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SPERM CHARACTERISTICS VARIATION OF LOCAL ALGERIAN RABBIT'S POPULATION UNDER DIFFERENT TEMPERATURES

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Abstract. The main study aim to evaluate the temperature 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 under an extensive rhythm. The average value of libido was 24,99±20,96 seconds (sec.). The ejaculate volume was 1,17±0,43 ml and the mean of pH was 7,45±0,39 and significantly affected ($p<0,05$). The analyses of semen show no significant for mass and individual motility (6,91±1,56 and 2,99±1 respectively). The rate of vitality was affected and equal to 61,78±17,03. However, the evolution of temperature has significantly affected the concentration and abnormal spermatozoa ($p<0,05$). In this study, most of semen parameters were influenced by temperature variation and better semen can be obtained under moderate temperatures.

Keywords: *Fertility, rabbit, semi-arid, spermogram, temperature.*

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1. Introduction

Rabbit meat production remains low in Algeria, even though it's a precious source of meat and protein of lower cost. The improvement of this production needs a better reproduction administration. Male plays a key role in the success and profitability of breeding (Alvarino, 2000), since numerous does are bred by a single buck. The male fertility evaluation prior to breeding is paramount of importance to achieve breeding success (Ranjan *et al.*, 2020).

The selection of adequate bucks depends on sperm assessment which acquires the efficiency of reproductive performance. Thus, semen evaluation must provide information on spermatozoa fertilizing capability (Boiti *et al.*, 2005).

To obtain an optimal quantity of sperm and spermatozoa, the conditions of use of the bucks have to be defined, so, it is necessary to identify its response to the collect and the factors of variation influencing the sperm production (Boulbina *et al.*, 2012). Moreover, the heat stress (HS) is the main factor that elicits notable alterations in the testes, which ultimately alters sperm structural and functional integrity (Maya-Soriano *et al.*, 2015; Pei *et al.*, 2012). Also, temperature essentially influences semen motility (Hahn *et al.*, 2019).

Thus, the aim of this work was to evaluate the sperm production characteristics evolution within temperature in Algerian local rabbit's population raised in the western region under semi-arid environment.

2. Material and methods

The study was conducted during 2019 at the experimental farm of Tiaret university in the western region of Algeria which under a semi-arid climate. Animals were the product of a crossing between local populations up to the 5th generation. They were housed individually in wire cages arranged in flat-deck layout on one level and fed *ad libitum* with granulated commercial diet. Automatic waterers were used for drinking. Ventilation and lighting were natural and daily ambient temperature was recorded. A total of 102 semen samples were analyzed in different shed's temperatures between 10°c and 32°c. Semen was collected from twenty adult rabbit bucks weighting between 3010 – 4540 g under an extensive rhythm of collection. Bucks were trained for collection from the age of 5 months (Garcia *et al*., 2004; Garcia-Thomas *et al*., 2006b; Theau-Clément *et al*., 2009). The collection was made with an artificial vagina, using a teaser doe at collection time (Boussit, 1989). A refusal of collection is recorded if the male could not be collected within ten minutes of contact with a first doe as well as with a second (Theau-Clément *et al*., 1991; 2009).Ejaculates with urine or blood drops and ruined samples were discarded. Libido was timed in seconds with a chronometer; from the time the doe was placed inside the buck's cage up to the point when the buck started to mount the doe (Castellini *et al*., 2006).

Immediately after collection, the volume of the ejaculate was assessed by reading the graduation of the collecting tube. The collection tube was immediately transferred into an electric oven at 37°C for the preservation of sperm characteristics until analyzing.

In this work, the effect of temperature on libido, sperm volume and pH, mass and individual motility, concentration, vitality and abnormal sperm rate in local rabbits were determined.

pH was determined using a pH paper. Mass motility (MM) was appreciated by placing a drop of pure sperm was observed at (x10) magnification, a note from 0 to 9 scale was attributed according to the grid of Petitjean (1965) (Boussit, 1989).

