

EFFECT OF BIRTH SEASON ON ONSET OF PUBERTY AND SEMEN CHARACTERISTICS IN MALE RABBIT OF ALGERIAN POPULATION (*Oryctolagus cuniculus*)

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ABSTRACT

The objective of this work was to determine the effect of birth season on the puberty age of male rabbit from local Algerian population (*Oryctolagus cuniculus*). Another objective was to study the quantitative and qualitative characteristics of semen during the same period. The mounting behaviour was controlled in 25 young males (14 were born in summer and 11 were born in winter) from the 11th week of age until the appearance of a positive mounting which resulted in the first ejaculate into the artificial vagina i.e. onset of puberty. Sperm collection started from the first ejaculate until 100% of the rabbits reached puberty. Since the onset of puberty, bucks were solicited one day per week two times separated by an interval of 10 to 20 minutes. Thus, 108 ejaculates were collected from the group of rabbits born in summer (S) and 82 ejaculates from those born in winter (W).

The onset of puberty was found to be between weeks 15 and 19 of age for rabbits born in winter and taking longer, between weeks 17 and 23 for young rabbits born in summer. The study of sexual activity and sperm production during the period of onset of puberty as a function of birth season showed that the libido was better in rabbits born in winter (14 s vs 22 s), nevertheless this difference was not statistically significant ($p > 0.05$). The same group of rabbits showed a significantly higher pH as well as the total and gel-free volume (7.46 vs 7.01; 0.74 ml vs 0.37 ml and 0.52 ml vs 0.32 ml respectively; $p < 0.001$). However, statistical analysis did not indicate any significant difference in color, sperm production, mass and individual motility, or the percentage of live spermatozoa between the two groups of rabbits considered in this study. Numerous anomalies of spermatozoa were observed in rabbits born in winter (32.6 %) compared to rabbits born in summer (27.6 %; $p < 0.01$). This study underlined the important effect of birth season on puberty age and semen characteristics.

Key words: Local male rabbit, puberty, semen, spermatozoa, summer, winter.

INTRODUCTION

Rabbit breeding in Algeria is mainly based on the use of local population. This requires knowledge of the biological and zootechnical abilities and the adaptability to breeding conditions. Several studies have been conducted to characterise and preserve the genetic patrimony of this population. Concerning reproduction, the majority of work focused on the physiological and hormonal aspects of female (Remas, 2001; Moumen et al., 2009; Boumahdi et al., 2009; Belabbas et al., 2011). Although reproduction aspects of male rabbit play an important role in the success and profitability of rabbit breeding, it has never been previously investigated in local population.

The enhancement of rabbit breeding requires the introduction of the artificial insemination technique in our farms. However, the application of this technology requires the determination of the reproductive capacities of both sexes. For the male, it is necessary to determine the age at puberty and sexual maturity, its response to artificial semen collection and the factors influencing sperm production. This allows the definition of optimal conditions to use bucks, thereby obtaining an optimal quantity and quality of sperm and spermatozoa.

The aim of the present study was to investigate the effect of birth season on onset of puberty in male rabbit of local population and study the quantitative and qualitative characteristics of their semen during the same period.

MATERIALS AND METHODS

Animals and experimental design

The experiment was performed in the rabbit experimental farm at the National Veterinary School of Algiers (ENSV). During the period between August and April, 25 young rabbits of local population 11 weeks old, were distributed in two groups as follows:

A group of 14 males born in summer (June), with an average weight of 1518 ± 190 g.

A group of 11 males born in winter (December), with an average weight of 1895 ± 221 g.

Rabbits were housed in individual cages, equipped with a feed hopper and an automatic watering system with nipples. They were watered and fed *ad libitum* with commercial rabbit pellets.

During the whole period of study, the lighting was natural and the diurnal air temperature and relative humidity inside the rabbit farm were measured daily. The temperature-humidity index (THI) was calculated using the equation modified by Marai *et al.* (2002) (Table 1).

Table 1: Experimental diurnal air temperature and relative humidity and calculated THI values (Mean \pm SD).

