HISTOFUNCTIONAL CHARACTERISTICS OF THE MAMMARY GLAND IN A SYNTHETIC RABBIT STRAIN

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ABSTRACT

The objective of our experiment is to provide on the one hand interpretation elements on the milk production rates recorded during the lactation period in nulliparous rabbits, 4 months old, belonging to the synthetic strain "SS" and on the other hand, to determine the functional modifications of the mammary gland in rabbits at the end of pregnancy. To do this, an evaluation of milk production during the three weeks of lactation and a histomorphometric study to evaluate the structural modifications of the mammary gland at the end of gestation were carried out. Rabbits were weighed and sacrificed by decapitation. The work took place at the private rabbit breeding station in Tigzirt (Algeria). The mammary glands were collected and treated for histological study with a standard topographical haematoxylin-eosin stain. A morphometric study was carried out, targeting the measurement of the different structures of the sampled organ. The results obtained show that in the pregnant rabbit an important development of the mammary gland is observed, in this case the diameter (107.47±5.48 µm) and the surface of the acini (10419.06 ± 999µm) as well as a milk production of 3506±444 g during the 21 days of lactation. These results show variations in the mammary structure that prepares for lactation during gestation to ensure growth and survival of the offspring.

Keywords: Rabbit, Synthetic strain, Mammary gland, Milk production, Histomorphometry.

INTRODUCTION

The mammary gland is the central organ of an essential biological function which is lactation. For this reason and for a long time, it has been the object of much research. Breast milk contains the essential elements for the development of the newborn and provides immunological and hormonal elements that protect it and promote its growth and survival. The rabbit strain used in the present study is the synthetic strain. 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. Its 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). Indeed, since its diffusion in 2012, this synthetic strain has been the subject of numerous works which first consisted in describing its reproduction and growth characteristics. The aim of this work was to study the histological and functional characteristics of the mammary gland at the end of gestation in these rabbits, in relation to the rabbits' milk production capacities.

MATERIALS AND METHODS

The study took place at the private rabbit breeding station of Tigzirt, Tizi-Ouzou (Algeria) (station which received the selection nucleus of the synthetic strain to continue the diffusion phase). Two experiments were carried out: The first one, concerns a follow-up of rabbits from birth until the end of the lactation phase (21 days) in order to measure the milk production of the females. The second aims to study the histomorphometric characteristics of the mammary gland taken from a few females (06) that were sacrificed. A total of thirty nulliparous females, aged 4 months and weighing 3300±141 (g) were inseminated. Abdominal palpation was performed 12 days after AI, revealing 4 negative and 26 positive rabbits. The 6 rabbits were destined for the histomorphometric study after sacrifice on day 24 of gestation. At parturition, the 20 rabbits were weighed and the live born litters were weighed after counting the litter sizes in terms of total number of young rabbits, both alive and stillborn. The rabbits were fed with a special commercial maternity food (SANDERS-SIM) with digestible energy (2575 kcal/kg), crude protein (17%), Fat (3.5%), Ca (0.6%), P (0.8%) and Vitamin A (10000UI). Access to water was free and ensured by an automatic watering system.

Milk production measurement: 20 rabbits and their litters were monitored throughout the lactation period. The contact between mother and young rabbits takes place only once a day for 3 minutes. The quantitative evaluation of the milk produced by the dams was done according to the method described by Lebas and Zerrouki (2011), by measuring the weights of the dams and litters before and after suckling. Weighing was carried out daily using an automatic 15 kg scale, with an accuracy of ± 5g.

Histo Morphometry: The histological study of the mammary gland in SS rabbits pregnant on day 24 was carried out on slices, from mammary fragments and stained with haematoxylin-eosin. The histological photos were taken under a photomicroscope (VioLA MC2Oi version 19.12.17) equipped with a digital tablet (at magnifications x 4, x 40 and x 400). For the morphometry, we used AxioVision 4.8, computer software developed by Carl Zeiss.

Statistics: The milk production averages of rabbits during the whole lactation phase are expressed as averages affected by the standard deviation for milk production (X ± δ) and the standard error to the mean (X ± ESM) for morphometry, they were obtained using the software "R" which allows comparing the averages for each parameter studied.

RESULTS AND DISCUSSION

The result of palpation of the thirty inseminated females revealed 26 positive rabbits of which 6 rabbits were destined for histomorphometric study after sacrifice towards the end of gestation and 20 rabbits were devoted to milk production evaluation.

