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SPERM QUALITY IN RESPONSE TO AGE IN LOCAL RABBITS REARED IN SEMI-ARID ENVIRONMENT (TIARET, ALGERIA)

Berrouaguia Karim*, Meliani Samia, Guemour Djilali, Khelil Sofiane Raouf, Selmani Moulkheir

Nature and Life Sciences Faculty, Ibn Khaldoun University, Tiaret, Algeria

Abstract. The aim of this study was to evaluate the age effect on quantity and quality of rabbit semen raised in semi-arid environment of Tiaret region. The study was conducted at the experimental farm of Ibn Khaldoun university of Tiaret. A total of 20 rabbit bucks of the local Algerian population (5-11 months of age) weighting between 3010g and 4540g were collected randomly and exposed to an extensive rhythm. The average value of libido was 25,17±20,94 seconds (sec.). The ejaculate volume was 1,48±0,33ml and the pH 7,67±0,36for bucks of 11 months of age. The analyses of semen show no significant for mass and individual motility (6,84±1,70 and 2,96±1,04 respectively). The rate of vitality was 61,18±18. However, the age of bucks significantly affected the concentration and abnormal spermatozoa ($p<0,05$). In this study, most of semen parameters were influenced by the age and rabbit bucks of the Algerian local population seems desirable for reproduction in compare with other strains.

Keywords: *Rabbit, fertility, age, semen, spermogram.*

Corresponding Author: *Berrouaguia, Karim, Nature and Life Sciences Faculty, Ibn Khaldoun University, Karman, Tiaret, Algeria, Phone: +213793314343, e-mail: karim.repro@gmail.com*

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1. Introduction

The best rabbit's performances use depends entirely on good reproduction management. According to Alvariño (2000); male is the basis of breeding success playing a key role in the achievement and profitability of the rabbit's breeding. The buck in natural breeding is used to fertilize 8 to 11 does (Roca, 1994; Osechas & Becerra, 2006) in contrast by artificial insemination it can be used to fertilize 100 does at a time, because of the fertilizing capacity of semen (Eid, 2008).

Semen evaluation traits informs about the spermatozoa fertilizing capability (Boiti *et al.,* 2005). Moreover, there are a wide variety in semen traits, and different factors such as the collection frequency, lighting programs, buck age, might influence qualitative and quantitative sperm production (Boiti *et al*., 2005). To obtain an optimal quantity of sperm and spermatozoa, it is necessary to define the conditions of use of the bucks (Boulbina *et al*., 2012). The analyses of sperm production is significantly highly correlated with sexual activity (Benia *et al*., 2018), it is necessary to identify the puberty age, sexual maturity, the response to the collect and factors affecting the sperm production. In order to characterize male reproductive performance of the Algerian local rabbit population; the aim of this study was to assess the sperm quality evolution with age in local rabbits raised in the semi-arid environment.

2. Materials and methods

The study was conducted at the experimental farm of Ibn Khaldoun University of Tiaret (western Algeria) during 2019. Rabbits were the product of a crossing between local populations up to the 5^{th} generation. Twenty rabbit bucks of the local population weighting between 3010 – 4540 were collected (from the 5^{th} to the 11^{th} month of age) to evaluate the semen quantity and quality evolution. Animals were housed individually in wire cages arranged in flat-deck layout on one level. Ventilation and lighting were naturally provided. Automatic waterers were used, and the rabbits were fed *ad libitum* with granulated commercial diet (13,81% crud protein, 2820 kcal digestible energy/kg).

