

Full Length Research Paper

Histological exploration of follicular population of the Moroccan bovine (Oulmes-Zaers) breed

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Follicular population and repartition in the ovarian cortex was investigated in the ovaries of the Oulmès-Zaers cattle breed and its crosses. A total of 30 ovaries were collected at slaughterhouse in Casablanca and Rabat from Oulmes-Zaers breed and its crosses (2 genotypes) of 3 age groups (<3; 3 to 5; >5 years). The histological study of the ovary revealed that the ovarian cortex is composed of different areas (from the periphery to the medulla), the non stratified epithelium of the ovary, the tunica albuginea composed of two areas rich of collagen fibers directed into different ways white variable thickness. The primordial and antral follicles appear in underlying areas. Therefore, the follicles cannot appear on the ovarian surface when situated under thicker Layers. At the quantitative level, the ovary of the Oulmes-Zaers breed seems to contain less follicles in animals less than 3 years of age as compared to the crossbreed ($P < 0.05$). Between 3 and 5 years of age, the follicles occupied similar areas of the ovaries (17% and 18% for oulmes-Zaers and its crosses, respectively). This study shows that the ovary of the Moroccan local cattle breed (Oulmes-Zaers) is equipped with a whole plain follicular population that is more important than the one visible on the surface of the ovary and which is exploited by aspiration and picking techniques.

Key words: Ovary, Oulmès-Zaers, follicular population, bovine, age.

INTRODUCTION

The oocytes represent the baseline of the blastocyst chain production via the *in vitro* production technique (IVP). Thus, their number and quality determine the efficiency of this operation. Different methods of data collection exist to enhance the oocytes' output: picking, aspiration (Kumar et al., 1997) and slicing (Hochi et al., 1993; Ward et al., 2001; Annemarie et al., 2003). These methods are adaptable to the structure of the ovary, in relation to a sub-superficial or deep localization of the follicles.

Follicles always develop in the cortical part of the ovary. Studies have revealed the existence of a variation in their distribution in this area (Ingrid et al., 1996). Thus,

among certain breeds, the majority of these follicles are located sub superficially and are, therefore, visible on the surface (to 1 mm of depth; Miyamura et al., 1996). In some other breeds, as it is in the case of the Buffalo for example, they are distributed at different levels of the cortex, but at a deep level for most of them and consequently they are not visible on the surface (Kumar et al., 1997).

The rate of oocyte reprocessing (via direct ovarian picking) among the Moroccan cattle breed (Oulmes-Zaers) in the framework of an inner work (Fassi Fihri et al., unpublished), was revealed to be weak (1-2 oocytes per cow collection and every datum collection). The objective of this study is to investigate the repartition of the follicles in the ovary through the study of the ovarian stroma percentage occupied by the follicles as well as its

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distribution in Oulmes-Zaers Moroccan local cattle breed and its crosses.

MATERIALS AND METHODS

Ovaries used in this study were collected at the local slaughterhouses (Rabat and Casablanca) from cows of 2 genetic groups and 3 different ages; Oulmès-Zaers cattle breed and its crosses (Oulmes-Zaers x Frisonne, Oulmes-Zaers x black pied and Oulmes-Zaers x Holstein). Breed and age were controlled before the slaughtering. The ages taken into account were: <3 years, between 3 and 5 years and >up to 5 years. The number of the ovaries used in this study is 30 in which 15 ovaries belonged to the local breed and the other 15 belonged to the mixed breed, (one ovary per animal because there is similarity between left and right ovaries (Rajakoski, 1960)); and 5 ovaries per age group. The ovaries were taken soon after the slaughter and washed one time in 0.9% of saline solution and then with 70% alcohol. They were transported to the laboratory in isotherm bottles at a temperature of between 30 to 35°C.

The Histological technique

We used the classical technique. The entire ovary was fixed in the sublimated Bouin of the Netherlands for 7 to 10 days. The dehydrated process is done with successive washing in alcohol solutions with increasing concentrations (the dilution of alcohol is carried out according to Gay Lussac table (Langeron, 1949)); 3 baths for 4 h each in alcohol at 70, 95 and 100%. The last step of dehydration is the incubation of the sample for 16 hours in toluol or butylic alcohol.

