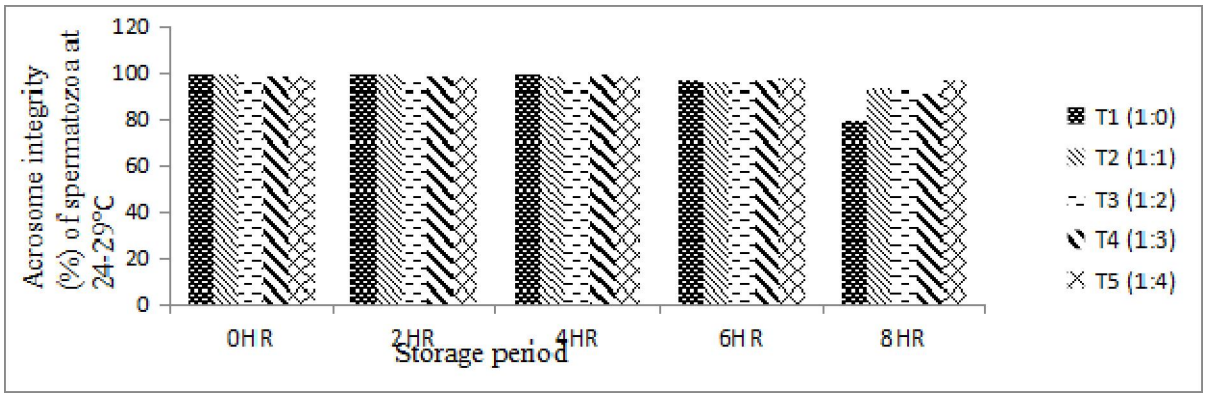

Figure 2. Acrosome integrity (%) of WAD goat semen diluted with normal saline at room temperature (24 -29℃)

At 0, 2 and 4 hours, there was no significant (P>0.05) differences in spermatozoa livability among the treatments (Figure 3). At 6 hours, spermatozoa livability in T2, T3 and T5 were significantly (P<0.05) higher than T1 and T4. At 8 hours, T2 was significantly (P<0.05) higher than T1 and T5, however, it was similar to T3 and T4. Normal saline as a diluent sustained the livability of the spermatozoa up to dilution ratio of 1:4, however, beyond 6 hours, livability declined across the treatments and this could be attributed to gradual depletion of nutrients required for high metabolic demands of sperm transport and survival (Peterson *et al.*, 2007; Ude and Oghenesode, 2011). Reduction in spermatozoa livability will result in availability of small proportion of live spermatozoa for fertilization (El-Mulla *et al*, 1996).

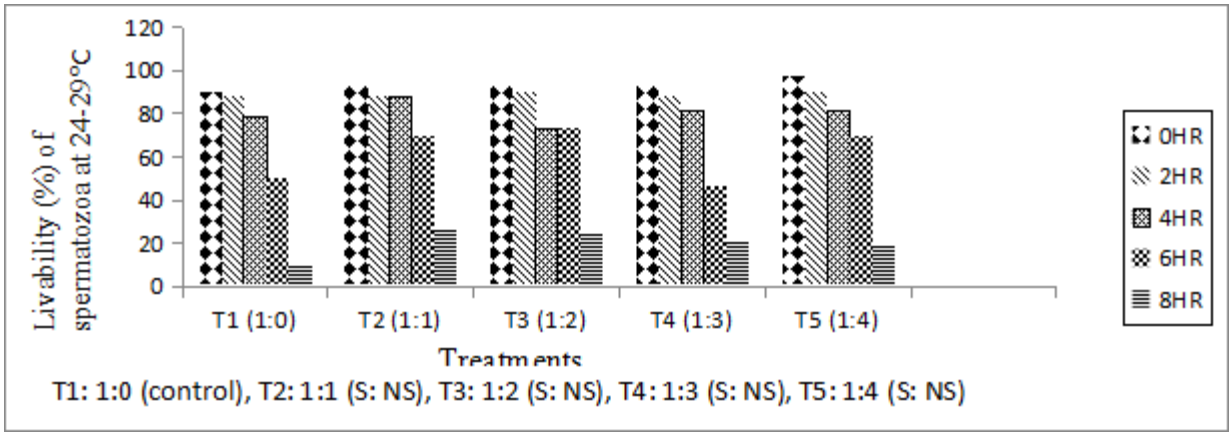

Figure 3. Livability (%) of WAD goat semen diluted with normal saline at room temperature (24 -29℃)

