Nig. J. Anim. Prod. 2018, 45(3): 115 - 120
Nigerian Journal of Animal Production

Viability of caprine spermatozoa in sodium citrate-egg yolk extender at room temperature

*Ewuola, E. O., Ihejirika, U. D. G. and Adepoju, T. F.
Animal Physiology and Bioclimatology, Department of Animal Science, Faculty of Agriculture, University of Ibadan, Ibadan, Oyo State, Nigeria.
Animal Physiology and Bioclimatology, Department of Animal Science, Faculty of Agriculture, University of Ibadan, Ibadan, Oyo State, Nigeria.
*Corresponding authors email: eoewuola@gmail.com, giftihejirika2017@gmail.com, tfactor10@gmail.com +2348063967195

Abstract

This work was aimed at evaluating the ability of sodium citrate-egg yolk extender to sustain the viability of spermatozoa in semen stored at room temperature over a period of time as well as determining the best dilution ratio for on-farm artificial insemination. Seven matured West African dwarf bucks were used for this study; semen was collected once weekly with electro ejaculator, maintained at 37°C in the water bath during extension and was evaluated immediately after extension at room temperature until motility dropped below 20%. The semen collected were pooled and divided into 5 portions. Each portion was extended with sodium citrate-egg yolk extender at 1:0 (without extender, control) as T1, (1:1) T2, (1:2) T3, (1:3) T4 and the 5th portion was extended with normal saline at 1:1 (T5) which served as the negative control. Sperm motility, spermatozoa concentration, livability, acrosome integrity and morphological abnormalities were assessed and data obtained were subjected to analysis of variance. Results showed that spermatozoa motility in treatments 1, 2, and 5 differed significantly (P<0.05) from treatments 3 and 4 at 0 hour assessment. At two hours, sperm motility was 0% in T3 and T4, while sperm motility in T2 (81.67%) was not significantly different from T5 (76.67%) and T1 (86.67%). Percentage of normal sperm cells was significantly (P<0.05) lower in T4 than treatments 1, 2, 3 and 5. At four hours of assessment, sperm motility in T1 and T5 were significantly (P<0.05) higher than T2, while livability was significantly higher in T2 than T4 but was similar to T1, T3 and T5. There was also significant (P<0.05) reduction in livability as the extension ratio increases among treatments. Sodium citrate-egg yolk extender was able to preserve the viability of spermatozoa up to two hours at 1:1 extension ratio at room temperature. This study suggests that the dilution ratio of 1:1 is recommended for on-farm artificial insemination in goats when sodium citrate-egg yolk extender is employed at room temperature.

Keywords: West African dwarf buck, Sperm viability, Sodium citrate-egg yolk extender, Semen extension

Introduction

West African dwarf goats are important in the rural village economy of West Africa and are trypanotolerant and haemonchotolerant, they resist infections and gastrointestinal parasites such as nematode *Haemonchus contortus* more effectively than other breeds of domestic goat (Chiejina *et al.,* 2015). However, in rural areas, farmers usually have a small number of goats per household, thus having limited access to a high quality sire or even no sire at all. Thus result in reduced or zero breeding of goats in such environments, thus the use of artificial insemination to improve their breeding efficiency and population. Beneficial use of superior germ cells can be made only when there is

extension of life of germ cells for longer periods, maintenance of motility of spermatozoa and increase in number of doses per ejaculate of semen (Etches, 1996; Leboeuf *et al.*, 2000). Significant temperature changes are considered detrimental to semen quality and under practical conditions, fluctuations in temperature may be easier to avoid at higher storage temperature than at 4°C (Lafalci *et al*., 2002). The extent to which the quality of extended semen can be sustained under ambient temperature for on-farm artificial insemination in goats has not been adequately documented. 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). Therefore, it is vital to develop reliable extenders for goat semen to facilitate the use of artificial insemination for goat reproduction in Nigeria. Thus, this study is aimed at investigating the length of time sperm cells can survive under ambient temperature when extended with sodium citrate-egg yolk and evaluate the optimum dilution ratio that can be used for on-farm artificial insemination.

Materials and methods

This study was conducted at the goat unit of the Teaching and Research Farm, University of Ibadan located at latitude 7° 26 North and latitude 3°54 East, at a mean altitude of 277 meters above sea level.