Individual motility (IM) was assessed after dilution of the sperm with a commercial diluent at 1/5 and 4/5 diluter volumes. A drop of diluted semen was observed at (x40) magnification, and a note from 0 to 4 was attributed using the Andrieu scale (1974) (Boussit, 1989).

The concentration of spermatozoa (C) (in millions/ml) was determined using a Malassez cell counter from a drop of sperm diluted to 1/200 with the diluter. Counting was performed under the microscope at ×40 magnification (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 a dye and was gently spread along the blade. The smear was left for a few seconds, then it was observed at (x100) magnification. Dead sperms spread the dye through their damaged membrane, while living spermatozoa remain colorless. A random count of 150 spermatozoa was performed along the smear, from which dead spermatozoa were distinguished from the living ones (Boussit, 1989). The abnormal spermatozoa (AS) rate was assessed on the same sample of the stained smear. 150 spermatozoa were randomly counted and abnormal spermatozoa were distinguished (Boussit, 1989).

Data collected, were studied statistically using the IBM® SPSS 25 software and one-way ANOVA was performed.

3. Results

Ejaculates volume, libido and pH mean values are expressed in Table 1. In this this work, mean term reaction was 24,99±20,96 s, the different temperatures had no significant effect on libido while the volume of the ejaculates remained unchanged with 1,17±0,43 ml.

In this work, pH was significantly affected by temperature (*p<0,05*). In 35% of the samples, pH was about 7,7–8,4 in the buck semen, however, 65% of the samples had a decreased pH value of 7,2.

Concentration, mass and individual motility at different temperatures are expressed in Table 2. The mean concentration value was 459,02±149,62×10^6 /ml while the average mean mass and individual motility were respectively 6,91±1,56 and 2,99±1.

Mass and individual motility in collected samples from males under 25°C were lower *(p<0,05)* than other temperatures. Concentration was significantly higher *(p<0,05)* in bucks kept under 27°C and decreased in low temperatures. Living spermatozoa rate was significantly higher (p<0,05) in rabbits kept under 29°c. However, in this work, abnormal spermatozoa increased within temperatures (p<0,05).

Table 1. Mean±SD libido, volume and pH values of fresh ejaculate under different temperatures

Temperature (°C)	N	Libido (s)	Volume (ml)	pH
10	5	19±10,49	1,5±0,37	7,46±0,42
12	5	22,8±14,96	1,22±0,26	7,28±0,19
14	7	15,29±8,73	1,21±0,48	7,2±0,22
15	4	26,75±17,87	1,23±0,61	6,93±0,05
16	5	23,4±16,64	1,34±0,59	7,28±0,13
17	3	26,67±17,67	1,23±0,25	7,03±0,32
18	2	37±31,11	1,45±0,64	7,25±0,21
19	3	36,67±24,5	1,37±0,47	7,7±0,26
20	5	35,4±26,52	1,62±0,26	7,2±0,45
23	7	43,71±36,34	1,07±0,31	7,51±0,32
25	6	27,17±15,11	1,32±0,81	7,68±0,66
26	4	36±40,01	1,28±0,54	7,63±0,43
27	5	25,2±2,6	0,9±0,28	7,74±0,42
28	10	32,1±29,46	0,89±0,27	7,44±0,38
29	9	12,44±6,27	1,01±0,29	7,63±0,36
30	6	19,17±11,72	1,1±0,41	7,43±0,29
31	11	13,27±5,08	1,07±0,34	7,75±0,2*
32	5	27±19,39	1,08±0,27	7,26±0,33
Total	103	24,99±20,96	1,17±0,43	7,45±0,39

*indicates a significant difference p<0,05 in the same column.

