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Original article

Effect of different concentrations of coconut water on ram sperm kinematic parameters

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Abstract

Romania's shepherding heritage is widely recognized. Sheep, shepherding and transhumance are ubiquitous in Romania, as fundamental for Romanian identity and economy. An overwhelming percentage of the total sheep population consists of, or is linked to the very old, hardy and resistant Turcana breed. Our aim is to improve growth rate, production and prolificity by maximizing the impact of high genetic value rams on local populations. The objective of our study was to evaluate the effect of two different concentrations of coconut water (*Cocos nucifera* L.) on the kinematic parameters of ram semen. Six ejaculates were collected from Turcana rams by electro-ejaculation. After microscopic analysis, two aliquots per ejaculate were diluted with Tris based extender (Tris, Citric acid, Fructose, D-glucose), supplemented with 10% and 20% commercial coconut water. The control aliquots were diluted with unsupplemented Tris extender. After equilibration, the kinematic parameters of semen were evaluated every 48 h. The ram sperm parameters were significantly higher ($p<0.05$) in the 10% and 20% coconut supplemented groups compared with control group. Based on CASA analysis sperm, motility, membrane integrity and acrosomal intactness were best preserved using the 20% coconut water supplemented extender. This study evaluated the potential of natural coconut water based extenders as an alternative to well established, commercial sperm mediums. Coconut water enriched media offer a safe and cost-effective improvement to classic/commercial extenders for ram semen preservation.

Key words: Ram, CASA, semen, coconut water, *Cocos nucifera* L.

1. Introduction

Official data suggested that up to 90% of the estimated 9 million Romanian sheep population consists of, or is linked to the hardy and resistant Turcana breed (Vasile *et al.*, 2011; Gavojdian *et al.*, 2013; Ministry of Agriculture and Rural Development-National Agency for Animal Husbandry, 2013; National Institute of Statistics, 2016). Much of the sheepherding business total income is represented by the capitalization on lamb trading. Up to 66% of the total returns are represented by this activity (Gavojdian *et al.*, 2013).

Considering the economic element, improving prolificity and average gain by maximizing the impact of high value rams is of utmost importance. Genetic value aside, ram fertility is a major factor regarding prolificacy in sheep. Improving semen preservation, particularly the post storage kinematic parameters is essential and could also improve the outcome and success rate of peri cervical artificial insemination (AI), a more accessible approach than the laparoscopic, transcervical, intrauterine deposition of semen. Being able to frugally preserve high quality semen could be a way towards economic success.

Coconut water-based extenders can provide a buffering, non-toxic, low cost, practical, and effective alternative to proven semen extenders (Cardoso *et al.*, 2005; Silva *et al.*, 2012). Coconut water is well known for its versatility, being used in a wide array of fields. Surprisingly, considerable confusion around the terms 'coconut water' and 'coconut milk' has led to their interchangeable use, the cause being the interweaving, country specific definitions regarding the same or similar products (Seow *et al.*, 1997).

The Standards Task Force of the APCC consider the term 'coconut water' to strictly stand for the natural aqueous liquid endosperm of the drupe of *C. nucifera* L., while the terms 'coconut milk' or 'coconut cream' should refer to the aqueous products, essentially fibre free, extracted from solid coconut endosperm, but which optionally may include traces of coconut water. In short, the term coconut water is used for the aqueous part of the endosperm, whereas coconut milk for the liquid products obtained from solid endosperm (Standards Task Force Asian and Pacific Coconut Community (APCC), 1994; Yong *et al.*, 2009). Necessary cell conservation elements, such as salts, proteins, sugars, vitamins, neutral fats, cell division inducers and electrolytes are richly found in coconut water (Blume and Marques Jr, 1994). Intense metabolism is a cause of an increase in reactive oxygen species levels (free radicals), as a result of oxidative metabolism. Coconut water

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micronutrients such as inorganic ions and vitamins could play an important antioxidant role to prevent free radical cell oxidative damage (Evans and Halliwell, 2001). By electron donation, micronutrients have a vast contribution preventing this kind of damage, actively help quenching free radicals or indirectly acting as part of enzymes that are conditioned by catalytic metal ions for their biological activity. The removal of oxidizing species is catalysed by such metalloenzymes as glutathione peroxidase (selenium dependent) or superoxide dismutase (zinc, copper, as cofactors) (Shenkin, 2006). Various amounts of sugars, sugar alcohols, lipids, amino acids, nitrogenous compounds, organic acids and enzymes are contained within coconut water (Tulecke *et al.*, 1961; Arditti, 1994; Santoso *et al.*, 1996; USDA, 2011). The ability to protect against cold shock relies on the strong antioxidant capacity, thus protecting spermatozoa from oxidative damage during low temperature processes (Seeram *et al.*, 2005; Tezcan *et al.*, 2009). Egg yolk provides much needed sperm membrane protection, restoring phospholipids lost due to cold exposure and thermal shock (Hammerstedt *et al.*, 1990).

The objective of our study was to evaluate the effect of two different coconut water (*C. nucifera*) concentrations on the kinematic parameters of ram semen during liquid storage.

