APRW -59

Spermatozoa Quality in Refrigerated Normal Saline Diluted Caprine Semen

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Abstract
This study aimed at evaluating the ability of normal saline diluent to sustain spermatozoa viability over a period of time at refrigerated temperature (4-6°C). Semen was collected from 17 West African Dwarf (WAD) bucks, 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 unextended portion (T1) served as a control in a completely randomised design. Spermatozoa characteristics were examined 2 hours interval at 4-6°C and Data were analysed using descriptive statistics and ANOVA. Results showed that at 0 hour, spermatozoa motility was significantly ($p<0.05$) higher in T1, T3 and T4 than T5, although similar to T2. At 6 hours, T2, T4 and T5 were significantly ($p<0.05$) higher than T1, but similar to T3. At 8 hours, the motility in T1 was significantly ($p<0.05$) reduced than in other treatments. Spermatozoa livability and acrosome integrity at 0, 2, 4 and 10 hours were not significantly different among the treatments. Plasma membrane integrity at 4 and 8 hours, T3 was significantly ($p<0.05$) lower than T2, however it was similar to T1, T4 and T5. At 6 hours, plasma membrane integrity of sperm cells in T1 was significantly ($p<0.05$) lower than T2, T3, T4 and T5. Percentage normal sperm cells at 0 and 6 hours was significantly ($p<0.05$) lower in T4 than T1, T2 and T3, but similar to T5 and T3 at 6th hour. This suggests that quality of the sperm cells was sustained up to 8 hours in normal saline diluted semen when refrigerated.

Keywords: Semen qualities, normal saline, goat semen extension

Introduction

Increasing need for use of artificial insemination (AI) in West African dwarf (WAD) goats has necessitated extension or dilution of semen and development of reliable on-farm goat semen diluents to make A1 economically beneficial. In order to satisfy the demand for animal products by the growing human population, it becomes necessary to adopt new techniques like artificial insemination (AI) that will increase the reproductive efficiency of the few superior individuals selected as a producer of the next generation (Baillargeon, 2000). The semen to be used for AI requires diluent to increase the volume to be able to serve multiple females. The advantages of semen dilution include a reduction in ratio of males to females and valuable sires with low semen quantity can be used for many females.

Keeping this in view, the present study was designed to explore the possibility of investigating the ability of cheap diluent like normal saline which is readily available to sustain the viability of caprine spermatozoa at refrigeration temperature for on-farm AI.

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, University of Ibadan, Ibadan, Nigeria. Seventeen adult bucks aged 1-3 years old with an average body weight of 12.15 ± 1.51 kg were used for the study. Semen was collected using electro ejaculator (Tingari *et al.,* 1986). The semen was collected into a collecting tube in a warm flask and temperature was maintained at 37°C , 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 4 - 6°C. 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).

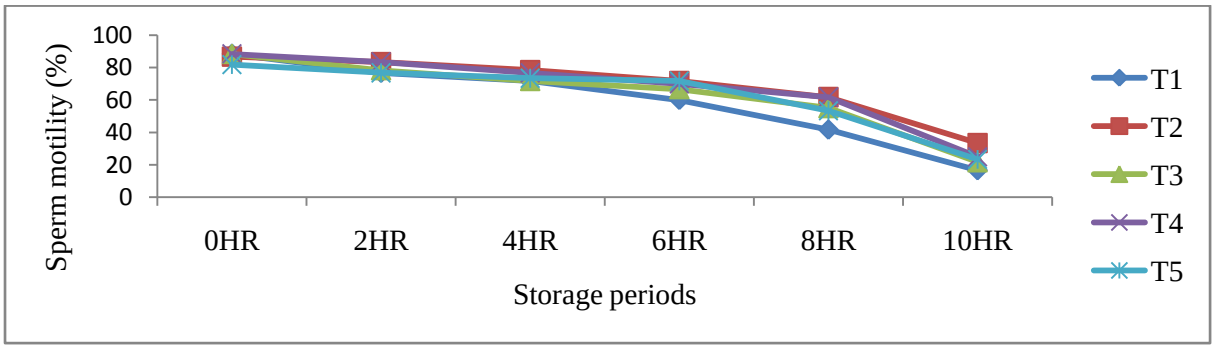
Data collected were subjected to descriptive statistics and one -way analysis of variance procedure of SAS (2011) and means were compared using Duncan's multiple range test of the same software.