Period	Temperature ($^{\circ}$ C)	Humidity (%)	THI
Summer (from July till September)	31.9 ± 1.9	58.8 ± 7.3	29.6 ± 1.5
Autumn-winter (from October till January)	17.6 ± 4.4	75.3 ± 7.6	17.3 ± 4.0
Spring (March and April)	20.4 ± 1.6	68.1 ± 6.9	19.8 ± 1.4

The mounting behaviour was tested weekly by placing a female in the bucks' cage for 15 minutes. The criterion used to determine the onset of puberty was the appearance of a positive mounting behaviour which resulted in the first ejaculate into the artificial vagina (Macari and Machado, 1978). Sperm collection started from the first ejaculate until 100% of the rabbits reached puberty.

Collection and estimation of semen characteristics

Semen collection was performed during the morning using an artificial vagina once a week. Two successive ejaculates per day (10 to 20 min apart) were obtained. Libido was estimated by the time between introduction of the female into the male's cage and ejaculation. Immediately after collection semen was kept in a water bath at 37° C. Semen color (scale of 0 to 3), pH, total and gel-free volume, mass motility (scale of 0 to 9), individual motility (scale from 0 to 4), percentage of live spermatozoa and abnormal spermatozoa were estimated according to the methods described by Alvarino (1993). Concentration was estimated using a Thomas-Zeiss cell counter (final dilution 1:50). From the previous elementary traits, two synthetic criteria were calculated: the total number of spermatozoa per ejaculate (TSE), calculated as the product of volume times concentration and the number of live sperm per ejaculate (LSE), calculated as the product of TSE times percentage of live spermatozoa. Semen was discarded from the analysis when volume was lower than 0.1 ml or contaminated by urine or blood.

Statistical analyses

Statistical analysis was performed using analysis of variance (ANOVA) to determine the effect of season of birth on the various parameters studied. Analyses were performed using the program StatView (Abacus Concepts, 1996, Inc., Berkeley, CA94704-1014, United Kingdom). The significance level is 5%.

RESULTS AND DISCUSSION

Age of puberty onset according to birth season

Results reveal that onset of puberty, corresponding to the first ejaculate, in young male rabbits of local population differs according to the season of birth (Figure 1). It was found to be between 15 and 19 weeks in rabbits born in winter and a little later, between the 17th and 23rd week of age for those born in summer. The effect of birth season on onset of puberty has already been shown by Garcia-Tomas *et al.* (2009b) in Spain. These authors located the puberty age of two rabbit lines Caldes and Prat by estimating the percentage of seminiferous tubules with presence of lumen, a criterion used frequently as an indicator of the onset of puberty. The study showed that the two lines reached puberty at 14 weeks of age for rabbits born in winter and 16 weeks of age for rabbits born in summer. The difference in the onset of puberty has been explained by the plasma testosterone concentration, which was low in rabbits born in summer compared to those born in winter (79.0 ± 29.5 ng/dl vs 184.0 ± 39.7 ng/dl at 14 weeks of age; 125.6 ± 35.1 ng/dl vs 284.9 ± 55.2 ng/dl at 16 weeks of age, respectively) (Garcia-Tomas *et al.*, 2010).