2. Materials and Methods

Semen from a total of six sexually mature (22-29 months), Turcana rams was used. The individuals belonged to a private farmer, located in Hida, Zalău, Romania. During the extent of the study, animal manipulations were performed within strict accordance with the Romanian animal protection regulation, which conforms to European Union Directive 2010/64/EU(EU, 2010). The animals were isolated from the rest of the flock before the start of the study and housed in an outdoor paddock. Water and high-quality feed were provided *ad libitum*. Feed was withheld the night before (12 h) induction of anaesthesia. General anaesthesia was induced with a single dose of xylazine (0,1 mg/kg, intramuscularly) + ketamine (4 mg/kg, intravenously) (Narcoxyl 2%; Intervet International, The Netherlands and Ketaminol 10; MSD Animal Health, The Netherlands). The animals were kept in lateral recumbency and the preputial area was groomed, washed with physiological saline serum and dried with a clean cloth. Semen was collected using an electro ejaculator. Electric stimulation was applied using a 20 cm by 3 cm, 3-electrode probe. Each stimulus lasted for 5 sec, with intermittent breaks of 5 sec during 5 min cycles. Semen was collected in preheated glass tubes, pooled and evaluated. Semen samples (control) were diluted 1:50 at 37°C with a tris-glucose-citric acid extender supplemented with egg yolk (10%, v/v) and stored at 4°C. Coconut water Tris supplemented extender (10%, v/v; 20% v/v) samples were diluted using the same protocol. Sperm kinetic parameters and vitality were reassessed using CASA system (Microptic, Barcelona, Spain) at 48 h.

3. Results and Discussion

The study was performed using a split-sample design. Data were analysed using GraphPad Prism 6 software (Manual Graph Pad Prism 5.0, GraphPad Software). Results were presented as Mean ± SEM. A value of $p < 0.05$ was considered statistically significant. After macroscopic evaluation and concentration assessment, the samples were diluted using three different extenders: Tris based extender (TE) - control group and experimental groups TEC10 and TEC20 (Tris based extender supplemented with 10% and 20% of coconut water). Sperm vitality and kinematic parameters (curvilinear velocity, straight line velocity, average path velocity, linearity index, amplitude of lateral head displacement, straightness index, oscillation index, amplitude lateral head, beat frequency) were evaluated in each sample using CASA system. The average of progressive motility in control group was 69.34% ± 1.07; 73.64% ± 3.40 in TEC10 group and 83.85% ± 1.35, respectively (Figure 1).

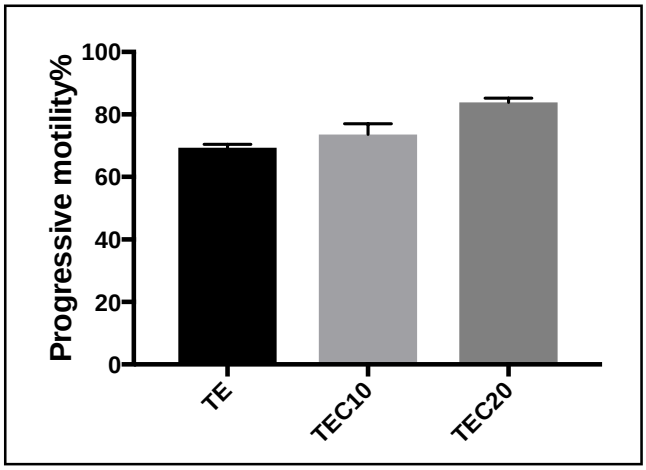

Figure 1: Progressive motility in ram spermatozoa diluted in three different extenders at 48 h.

The average of curvilinear velocity in control group was 60.34% ± 1.02, 71.51% ± 2.08 in 10% coconut water supplemented group and 83.68% ± 1.77 in TEC20, respectively (Figure 2).

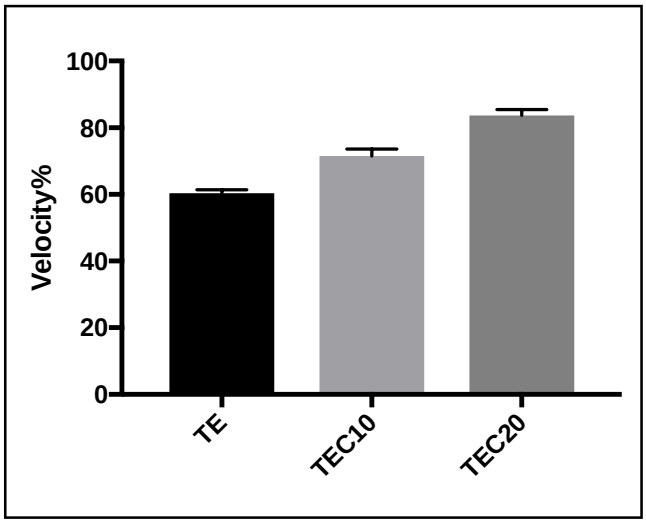

Figure 2: The average of curvilinear velocity in ram spermatozoa diluted in three different extenders.

