EVALUATION OF SEMEN QUALITY IN RABBIT OF LOCAL ALGERIAN POPULATION AND SYNTHETIC LINE IN THE SUMMER SEASON: A COMPARATIVE STUDY

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Abstract. The aim of our study was to compare the libido and semen characteristics in 24 rabbits of local and synthetic line during summer season. Results showed that both breeds had similar ($p > 0.05$) libido (13.92 vs 16.85 s). Gel free volume (0.88 vs. 0.87 mL), pH (7.51 vs. 7.65), and live sperm (56.21 vs. 55.88%) were similar. Local population had higher semen concentration (398.50 $x10^6$ /mL vs. 328.90 $x10^6$ /mL) and percentage of abnormal spermatozoa (36.54 vs 30.28%). Massal and individual motility ($p=0.006$ and $p=0.008$) were significantly increased in local population. Kinetic traits for Local population were significantly greater ($P<0.05$), except for VCL, ALH and BCF. We conclude that, rabbit bucks of local population had a good ability of adaptation to produce in a hot climate.

Keywords: Rabbit, semen, temperature, CASA.

INTRODUCTION

The domestic rabbit has been associated with man, for many centuries, as a source of food and fur. Besides, it has always been used as an experimental subject to carry out scientific researches. In Algeria and many other hot climate countries, especially during summer, heat constitutes a major constraint for rabbit production (Zerrouki et al., 2014). In the summer season, the surrounding temperature increases, causing negative effects on rabbit productivity and physiological status. These disorders are related to the inability of rabbits to eliminate excess body heat due to their un-functional sweet glands (Marai et al., 1991).

The reproductive capacity of male rabbits decreases during July, August, and September, as hot conditions reduce the ejaculate volume, sperm cell concentration, and total sperm output (Marai et al., 2003). Indeed, high temperature in summer decreases libido, affects fertility by increasing semen pH values and morphological alterations (Safaa et al., 2008a). Heat stress is one of the most environmental disruptors to the reproductive functions of male rabbits as inferred by declining conception rate and litter size at birth after mating with males exposed to heat stress during summer season (Marai et al., 2003).

Rabbit breeding in Algeria has mainly been based on the use of local population for family production. Studies highlighted their good ability of adaptation to local conditions, particularly their suitability for production in hot stress conditions. However, the weight of these adult rabbits and their litter size are too low to allow

them to be considered for intensive production. To try to promote rabbit production in the country, a new synthetic rabbit line was created since 2003 by cross-breeding females from the local population with males from the French INRA 2666 strain at ITELV (Gacem, and Bolet, 2005). The creation of synthetic lines has been adopted as a new strategy to improve rabbit production in hot climate countries, such as in Egypt and Saudi Arabia by cross-breeding between foreign commercial lines and the local population (Youssef et al., 2008).

In the Algerian farms the development of rabbit breeding will require the introduction of artificial insemination (AI) (Boulbina et al., 2012). The objective of the AI centers is to obtain a greater number of doses at lower cost, and at the same time it should allow semen production to be increased while maintaining a high level of male fertility and prolificacy. The success of rabbits AI program depends, to a great extent, on male health and reproductive performance at the time of semen collection; lack of seasonal impacts on libido, producing large volume of sperm, sperm motility and viability with a few numbers of abnormalities (El-Tarabany et al., 2015).

However, few studies have been investigated to characterise reproductive performance of rabbit male of local Algerian population (Boulbina et al., 2012). Moreover, only the effect of the collection rate of sperm has been reported by Lankri et al. (2019) in the rabbit synthetic line. To the best of our knowledge, no comparative study of the reproductive performance in the Algerian local rabbit population neither the synthetic line under heat stress conditions has been done. The objective of this paper was to compare the differences between rabbit bucks of local population and those of the synthetic line on semen characteristics and physiological response under high ambient temperatures.