The inclusion in paraffin took place after the soaking of the sample in two baths of paraffin (8 h in each bath). Half of each ovary (Blondin et al., 1995; Ingrid et al., 1996) is sliced transversally in serials of 5 μm thick. One slice out of 5 is used for the hematoxyline and eosin coloration assay. This method allows for the observation of the granulosa cells and the identification of the follicles and their oocytes precisely (Pearse, 1996; Jorio, 1999).

The structure of the cortex and the distribution of the follicles were studied using a regular microscope. In order to identify the percentage of the areas occupied by the follicles and the stroma, the surface of the follicles is evaluated by the measure of the diameter with the help of ocular micrometer, (Kumar et al., 1997), the follicles being considered circular (Maurasse et al., 1985). The surface areas occupied by the follicles are calculated via the accumulation of the surface areas of different follicles observed. The statistical analysis was made by student "t" test.

RESULTS

The general structure and follicular population of the ovarian cortex of the oulmès-zaers cattle breed was compared to that of its crosses.

Structure of the ovary

The histological slices of the ovaries reveal a structure of polarity distinguished from the medulla to the surface, made up of 5 identifiable areas (Figure 1). Area 1 (Figures 1a, b and c): corresponds to a non-stratified epithelium of the surface area where the cells either cubic or stretched out are parallel to the surface of the ovary.

The areas 2 and 3 (Figures 1a, b, and c) represent the external and the internal parties of the *Tunica albuginea*, and which is populated with somatic cells. The matrix between these cells is very rich with fiber (Figure 1b). In area 2, cells are generally similar to the epithelium of the ovary surface whereas the cells of area 3 are directed perpendicularly toward this surface. (Figures 1b and c). The thickness of areas 2 and 3 is extremely variable: these areas can be thick (Figure 1b and c) or sometimes are reduced to a thin layer (Figure 1a).

Area 4 (Figure 1b) is denser in cells and fiber and which seems to be of the same nature as the ones observed in areas 2 and 3. A big number of primordial and primary follicles, are respectively recognized by their flattened low number of follicle cells and the cubic cells arranged like a layer, are situated in the upper part of area 4, the primary follicles are more internal (Figures 1b and c).

The distance between the primordial follicles and the surface area of the ovary is very variable; it depends on the thickness of areas 2 and 3, but also of the depth to which they are inserted in zone 4 (Figure 1c). The antral follicles which are older and larger meet in deep area 5 (Figure 1d), which is in contact with the medulla and whose structure remind us of area 4 with a difference of being not firm.

Follicle population

For the local breed, the percentage of the ovarian area occupied by the follicles increase in a significant way from the young cows to the old ones (15% to 17%; $P < 0.01$). Then it decreases in cows aged more than 5 years (9.4%). For the mixed breed the percentage of the ovarian area occupied by the follicles is the same for ages <3 and 3 to 5 years (17%, $p < 0.01$). It decreases significantly ($P < 0.01$) if the cows are older than 5 years (9.4%).

DISCUSSION

The appearance of the follicles on the surface area of the ovary seems to depend on the depth of the cortex where they are localized. In fact, our exploration of the structure of the ovary reveals that the thickness of the cortex corresponds to stratification with a variation in the different constituent layers. Similar observations have made elsewhere on the bovine breeds of Australia and America (Ingrid et al., 1996; Vigne et al., 1994). The majority of the follicles being located in the most inner layers (areas 4 and 5). This disposition connected to the general architecture of the cortex might be responsible of the exposition of certain follicles on the surface area. The histological figures reveal a phenomenon called "emergence" of these follicles during their development