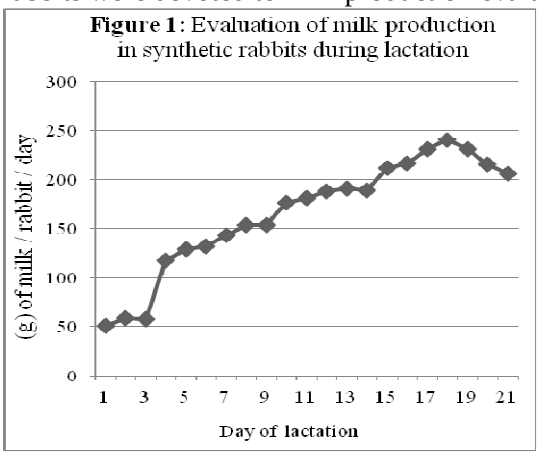

Figure 1: Evaluation of milk production in synthetic rabbits during lactation

Dairy ability: The milk production curve of the 20 rabbits of the synthetic strain that gave birth and was followed over 21 days of lactation is shown in figure 1. During the three weeks of lactation, a female rabbit produces 3506 ± 444 g of milk for a litter with an average litter size ranging from 8 to 5.4 from the beginning to the end of the lactation period. Thus, each weaned litter receives an average of 3206 ± 422 g of breast milk, or about 152.7 g/day. The milk production results obtained in the present study are better (3506 g) than those reported by Zerrouki and *al.* (2005) on the local population (2180 g) and Lebas and Zerrouki (2011) on the white Algerian population (3047.6 g). The milk production rate evolves progressively during the 3 weeks of lactation (694±41.8/S1, 1240±14.5/S2 and 1572±14.4/S3) as well as the average daily gain of the litter (35.4 ± 105/S1, 69/S2 and 73/ S3). Milk production peaks in the 3rd week at about 17-19 days of lactation and then decreases. Milk is the only food for the bunnies during the first seventeen days of life (Chibah and Zerrouki, 2015) and around 18 days of age they start to ingest solid food in addition to the milk provided by the mother (Fortun-Lamothe and Gidenne, 2003).

Table 1: Performance of rabbit does during lactation period (average weight of doe and size of litters born and suckled)

	Mean ± Standard Deviation			
	Day of birth	**7 days**	**15 days**	**21 days**
Average weight of the rabbit (g)		3341.4±116.4	3576.1±66.1	3627.3±50.6
Total of born	9.7±2.4	5.4±3.8	5.4±3.8	5.4±3.8
Number of alive born	8±2.9	5.4±3.8	5.4±3.8	5.4±3.8
Average milk production/female	-	694±41.8	1240±14.5	1572±14.4

At birth, the average litter size of rabbits is 8±2.9 born alive, with a minimum of 7 and a maximum of 15 rabbits. These females have a better litter size at birth than that reported by Zerrouki and *al.* (2007) for the local population of rabbits (6.21 live births). With the exception of the first week, mortality of the bunnies is low. Our results are in agreement with those of Amroun (2018), who points out that the second and third weeks of lactation are characterised by stable numbers in SS litters. The litter sizes thus maintained would be directly related to the stimulation of prolactin production leading to a correct total protein level. The high mortality rates recorded during the lactation period (41.3%) could be linked to the difficulties of adaptation of the bunnies to the transition from intrauterine foetal life

(thermoregulated environment) to autonomous existence which is an enormous challenge (Combes and *al.*, 2013) as well as to the disappearance of the lightest bunnies during the first days (Poigner and *al.*, 2000; Szendrö, 2000).

Histology of the mammary gland: During gestation, breeding females undergo great variations in body composition, reserve tissue deposits and energy. Indeed, Rommers and *al.* (2002), showed a large fluctuation in the body weight of rabbits during the breeding period. The histological sections photographed at three magnifications (Figure 2), focus on the structural development of the mammary gland of a pregnant rabbit, which results in a density of mammary acini, which undergoes a marked evolution, regression of adipose tissue as well as connective tissue in contrast to an empty rabbit, a result observed by Amroun (2018) in rabbits of the synthetic strain and the white population in the physiological stages; empty and mid-gestation, thus reflecting a higher proportion of epithelial tissue and an opening of the mammary acini at the gestational stage allowing a good preparation of the mammary gland during this phase and it is accompanied by a better milk production in quantity and quality and the results of the milk production of rabbits of the same strain confirm this.

