STUDY OF THE HISTOFUNCTIONAL CHARACTERISTICS OF THE OVARIAN STRUCTURES OF RABBITS OF THE SYNTHETIC STRAIN IN THE STATE OF PREGNANCY

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ABSTRACT

The objective of our study is to determine the modifications of ovarian structures in rabbits belonging to the synthetic strain "SS" at the end of pregnancy, in order to evaluate the prolificity and its main biological components. A total of thirty (30) 4-month-old females (nulliparous) with an average weight of 3300±141g were inseminated, twenty-six (26) of them were positive on palpation (12th day of gestation), and six (6) of them were sacrificed by decapitation on the 24th day of gestation. The ovaries were removed and fixed in 10% formaldehyde and then treated for histological study with a standard topographical haematoxylin-eosin stain. A morphometric study was carried out using AxioVision software to measure the different structures of the ovary (follicular populations). The classification of fetuses of the synthetic strain according to their status (dead, alive, resorbed) showed that of the total number of fetuses implanted at the end of pregnancy (195 in total), the average number of live births determined in live females (20) was 8.2 per rabbit and the number of resorbed fetuses was almost nil. The number and measurements of follicular and oocyte components (diameters) revealed that rabbits of the synthetic strain in terms of fertility, productivity and ovulatory potential are better compared to local populations.

Key words: Rabbit, Ovary, Gestational histomorphometry.

INTRODUCTION

The rabbit is a coitus-induced ovulating species which accepts mating a few hours after giving birth (Lebas, 1994; Theau-Clément, 2008). She can thus be simultaneously pregnant and nursing (Fortun Lamothe and Bolet, 1995). Gestation does not lead to an interruption of follicular growth. It is therefore possible to observe, in addition to the corpus luteum, follicles at different stages at the ovarian level (Adams, 1968).

The synthetic line rabbit was resulting from an insemination of 81 females of local population with male rabbit semen of the INRA 2666 strain in 2003 (Gacem and al., 2009) selected with a high prolificacy at birth and at weaning and a production in hot climat. The characterization of the synthetic strain has indicated that it is advantages; a higher adult weight (about 450 g than local rabbits), a higher litter size at birth and weaning (+2.12 total born and +1.46 weaned rabbit / female per litter) and a hot season production (Gacem and al., 2009; Bolet and al., 2012; Zerrouki and al., 2014). The origin of the higher prolificity registered in this strain may be related to a higher ovulation potential than that determined in females of the local population characterized by a low prolificity (6.2 live births/carried/female) and an ovulation potential of 9.13 oocytes laid (corpora lutea) counted at 12 days gestation (Zerrouki et al., 2009). It should be noted that studies on ovarian morphometry and follicular growth are often old or few (Kranzfelder et al., 1984; Boumahdi et al., 2013).

The aims of this work is to characterize the productivity of rabbits of the synthetic strain by measuring ovulatory potential by quantifying the follicles at different stages, thus allowing to explain in part the better prolificity recorded in this strain.

MATERIELS AND METHODS

The experiment was carried out at the Tigzirt breeding station in Kabylia in northern Algeria. The females are from the genetic core of the synthetic strain established at the Tigzirt breeding farm in 2011 (Zerrouki et *al.*, 2014; Bouziad-Chibah and Zerrouki, 2015). The rabbits belong to the 10th generation from the selection nucleus established at this station (Zerrouki, 2019). These rabbits are bred according to a breeding protocol that allows a closed nucleus of this strain to be maintained while applying an insemination programme that minimises inbreeding. A total of thirty (30) 4-month-old females (nulliparous) with an average weight of 3300±141g were inseminated, twenty-six (26) of them were

positive on palpation (12th day of pregnancy), and six (6) of them were sacrificed on the 24th day of gestation. Six (6) of them were sacrificed on the 24th day of pregnancy. The females fasted before the sacrifice. After anaesthesia with chloroform by inhalation, they were decapitated, the ovaries were removed and fixed in a 10% formaldehyde solution and cut according to the method described by Martoja and Martoja 1967 to observe the stages of follicular development and their quantification (figures 1 and 2), and the classification of fetuses of the synthetic strain according to their status (dead, alive, resorbed) in nulliparous rabbits of heterogeneous coat color was determined.