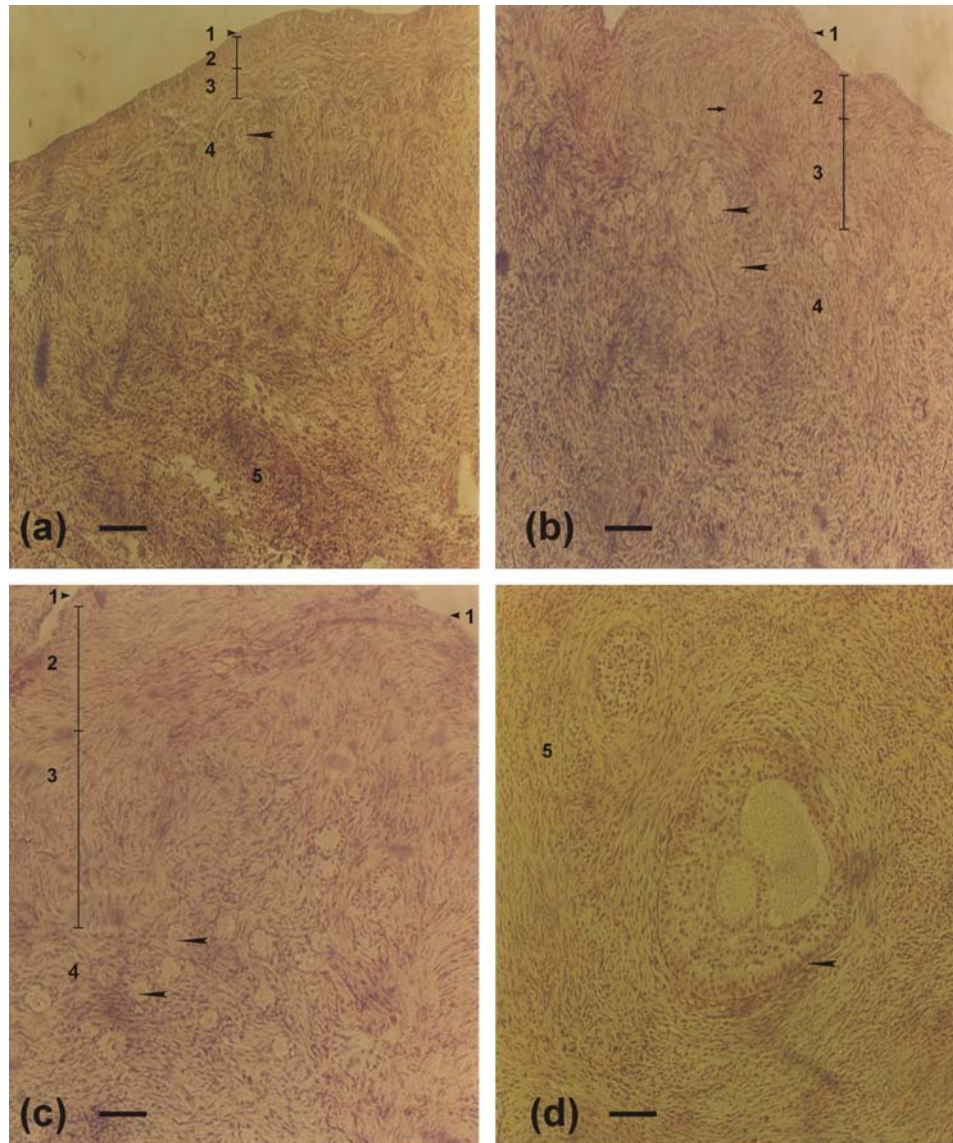


Figure 1. Transversal sections through the ovaries of the Oulmes-Zaers cattle breed. Five identifiable areas are described under results.

into the direction of the surface area, in a very intimate relationship with the thickness of the superficial areas 2 and 3. The development of the follicles at these levels seems to be random, because the stock is distributed in a heterogenic manner in area 4 according to our results, and as described by Sforza et al (2003).

