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Oogênese e Foliculogênese em Bovinos

Oogenesis and Folliculogenesis in Cattle

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Resumo

Os ovários desempenham papéis relevantes para o sistema reprodutivo, no qual a função exócrina (ou gametogênica) realiza a maturação e liberação do oócito para a fecundação e a função endócrina (ou esteroidogênica) afeta a síntese, secreção de hormônios e fatores de crescimento. Além disso, existe uma interação entre os fatores endócrinos, autócrinos e parácrinos, atuando em associação com o processo de desenvolvimento folicular e oocitário durante a vida reprodutiva da fêmea. O córtex, porção mais externa do ovário, representa a região funcional onde se localizam os folículos ovarianos e as estruturas lúteas, em diferentes estágios de desenvolvimento e/ou atresia. A região medular localiza-se mais internamente, na maioria das espécies, e sua principal função é nutrir e sustentar o ovário. Apesar da numerosa população folicular presente nos ovários, estabelecida durante a vida fetal, quase todos os folículos encontrados no *pool* de reserva ovariana, ou seja, 99,9%, não atingem a ovulação. Esses folículos passam por um processo de morte celular conhecido como atresia folicular, tornando o ovário um órgão de baixa produtividade. A elucidação dos mecanismos que regulam a foliculogênese, incluindo o processo de atresia, é importante para o melhor aproveitamento dos folículos na melhoria da eficiência reprodutiva de animais de produção.

Palavras-chave: Atresia Folicular; Folículos Antrais; Folículos Pré-Antrais; População Folicular Ovariana.

Abstract

The ovaries play roles relevant to the reproductive system, in which the exocrine (or gametogenic) function carries out the maturation and release of the oocyte for fertilization and the endocrine (or steroidogenic) function effects the synthesis and secretion of hormones and growth factors. In addition, there is an interaction between endocrine, autocrine and paracrine factors, acting in association with the follicular and oocyte development process during the female's reproductive life. The cortex, the outermost portion of the ovary, represents the functional region where the ovarian follicles and luteal structures are located, at different stages of development and/or atresia. The medullary region is located more internally, in most species, and its main function is to nourish and support the ovary. Despite the numerous follicular population present in the ovaries, established during fetal life, almost all follicles found in the ovarian reserve pool, i.e., 99.9%, do not reach ovulation. These follicles undergo a process of cell death known as follicular atresia, making the ovary a low-productivity organ. The elucidation of the mechanisms that regulate folliculogenesis, including the atresia process, is important for the better use of follicles in improving the reproductive efficiency of production animals.

Keywords: *Follicular Atresia; Antral Follicles; Preantral Follicles; Ovarian Follicular Population.*

Introduction

The ovary of mammals has two essential functions: one endocrine and the other gametogenic, the latter being responsible for the production of thousands of oocytes and follicles (Dalbies-Tran et al., 2020). Thus, the ovary, through the production of hormones, as well as being the place that supports the processes of folliculogenesis and oogenesis. Such processes culminate in the release of a mature oocyte

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for fertilization (Cox and Takov, 2020). In ruminants, folliculogenesis begins at the fetal stage, when primordial germ cells migrate from the yolk sac to the primordial gonads, and then, the sequential mitotic production of germ cells occurs, establishing many groups of oogonia. Subsequently, these groups of oogonia are surrounded by somatic cells to form the cortical cords, which are the precursors of primordial follicles.

The interactions between the somatic cells of the follicle and the oocyte (i.e., communication between the granulosa and theca cells with the oocyte) are of paramount importance for the folliculogenesis process (Bernabé et al., 2020). For the formation of primordial follicles, oogonias are differentiated, forming oocytes, in which they are surrounded by granular cells with a pavement shape. It is estimated that in cows, the population of primordial follicles is about 120,000, being reported a notable difference in the number of preantral follicles present at the birth of *Bos taurus taurus* and *Bos taurus indicus* females (Silva-Santos et al., 2011). The meiotic division of oocytes begins and stops at the prophase stage of meiosis I, also known as the diplotene stage. Thus, meiosis resumes only at puberty, with follicular recruitment.

After activation, the primordial follicle develops into a primary follicle characterized by an oocyte surrounded by a layer of cuboidal granulosa cells. At that time, the zona pellucida appears, a structure that covers all the oocytes present throughout the follicle development (Scaramuzzi et al., 2011). Then, the primary follicle passes to the secondary follicle stage when the granular cells multiply and form two layers of cubic morphology, in addition to the emergence of the first theca cells (Van Den Hurk et al., 2000). The hormone responsible for inducing follicle growth is called follicle-stimulating hormone (FSH). In addition, there is the appearance of follicular waves in cattle. For the follicular waves to occur, it is necessary to activate the activation of the primordial follicles. This occurs during follicular recruitment of the estrous cycle due to the contribution and increased FSH release (Padmanabhan and Cardoso, 2020).

The next stage consists of the tertiary follicle. The follicle has an antral cavity filled with follicular fluid, multiple layers of granular cuboidal cells, and an entire organization of theca cells. FSH assumes an important role at this stage of development due to its action on endocrine and paracrine growth factors (Buratini et al., 2005). The presence of follicular fluid enables ultrasonographic studies, making it possible, in this way, to monitor follicular *in vivo* until its final destination: either the atresia or ovulation process. Activation of primordial follicles constantly occurs, reflecting in the pool of antral follicles that remain in growth, mainly in estrus.

In this context, the ovarian follicular population contains thousands of ovarian oocytes, most of which are included in the preantral follicles (90-95%). However, only a small portion of the ovarian follicles culminates in ovulation; the rest of these follicles undergo an apoptotic process, called atresia - a physiological event, of unknown duration. Thus, the quantity of primordial follicles (ovarian follicular reserve) is an important indicator of reproductive capacity, which may influence the development of reproductive biotechniques.