Results and Discussion

Progressive motility of WAD goat semen extended with normal saline stored at refrigerated temperature is presented in figure I. At zero hour, spermatozoa motility was significantly ($p<0.05$) higher in T1, T3 and T4 than T5, but similar to T2. At 6 hours, spermatozoa motility in T2, T4 and T5 were significantly ($p<0.05$) higher

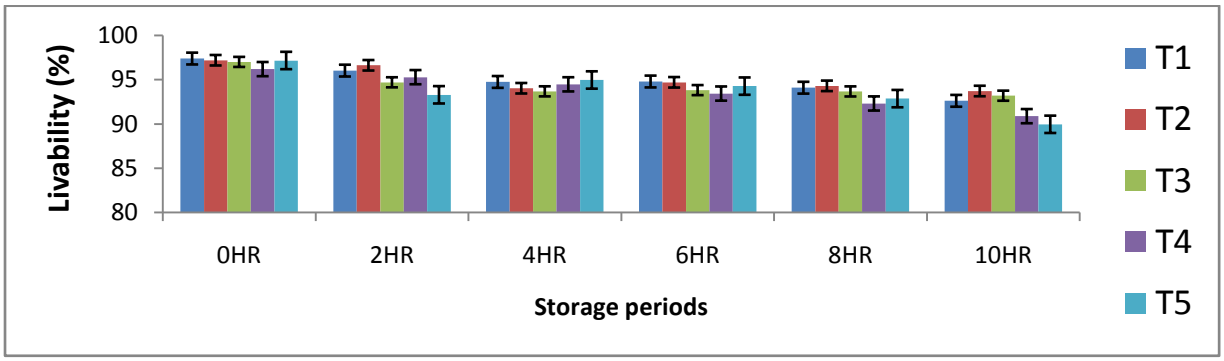
than T1, but similar to T3. At 8 hours, T2 and T4 had higher sperm motility than treatment 5, but similar to T4, while sperm motility in treatment 1 was significantly ($p<0.05$) reduced than in other treatments. From collection time to 8 hours, motility of diluted semen was significantly high. This is because refrigeration helped to slow down the metabolic process in stored goat semen which resulted in the reduced utilisation of nutrients such as fructose by the sperm cells, thereby sustaining the cells for a longer period (Aboagle and Terade, 2003). This report supports Ude and Oghenesode (2011), Bayemi *et al*. (2010) observed that refrigeration at 4-6°C recorded the highest viability of stored bull semen with a general motility of 44.5% over the whole experimental period.

The livability of spermatozoa of goat semen extended with normal saline at 4-6°C is presented in Figure II. The livability of spermatozoa at 0, 2, 4, 8 and 10 hours were not significantly different across the treatments. At 6 hours, spermatozoa livability in T1, T2, T3 and T5 were significantly ($p<0.05$) higher than T4. The acrosome integrity of spermatozoa in normal saline diluted semen at 4-6°C is presented in Figure III. At 0, 2, 4 and 10 hours, acrosome integrity was not significantly ($p>0.05$) different across the treatments. At 8 hours, T2 was significantly ($p<0.05$) higher than T3 and T5, but similar to T1 and T4.