At 0 and 2 hour, there were no significant (P>0.05) differences in plasma membrane integrity among the treatments (Figure 4). The values ranged from 63.80±7.38% in control (T1) to 73.10±4.10% in T3. At 4, 6 and 8 hours, Plasma membrane integrity was not significantly (P>0.05) different among the treatments. Plasma membrane integrity level is a good indicator to guarantee fertility. Given that the hypo-osmotic condition that spermatozoa were subjected to is considered to be a stressful factor, it could be inferred that the response of buck spermatozoa to the hypo osmotic swelling test could be a good indicator of the reproductive ability of the buck. This test can be carried out in order to obtain an improved evaluation of spermatozoa. The range of plasma membrane integrity observed in this study indicated high fertilizing ability of the ejaculate. 60% and above has been recommended to be the desirable plasma membrane integrity required to guarantee fertility (John *et al*., 2003). From 0 – 4 hours, plasma membrane integrity were intact and suitable to initiate fertility after which it declined below 60%.

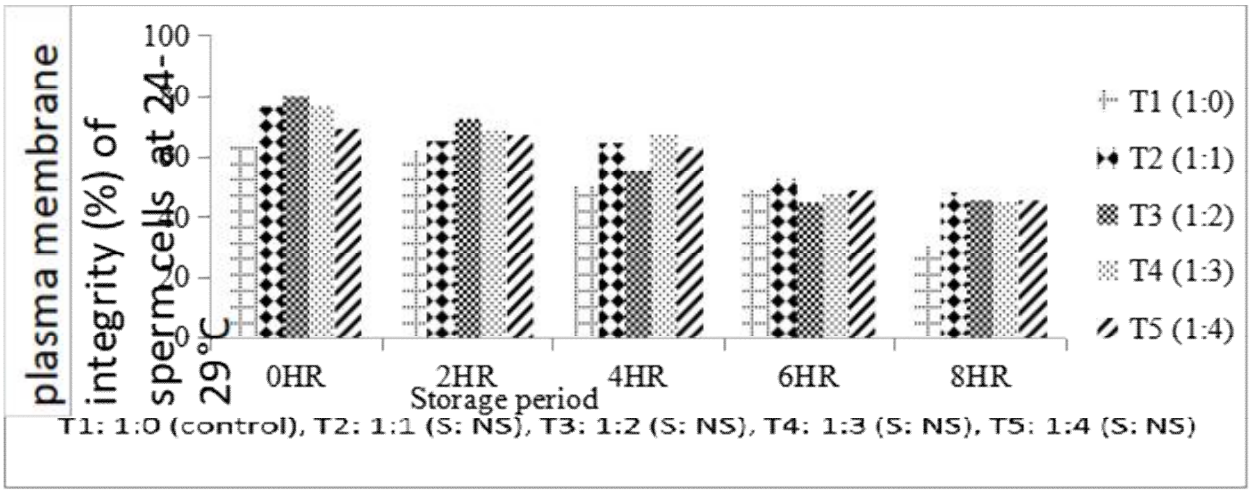

Figure 4. Plasma membrane integrity (%) of WAD goat semen diluted with normal saline at room temperature (24 -29℃)

CONCLUSION AND RECOMMENDATIONS

Spermatozoa motility, acrosome integrity, plasma membrane integrity and livability of sperm cells in normal saline diluted semen were adequately maintained and improved spermatozoa viability at room temperature up to four hours of storage at ambient temperature. Goat semen diluted in normal saline at ratio of 1:1 – 1:4 under room temperature are therefore recommended for artificial insemination within four hours.

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