MATERIAL AND METHODS

Animals and management. The experiment was carried out at the Rabbitry Unit of the Teaching and Research Farm of the University of Blida1, Algeria, from June to July 2017. A total of twenty four clinically healthy ten months old rabbit bucks were used (12 from the local Algerian population and 12 from the synthetic line). Those rabbit have an average body weight of 3.075±0.35 Kg and 3.119±0.32 Kg for the local population and the synthetic line, respectively. All animals were kept under the same managerial and hygienic conditions and raised in individual galvanized wire cage batteries. The rabbit building was naturally ventilated with fresh air through wired windows. The averages of maximum and minimum ambient temperature in summer seasons (June-July) were 37 ºC and 26.5 ºC. Animals were fed and watered *ad libitum* during the whole experimental period.

Collection protocol. Rabbit bucks were trained to mount teaser female and then ejaculated in artificial vagina the 9th month of age. Males used for the study were selected on the basis of their ability to respond to semen collection. Animals have been subjected to the experimental evaluation at the 10th month of age. Two ejaculates per male were collected weekly by artificial vagina, with an interval of 15-30 min between successive ejaculates, as described by Mocé et al. (2000) for 9 consecutive weeks. A matured cyclic doe (teaser doe) was introduced to tease the experienced buck and to ensure natural stimulation for ejaculation. All ejaculates were stored at 37°C in a water

bath until evaluation, non-later than 15 minutes after collection. Ejaculates under 0.2 ml or comprising urine, blood, calcium carbonate deposits were neglected.

Libido and semen analysis. Libido (Reaction time) was estimated by the time interval in seconds between the introduction of the teaser female into the buck's cage and ejaculation (Daader et al., 1999). It was recorded with a stop watch. After semen collection from each male, the volume without gel fractions (mL) of each ejaculate was measured in a graduated conical tube. Semen pH was determined by a litmus paper (universal simplex health pH ranging from 0-14) and corresponding color changes were observed and recorded. The rabbit semen collected from the field was transferred to the laboratory using a flask containing warm water to maintain the semen temperature at 37 °C. Sperm viability and sperm cell morphological abnormalities were evaluated using eosin-nigrosin staining technique (Bamba, 1988). Sperm concentration (spermatozoa million/mL) was evaluated by Thoma–Zeiss cell counting chamber after extending fresh semen from each buck to a final dilution of 1/100 with a (10 ml 35% v/v formalin in 1 l of 0.9% NaCl). Mass motility was evaluated according to a subjective scale ranging from 1 to 9 (Petitjean, 1965). 10 µl of the semen sample was placed under a cover slip in the center of a pre warmed (37 °C) clean glass slide and examined with a microscope under x10 objective lens to determine mass motility. Sperm individual motility and kinetic traits were assessed by extending a 10µl of raw semen with 290 µl (1:30 v/v) of rabbit extender Galap (IMV, France) then 3 µl from homogenized solution were placed into a pre-warmed chamber of a Leja 20-micron four chamber slide (Leja Products BV, NieuwVennep, The Netherlands) using Sperm Class Analyzer (SCA, version 5.1, Microptic, Barcelona, Spain), a light microscope (Nikon Eclipse E200), with a x10 negative phase objective. In each sample, the kinetic parameters were: the percentages of total motile sperm cells, curvilinear velocity (VCL, µm/s; the average velocity measured over the actual point to point track followed by the cell), straight-line velocity (VSL, µm/s; the average velocity measured in a straight line from the beginning to the end of the track), average path velocity (VAP, µm/s; the average velocity of the smoothed cell path), linearity index (LIN, %; the average value of the ratio VSL/VCL), straightness (STR, %; the average value of the VSL/VAP ratio), amplitude of lateral head displacement (ALH, µm; the mean width of the head oscillation as the sperm cells swim) and beat cross-frequency (BCF) the frequency of sperm head crossing the average path in either direction) were evaluated.

Statistical analysis. All statistical analyses were done using R (version 3.5.1; R Foundation for Statistical Computing, Vienna, Austria) via RStudio (version 1.1.383, RStudio Inc., Boston, MA). Repeated-measures analysis of variance (ANOVA) in R was performed for all measured variables using compound symmetry (CS) structure. Diagnostic plots for assessing the normality of residuals and effects in models fit were obtained using ''qqnorm'' function of R. If the model not be considered normally distributed; therefore, logarithm transformation of independent variable were used. Post-hoc comparisons were computed using the ''lsmeans'' function from the lsmeans package in R (Lenth, 2016). The results were represented by the last square means (LSM) ± standard error of mean (SEM).