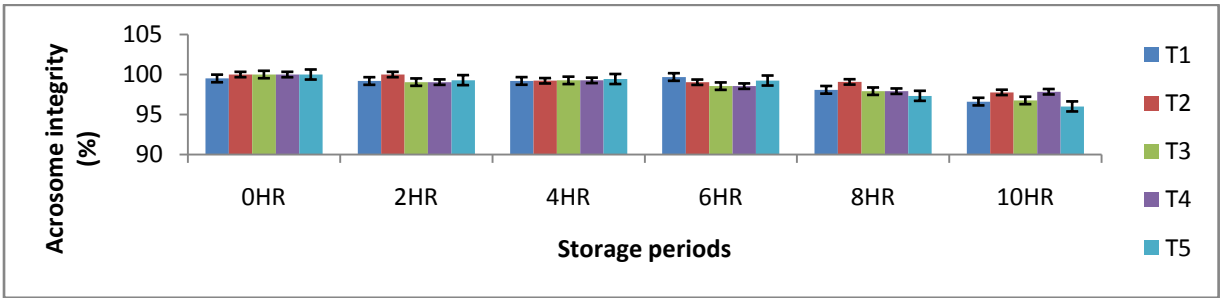
T1: 1:0 (control), T2: 1:1 (S: NS), T3: 1:2 (S: NS), T4: 1:3 (S: NS), T5: 1:4 (S: NS) where S: semen, NS: normal saline

Fig. I. Progressive spermatozoa motility (%) of goat semen extended with normal saline at refrigerator temperature (4 -6°C)



T1: 1:0 (control), T2: 1:1 (S: NS), T3: 1:2 (S: NS), T4: 1:3 (S: NS), T5: 1:4 (S: NS) where S: semen, NS: normal saline

Fig. II. Spermatozoa livability (%) of WAD goat semen diluted with Normal saline stored at refrigerator temperature (4-6°C)

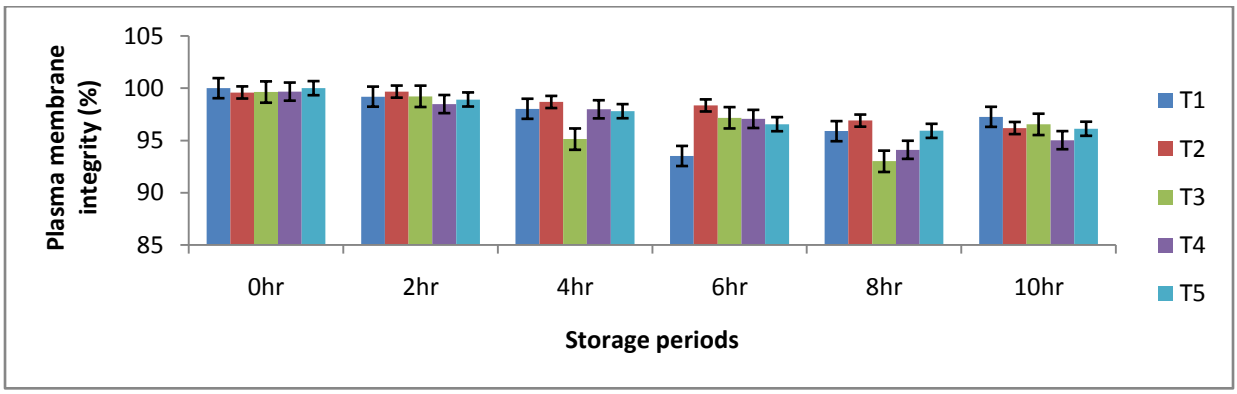


T1: 1:0 (control), T2: 1:1 (S: NS), T3: 1:2 (S: NS), T4: 1:3 (S: NS), T5: 1:4 (S: NS) where S: semen, NS: normal saline

Fig. III: Acrosome integrity (%) of WAD goat semen extended with Normal saline stored at refrigerator temperature (4-6°C)