Experimental animals and management

Seven matured West African dwarf goats were used for this experiment. The bucks were about 1- $1^{1}/_{2}$ years old with an average body weight of 12.15±1.51kg. Prior to the arrival of the bucks, the pen was thoroughly swept and cleaned. The pen was also disinfected with antiseptics. Wood shavings were spread on the floor to serve as soft bedding for the bucks and to protect them from the cold floor at night. They were semi-intensively managed in clean, well-ventilated pen. The goats were fed 60% concentrate containing 12.60% crude protein, 10.0% crude fibre and 3202.28 kcal/kg digestible energy and 40% guinea grass and *Gliricidia sepium*. Water was provided regularly. The experiment lasted for a period of six (6) weeks in a completely randomised design.

Composition of sodium citrate-egg yolk extender

The extender comprised of 100mL of distilled water, 58mg of glucose, 5gm of sodium citrate, 20 mL of egg yolk, 1mg of penicillin G in 100mL and 1mg of streptomycin per mL as was reported by Ewuola *et al*. (2014).

Semen collection and evaluation

Semen was collected using electro ejaculator (Tingari *et al.*, 1986). The semen was collected and pooled into a collecting tube in a warm flask and temperature maintained at 37

Table 1: Goat semen characteristics at collection (0 hour) under room temperature

Parameter	T1	T2	T3	T4	T5
Sperm motility (%)	91.67±2.89^{a}	91.67±2.89^{a}	81.67±2.89^{b}	80.00±0.00^{b}	91.67±2.89^{a}
Sperm concentration (x10^{6}/mL)	305.81±23.47^{a}	265.81±106.90^{a}	262.4±84.40^{ab}	192.43±26.37^{ab}	143.12±68.86^{ab}
Livability (%)	98.35±1.50	89.44±13.98	93.20±1.56	89.83±1.76	94.39±2.25
Normal sperm cells (%)	97.35±0.26^{a}	96.99±2.71^{ab}	94.00±1.51^{bc}	97.91±1.81^{a}	93.33±1.07^{c}

a, b - Means within the same row with different superscripts are significantly different (P<0.05),
T1 = Undiluted semen, T2 = 1:1, T3 = 1:2, T4 = 1:3, T5 = Negative control.

The characteristics of extended caprine semen at different dilution ratio two hours after extension under room temperature are presented in Table 2. It was observed that sperm motility, livability and normal sperm cells were significantly (P<0.05) influenced by the dilution ratio. The mean values obtained for sperm motility in treatment 1 (86.67±5.77%) was significantly (P<0.05) higher than treatments 3 (0.00±0.00%), 4 (0.00±0.00%) and 5 (76.67±5.77%), although similar to treatment 2 (81.67±2.89%). The percentage of normal sperm cells in T4 (76.49±11.42) was significantly (P<0.05) lower than T1 (95.37±2.03), T2 (95.22±2.87), T3 (93.01±7.15) and T5 (89.66±2.84%).

Table 2: Extended goat semen characteristics at two hours of storage under room temperature

Parameters	T1	T2	T3	T4	T5
Sperm motility (%)	86.67±5.77^{a}	81.67±2.89^{ab}	0.00±0.00	0.00±0.00	76.67±5.77^{b}
Livability (%)	95.78±4.36^{ab}	93.77±1.36^{a}	72.22±25.46^{ab}	69.33±7.24^{b}	75.64±5.12^{ab}
Normal sperm cells (%)	95.37±2.03^{a}	95.22±2.87^{a}	93.01±7.15^{a}	76.49±11.42^{b}	89.66±2.84^{a}

a, b - Means within the same row with different superscripts are significantly different (P<0.05)
T1 = Undiluted semen, T2 = 1:1, T3 = 1:2, T4 = 1:3, T5 = Negative control.

The effect of semen extension on the viability of caprine semen at four hours after extension under room temperature is presented in Table 3. It was observed that sperm motility in T2 (26.67±5.77%) was significantly (P<0.05) lower than sperm motility in T1 (53.33±11.55) and T5 (66.67±15.28%). Livability of sperm cells in T4 (44.17±30.17) was significantly (P<0.05) lower than T1 (82.72±8.67), T2 (89.28±1.06) and T5 (82.50±0.77), although T3 (62.63±7.00) and T4 (44.17±30.17) were statistically similar. There was no significant (P>0.05) difference in the percentage normal sperm cells across all the treatments

Table 3: Extended goat semen characteristics at four hours under room temperature

Parameter (%)	T1	T2	T3	T4	T5
Sperm motility	53.33±11.55^{a}	26.67±5.77^{b}	0.00±0.00	0.00±0.00	66.67±15.28^{a}
Livability	82.72±8.67^{ab}	89.28±1.06^{a}	62.63±7.00^{bc}	44.17±30.17^{c}	82.50±0.77^{ab}
Normal sperm cells	95.82±0.29	95.74±0.46	91.82±10.48	94.08±6.28	91.20±3.11

a, b, c - Means within the same row with different superscripts are significantly different (P<0.05)
T1 = Undiluted semen, T2 = 1:1, T3 = 1:2, T4 = 1:3, T5 = Negative control.