RESULTS AND DISCUSSION

In our experimental conditions, samplings were carried out during the summer. The data on the reaction time (libido) and certain seminal parameters in the two groups of rabbit bucks are presented in Table 1.

Reaction time (Libido). The results showed no significant effects of line and line-by-time interactions that were found for reaction time (libido). However, sampling time had a significant effect ($p<0.0001$). Our results in libido (13.92 *vs.* 16.85 s for local population and synthetic line, respectively) were closely similar to those of Rodriguez–De Lara, (2010) (13.2 s) in New Zealand white rabbit, but less than the mean value of 27.9 s obtained by Brun et al. (2006) and 20.6 s in New Zealand White and Baladi rabbits (Safaa, et al., 2008a) during the summer season. Furthermore, our values in libido were much higher than those reported by El-Tohamy et al. (2012): (11.49 s), 4.2 s by Ogbuewu et al. (2009) in New Zealand White, Chinchilla rabbit bucks, 4.45, 4.31 and 4.25 s in New Zealand Red rabbit, followed by New Zealand white and Chinchilla respectively by Isidahomen and Oguntade (2018). According to Gado et al. (2015) estradiol may be stimulated by frequency of mountings and reduced mount latency, while testosterone acts in part through *in situ* conversion to estradiol by aromatase in the preoptic area stimulating mounting, ultimately improving the copulatory behaviour.

The assessment of the seminal characteristics of the rabbits gives an excellent indication of the reproductive capacity of the animal (Isidahomen and Oguntade, 2018).

Gel free volume and pH. No significant effects of line and line-by-time interactions were found for the volume of the ejaculum (without any gel-mass). Sampling time, nonetheless, had a significant effect ($p<0.0001$). Also, there was no significant change in pH, either through the line, time or in a line-by-time interaction. The overall means of our results in free volume (0.88 *vs.* 0.87 mL for local population and synthetic line, respectively) were higher compared to (0.52 mL) ejaculate volume (Safaa et al., 2008a) in the Black Baladi and New Zealand White rabbit bucks, and in local population (0.67mL) (Ain-Baziz et al., 2012) during summer. In addition, El-Tohamy et al. (2012) reported lower values in the R line and L line selected on body weight at 63-d of age (60.1 and 46.2 mL, respectively), Iraqi et al. (2012) have registered in Gabali, V-line and M-line, means of the ejaculate volume were 0.60, 0.66 and 0.72 ml and Isidahomen and Oguntade (2018) showed that semen volume for New Zealand White, Chinchilla and New Zealand Red was 0.72, 0.59 and 0.52 ml, respectively. The studies of Marai et al. (1991) and Okab, (2007) on rabbits showed that semen-ejaculate volume decreased with the elevation of temperature. The variation in the ejaculate volume between breeds induced by summer ambient temperature may be due to differences in the rate of the accessory sex glands activity in response testosterone hormone (El-Masry et al., 1994, El-Kamash et al., 2000). The measurement of semen pH is of great importance because any semen extender used should be of approximate similar pH value as semen should act as a buffer against excessive acidity or alkalinity. Also, it acts as an indicator for the normal accessory glands secretion and the livability of spermatozoa (Abd El-Ghaffar, 1992). Indeed, pH is considered a good indicator for semen quality. In our experimental conditions, we

did find non-significant effect on pH of semen's buck. Similar results were reported by Ain-Baaziz et al. (2012) in the local population (pH: 7.2), by Iraqi et al. (2012) in Gabali, V-line and M-line (7.76, 7.72 and 7.75, respectively) and by Isidahomen and Oguntade (2018) in New Zealand White, New Zealand Red and Chinchilla (7.49, 7.47 and 7.13 respectively). The genotype and summer season did not influence pH measured in the semen's buck.

