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Assessment of different extenders for the cryopreservation of Moroccan Beni Arouss buck semen with no sperm washing

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Abstract. This study aimed at investigating the effects of two commercial extenders and skimmed milk extender on the quality of cryopreserved buck semen without washing. Ejaculates were collected from four Beni Arouss bucks using an artificial vagina, pooled and split into equal parts in three tubes. Tubes were diluted in parallel at 37°C with Optixcell® semen extender already containing glycerol, INRA96® semen extender and skim milk-based extender. After cooling to 4°C, glycerol (7%) was added to samples diluted with INRA96® and skimmed milk extenders and all samples were equilibrated, cryopreserved and stored in liquid nitrogen. CASA motion parameters, viability and normal morphology of sperm were assessed after dilution, equilibration and freeze–thawing. The cryopreservation process had a significant effect on semen quality. All parameters decrease over time of the storage process ($P < 0.001$). Optixcell® extender provided more effective preservation after freeze-thawing followed by skimmed milk extender and then INRA96® extender ($P < 0.05$) of progressive motility (47%, 35% and 16%, respectively), velocity parameters (RAPID: 48%, 33% and 14% respectively and VCL: 100 µm/s, 83 µm/s, 71 µm/s and VSL: 58 µm/s, 56 µm/s and 45 µm/s, respectively) and viability (41%, 32% and 16%, respectively). No significant differences were observed between extenders regarding the normal morphology ($P > 0.05$). It was concluded that Optixcell® extender allowed better cryoprotection to Beni Arouss buck spermatozoa as showed by semen quality parameters.

Keywords. Extender – Buck semen – Cryopreservation – Semen quality.

Evaluation de différents dilueurs pour la congélation de la semence du bouc Beni Arouss sans lavage préalable de la semence

Résumé. *Cette étude a pour objectif d'évaluer l'effet de deux dilueurs commerciaux et d'un dilueur conventionnel à base du lait écrémé sur la conservation en congelé de la semence du bouc sans lavage préalable de la semence. Les éjaculats prélevés à l'aide d'un vagin artificiel de quatre boucs Beni Arouss ont été mélangés et repartis en portions égales dans trois tubes. Ces derniers ont été dilués en parallèle, à 37°C, avec un dilueur commercial qui contient du glycérol (Optixcell®), un deuxième dilueur commercial (INRA96®) et un dilueur à base de lait écrémé. Après refroidissement progressif à 4°C, le glycérol (7%) a été ajouté dans les échantillons dilués avec l'INRA96® et le lait écrémé. L'ensemble des échantillons ont été soumis à une équilibration avant d'être congelés et conservés en azote liquide. Les paramètres de motilité générés par le CASA, la viabilité et la morphologie normale des spermatozoïdes ont été évalués après dilution, après équilibration et après congélation-décongélation. D'après les résultats, la congélation a un effet significatif sur la qualité de la semence. L'ensemble des paramètres diminue au cours du processus de la congélation ($P < 0,001$). Optixcell® a permet une conservation plus efficace de la qualité de la semence après congélation-décongélation suivi de lait écrémé, puis de l'INRA96® ($P <0,05$). Les pourcentages de la motilité progressive ont été respectivement 47%, 35% et 16% pour les trois dilueurs. Des pourcentages respectifs de 48%, 33% et 14% ont été enregistrés pour RAPID, 100 µm/s, 83 µm/s et 71 µm/s pour VCL, 58 µm/s, 56 µm/s et 45 µm/s pour VSL et 41%, 32% et 16%, pour la viabilité. Aucun effet du dilueur sur la morphologie normale n'a été enregistré ($P> 0,05$). En conclusion, Optixcell® a permet une meilleure conservation en congelé de la semence du bouc Beni Arouss.*

Mots-clés. *Dilueur – Sperme du bouc – Congélation – Qualité de la semence.*

I – Introduction

In the North of Morocco, Beni Arouss goat farming contributes approximately to 70% of rural population incomes (Chentouf *et al*., 2011). In order to preserve this local goat breed and improve farm productivity, a genetic improvement program is implemented by a professional organization (ANOC) and supported by the Ministry of Agriculture. Artificial insemination is of crucial importance to identify and disseminate the genetic progress of this breed. The development of an optimal and easy protocol for semen cryopreservation is necessary to accompany this genetic improvement program. In goats, sperm washing step is largely recommended to improve the quality of frozen-thawed buck semen when diluents containing egg yolk or skimmed milk are used (Leboeuf *et al*., 2000; Purdy, 2006). However, this step is a time consuming and can be responsible of sperm damaging or lost (Miro *et al*., 2009). Still, many studies report that removing the seminal plasma when freezing buck semen is not necessary (Leboeuf *et al*., 2000; Azeredo *et al*., 2001; Purdy, 2006). The aim of the present research was to study the effect of two commercial extenders (Optixcell® and INRA96®) and skimmed milk extender on the quality of cryopreserved buck semen without washing.

II – Material and methods

1. Animals and semen collection

Four Beni Arouss bucks aged between 3 and 7 years and weighted between 40 and 52 kg were used in this study. They were maintained at the experimental station of INRA, regional center of Tangier (35°N of latitude) under natural conditions of photoperiod and optimum feeding conditions. Semen was collected during breeding season using an artificial vagina. A total of 24 ejaculates were collected in 6 replicates throughout the study period. After collection, semen was placed in a water bath at 37 °C and evaluated for volume, colour, mass motility and sperm concentration. Only ejaculates between 0.6 and 2 ml of volume with a concentration greater than 2 x 10^9 spermatozoa/ml and having a mass motility higher than 4 were selected and pooled for cryopreservation.