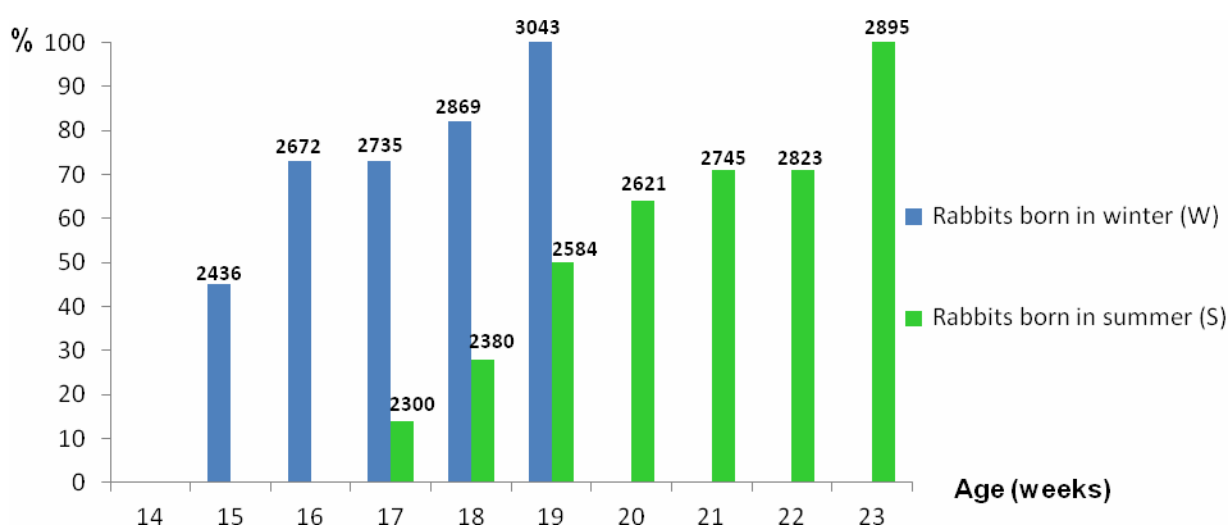


Figure 1: Progressive evolution of onset of puberty of young rabbits (%) according to birth season
Group S: n=14 males; group W: n=11 males. Number on bars: Average weight of young rabbits in grams.

The duration of onset of puberty was 5 weeks for group W and 7 weeks for group S (Figure 1). In addition, 45% of rabbits born in winter were considered pubertal from the first week of onset of puberty whereas the first week represented only 14% of rabbits born in summer. This can be probably related to greater individual variability in rabbits born in summer compared to that found in rabbits born in winter. Indeed, Berger *et al.* (1982) reported a significant individual variability in buck's fertility between 3 and 6 months of age. Variability was also reported in weight, volume of the testis and epididymis, percentage of seminiferous tubules with presence of elongated spermatids and spermatozoa, which could possibly explain the variability of the onset of spermatogenesis or onset of puberty between males (Garcia-Tomas *et al.*, 2009a).

Also, it is important to note that for the same age (particularly 17, 18 and 19th week), the body weight of the young bucks of the group W was significantly higher than that of the young bucks of the group S ($p < 0.001$, Figure 1). The average difference in body weight between the two groups recorded between the 17th and 19th weeks of age was 16%. Indeed, rabbits born in winter have passed a growth in favourable environmental conditions (mid-December to March), ensuring their proper physical development. In contrast, those born in summer are subjected to heat stress (from June to September), which induces a decrease in feed intake and consequently an alteration of body weight (Marai *et al.*, 2002). This higher body weight observed in rabbits of the group W could explain the early onset of puberty. The effect of the difference in body weights induced by the season of birth on the onset of puberty of young rabbits was also reported by Garcia-Tomas *et al.* (2009b) to be 12.5%.

However, the differences between the weights of the two groups of rabbits recorded during the first week of onset of puberty and during the last week when 100% of the rabbits reached puberty were not statistically significant ($p > 0.05$). This suggests that puberty in rabbits is related more to body weight than age.

Sexual activity and semen characteristics

The useful ejaculates rate was estimated for rabbits of local population (born in summer and winter) to be 94.4%. Similar results were reported by Nizza *et al.* (2003) with average rates of 91.5% among commercial strain Hyla. However, Brun *et al.* (2002) and Bencheikh (1995) obtained lower rates of 78.7% in strain INRA1601 and 82.9% in the commercial strain Hyplus, respectively. The overall rate of ejaculates with presence of gel was higher compared to that reported in different breeds and lines of rabbits (Roca *et al.*, 1993; Garcia-Tomas and *al.*, 2006). Moreover, ejaculates with presence of gel showed a significantly higher rate ($p < 0.05$) in the group W, compared to the group S (Table 1).

Table 1: Effect of birth season on sexual activity and semen characteristics throughout the period of onset of puberty (weeks 15 to 19 for group W and 17 to 23 for group S) (Mean \pm SE).