It has been reported that more than 95% of the cancer of the ovary originate from the area 1 ovary envelope (Jeff et al., 2000; Choi et al., 2003). Concerning areas 2 and 3 whose structure is different, previous study to determine their functional properties has concluded that there is a secretory activity of specific proteins different from those in area 1 (Vigne et al., 1994). Moreover, areas 2 and 3 are characterized by a particular aspect of cells different from the one of under layer areas 4 and 5

(Ingrid et al., 1996). In area 4 where the follicles start to appear, the stroma is rich with collagen fiber, but it is not vascular. The follicles without antrum (primordial, primary and secondary) are oriented differently (Ingrid et al., 1996). The avascularisation of area 4 with the predominance of the primordial follicles (Sforza et al., 2003) compatible with the fact that the activation of the primordial follicles seem to be non- hormone dependent (Wezel et al., 1995). Also, these follicles being quiescent require a minimum amount of oxygen and energy. As for area 5, it can be distinguished by its developed vascularization (Zheng et al., 1993) and for being an inhabited by follicles with antrum. This configuration is confirmed by the exchange that is established normally between this type of follicles and blood.

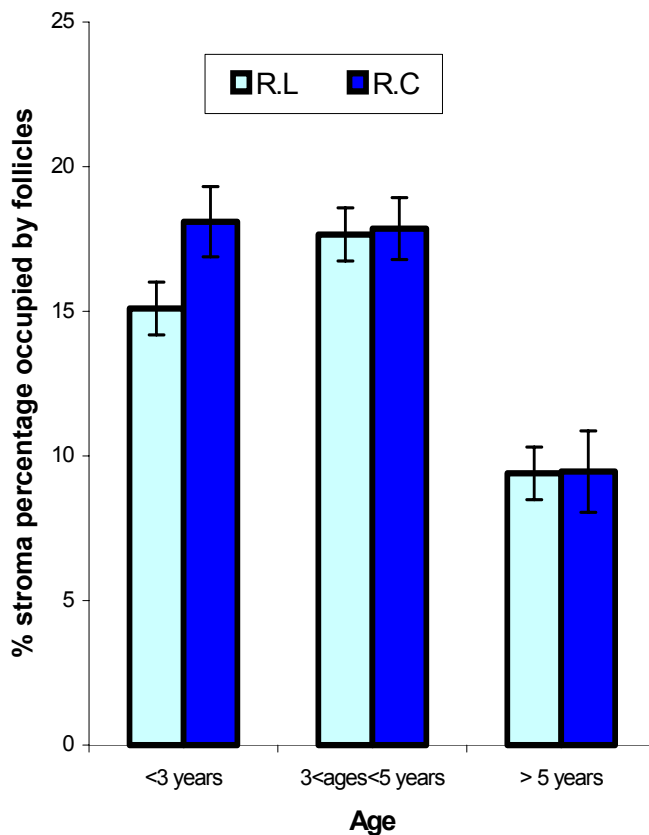


Figure 2. Follicular population in function with age and breed.

This spatial heterogeneous distribution of follicles within the ovarian cortex has also been reported on the human (Sforza et al., 2003). This disposition might be a consequence of the follicles migration during the final stage of histogenesis of the normal ovary acquired at the premature phase of the ovarian morphogenesis. A similarity of this distribution between human and bovine has been previously reported (Vigne et al., 1994).

At the quantitative level, the histological exploration allowed us to reveal a variation of the follicle population depending on age. The females aged more than 5 years have the lowest rate of follicles. We observed that within the age group of less than 3 years, the primordial follicles predominate. On his part, Schmidh et al. (2003) observed that the follicle stock is weaker in the fragments taken from older individuals. On the other hand, the local breed shows less primordial follicle stock less important compared to the improved breed at an age less than 3 years. This result is confirmed by evaluating the percentage of areas occupied by the follicles and the ovarian stroma that showed a progressive plain decrease of the follicle population of cows. This is similar with the results of Kumar et al. (1997) for buffalo.

At the practical level, it can be inferred from our study on the Moroccan local breed, that the ovaries are

endowed by a plain total follicular population that is more important than the one exploited via aspiration and picking techniques which are visible on the surface area. A technique which is more aggressive slicing would allow more access to the deepest follicles that are not visible on the surface area but can constitute an oocytes source for the production of bovine embryos *in vitro* (Miyamura et al., 1996).