Table 1

Overall mean values and the standard deviation (SD) of libido and certain seminal parameters in the two groups of rabbit bucks

Variable		(Mean ±SD)		P values		
		Local Population	Synthetic Line	Line	Week	Line Week
Libido (sec)		13.92±1.82	16.85 ±1.53	0.103	<0.0001	0.726
Gel free volume (mL)		0,88±0,03	0,87±0,04	0.920	0.0004	0.245
pH		7,51±0,08	7,65±0,04	0.440	0.286	0.649
Concentration (x10^6/mL)		398,50±25,10	328,90±24,20	0.016	<0.0001	0.371
Vitality (%)		56,21±1,73	55,88±2,29	0.743	0.001	0.779
Morphological abnormalities (%)	Total	36,54±36,54	30,28±30,28	0.003	0.001	0.968
	Head	11,58±11,58	11,91±11,91	0.717	<0.0001	0.612
	Midpiece	0,21±0,21	0,11±0,11	0.303	0.280	0.973
	Tail	24,99±24,99	18,16±18,16	0.0001	<0.0001	0.862
Motility	Massal motility	6,82±6,82	5,96±5,96	0.006	0.147	0.901
	Individual motility (%)	40,41±18,64	34,16±20,63	0.016	0.034	0.943
Sperm motion traits	VCL (µm/s)	62,44±62,44	59,23±59,23	0.179	0.129	0.826
	VAP (µm/s)	46,62±46,62	39,88±39,88	0.0002	0.278	0.957
	VSL (µm/s)	37,87±1,04	31,26±1,33	0.0001	0.459	0.977
	STR (%)	60,70±1,79	45,47±2,73	<0.0001	0.897	0.405
	LIN (%)	74,72±1,78	59,66±3,18	<0.0001	0.794	0.315
	WOB (%)	73,67±1,86	56,84±3,07	<0.0001	0.822	0.252
	ALH (µm)	2,51±0,08	5,43±2,88	0.321	0.85	0.558
	BCF (Hz)	5,27±0,14	5,68±0,22	0.140	0.793	0.732

VCL: curvilinear velocity, VSL: straight-line velocity, VAP: average path velocity, LIN: linearity index, STR: straightness, ALH: amplitude of lateral head displacement, BCF: beat cross frequency.

Concentration and vitality. The total spermatozoa per mL was significantly different (p=0.016) at week 5 and 8 in local population compared with synthetic line. However, there was no significant change in vitality between the local population and synthetic line. Sampling time had a significant effect on semen concentration and vitality. Line-by-time interaction, though, was not significant.

Means of sperm concentration were 398.50 x10^6/mL *vs.* 328.90 x10^6/mL for local population and synthetic line respectively. Our results are lower than those recorded by Brun et al. (2006) in the L line (634 x10^6/mL) and line H (738 x10^6/mL) and those reported by Safaa et al. (2008a) in the Black Baladi (703.1 x10^6/mL) and White New Zealand bucks (596.7 x10^6/mL). However, sperm concentrations of local population were closely similar with those of Iraqi et al. (2012) for gabali line (405.83 x10^6/mL) and those reported by Lankri et al. (2019) for Synthetic line (415 x10^6/mL). Furthermore, our values in sperms concentrations were higher than those reported by

Safaa et al. (2008b) in the A line (232 x10^6/mL) and R line (220 x10^6/mL). Other studies showed detrimental effects on the concentration of spermatozoa in rabbits during the summer season (Marai et al., 1991, Ahmed et al., 2006, Okab, 2007, Ain-Baziz et al., 2012). Ayyat and El-Aasar (2008) stated that the sperm cell concentration associated with the hot climate, summer season, could be attributed to the decline in levels of testosterone and gonadotrophins essential for maintaining the testicular sperm producing potential.

In our study, it was found that means of live sperm per ejaculate were 56.21 *vs.* 55.88 % for local population and synthetic line, respectively. In our conditions, this did not reflect a difference in such group; the genotype did not affect the live sperm. Similar data for viability were recorded previously by Safaa et al. (2008b). Whereas, Iraqi et al. (2012) found a higher value 80.63, 82.38, 81.99% for Gabali, V-line and M-line, respectively, Okab (2007) found that there is an increase in live spermatozoa in the summer season and Khalil (1996) reported that the percentage of dead sperm was lower in Gabali (21%) than that in New Zealand White (24%) rabbits. While, Ain-Baziz et al. (2012) and Ahmed et al. (2006) found a significant negative effect of high ambient temperature on live normal sperms.