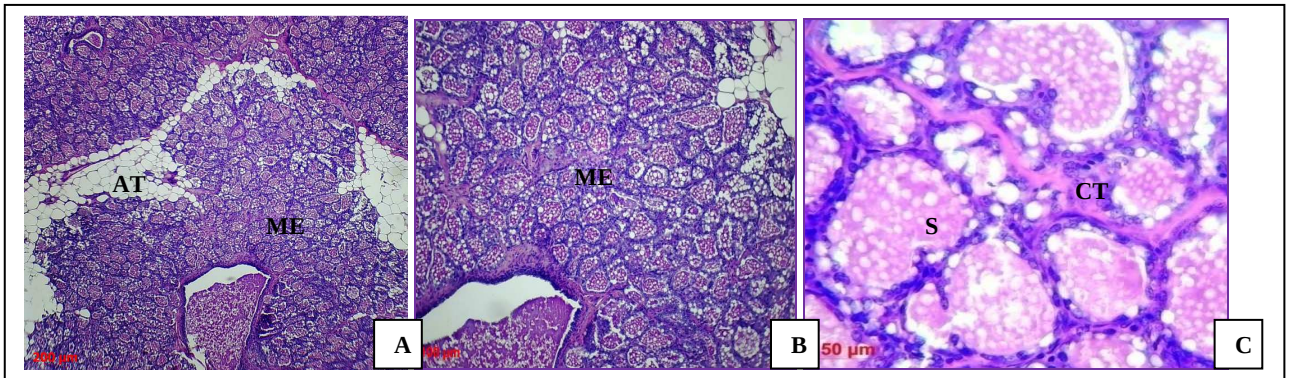

Figure 2: Histological sections of mammary glands of rabbits pregnant on day 24, stained with Haematoxylin Eosin (HE) and observed at different magnifications (A= x40, B= x100, C= x400). ME: Mammary epithelium, AT: Adipose Tissue, CT: Connective Tissue, S: Secretions (milk constituents).

Morphometry of the mammary gland: The morphological study of the SS mammary gland, which was undertaken by means of histological analyses, revealed a significant evolution of the mammary epithelium (ME) to the detriment of the surface occupied by the connective (CT) and adipose (AT) tissues. The results are presented in table II**.**

Tableau 2: Evaluation of mammary acini diameter, surface area, lumen area, nucleo-cytoplasmic ratio and surface area of acini epithelium in a synthetic strain of rabbits, at gestation.

Parameters	Pregnant rabbits
Diameter of acini (µm)	107.5±6
Acini surface area (µm²)	10419.06 ± 999
Acini light area (µm²)	6532.63 ± 819
Epithelial surface area of acini (µm²)	4160.83 ± 254
Nucleocytoplasmic ratio	0.77 ± 0.02

After analysis of the observations concerning the structural and histo-morphometric modifications of the mammary gland of rabbits on day 24 of gestation, we note a significant variation in the structure of its different components, which is reflected in an intense development of the mammary epithelial tissue despite the adipose and connective tissue, confirming the histological observations made. In fact, the growth of the mammary gland is important during the first half of gestation, indicating a preparation of the rabbit for the lactation function. Houdebine (2007) reports that it is from gestation on wards that the mammary gland prepares for lactation. We then note the presence of an important lobulo-alveolar development, the same observation reported by Catherine Hue-Beauvais (2014) in rabbits of INRA strain. The structural modifications of the mammary gland are ensured by a neuro-hormonal regulation which mainly results in the intervention of several hormones. Indeed, under the influence of oestrogen and progesterone, the epithelium of the duct proliferates to form a large number of acini (De Boeck and Larcier, 2001). Progesterone ensures the expansion of the epithelial compartment and the development of the alveoli. It has been shown that progesterone has two receptors in the mammary epithelial cells PR-a and PR-b. However, only PR-b is necessary for the proliferative action of progesterone on the mammary epithelial cells, as the inactivation of the genes coding for PR-a has no effect on mammary development (Mulac-Jericevic and *al.*, 2003). Prolactin secreted by the anterior pituitary gland plays a major role in the development of the mammary gland (Brisken and O'Malley, 2010) by highlighting the crucial role played by the prolactin receptor (PRL-R) during mid-gestation by stimulating alveolar differentiation (Ormandy and *al.*, 1997). Interestingly, progesterone induces the expression of PRL-R while

prolactin induces the expression of PR, suggesting that these hormones interact synergistically to control breast development.

CONCLUSION

The results obtained during this study confirmed that the dairy capacities of rabbits of the synthetic strain released since 2011 remain higher than those reported for the two other genetic types existing in our country (local and white population). An average difference of about 400g of milk produced by the rabbit is obtained in this study, compared to the values reported on females of the same genetic type during the first years of diffusion and also compared to the two other local populations. The histomorphometric study of the mammary gland carried out at the end of gestation confirms this result as it allowed the observation of a better preparation of the mammary gland ensuring a better preparation of the rabbit to ensure the lactation function, which conditions the survival and growth of the young rabbits, thus reducing their mortality rate during the lactation period. A continuation of the study on rabbits of this strain is in progress in order to deepen this research on the hormonal and biochemical aspects of milk.

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