The characteristics of extended caprine semen at different dilution ratio six hours after extension under room temperature are presented in Table 4. The mean values obtained for sperm motility in treatments 1 (45.0±7.07%) and 5 (46.67±5.77%) were significantly (P<0.05) higher than treatments 2 (0.00±0.00), 3 (0.00±0.00) and 4 (0.00±0.00%) whose motility had dropped to zero. Livability of sperm cells in T4 (71.30±7.34%) was significantly lower than T1 (89.85±3.22) and T5 (86.87±7.07), but similar to T2 (76.47±9.94) and T3 (78.98±4.89), while the percentage of normal sperm cells ranged from T5 (93.33±11.55) to T4 (97.74±2.03).

Table 4: Extended goat semen characteristics at six hours after collection under room temperature

Parameter (%)	T1	T2	T3	T4	T5
Sperm Motility	45.0±7.07^{a}	0.00±0.00	0.00±0.00	0.00±0.00	46.67±5.77^{a}
Livability	89.85±3.22^{a}	76.47±9.94^{bc}	78.98±4.89^{abc}	71.30±7.34^{c}	86.87±7.07^{ab}
Normal sperm cells	94.43±4.84	95.83±7.22	96.87±3.13	97.74±2.03	93.33±11.55

a, b, c - Means within the same row with different superscripts are significantly different (P<0.05)
T1 = Undiluted semen, T2 = 1:1, T3 = 1:2, T4 = 1:3, T5 = Negative control.

Discussion

At room temperature (24 – 29^{o}C motility of WAD goat semen extended with sodium citrate-egg yolk at ratio of 1:1 preserved goat semen up to 2 hours before the sperm motility dropped to 26.67%. This implies that extended 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 was in line with the observation of Peterson *et al.* (2007), Ude and Oghenesode (2011) on progressive decline in percentage of motile spermatozoa of buck semen stored at room temperature condition over time. This study showed that the use of sodium citrate–egg yolk (SCEY) and normal saline as extenders for goat semen at a dilution ratio of 1:1 compared favourably with the control (undiluted semen) at collection (zero hour) and two hours post collection. It was observed that the higher the ratio of extender to semen, the lower the sperm motility as depicted in treatments 2 to 4. This corroborated the findings of Karabinus *et al.* (1991) that sperm motility reduced when egg yolk-citrate was used as extender in bull semen. The decrease in sperm concentration could be attributed to the increase in extension ratio in treatments 2, 3, and 4 since the same semen volume was used for the treatments. At 2 and 4 hours, semen extended with sodium citrate-egg yolk at ratios 1:2 and 1:3 had 0% motility. Also at 6 hours, semen extended with sodium citrate-egg yolk at ratios 1:1, 1:2 and 1:3 had 0% motility. Extension ratio beyond 1:1 is not recommended if egg yolk is employed as the component of extender and this observation was similar to Pellicer–Rubio and Combarnous (1998) that certain enzymes in the seminal plasma originating from the bulbourethral gland secretion probably catalysis the hydrolysation of lecithin and triglyceride of the egg yolk extender releasing sperm-toxic products (lysolecithin and fatty acids) thus led to subsequent spermatozoa deterioration (Nunes *et al.*, 1982; Pellicer – Rubio and Combarnous, 1998). The consistence in the high proportion of normal sperm cells with time across the treatments indicates that the presence of egg preserves the morphology of the sperm cells. This is in accordance with the findings of Valéria *et al.* (2014) that extender containing higher egg yolk provided better result. Egg yolk-based extenders have been widely used for dilution and cryopreservation of semen from farm animals (Aboagla and Terada, 2004). Furthermore, it has been reported that the addition of egg yolk affects the acrosome integrity and the post-thawing viability of ejaculated spermatozoa in goats (Aboagla and Terada, 2004), rams (Watson and Martin, 1975) and water buffaloes (Kumar *et al*., 1993). The potential cause of the decline in motility and fertility during hypothermic storage of liquid semen is an oxidative damage of spermatozoa (Ball *et al.*, 2001).