The semen collection was made by using an artificial vagina, using a teaser doe. Libido was recorded in terms of reaction time in seconds and was estimated from the time the doe was placed inside the buck's cage up to the point when the buck started to mount the doe (Daader *et al.,* 1999). After collection, the volume of the ejaculate was assessed by reading the graduation of the collecting tube. Sperm volume is deducted after removal of the gel fraction. The collection tube was immediately put into an electric oven at 37 °C. The pH of the semen is determined by a pH paper. Then, the mass (MM) and individual (IM) motility of the spermatozoa were determined under a phase contrast microscope. Mass motility was appreciated by placing a drop of pure sperm between slide and lamella observed at magnification (x10), a note from 0 to 9 was attributed to the movement of sperm mass observed on the Petitjean scale (1965) mentioned by (Boussit, 1989). Individual motility was assessed after dilution of the sperm with a commercial diluent at the rate of 1/5 and 4/5 diluter volumes. A drop of diluted semen was observed between slide and lamella at magnification (x40), a note from 0 to 4 was attributed to the individual movement of spermatozoa observed on the Adrieu scale (1974) according to (Boussit, 1989).

The concentration (C) in spermatozoa (10^6/ml) was determined using a malassez cell from a drop of seed diluted to 1/200 with the diluter. Counting was performed under the microscope at magnification (x40) (Boussit, 1989).

The vitality was determined by the preparation of a smear using eosin-nigrosine vital staining, a drop of semen was mixed with a drop of the dye, and then the mixture was gently spread along the blade. The smear was left for a few seconds, then it was observed under magnification microscope (x100). Dead sperms spread the dye through their damaged membrane, while living spermatozoa with their functional membranes do not diffuse the dye and therefore remain colorless. A random count of 150 spermatozoa was performed along the smear, from which dead spermatozoa were distinguished from the living (Boussit, 1989).

The percentage of abnormal spermatozoa (AS %) was studied on the same sample of the stained smear. 150 spermatozoa were randomly counted and abnormal spermatozoa were distinguished (Boussit, 1989).

Data were collected and statistically analyzed using one-way ANOVA (IBM® SPSS 25 software). The variables analyzed were macroscopic sperm parameters (weight, Libido, volume, and pH) and microscopic parameters (mass motility, individual motility, concentration, vitality, and percentage of abnormal sperm) and the effect of age on these parameters.

3. Results and discussion

In this work, a total of 103 semen samples from the 5th month until the 11th age were collected and analyzed Table 1.

Table 1. Mean±SD values of the semen parameters analyzed

Age (Months)	*5 (n=10)*	*6(n=15)*	*7(n=16)*	*8(n=17)*	*9(n=16)*	*10(n=15)*	*11(n=14)*	*Total (n=103)*
weight (g)	3557±367,9	3675±332,7	3745±336,9	3858±354,5	3955±304,1	3814±287,5	3736±354,3	3777±331,2
Libido (s)	33,7±16,29	26±19,01	23,38±27,29	34,82±26,97	24,56±19,83	16,27±12,80	19,14±12,98	25,17±20,94
pH	7,13±0,26	7,10±0,20	7,52±0,30	7,61±0,50	7,38±0,30	7,59±0,34	7,67±0,36**	7,44±0,39
Volume (ml)	0,98±0,45	1,48±0,33*	1,44±0,33	1,12±0,59	1,03±0,36	1,05±0,28	0,99±0,27	1,17±0,43
Concentration (10^6/ml)	314±52,5	412±86,5	405±94,5	426±158,4	456±175,2	548±151,2	599±148,9*	456±154,4
Mass motility	6,5±0,71	6,27±0,80	6,38±1,36	6,59±2,45	7,19±2,04	8,07±1,10	6,86±1,79	6,84±1,70
Individualmotility	2,8±1,03	3,13±0,64	2,88±1,20	2,41±1,18	3,19±1,17	3,4±0,74	2,93±1	2,96±1,04
Abnormalsperms (%)	46,5±6,54	41,13±6,66	38,25±8,06	29,29±12,67	30,69±7,58	39,33±8,06	47±3,40*	38,17±10,21
Vitality (%)	53,05±18,1	46,53±8,9	58,94±10,4	60,71±23,5	66,5±21,1	75,87±9,7	64±14,7	61,18±18

**Refers to a significant difference (p<0,05). **Refers to a significant difference (p<0,005).*

In our work, the average value of libido was 25,17±20,93 , lower than 14,5 and 21,9 respectively reported for the Black Baladi and White New Zealandies rabbits by Safaa et al. (2008). These differences appear to be related to the genetic origin of the rabbits and the breeding program to which they have been subjected (Lankri *et al.*, 2019). While the pH of semen were significantly higher *(p<0,05)* at 11th month of age with 7,67±0,36, this can be due to the increased secretions of the vesicular glands (Rigal, 2008).