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REFERENCES

- Annemarie Z, Karin M; Karen R, Katarina J(2003). Apoptosis within bovine follicular cells and its effect on oocyte development during in vitro maturation. *Theriogenology* 59:1421-1433.
- Blondin P, Sirard MA (1995). Oocyte and follicular morphologie as determining characteristics for developmental competence in bovine oocytes. *Mol. Reprod. Dev.* 41(1): 54-62.
- Choi KC, Auersperg N(2003). The ovarian surface epithelium: simple source of a complex disease. *Minerva Ginecol.* 55(4): 297-314.
- Hochi-S, Choi YH, Braun JW, Sato K, Oguri N (1993). Factors affecting the recovery of follicular oocytes from horses and their in vitro maturation. *Japanese-journal-of Equinine-Science.* 4(2): 145-150.
- Ingrid L, van Wezel, Raymond J. Rodgers (1996). Morphological characterization of bovine primordial follicles and their environment in vivo. *Biol. Reprod.* 55: 1003-1011.
- Jeff A. Parott, Michael K, Skinner (2000). Expression and action of hepatocyte growth factor in human and bovine normal ovarian surface epithelium and ovarian cancer. *Biol. Reprod.* 62 : 491-500.
- Jorio A (1999). Etude comparative de la dynamique folliculaire ovarienne avant la puberté chez des races ovines Marocaines de prolificité différente. Thèse d'Etat es-Science, Faculté des sciences de Fès.
- Kumar A., Solanki S.K., Tripathi V.N., Jain G.C (1997). Oocyte retrieval and histological studies of follicular population in buffalo ovaries. *Animal Reproduction Science* 47: 189-195.
- Langeron,M.1949.Methodes d'inclusion. In Masson et Cie, Edt. *Precis de Microscopie.* Collection, Précis Medicaux. Edition Masson et Cie. 452-453.
- Maurasse C, Matton P, Dufour J.J (1985). Ovarian follicular populations at two stages of an estrous cycle in heifers given high energy diets. *J. Anim. Sci.* 61 (5): 1194-1200.
- Miyamura M, Kuwayama M, Hamawaki A, Eguchil Y(1996). Total number of follicles in the ovaries of Japanese black cattle. *Theriogenology* 45 : 300.
- Pearse A.G.E. (1969). *Histochemistry, Theroretical and applied*, Ed. Churchill, London, Vol.I. pp. 607-611.
- Rajakoski E.1960.The ovarian follicular system in sexually mature heifers with special reference to seasonal, cyclical and left-right variations. *Acta Endocrinol.* 34(suppl 52):6-68.
- Schmidt KLT, Byskov AGB, Nyboe Andersen A, Muller J, Yding Andersen C (2003). Density and distribution of primordial follicles in single pieces of cortex from 21 patients and in individual pieces of cortex from three entire human ovaries. *Human Reproduction* 18 (6): 1158-1164.
- Sforza C, Vizzoto L, Ferrario VF, Forabosco A (2003). Position of follicles in normal human ovary during definitive histogenesis. *Early Hum. Dev.* 74 (1):27-35.

- Vigne J-L, Halburnt LL, Skinner MK (1994). Characterization of bovine surface epithelium and stromal cells: identification of secreted proteins. *Biol. Reprod.* 51: 1213-1221.
- Ward F, Rizos D, Corridan D, Quinn K, Boland M, Lonergan P (2001). Paternal influence on the time of first embryonic cleavage post insemination and the implication for subsequent bovine embryo development in vitro and fertility in vivo. *Mol. Reprod. Dev.* 60:47-55.
- Wezel IL, Umaphysivam K, Tilley WD, Rodgers RJ (1995). Immunohistochemical localization of basic fibroblast growth factor in bovine ovarian follicles. *Mol. Cell Endocrinol.* 115: 133-140.
- Zheng J, Redmer DA, Reynolds LP (1993). Vascular development and heparin-binding growth factors in the bovine corpus luteum at several stages of the estrous cycle. *Biol. Reprod.* 49: 1177-1189.