Table 2. Concentration, mass motility and individual motility of bucks' semen kept under different temperatures (Mean±SD)

Temperature (°C)	N	Concentration (10/ml)	Massal motility	Individual motility
10	5	344±94,23	5,6±1,14	2,6±0,55
12	5	368±86,72	6,2±0,45	2,8±1,10
14	7	377,14±94,82	6,71±0,67	2,71±1,25
15	4	385±114,75	6,5±0,58	3,25±0,5
16	5	420±96,95	6,8±0,45	3,8±0,45
17	3	366,67±11,55	7,33±0,58	3,67±0,58
18	2	280,00	6,00	2,5±0,71
19	3	446,67±83,27	7,00	3,67±0,58
20	5	364±118,66	6,2±0,45	2,6±0,55
23	7	491,43±106,99	7,43±0,98	2,71±0,76
25	6	420±125,86	5,17±2,93*	1,67±1,37*
26	4	460±118,88	7,5±1	2,75±0,96
27	5	620±171,46*	6,4±2,7	2,6±1,34
28	10	510±102,09	7,4±1,26	3,4±0,84
29	9	575,56±149,26	8±0,71	3,67±0,5
30	6	373,33±147,87	7,17±1,47	3±0,89
31	11	605,45±97,61	8,18±0,87	3,54±0,52
32	5	416±276,91	5,8±3,11	2±1,41
Total	103	459,02±149,62	6,91±1,56	2,99±1

**indicates a significant difference p<0,05 in the same column.*

Table 3. Percentage of viability and abnormal spermatozoa evolution within temperatures (Means±SD)

Temperature (°C)	*N*	*Viability (%)*	*Abnormal spermatozoa (%)*
10	*5*	51,7±20,32	44,2±8,38
12	5	54±11,22	45,4±6,23
14	7	61,71±15,13	36,86±10,87
15	4	52±1,63	41,75±7,68
16	5	46±7,62	45,2±7,69
17	3	46±21,07	46±5,57
18	2	42,5±27,58	41,5±3,54
19	3	58±4	40±5,29
20	5	54±5,48	37,6±4,77
23	7	70,57±9,43	28±6,63
25	6	50,33±22,99	36±14,03
26	4	72,5±13,6	30±9,09
27	5	61,6±20,85	47,6±3,58*
28	10	72±10,79	33,8±10,04
29	9	72,89±13,64*	39,56±8,82
30	6	63,33±15,93	30,67±6,15
31	11	72,36±11,79	40,45±8,12
32	5	58±29,63	42±9,59
Total	103	61,78±17,03	38,54±9,53

**indicates a significant difference p<0,05 in the same column.*

4. Discussion

It is well known that there is a wide variety in semen traits and different factors that can influence qualitative and quantitative sperm production such as collection frequency, temperature, lighting programs and buck age (Boiti *et al.,* 2005).

In this work, reaction time decreased slightly during high temperatures exposure but with no significant difference was observed. Indeed, libido is controlled by many factors, such as hormone, sexual pheromone and the hypothalamus–pituitary–testis axis (Yang *et al.,* 2005). According to Pei *et al.* (2012) the decrease of testosterone in high temperature is consistent with reduced libido under heat stress, which indicate the involvement of testosterone in the regulation of libido.

In our study, no significant differences in semen volumes were recorded between bucks collected under different temperatures. However, with an average volume of 0,86 ml on the local rabbit population was higher than values reported by Lebas *et al.* (1996) and Boulbina *et al.* (2011). According to Alvariño (2000) feeding *Ad libitum* increase the libido and the volume of the sperm. Extreme heat stress get animal physically exhausted and reduce eagerness which might result in higher reaction time and increase total time for successful ejaculation, thus having an ultimate effect on sperms production (Mandal *et al.*, 2000).