The ejaculate volume of the rabbits' semen, in this work was about 1,17±0,43 ml, higher than values from 0,3 to 1 ml reported by Orgebin-Crist (1968), Cole and Copps (1977) and Lebas et al. (1996) in adult rabbit sperm, and Boulbina et al. (2011) with 0,86 ml on the local rabbit population. Feeding *Ad libitum* increase the libido and the volume of the sperm according to (Alvarino, 2000). In the other hand; the volume of the semen, gel fraction, sperm motility, sperm concentration and morphological alterations, show high variations among the different breeds (Dubiel *et al.*, 1985; Abo El-Ezz *et al.*, 1985). The highest semen volume was recorded at six months of age which coincided with March and according to Boussit (1989) the volume of ejaculate reaches its high values from March to June.

Joly and Theau-Clément (2000) reported that the biological characteristics of the semen (volume, concentration, motility, morphological alterations ...) are very variable between and intra-breeds, but on average the values of these parameters increase with the age of bucks (from 5 months to 24 months).In this work, the adult's semen concentration was significantly higher with 599±148,94x10^6/ml. According to Theau-Clement et al. (2003) the age of males influences significantly the sperm concentration and production. Our results are higher than 232 x10^6/ml and 220 x10^6/ml reported by Safaa et al. (2008b) of two selected lines of New Zealand rabbit bucks, otherwise, it was recommended to make a collect one a week (Tacke *et al*., 1995; Bencheikh 1993; 1995), however, our rabbits were collected 2 to 3 times in a month. The different studies were

based on observation and counting, which are probably an additional source of variability in the results (Cabannes, 2008).

Sperm motility is a very important parameter that reflects the quality and has a significant effect on egg cell fertilization (Wysokińska *et al*., 2013). The higher rate of motility in this work was recorded in the 10th month of age. However, the average of total values of mass and individual motility were about 6,84 and 2,96 respectively, lower than values reported by Boulbina et al (2011) in male rabbits of the local population with a mass motility of 7,68 and an individual motility of 3,57. It was estimated that semen had a good mobility with mass motility with an appearance of waves (≥ 6/9) and individual motility with a fast progression (≥3 / 4)) (Boussit, 1989).

The vitality rate of the sperm collected increased with age and the highest value was recorded at 10th months of age with 75,87±9,69%. According to Boulbina et al (2012) delayed puberty gives more time for testicular and epididymal function to settle, and consequently a higher quality semen from the first ejaculates. Moreover it start decreasing after the 10th month because semen quality generally decreases in older rabbit bucks (Castellini, 2008).

The total sperm abnormalities rate was significantly higher at 11th months with a value of 47±3,40%, it was explained by the high environmental temperatures recorded at the moment of collection, because the age of 11 months coincided with August. Which was August characterized in the region, these results showed that temperature changes that correspond to the month of August. However, a significant interaction was observed between the breed and season (Safaa *et al*., 2008a).

4. Conclusion

It could be concluded from the current study that the age has a huge effect on semen production and characteristics.

The Algerian local bucks remain good and desirable for reproduction (natural breeding and artificial insemination)in compare with other strains like New Zealand rabbit bucks although it raised in harsh environment.

It appears that rabbit local population bucks of 10th month of age are the best reproductive and desirable.

We recommend to improve livestock under controlled conditions to have better results.

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