Conclusion and recommendations

Based on the results obtained from this experiment, it can be concluded that goat semen extended in sodium citrate-egg yolk extender under ambient condition at extension ratio of 1:1 maintained the highest viability of sperm cells and can be

recommended for on-farm artificial insemination to achieve good conception rate. In addition, extended caprine semen for on-farm artificial insemination should be used up within 2 hours post extension with sodium citrate-egg yolk extender under ambient condition.

References

Aboagla, E. M. and Terada, T. 2004. Effects of egg yolk during the freezing step preservative onthe viability of goat spermatozoa. *Theriogenology*, 62:1160-1172.

Ball, B. A., Medina, V., Gravance, C. G. and Baumber, J. 2001. Effect of antioxidants on preservation of motility, viability and acrosomal integrity of equine spermatozoa during storage at 5°C *Theriogenology* 56: 577-589.

Chiejina, S. N., Behnke, J. M., Fakae, B. B. 2015. "Haemonchotolerance in West African Dwarf goats: contribution to sustainable, anthelmintics-free helminth control in traditionally managed Nigerian dwarf goats"

Duncan, D. B. 1955. Multiple range and multiple F-test. *Biometrics*, 11: 1-42.

Etches, R. J. 1996. Reproduction in Poultry. *CAB International*, Wallingford, UK, pp: 241-250.

Eva-Marie, S.E., Johnson, L. and Philipson, J. 1989. Genetic studies on fertility in AI bulls. 1. Age, season

Ewuola, E. O. and Egbunike, G. N. 2010. Effects of dietary fumonisn B1 on the onset of puberty, semen quality, fertility rates and testicular morphology in male rabbits. *Reproduction 139: 439 – 445*

Ewuola, E. O., Lawanson, A. A. and Adeyemi, A. A. 2014. An improvised artificial vagina for rabbit semen collection and the characteristics of the extended rabbit semen as panacea for artificial insemination. Trop. Anim. Prod. Invest. 17(1):19-24

Karabinus, D. S., Evenson, D. P. and Kaproth, M. T. 1991. Effects of egg yolk citrate and milk extenders on chromatin structure and viability of cryopreserved bull sperm. *J. Dairy Sci*. 74, 3836–3848.

Kumar, S., Sahni, K. L. and Mohan, G. 1993. Effect of different extender formulations on acrosomal maintenance of buffalo spermatozoa frozen in milk, Tris, and sodium-citrate dilutors. *Ind. J. Anim. Sci., 63: 1233-1239*

Lafalci, V. S. H., Tortorella, J. L., Rodrigues, A. and Brandelli, B. 2002. Seasonal variation of goat seminal plasma proteins. *Theriogenol*., 57(3), 1033-1048.

Leboeuf, B., Restall, B. and Salamon, S. 2000. Production and storage of goat semen for artificial insemination. *Animal reproduction science* 62. 113-141

Nunes, J. F., Corteel, J. M., Combarnous, Y. and Baril, G. 1982. Role du plasma seminal dans la survie *in vitro* des spermatozo¨ıds de bouc. *Reprod. Nutr. Dev.* 22: 611–620.

Pellicer-Rubio, M. T. and Combarnous, Y. 1998. Deterioration of goat spermatozoa in skimmed milk based extenders as a result of oleic acid released by the bulbourethral lipase BUSgp60. *J. Reprod. Fert*., 112(1): 95-105.

Peterson, K., Kappen, M. A. P. N., Ursem, P. J. F., Nothling, J. O.,

Colenbrander, B. and Gadella, B. M. 2007. Microscopic and flow cytometric semen assessment of Dutch AI-bucks: Effect of semen processing procedures and their correlation to fertility. *Theriogenol.*, 67: 863-871.

SAS. 1999. SAS /STAT User's Guide Version 8 for windows SAS Institute Inc. cary NC

Tingari, M. D., El-Manna, M. M., Rahim, A. T. A., Ahmed, A. K. and Hamed, M. H. 1986. Studies on camel semen. I. Electroejaculation and some aspects of semen characteristics.

Udeh, I. and Oghenesode, B. 2011.Effects of type 0f extender and storage conditions on the motility of goat spermatozoa. *International Journal of Animal and veterinary advances* 3(5):282-286.

Valéria, **S. F., Marco**, **R. B. M., Carlos**, **E. M. F., Állan**, **C. F. D., Jéssica**, **M. C., Rebecca**, **B. S. 2014.** Effects of seminal plasma and egg yolk concentration on freezability of goat semen. *Bras. Zootec*. 43 (10)

Watson, P. F. and Martin, I. C. 1975. The influence of some fractions of egg yolk on the survival of ram spermatozoa at 5

Received: 12th May, 2018
Accepted: 31st August, 2018