The rest of evaluated kinematic parameters as well as straight-line velocity (VSL), average path velocity (VAP), the straightness index, the linearity index, the amplitude of lateral head displacement, beat-cross frequency values show significantly improved results in 10% and 20% coconut water supplemented groups (Table1).

Table 1: Ram semen kinematic parameters

Avg.valuesofspeed	Average			Slow			Medium			Rapid			Units
	TE	TE10%	TE20%	TE	TE10%	TE20%	TE	TE10%	TE20%	TE	TE10%	TE20%	
Curvespeed-VCL	87.32	96.51 43.35 74.57	107.48	18.83	18.46	19.82	102.64	106.96	121.78	104.08	115.25	111.14	mm/s
Linearspeed-VSL	29.63	43.35	33.02	6.41	4.67	4.23	29.96	35.95	32.88	64.22	81.78	65.86	mm/s
Avg.value-VAP	53.28	74.57	65.61	11.18	9.52	9.80	60.94	83.12	73.68	74.02	90.55	74.70	mm/s
Linearityindex-LIN	35.24	42.51	30.46	37.74	26.99	21.96	30.04	33.75	27.39	62.15	71.34	60.01	%
Straightnessindex-STR	55.71	56.88	49.74	57.63	49.01	40.66	49.98	44.41	45.42	86.73	90.12	88.08	%
Oscillationindex-WOB	61.78	74.72	59.77	62.35	53.28	50.34	59.99	77.96	60.10	71.52	79.01	68.03	%

Avg.valuesofotherparameters	Average			Medium			Rapid progressive			Units
	TE	TE10%	TE20%	TE	TE10%	TE20%	TE	TE10%	TE20%	
Amplitudelateralhead-ALH	3.68	3.35	4.40	4.29	3.63	4.95	3.95	3.89	4.28	mm
Beatfrequency-BCF	7.25	5.83	7.78	8.04	5.85	8.42	8.56	7.70	9.52	Hz

The sperm vitality in control groups were 70.18 ± 1.51%, in TEC10 group 76.99 ± 0.69% and 85.85 ± 3.71% in TEC20, respectively (Figure.3).

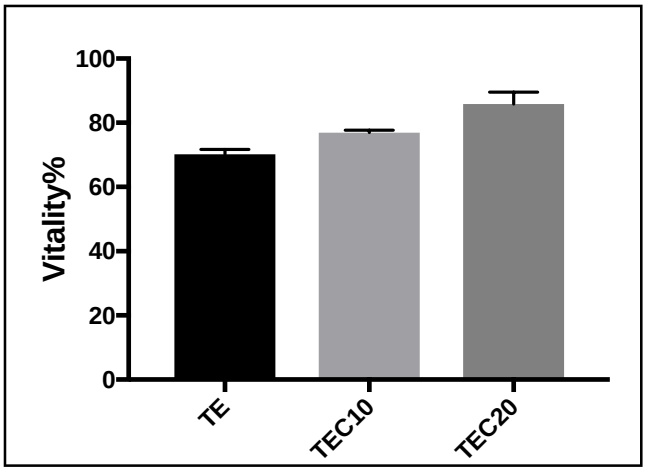

Figure 3: The average of vitality distribution in ram spermatozoa diluted in three different extenders.

Regardless of the used extender, semen endpoints were reduced after low temperature exposure. Cell viability loss and a decrease in motility as a cause of exposure to refrigeration temperatures, extenders and during the return to isosmotic conditions must be taken into account during liquid storage.

Results for the control samples are similar to those previously reported by Câmara *et al*. (2011) and Rajashri *et al*. (2017) and also comparable to the results obtained by a standard estimation method using a phase contrast microscope (Gündoðan, 2009).

The addition of 10% coconut water had a positive effect on sperm liquid storage quality and outperformed the unsupplemented media. The percentage of viable and motile spermatozoa improved along with the increase in coconut water concentration in the tris-based extender. Results shown that enhancing the used extender with 20% coconut water provided the best results regarding sperm characteristics after cold storage, being an improvement over the unsupplemented media and the 10% coconut water extender (p<0.05).

In order of achieving satisfactory liquid storage sperm vitality and kinematic parameters we recommend the addition of 20% coconut water. The applied electroejaculation method, provided good quality ejaculates from all individuals, with no negative impact on semen quality with the added benefit of eliminating the need for a training period for the rams, as required for artificial vagina use.

4. Conclusion

The results of the present study showed that the addition of coconut water to a TRIS based extender is beneficial to sperm kinematic parameters after liquid storage. The supplementation of the TRIS media with 20% coconut water constantly provided better results. Increasing the semen liquid storage kinematic parameters is a step towards increasing the success rate of peri cervical insemination, a much simpler and accessible method of artificial insemination. The tested coconut water enhanced tris extender proved effective for the liquid storage of Turcana ram semen improving sperm viability and kinematic parameters during storage.

However, the results presented here are based on *in vitro* evaluations and further studies must be performed in order to evaluate whether the extenders and cooling conditions tested have any influence on the fertility results after artificial insemination under field conditions.

Conflict of interest

We declare that we have no conflict of interest.

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