Morphometric study: All the histological photos were taken with a photomicroscope (VioLA MC2Oi version 19.12.17) equipped with a digital tablet at magnifications x 4, x 40 and x 400). We carried out measurements, using 'AxioVision 4.8, computer software developed by Carl Zeiss, after micrometer calibration.

Statistical analyses: The values presented are expressed as averages affected by the standard error to the mean. The latter was carried out using the "R" software, which allows the averages obtained for each parameter studied to be compared.

RESULTS AND DISCUSSION

Morphology, development and follicular quantification in the rabbit ovary according to the physiological stage.

The classification of fetuses of the synthetic strain according to their status (dead, live, resorbed) determined in our study showed that of the total number of fetuses implanted in late gestation (195 in total), the average number of live births determined in live females (20) was 8.2 per rabbit and the number of resorbed fetuses was almost nil (only one resorbed fetus), which is almost identical to the results on the (SS) of Bolet et *al*, (2012) with an average of 9. 13 total births and 8.40 live births. These rabbits of synthetic strain may have a higher ovulatory potential allowing better prolificity results than those reported for the local population (6.23 live births), (Gacem et *al*, 2009; Zerrouki et *al.*, 2014).

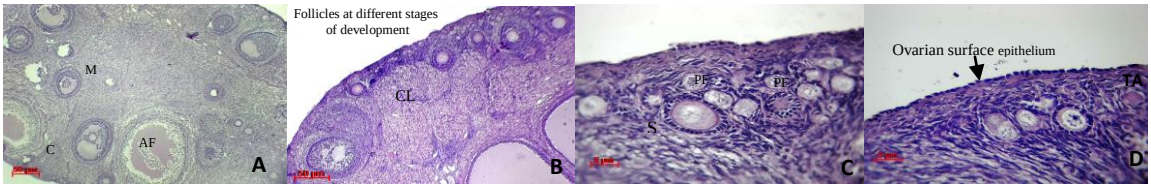

Figure 1: Photomicrographs of rabbit ovary sections stained with haematoxylin and eosin (HE) .
(A and B) Histological structures of the ovary of the rabbit which is mainly composed of a cortex and a medulla. The ovarian epithelium, in continuity with the peritoneum, surrounds the organ. The cortex, made up of a dense connective tissue of cells, houses the ovarian follicles. The medullary, the connective-vascular zone. (C and D). The ovarian epithelium consists of a bed of cubic squamous cells called the germinal epithelium. The cortical stroma is a connective tissue poor in fibres and rich in cells, arranged in swirls. The micrographs were taken at different magnifications Bar 5 and 50 µm. M : Medulla, AF :Atresic folicle, C : Cortex, CL: Corp luteum, TA: Tunica albuginea, PF: Primordial follicles

Table 1: Average diameter of oocytes and follicles at different physiological stages in pregnant rabbits.

Follicular stage [q]	Average diameter (µm) ±SE	
	Oocyte	Follicle
Rabbits [s]		
Primordial n = 151	39,17±1.38	44,65±1.57
Primary n = 41	55,07±2.58	80,44±3.68
Secondary n = 45.5	110,35±4.46	180,27±10.29
Tertiary **n = 25.5**	115±5.02	**598,39±34.63**
Degraf(preovulatory) **n=9**	121,22±8.32	**981,1±71.59**

[q] The total number of follicles noted for each category is indicated next to the follicular stage.
[s] Diameters were determined by examining 12 ovaries. At least six tissue sections for each ovary were analysed; all follicles in 10 fields of vision (magnification × 400 and magnification × 100).

Number of follicles (Table 1):
The average number of primary and secondary follicles determined in rabbits of the synthetic strain was higher than that reported by Boumahdi and Tarzaali, 2019 in both receptive and non-receptive rabbits of the local population that received GnRh (41 and 45.5 vs. 21.4 and 22.5, respectively). However, our results on pre-ovulatory follicles are close to those reported by Belabas et *al*, (2011) and are superior to those reported by Boumahdi and Tarzaali, (2019) in local nulliparous rabbits. Theau Clément et *al*, (2008) report lower values than ours (6,7) in primiparous rabbits INRA0067 . Ovarian changes due to decreased progesterone levels (Beyer and Mcdonald, 1973; Elsaesser, 1980; Hudson et al., 1990) associated with the levels of

oestrogen released by the prenatal follicles (Challis et *al.*, 1974; Nicosia et *al.*, 1975) explain the sexual behaviour of rabbits in the last days of gestation (Beyer and Rivaud, 1969). Boumahdi et *al*, (2009) show that at parturition, the antenatal follicles are ready to ovulate just after birth when the receptivity of the females is high. However, their number is low between 0 and 48 h and never higher than (2) in local rabbits, whereas in the synthetic strain our results show a high number of tertiary and pre-ovulatory follicles on the 24th day of gestation which are (25.5 and 9). Lefevre and Caillol (1978) and Kermabon et *al*, (1994), showed that the number of pre-ovulatory follicles is higher in receptive females compared to non-receptive females.