2. Semen cryopreservation

Immediately after collection and pooling, ejaculates were divided into equal parts in three tubes. Tubes were diluted in parallel, by two steps method. The first fraction added at 37°C of Optixcell® semen extender already containing glycerol, INRA96® semen extender and skim milk-based extender. After cooling to 4°C, a second fraction containing glycerol (14%) was added to samples diluted with INRA96® and skimmed milk extenders in order to achieve a final concentration of 400 x 10^6 spermatozoa/ml and 7% glycerol level in the final volume. The diluted semen was then equilibrated at 4°C for 2 hours. After equilibration, the semen was aspirated into 0.25 ml French straws and sealed with polyvinyl powder. The straws were cooled at a rate of 7 °C/min from 4 to –50 °C and 25 °C/min from –50 to –150 °C in a programmable freezer (Micro-Digitcool®, IMV, France), then plunged into liquid nitrogen for storage. Thawing was carried out in a water bath at 37 °C for 30 s.

3. Semen evaluation after dilution, equilibration and freeze-thawing

The sperm motion and normal morphology parameters were assessed immediately after dilution, equilibration and freeze-thawing using a computer-assisted sperm analysis system (ISAS®, Proiser R + D SL, Spain) using the procedures published by El kadili *et al*. (2019). The following motility parameters were assessed: percentage of progressive motile spermatozoa (PM, %), curvilinear velocity (VCL, µm/s), straight line velocity (VSL, µm/s) and rapid spermatozoa (RAPID, %). The viability of the sperm was evaluated following eosin–nigrosin staining (Evans and Maxwell, 1987).

4. Statistical analysis

Data were analysed using the GLM procedure of SAS 9.0. An ANOVA model for repeated measures was used for each parameter of sperm quality. The model included the fixed effects of extender and time of analyse. Differences between mean values were analysed by the LSD test. Data were presented as mean ± SD, and the level of significance was set at $P < 0.05$.

III – Results and discussion

Table 1 summarizes results obtained in this experiment. As expected, all parameters were reduced after semen freeze-thawing ($P < 0.001$). Cryopreservation that included dilution, cooling, equilibration, freezing and thawing induces detrimental effects in sperm cells, resulting in a reduction of motility, membrane integrity and fertilizing ability. Similar results were reported by Watson (2000) and Purdy (2006).

Table 1. Effect of different extenders on semen quality of post dilution, equilibration and freeze-thawing semen in Moroccan Beni Arouss bucks (mean ± SD)

Semen parameters	Extenders		
	Optixcell	INRA96	Skimmed milk
Progressive motility (%)			
After dilution	70.82±4.12a	71.95±6.16a	71.08±8.74a
After equilibration	67.28 ±12.28a	64.95±13.42a	62.83±11.64a
After Freeze-thawing	47.13±13.32bx	15.82±11.26by	34.92±12.84bx
RAPID (%)			
After dilution	85.28±7.36a	86.03±11.65a	80.67±14.92a
After equilibration	76.23±15.72a	67.60±16.02b	66.86±12.64a
After Freeze-thawing	48.50±9.32bx	13.95±10.72cy	32.75±16.15bx
VCL (µm/s)			
After dilution	124.81±20.25ax	125.61±14.88ax	106.56±16.19ay
After equilibration	118.81±8.97a	121.06±16.72a	103.96±16.33a
After Freeze-thawing	99.76±19.32bx	70.78±15.97by	83.38±20.41bxy
VSL (µm/s)			
After dilution	53.28±13.42	67.63±13.17b	61.81±10.80
After equilibration	56.08±9.66y	74.35±15.11ax	64.78±20.71y
After Freeze-thawing	58.55±15.16x	45.01±16.51cy	55.95±21.32xy
Viability (%)			
After dilution	70.75±6.54a	75.92±11.83a	72.68±9.96a
After equilibration	68.42±11.12a	65.83±13.85a	67.67±10.76a
After Freeze-thawing	40.67±8.61bx	15.58±8.28by	32.08±11.38bx
Normal sperm (%)			
After dilution	80.58±3.10	83.50±3.21a	80.33±3.30
After equilibration	79.33±3.61	78.00±3.85a	78.75±2.42
After Freeze-thawing	76.83±3.87	71.83±4.67b	77.58±6.84

a,b Means with different superscripts in the same column indicate significant difference for a parameter among times of evaluation($P<0.001$).

x,y Means with different superscripts in the same row indicate significant difference among treatment groups ($P<0.05$).

Significant differences were found between semen extenders in term of sperm quality. Most of the freeze-thawing sperm motility and viability parameters were significantly higher in samples diluted in Optixcell® extender followed by those diluted in skimmed milk extender and then those diluted in INRA96® extender. Whereas, there was no effect of extender on the percentage of normal sperm ($P > 0.05$). Optixcell® is a liposomes-based extender that demonstrated a better result than INRA96® extender supplemented with a purified fraction of caseins (native phosphocaseinate) in our experiment. The variations in findings using liposomes-based extender is might be due to the difference in protective effect of liposomes or to glycerol concentration which is unknown in Optixcell®. Regarding semen motility, viability and normal morphology a good semen quality was obtained without sperm washing, using skimmed milk extender. This implied that semen without washing could be extended in this extender for cryopreservation in Beni Arouss buck.

IV – Conclusions

In conclusion, results of the present study suggest that Optixcell® extender allowed better cryoprotection to Beni Arouss buck spermatozoa as showed by semen quality parameters. During the breeding season, skimmed milk can be used as a conventional and economic extender for cryopreservation of Beni Arouss buck semen without sperm washing.

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