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## Variations in semen characteristics rams of Ouled Djellal breed have received an important dietary supplement after regular and intensive collection

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### ABSTRACT

**Objective:** To evaluate the effect of an important dietary supplement on sperm quantity and quality breeding rams of artificial insemination center Belhandjir in region of Naama in Algeria. **Methods:** Ten breeding rams of the white arab breed, called Ouled Djellal are divided into two groups CR and SR, and subjected to two different diets, the first group CR followed a regime R based of barley and fodder and the second SR group to follow a regime R + a vitamin and mineral dietary supplement for a 15 weeks of regular collections followed by 12 weeks of intensive collections. Analysis of sperm quality and quantity were performed to normal and intensive collection. **Results:** The quantitative effect is more significant in rams supplemented SR, volume and concentration were higher in rams SR (respectively+40% and  $\pm 13\%$ ), therefore the doses produced differ significantly compared to the group of control rams CR (+70%). Results of qualitative parameters were more mixed, motility showed a significant difference in rams supplemented with against the rate of dead semen and the rate of anomalies did not show significant differences in sperm. **Conclusion:** supplementation could significantly improve the quantitative semen characteristics in rams breeding Ouled Djellal. Collections of semen on the rams may be daily without sperm quantity is affected.

## 1. Introduction

The sperm characteristics and producing protocol doses of semen in sheep artificial insemination centers (SAIC) are still subject to studies and experiences in order to have doses with reduced cost and optimal power fertilizing. The male fertilizing is therefore a critical point in the success of a breeding scheme[1].

Thus the concentration of semen doses increased from 400 million to 350 and this without the success rate of artificial insemination (AI) is reduced significantly[2]. Various causes could be the cause of impaired semen quality in sheep,

diet, xenobiotics, the pace of semen collection, stress, photoperiod, pathology[3].

Some of SAIC resort to other methods to improve the quality and/or quantity of semen from rams, some use of the compounds of vitamin supplements (water-soluble and fat-soluble) and dietary elements to boost sperm production in rams breeding, this is in order to ensure a larger scale dissemination of higher genetic potential of the selected rams, or also to ultimately reduce the costs of production of semen doses produced.

The objective of this study was to determine the effects of dietary supplements of important fat-soluble vitamins, water-soluble and dietary elements on semen characteristics in rams breed Ouled Djellal subjected to normal and intensive collection.

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## 2. Materials and methods

During 2012, ten breeding rams healthy, with no damage in their reproductive system, Ouled Djellal breed, previously trained for semen collection and regularly collected in artificial insemination center, were selected for experimentation. Rams were followed at the NAICGI (NAICGI: National Artificial Insemination Centre and Genetic Improvement). Belhandjir in the region of Naama, which is located in the semi-arid area at an altitude of 1147 m above sea level, latitude: 32° 42 ' 16 N, longitude: 0° 42' 07 W. Rams are aged from 24 to 40 months; they are medium weight, BCS : body condition scoring, between 2 and 3 (The results concerning the relationship between the BCS at the moment of AI and its success are variable across studies. For Grimard et al., there is no significant relationship between these variables[4], and Roche[5], the relationship is positive). Breeding rams are divided into two groups, the CR group or control rams ( $n=5$ ), and a SR group or supplemented rams ( $n=5$ ). Throughout the experiment, the group of control rams CT followed a regime R which is based of barley and fodder and the second group SR followed an R + vitamin and mineral regime (A mineral and vitamin complement or MVC, resulting from industry). This dietary supplement is made up of fat-soluble vitamins (B vitamins and vitamin C), fat-soluble vitamins (A, D, E and K) and minerals such as iron, copper, zinc, manganese, phosphorus and calcium, it also contains betaines, and methionine. The dietary supplement is used at 15 for 30 days at a dosage of 1.5% of the R (Barley + fodder) regime.

Given the strong positive correlation existing between scrotal circumference and sperm production[6], the two groups CR and SR were chosen so that the average scrotal perimeter will be very close, which are respectively ( $36.24\pm 1.36$ ) and ( $35.76\pm 0.85$ ), and in order to avoid bias in the analysis of our quantitative results.

Rams are stimulated by the presence of a sheep and collections are made using an artificial vagina. Three quantitative measures are deducted on all collections for each group of rams: the volume is read using a graduated tube, the concentration was determined by spectrophotometry and the number of doses produced counted at the end of collection. Three qualitative values are determined for all the collections for each group of rams: the percentage of dead sperm, the percentage of abnormal sperm (the rate of dead and abnormal sperm were determined after staining with eosin taking into account 200 cells per ejaculate) and positive mass motility, which is evaluated microscopically from an undiluted or colored sperm drop, it

is scored on a continuous scale from 0 to 5.