Changes in the semen pH and sperm morphological alterations increases during the summer (Amin *et al.*, 1987) which matches with our findings. The highest values of semen pH were recorded under 31°C, this can be due to the increased secretions of the vesicular glands (Rigal, 2008). Moreover, high temperatures (more than 27 ºC) can affect fertility due to increasing semen pH values and morphological alterations, as well as a decrease in sperm motility and libido (Brockhausen *et al.*, 1979; Bagliacci *et al.*, 1987). In this study, we observed a slight decrease in pH of 65% of bucks. This may be attributed to the spermatozoa metabolic activity releasing lactic acid that decreases pH while fructose is the semen major source of energy (Bencheikh, 1995; Klein *et al.*, 1963).

Sperm motility is a very important parameter that reflects its quality and has a significant effect on egg cell fertilization (Wysokińska *et al.*, 2013). In the current study, we have observed that bucks kept under 25°C showed a lower sperm motility. Similarly, in mouse (Pérez-Crespo *et al.*; 2018) with more intense testicular heat exposure (42°C for 30 min), at 14, 21 and 28 days after exposure, there were reductions in both total motility (from ~67% to 28, 8 and 37% respectively) and progressive motility (from ~39% to 5.5, 6.5 and 6.6%). Also, we have noticed that motility on average was higher in bucks kept under high temperatures comparably with low temperatures (≤ 15°C). Furthermore, Llamas-Luceño *et al.* (2020) indicated that total motility of fresh sperm from young bulls was higher (p<0,001) when spermatogenesis occurred in summer under high temperatures compared with other seasons, which agree with our findings.

While, a significant influence of temperature was observed on the proportion of fast-motile sperm, with the better results when animals were kept at 20 °C according to Hahn K. *et al.* (2019). The different studies based on observation and counting, which are probably an additional source of variability in the results (Cabannes, 2008).

The Algerian local bucks remain better and desirable for reproduction (natural breeding and artificial insemination) in comparison with other strains (Karim *et al.*, 2020). In our work, values for sperm-cell concentration with 459,02±149,62×10/ml

were higher than those reported by Safaa *et al.* (2008b) of two selected lines of New Zealand rabbit bucks with 232x10/ml and 220 x10/ml. These differences can be explained by genetic, environmental factors, the different criteria employed for the evaluation and the use of various semen processing technologies according to Safaa *et al.* (2008).

The values of sperm concentration increased with temperatures. Animal in summer season showed highest thermal regulation, which maintain live body weight, improve sexual desire and semen quality under heat stress condition (Abdel-Khalek *et al.*, 2019).

Finzi *et al.* (1995) reported that the daily exposure of rabbits in a climatic chamber to high ambient temperature (30°C) and humidity (70%) for 21 h over a 60 days period increased the number of abnormal spermatozoa. In the current study, the high rate of abnormal spermatozoa was recorded in a temperature equal to 25°C. Although, we have noticed that high temperatures had a less impact in comparison with bucks kept under 25°C. This may be due to the few days of exposure in high temperatures. Generally, when testicular temperature rises, sperm morphology often remains normal for a few days, if sperm in the epididymis are minimally affected, followed by appearance of morphologically abnormal sperm (Barth and Oko, 1989). According to Shahat *et al.*, (2020) heat stress alters sperm quality, with effects on extent and duration of testicular heating.

In contrast, Safaa *et al.* (2008) analyzed the seasonal effects on sperm parameters of Black Baladi and New Zealand's bucks reared in Egypt, and reported that quality parameters such as viability and acrosome abnormalities of semen collected in winter were better than those collected in summer. These differences appear to be related to the genetic origin of the rabbits and the breeding program (Lankri *et al.*, 2019).

The production of sperm was variable between the males and according to the ejaculates for the same male; the temperature influences directly the quantity and quality of semen.

5. Conclusion

Most of the analyzed parameters, that indicates semen quality and predicts its fertility potential, were affected by the variation of temperatures in our study. During high temperatures, which occurs in Algeria in summer, reaction time decreased slightly, the decrease of testosterone, semen pH and sperm morphological alterations increased. According to our finding, better semen can be obtained under moderate temperatures.

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