Morphometry of follicles and oocytes :

The mean diameters of primordial, primary, secondary, tertiary and pre-ovulatory oocytes and follicles (Figure 2) in rabbits on day 24 of gestation (Table 1) were almost similar to those of Hutt et al. (2006) who report values in 12 week old New Zealand rabbits of 32.7 ± 2.3 µm, 95.5 ± 5.0 µm, 144.7 ± 10.5 µm, 676.7±17, 2 µm with follicular oocyte diameters of 30.3 ± 0.2 µm, 59.0 ± 1.0 µm, 81.8 ± 2.1 µm, 84.0 ± 3.7 µm for all developmental stages. Our results show that the mean diameter of pre-ovulatory follicles in females of synthetic strain is equal to 941µm, higher than that of local rabbits, which is 640 µm (Boumahdi and Taarzali, 2019) and that obtained by Zitný et *al*, (2004), which is 682 µm. This value is close to that obtained by Kranzfelder et al, (1984) who consider pre-ovulatory follicles to be those with a diameter between 800 and 900µm. For Hulot and Mariana (1985), the diameters of 800 and 951µm, are characteristic of the pre-ovulatory stage. The diameter of the oocytes is on average equal to 99µm for Zitný et *al*, (2004). The relationship between the average diameters of the oocytes included in their follicles, reveals that the diameter of the oocytes increases proportionally to the diameter of the follicles from the primordial stage to the tertiary stage, due to the beginning of basal folliculogenesis (Figure2), which takes place in the presence of hormonal stimulation by FSH (Driencourt et *al.*,2001). These hormones probably modulate the synthesis and maturation capacity of the granulosa cells regulated by numerous growth factors, of oocyte or somatic origin, which control this stage and act essentially according to a paracrine regulation mode (Monniaux et al., 2009).

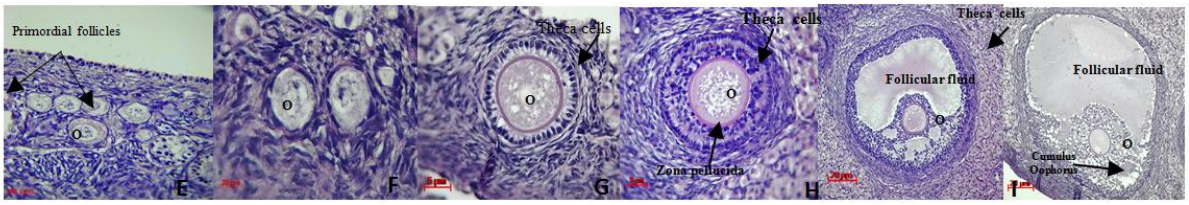

Figure 2: Classification of rabbit ovarian follicles. E. The primordial follicles of the rabbit are surrounded by a single layer of flattened follicular cells. F. The first primary follicles are surrounded by a mixture of flattened and cuboid follicular cells. G. The primary follicles are surrounded by a single layer of cuboid cells. H. One to two layers of cuboid follicular cells that form the granulosa are observed in the secondary follicle: the Theca cells that surround the follicular basement membrane, the zona pellucida can be clearly observed. I. Cavities filled with follicular fluid form between the cell layers of the granulosa of the small antral follicle and these merge into a single large pool of fluid in the large antral follicle surrounded by the internal and external theca (J). Bars 5 µm, 20 µm, 50 µm, 200 µm.

CONCLUSION

The study carried out on the histo-morphometric characteristics of the ovaries at the end of gestation in rabbits of Algerian synthetic strain showed that the ovarian structures evolve in a similar way in all species, from the primordial follicle stage to the pre-ovulatory follicle stage. However, the high number of tertiary and pre-ovulatory follicles asserts the performance of this strain, confirming its good qualities of adaptation to climatic conditions, maintaining its superiority over the two local populations in terms of litter sizes recorded at birth.

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