Traits	Group S (n=101)	Group W (n=75)	p
No. of solicitations	112	82	-
Response to solicitation (%)	96.4 ^a	100 ^a	0.775
Libido (s)	22 \pm 3.5 ^a	14 \pm 2.9 ^a	0.101
Useful ejaculates (%)	92.6 ^a	96.3 ^a	0.827
Gel presence (%)	21 ^a	40.5 ^b	0.013
Color (0 - 3)	1.36 \pm 0.05 ^a	1.43 \pm 0.06 ^a	0.373
pH	7.01 \pm 0.04 ^a	7.46 \pm 0.03 ^b	<0.0001
Total volume (ml)	0.37 \pm 0.03 ^a	0.74 \pm 0.06 ^b	<0.0001
Gel-free volume (ml)	0.32 \pm 0.02 ^a	0.52 \pm 0.04 ^b	<0.0001
Mass motility (0 - 9)	3.04 \pm 0.22 ^a	2.63 \pm 0.25 ^a	0.225
Individual motility (0 - 4)	1.96 \pm 0.15 ^a	1.94 \pm 0.16 ^a	0.910
Live spermatozoa (%)	42 \pm 3.2 ^a	36 \pm 3.1 ^a	0.176
Concentration 10 ⁶ spz/ml	234 \pm 26 ^a	87 \pm 17 ^b	<0.0001
TSE (10 ⁶ spz/ejaculate)	89 \pm 12 ^a	62 \pm 16 ^a	0.186
LSE (10 ⁶ spz/ejaculate)	61.4 \pm 9.8 ^a	39 \pm 11 ^b	0.001
Abnormal spermatozoa (%)	27.7 \pm 1.01 ^a	32.6 \pm 1.63 ^b	0.007

Means with different letters on the same row differ significantly at the 5% level.

Ejaculates from young rabbits born in winter were less concentrated and had less number of live spermatozoa (Table 1). This is due to ejaculates collected during the first two weeks (15th and 16th weeks of age) which were characterised by the absence of cells. However, ejaculates from rabbits born in summer had an acceptable sperm concentration and viability from the first collection age (17 weeks).

Rabbits born in winter have better libido compared to those born in summer. Similarly, total and gel-free volumes are significantly higher (Table 1). The increased ejaculate volume in rabbits born in winter could be explained by a significant secretion of glands stimulated by a higher testosterone levels (Garcia-Tomas *et al.*, 2010). Also, in the same group, sperm was characterised by a higher pH which could be related to their young age as previously reported in a study by Theau-Clément *et al.* (2009), which investigated the variation of pH of semen in rabbits of different ages. Moreover, under our experimental conditions, the rabbit age and duration of onset of puberty did not affect sperm production, motility and viability of spermatozoa in both groups of rabbits (Table 1). The rate of total spermatozoa abnormalities was significantly higher in rabbits born in winter ($p < 0.01$) (Table 1). This could be explained by the age difference between the two groups and by the prolonged duration of onset of puberty for males born in summer. In addition, the collection of sperm from both groups of rabbits was carried out under different photoperiods (spring for rabbits born in winter and autumn for

those born in summer), that may affect different male reproductive traits. Roca *et al.* (1995) reported that the semen of young rabbits reared under natural light showed more anomalies than young rabbits reared under low light intensity (<5 lux). Moreover, testis size in male wild rabbits was maximal during May and June and minimal between October and December. Regression occurred after the summer solstice and recrudescence occurred after the winter solstice (Boyd, 1985).

CONCLUSION

Following the results of this study, we can conclude that:

- Males of local population showed a good response to solicitations and a good rate of useful ejaculates compared to data found in literature.
- The age at onset of puberty in the local rabbit varied with birth season, it was located between the 15th and 19th weeks for the rabbits born in winter and between the 17th and 23rd weeks for the rabbits born in summer.
- The weight of rabbits born in the summer and rabbits born in the winter did not differ significantly in the first week of onset of puberty and last week when 100% of rabbits reached puberty despite the age difference between the two groups. This suggests that puberty in rabbits is related more to body weight than age.
- In general, rabbits born in winter (early and rapid onset of puberty) have better libido with an important volume of ejaculate, but characterised by a lower number of live sperm per ejaculate and many more abnormalities. Future work will investigate the effect of early and rapid onset of puberty showed by young rabbits born in winter on semen beyond 19 weeks of age.

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