The least significant difference test (z-test) and the Fisher's Z-Transformation were used. The  $P$  value  $<0.05$  was considered as significant.

## 3. Results

### 3.1. Quantitative study

A highly significant difference is found in the calculation of the average doses produced by SR group rams compared to rams CR group ( $P<0.01$ ), average  $29.23\pm 13.47$  respectively and  $16.83\pm 6.92$  (Table 1).

**Table 1**

Quantitative effect of the supplements on the semen.

Parameters	Control rams( $n=69$ )	Supplemented rams ( $n=69$ )
Average of volume of semen (mL)	$1.21\pm 0.37$	$1.79\pm 0.63^*$
Concentration	$4.96\pm 1.19$	$5.64\pm 1.23$
Sum of doses	1 161	2 017
Average doses produced	$16.83\pm 6.92$	$29.23\pm 13.47^*$
Volume of diluent (mL)/100 doses	$17.50\pm 1.39$	$18.51\pm 2.78^*$
Volume of semen (mL)/100 doses	$7.49\pm 1.39$	$6.48\pm 2.78^*$

\* $P<0.01$  comparing with the control group.

The effect of supplementation is very marked in rams from the SR group after normal collection, use dietary supplements increase the number of doses produced (2 017 against 1 161 doses) an increase of about 73% of doses produced SR group compared to CR group (Table 1). The volume of diluent required for the producing of 100 doses, or a volume of 25 mL was significantly different between the two groups of rams CR and SR ( $P=0.0014$ ). The volume of diluent used is ( $17.50\pm 1.99$ ) for 25 mL in controls against  $18.52\pm 1.70$  mL of 25 in breeding rams supplemented (Table 1), which returns us to a consumption 8.73 mL of diluent and more in rams SR group compared to CR group (2017 doses produced against 1 161), a difference in the volume of diluent 1%. The use of dietary supplements (vitamin compounds and minerals) does not allow us to reduce the volume of diluent required for producing semen doses, increasing the volume of diluent of 1% was observed after 15 weeks of regular collection.

The process of correlation breeding rams varies from 0.80 and 0.84 in CR an SR group respectively, positive and moderately strong correlation was found between the volume of semen and doses produced (Table 1), the correlations are very similar in the two groups of rams (the difference is not significant,  $P>0.05$ ,  $P=0.45$ ). The increase in the two groups, the volume of codes, is expected to increase the number of doses produced. The volume in the group SR

differs significantly from that observed in the CR group, a difference of about 47%, [(1.79±0.63) vs. (1.21±0.37) mL], ( $P<0.01$ ) (Table 1), which has consistently increased the number of doses produced given the correlation between the two parameters (Figure 1), the volume was higher in rams SR (+ 40%). The total sperm production in the SR group collected four times a week is 1830 doses, averaging a production of 28.59±8.48 doses per rams compared to 1 072 doses in the CR group those collected in the same frequency or producing an average of 16.75±5.32 rams doses (Table 2). This represents a production of 758 additional doses per SR group, a difference of 12 doses per ram made after 12 weeks

of intensive collections, and 15 weeks of regular collections.

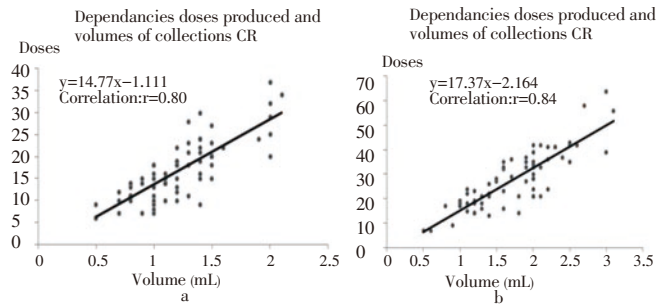


Figure 1. Correlation between doses produced and the volume of the

Table 2

Effect of collect frequency on seminal performance of control (RC) and supplemented rams (RS).