Morphological abnormalities. Spermatozoa morphology is an important parameter in the fertilization process. It is important to notice that the presence of large number of abnormal spermatozoa in semen seem to decrease its fertility (Iraqi et al., 2012). In our study, local population rabbit had significantly ($p= 0.003$) a high percentage of abnormal spermatozoa compared with synthetic line, with total means of 36.54 and 30.28% for local population and synthetic line respectively. This difference was mostly recorded in the tail ($p=0.0001$). However, there was no significant effect of line on the percentage of head and midpiece abnormalities. Sampling time had a significant effect ($p<0.0001$) on the percentage of head and tail abnormalities. However, there was no significant effect of sampling time on the percentage of midpiece abnormalities. Likewise, there was no significant effect of line-by-time interaction on the three of abnormality categories. Moreover, Ain-Baziz et al. (2012) found a very significant effect of the summer ambient temperature on the abnormal sperms rate in local population rabbits. On the other hand, Iraqi et al. (2012) observed sperm abnormalities with means of 11.79, 12.92 and 12.09% for Gabali, V-line and M-line, respectively and Błaszczyk et al. (2013) showed that the total number of pathological spermatozoa in adult New Zealand White rabbits was 14.2%, from all the pathological spermatozoa evaluated the highest number was tail abnormalities (12.31%). Mostly, Marai et al. (1991) attributed the increase of the abnormal sperms rate in the summer ambient conditions to defects of the spermatogenesis, particularly in the last stage of differentiation of spermatids.

Massal and individual motility. Mass motility score was 6.82 *vs.* 5.96 for local population and synthetic line respectively. Individual motility was 40.41 % *vs.* 34.16 % for local population and synthetic line respectively. In our study, both massal and individual motility were significantly increased in local population compared to synthetic line ($p=0.006$ and $p=0.016$ for massal and individual motility respectively). However, there was no significant effect of sampling time and line-by-time interaction. Whereas, Ahmed et al. (2006), Ain-Baziz et al. (2012) and Iraqi et al. (2012) found a significant negative effect of high ambient temperature on sperms motility. On the

other hand, Isidahomen and Oguntade (2018) showed that motility was higher for New Zealand white, followed by New Zealand Red and Chinchilla (87.27%, 86.24% and 85.08% respectively). Likewise, Błaszczyk et al. (2013) reported higher values of sperm motility in New Zealand white (81.17%). These differences in sperm motility may be due to the variations in pituitary gland activity that can affect the secretion of luteinizing hormone (LH) which affects the secretion of testosterone from the interstitial tissue (Leydig cells) of the testes (Seleem, 2005).

Sperm kinetic traits. The visual evaluation of the motility by an operator is rather subjective; therefore the use of the CASA system is necessary. Motility parameters, determined by this method can provide more accurate information about the fertilizing potential of rabbit spermatozoa (Lavara et al., 2005). Concerning the motility parameters assessed by the CASA system, in our experimental kinetic traits for local population, they were ($P<0.05$) significantly greater than those for the synthetic line, except for VCL, ALH and BCF. However, there was no significant change in all the kinetic traits, either through the time or in a line-by-time interaction. Safaa et al. (2008b) achieved similar results and detected no differences between A and L lines except for BCF. On the other hand, Lukac et al. (2009) reported lower values on transgenic and non-transgenic rabbit bucks for detailed analysis of spermatozoa motion than those observed in the current research. However, Błaszczyk et al. (2013) reported higher values in New Zealand White rabbit bucks for detailed analysis of spermatozoa motion (VCL :120.99 μm/s, ALH: 4.09 μm/s, BCF : 33.41 Hz).

CONCLUSIONS

The assessment of semen characteristics is very important and useful especially for breeders in diagnosing fertility problems. In our conditions, the results demonstrated that the rabbit buck of local Algerian population had similar libido compared to the synthetic line. Also, no difference in sperm volume, pH and in live sperm was observed between the two groups of rabbit bucks. However, semen concentration percentage of abnormal spermatozoa was higher in the local population. The motility parameters assessed in our experimental showed that both massal, individual motility and sperm kinetic traits were significantly higher in local population compared to synthetic line. Eventually, more studies could be considered to focus on the relationships between semen characteristics and fertilising ability from groups of rabbit bucks under the influence of different factors.

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