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Enhancement of the motility during the liquid storage of ram semen by argan oil

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Abstract. The aim of this study was to evaluate the motility of fresh ram semen after addition of different concentrations of argan oil. Semen was collected from five adult fertile Boujaâd rams (3-4 years of age) with artificial vagina. Ejaculates were diluted to a final concentration of 0.8 x 10^9 sperm/ml with TRIS-egg yolk diluent supplemented with argan oil at different concentrations: 0 (control), 1, 5, and 10% v/v. Semen was then stored at 15°C and sperm motility was evaluated at 0, 8, 24, 48 and 72 h post dilution with microscope (40 X magnification). Semen diluted and stored in the TRIS-egg yolk diluent supplemented with 1% of argan oil had higher sperm motility up to 3 days after collection as compared to the other concentrations used in this experiment. In conclusion, our results show that argan oil, which is rich in antioxidants, may have a protective effect allowing widening the duration of fresh ram semen conservation in Boujaâd breed.

Keywords. Ram – Semen – Argan oil – Antioxidant – Motility.

L'amélioration de la motilité du sperme fraiche chez le bélier par l'emploi d'huile d'argan

Résumé. *Le but de cette étude était d'évaluer l'effet de l'huile d'argan à différentes concentrations sur la motilité des spermatozoïdes chez bélier. Le sperme de cinq béliers adultes et fertiles de race Boujaâd âgés de 3-4 ans a été prélevé à l'aide d'un vagin artificiel. Les éjaculats ont été dilués pour atteindre une concentration finale de 0,8 x 10^9 spz /ml dans un dilueur Tris – jaune d'œuf supplémenté avec de l'huile d'argan à différentes concentrations : 0 (contrôle), 1, 5, et 10% v/v. La semence diluée a été stockée à 15°C, et la motilité des spermatozoïdes a été évaluée à 0, 8, 24, 48 et 72 h après dilution au microscope (grossissement 40 ×). Les résultats obtenus ont montrés que le dilueur Tris-jaune d'œuf supplémenté à 1% d'huile d'argan permettait de maintenir une meilleure motilité du sperme jusqu'à 3 jours de conservation. En conclusion, l'huile d'argan, riche en antioxydants, pourrait avoir un effet protecteur permettant de prolonger la durée de motilité du sperme conservé à 15°C chez les béliers de race Boujaâd.*

Mots-clés. *Bélier – Sperme – Huile d'argan – Antioxydant – Motilité.*

I – Introduction

One of the main problems that held back the use of artificial insemination in sheep on a large scale is the need to improve the conservation of fresh ram semen. It is well known that during storage, the accumulation of reactive oxygen species and the oxidation of sperm plasma membrane cause changes in the spermatozoa. Many studies have demonstrated membrane lipid peroxidation (LPO) as one of the causes of defective sperm function after liquid conservation at 4ºC (Vishwanath and Shannon, 2000). However, ram semen contains appreciable amounts of superoxide dismutase and also, in much lower concentrations, glutathione peroxidase and catalase (Abu-Erreish *et al.,* 1978; Mann and Lutwak, 1981), but these concentrations may be considerably reduced by the semen dilution. Thereby, the storage of semen in a liquid state can be achieved by methods reducing sperm metabolism and prolonging their fertile life (Maxwell and Stojanov, 1996). Among these methods, the addition of various antioxidants to sheep semen diluents has

been shown to extend the period of semen storage, improve sperm motility, reduce the degree of cellular damage, improve the acrosomal integrity and increase the viability and fertilization capacity of sperm in vitro (Jones and Mann, 1976; Maxwell and Stojanov, 1996; Ollero *et al.,* 1996; Sánchez-Partida *et al.,* 1997; Upreti *et al.,* 1997; Rzyzosiak *et al.,* 2000; Sarlós *et al.,* 2002).

In the present work, the antioxidant that has been chosen is argan oil. It is harvested from the fruits of the argan tree (*Argania spinosa*) that is endemic to Southwest of Morocco. This oil is known as a rich source of linoleic and oleic acids (37% and 45% respectively) and minor compounds such as tocopherols, polyphenols, sterols, carotenoids, xanthophyls and squalene (Khallouki *el al.,* 2003). The unsaponifiable fraction is very abundant in these compounds and has powerful antioxidant effects (Drissi *et al.,* 2004). The purpose of this study was to determine whether supplementation of ram semen extender with Argan oil could improve the quality of liquid semen stored at 15°C.

II – Material and methods

1. Animals and semen collection

Five adult fertile Boujaâd rams (3-4 years of age) were used in this study. They were maintained in the laboratory of reproduction biotechnology at the Regional Center of Agriculture Research of Settat (INRA Morocco). All rams were maintained with proper balanced diet and had a free access to water. A total of 20 ejaculates were collected with artificial vagina (40-42°C) during the non-breeding season 2012 (December-January- February). Only ejaculates between 1 and 2 ml in volume, spermatozoa with >70% progressive motility, and a concentration higher than 2.5 x 10^9 spermatozoa were used for this study.