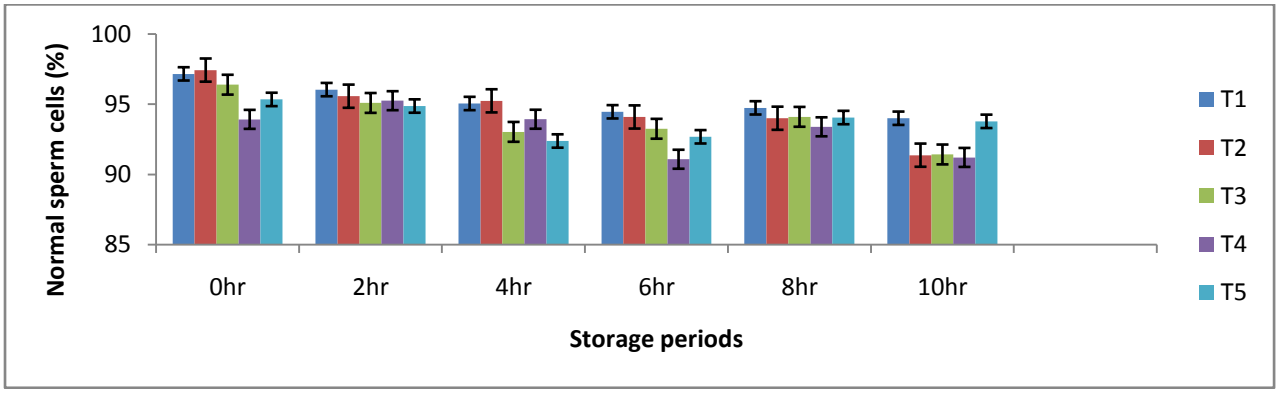
Plasma membrane integrity of goat spermatozoa diluted with normal saline at 4-6°C is shown in Figure IV. Plasma membrane integrity of spermatozoa at 0, 2 and 10 hours were not significantly ($p>0.05$) different. However, at 4 and 8 hours, plasma membrane integrity in T3 was significantly ($p<0.05$) lower than T2 which was similar to T1, T4 and T5. At 6 hours, plasma membrane integrity of T1 was significantly ($P<0.05$) lower than other treatments. However, treatments 2, 3, 4 and 5 were not significantly different from one another.

Percentage of normal sperm cells in normal saline diluted caprine semen at 4-6°C is shown in Figure V. Results on the percentage of normal sperm cells showed that at 0 hour, T4 was significantly ($p<0.05$) lower than other treatments except T5. At 2 hours, there were no significant differences among the treatments. At 4 hours, T5 was significantly ($p<0.05$) lower than T1 and T2, but not significantly different from T3 and T4. At 6 hours, percent of normal sperm cells in T4 was significantly ($p<0.05$) lower than T1 and T2. However, T4 was not significantly ($p>0.05$) different from T3 and T5. At 8 hours, all the treatments were similar and at 10 hours, T2, T3 and T4 were significantly ($p<0.05$) lower than T1, but similar to T5. Based on the report of John *et al*. (2003) that high-quality semen contains a minimal number of 5 to 15% of abnormal spermatozoa whereas low-quality semen frequently contains a large number (30% or more) of morphologically abnormal spermatozoa.



T1: 1:0 (control), T2: 1:1 (S: NS), T3: 1:2 (S: NS), T4: 1:3 (S: NS), T5: 1:4 (S: NS) Where S: Semen, NS: Normal saline

Fig.IV: Plasma membrane integrity (%) of WAD goat semen diluted with normal saline at refrigerator temperature (4-6°C)



T1: 1:0 (control), T2: 1:1 (S: NS), T3: 1:2 (S: NS), T4: 1:3 (S: NS), T5: 1:4 (S: NS) Where S: Semen, NS: Normal saline

Fig. V: Normal sperm cells (%) in WAD goat semen diluted with normal saline at refrigerator temperature (4-6°C)

Conclusion and recommendations

Motility, acrosome integrity, plasma membrane integrity, livability, percentage of normal sperm cells in caprine semen diluted with normal saline was not adversely affected at 4-6°C. In order to ensure good motility, semen diluted with normal saline should not be kept beyond 8 hours at 4-6°C for artificial insemination

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