Collecting frequency	Doses /Ejaculate		Doses produced		Volume /Ejaculate (mL)		Production /Ejaculate (10 <sup>9</sup> sperm cell)	
	RC	RS	RC	RS	RC	RS	RC	RS
2 times/week	16.62±5.52 <sup>a</sup>	27.25±6.57 <sup>b</sup>	665	1 090	1.21±0.32 <sup>a</sup>	1.71±0.32 <sup>b</sup>	6.24±1.79 <sup>a</sup>	9.49±2.42 <sup>b</sup>
3 times/week	16.79±5.35 <sup>a</sup>	27.56±7.49 <sup>b</sup>	806	1 323	1.20±0.30 <sup>a</sup>	1.74±0.30 <sup>b</sup>	5.89±1.56 <sup>a</sup>	9.83±2.83 <sup>b</sup>
4 times/week	16.75±5.32 <sup>a</sup>	28.59±8.48 <sup>b</sup>	1 072	1 830	1.19±0.30 <sup>a</sup>	1.83±0.39 <sup>b</sup>	5.85±1.81 <sup>a</sup>	10.30±3.10 <sup>b</sup>

-6 Weeks = start of supplementation; Weeks 0 = Start of semen collection; 15 = end of the regular collection; 28 = end of the intensive collection. Values with same letters in the same column are not different significantly therebetween at  $P < 0.05$ .

collection in CR and SR.

### 3.2. Qualitative study

Observed in the two groups of rams CR and SR motility showed a significant difference after dietary supplementation in rams of the study cohort (4.28±0.36 in the CR group, against 4.48±0.08 in the SR group after supplementation.  $P<0.01$ ). The rate of dead sperm and the rate of anomalies counted in two groups CR and SR sperm before and after supplementation showed no significant differences after statistical analysis (Table 3).

Table 3

Qualitative characteristics of the seed of the study cohort (mean ± standard deviation).

Groups of rams	Motility	Dead sperm (%)	Abnormal sperm (%)
Group CR (n=69)	4.28±0.37	35.14±9.01	4.68±1.81
Group SR before (n=25)	4.35±0.23	35.08±6.24	4.59±1.65
Group SR after (n=69)	4.49±0.08*	35.56±7.89	4.85±0.84

\* $P<0.05$  comparing with data in the same column.

The values are respectively: 35.14±9.01 vs. 35.56±7.89 ( $P>0.6$ ) for the rate of dead sperm, and 4.68±1.81 vs. 4.85 ± 0.84 ( $P>0.48$ ) for the rate of sperm with abnormalities.

The results are mixed as to the qualitative effect of dietary supplements on the seed produced in rams, the percentage of dead and abnormal sperm showed no significant while rates motility differ significantly compared and differences in both groups of the study cohort. Collections taken before

and after supplementation showed no significant values except for motility differences (Table 3). Motility is an evaluation of sperm movements. According to some authors, these movements reflect the fact that the sperm membrane is intact and functional; which implies that the sperm is fertilizing[7].

### 4. Discussion

Similar studies have shown that massive supplements fat-soluble vitamins (A, D, E and K) and water-soluble (B-complex vitamins), which were administered to male pigs, can increase the total amount of sperm and the products, especially after daily intensive collections so-called stress[8] periods. Some studies of vitamin E in the diet can improve the density of cells in spermatogenesis, Sertoli cells, tube diameter and thickness of the germinal epithelium seminiferous especially at a rate of 200 IU[9].

The frequency of collections had no significant effect after 2, 3 or 4 collections per week. The effect of dietary supplements seems more useful for collection higher than normal, a slight improvement in sperm quantity was observed but there were no significant differences in significant volume, the number of doses produced or sperm production (Table 2).

The use of dietary supplements has no beneficial effect on the extensive collection, it seems that no supplements

in CR group rams had no impact on the frequency of collections whether two three or four times a week. the number of doses. the volume and concentration had average very close (the differences are not significant). The effect of intensive collection on the quality of the seed is completely nonexistent. Collections of semen on the rams may be daily without sperm quantity is affected.

An abnormal decrease in zinc leads to a particular decrease in motility of spermatozoa<sup>[10]</sup>. the presence of added zinc through the mineral and vitamin supplement could have a direct effect on the observed results.

Vitamin C may also have an effect on sperm motility. Injections of vitamin C for 90 days increased sperm motility and the effect was still evident up to 30 days after stopping the injections in the goat<sup>[11]</sup>. The percentage of live sperm showed similar trends. doses were equally effective in reducing the percentage of abnormal sperm according to the same study. This was not confirmed by our study. These current data indicate the importance of vitamin C in the breeding of goats. as also shown for several species of mammals.

In conclusion the quantitative effect is more significant in rams supplemented. Supplementation has allowed us to have a greater seminal performance. Apart motility. supplementation does not improve the quality of seed produced. namely the rate of live sperm and the rate of anomalous which showed no significant differences. Collections of semen on the rams may be daily without sperm quantity is affected. The supplementation may allow us a larger scale dissemination of superior genetic potential breeding rams selected. but it does not allow us to reduce the cost of production of semen.

### Conflict of interest statement

The authors declare that there are no conflicts of interests regarding the publication of this article.

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