2. Semen processing

Immediately after collection, the ejaculates were placed in a water-bath at 37°C. Sperm concentration was evaluated using a calibrated spectrophotometer. The mass movement, motility score, and percent of motile sperm (motility %) were estimated with a microscope. To evaluate sperm motility, a semen sample (5µl) was placed under a cover slip on a pre-warmed (37°C) slide and subjectively assessed using a phase microscope (40X magnification) as described by Evans and Maxwell (1987). A Tris-egg yolk extender based containing Tris (2.666 g), citric acid (1.398 g), fructose (0.44 g), egg yolk (12%) at pH 6.8 was used to dilute the semen. Each ejaculate was divided into four equal aliquots. Each was diluted in the above cited extender supplemented with 0% (control), 1%, 5% and 10% argan oil to reach a final concentration of 0.8 10^9 spermatozoa/ml. The semen was then stored at 15°C. Sperm motility was further evaluated at 0, 8, 24, 48, 72 h post dilution with microscope (40 X magnification).

3. Statistical analysis

Results are expressed as the mean ± SEM. Means were analyzed by Tukey's post-hoc test to determine significant differences between groups in motility using SAS (version 10). Differences with values of $P < 0.05$ were considered to be statistically significant.

III – Results

As shown in Table 1, there was no difference in quality between the 4 fractions of Boujaâd ram semen at the time of dilution. Supplementation of the tris-egg yolk extender with argan oil at 1% maintained a motility equivalent to that obtained at 0h of storage until 72 h of storage. Semen stored with 1% argan oil showed also a significantly higher motility of spermatozoa after 8, 24,

48 and 72h of storage when compared to 0%, 5% or 10% of argan oil (P<0.05). The quality decreased after 8h in all other fractions (0%, 5%, 10%) when compared to 0h storage time. Motility was not different from 8 h until 72 h in the fractions containing 0% (control) and 5% argan oil. However, the extender supplemented with 10% argan oil seemed to have a deleterious effect on motility which significantly lower between 8 h and 24 h of storage and was significantly lower than that of the control fraction (0%) at 24, 48 and 72 h of storage (P < 0.05).

Table 1. Means of motile spermatozoa percentage of semen preserved in tris-egg yolk extender with differents concentration of argan oil

Storage time (h)	Argan oil concentrations			
	0%	1%	5%	10%
F0	80 ± 5.1^{a}	81.1 ± 4.9^{a}	79 ± 5.9^{a}	80 ± 8.1^{a}
8	72.95 ± 7.2^{b}	82.91 ± 7.82^{a}	72.08 ± 8.3^{b}	70.4 ± 6.20^{b}
24	68.75 ± 7.72^{b}	78.7 ± 6.44^{a}	67.9 ± 4.98^{bc}	59.59 ± 8.9^{c}
48	64.58 ± 9.15^{b}	74.5 ± 4.98^{a}	59.9 ± 3.96^{bc}	51.36 ± 6.74^{c}
72	61.15 ± 2.99^{b}	71.15 ± 2.99^{a}	55 ± 6.06^{bc}	40.76 ± 9^{bc}

^{a,b,c} Values in rows with different superscripts differ significantly (P < 0.05).

IV – Discussion

Oxidative stress is one of the most important factors associated with fertility decrease over sperm preservation. The plasmatic membrane of spermatozoa contains a great number of unsaturated fatty acids which are susceptible to lipid peroxidation, and the consequences are numerous, ranging from membrane damage to cell functions disorder and low motility (Aurich *et al.,* 1997; Ball *et al.,* 2001).

This study is the first evaluation of the influence of argan oil concentration on ram sperm motility during liquid storage. Our results showed that the addition of argan oil at 1% improve sperm motility of ram spermatozoa during liquid preservation at 15°C for 3 days. Such effect has been recorded in many previous studies based on other antioxidants. In fact, it was proven that superoxide dismutase, catalase, and cytochrome C and glutathione peroxidase could improve the motility of the liquid-preserved ram semen (Maxwell and Stojanov, 1996; Rzyzosiak *et al.,* 2000; Bilodeau *et al.,* 2001; Sarlos *et al.,* 2002).

V – Conclusions

To conclude, our results on Boujaâd ram showed that the addition of argan oil at 1% as an antioxidant improve sperm motility during liquid preservation at 15°C for up to 3 days. Further investigations will be run to assess the effect of argan oil addition on lipid peroxidation and generation of H_2O_2. The effect of different single components of argan oil on ram semen motility and viability should also be evaluated.

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