Thus, we aimed evaluate the follicular development from the moment of its initial formation to the moment of ovulation and/or atresia, elucidating the main aspects involved in folliculogenesis and oogenesis.

Oogenesis and folliculogenesis

The oocyte stock of mammalian females is formed over fetal life, through two processes: oogenesis and folliculogenesis (Collado-Fernandez et al., 2012). The physiological process of oogenesis leads to the growth and distinction of the female's primordial germ cells, which results in the fertilized haploid oocyte. On the other hand, folliculogenesis refers to the formation of follicles. It starts with the primordial follicle, and development can occur up to the stage of the mature follicle (also known as De Graaf follicle or preovulatory).

During folliculogenesis, many morphological and cytological events occur in the oocytes, which contribute toward the acquisition of developmental competence. It is these changes that enable the oocyte to progress in the process of folliculogenesis, to be subsequently fertilized, until the stage of embryo development (Walker and Biase, 2020). In the uterine environment, during fetal development, primordial germ cells are formed. These cells migrate from the yolk sac to the gonadal ridges, undergo successive mitoses giving rise to oogonia. These remain linked by cytoplasmic processes, also known as germinal cysts or nests. During this stage, somatic cells in mesonephros surround oogonias, forming a cord-like structure called cortical cords. This later form the primordial follicles. Thus, for a composition of the oocyte population that will be used throughout reproductive life, mitotic multiplication is indispensable.

There is the formation of a limited number of oocytes available for use in adulthood. The balance between the production of oogonias and apoptosis determines the availability of this range in the reproductive life of mammals (Aitken et al., 2011). After successive mitoses, in which oogonias are differentiated into oocytes, the process of meiotic division begins. There is an interruption in the diplotene stage, which belongs to the prophase stage of meiosis I. The beginning of follicular recruitment can occur at puberty or during the proximity of the end of reproductive life.

The primary or immature oocyte lasts in the stage of prophase I until just before ovulation. The meiosis process is resumed in response to FSH and luteinizing hormone (LH). Then, the other phases of meiosis occur, such as metaphase I, then anaphase I and finally telophase I. The first polar body is released and the secondary oocyte is formed. The meiotic maturation process, *in vivo*, can occur only in the oocyte of the preovulatory follicle and results, among other factors, from specific stimulation by the preovulatory peak of LH and FSH.

Furthermore, other hormonal interactions that occur during follicular development contribute to the growth of the antral follicle by mimicking or enhancing the effect of FSH on bovine granulosa cells. Among them, the main negative feedback hormones for FSH, estradiol and inhibin, in addition to insulin-like growth factor I (IGF-1). In the metaphase II stage, there is a second interruption of meiosis. In most domestic species, the oocyte remains in metaphase II until it is ovulated and transported to the oviduct, where it can be fertilized. If fertilization occurs, the oocyte resumes and culminates in the elimination of the second polar corpuscle, thus marking the end of oogenesis. Finally, both the folliculogenesis and oogenesis process ensure a female gamete capable of being fertilized. In short, the end of folliculogenesis process happens at the time of ovulation of the mature follicle, while oogenesis ends only after fertilization.

Ovarian follicular population

The size of the ovarian follicle reserve (ovarian follicular population) greatly influences applied aspects of reproduction, such as fertility and pregnancy rates, thus extending the conventional concept of the ovary as a static structure. The follicular population of the ovaries may vary individually, according to species, breed, genetics, age, hormone levels, and reproductive status. Such factors can influence at quantity and distribution of ovarian follicles. It is estimated that the ovarian follicular population of bovine females, at birth, is about 235,000 follicles, ranging from 0 to 720,000 follicles per ovary (Betteridge et al., 1989).

Studies on follicular population have shown similarity in the quantity of preantral follicles in the ovaries of female zebu and taurine females. These surveys showed a follicular population for fetuses, heifers and Zebu cows, respectively, 143,929 ± 64,028; 76,851 ± 78,605 and 39,438 ± 31,017 preantral follicles. For Taurine females, the population of preantral follicles was 285,155 ± 325,195; 109,673 ± 86,078 and 89,577 ± 86,315, fetuses, heifers and cows, respectively (Silva-Santos et al., 2011).

The correlation of the population of preantral and antral follicles in the ovaries of female zebu and taurine females was also studied, in which cows with a high antral follicle count (AFC) showed a smaller amount of preantral follicles compared to animals with a low count of antral follicles. Thus, cows with low AFC had the largest population of preantral follicles (Silva-Santos et al., 2014). The authors of this study reported individual differences in the amount of preantral follicles in the ovaries of bovine females.

In relation to other species, these differ in the population of preantral follicles, such as the sheep, which has approximately 160,000 follicles, the mare about 30,000 to 150,000, and in women, about 2,000,000 preantral follicles. In short, this is an important field of study for understanding female reproductive physiology and assisting in the use of reproductive tools.

Ovarian follicles

The ovary is a dynamic structure in which several events occur simultaneously, including follicular growth and development. The follicles have an oocyte surrounded by granulosa cells, in addition to the teak cells, being considered the morpho functional unit of the ovary. The follicles have endocrine (production and release of steroid hormones and other peptides) and exocrine or gametogenic functions, presenting itself as an essential element for maintaining oocyte viability. Thus, the follicle provides an ideal environment for the growth and maturation of the immature oocyte and allows the mature oocyte to reach ovulation. Ovarian follicles are distributed in the outermost portion of the ovary, also called the cortex. However, females of the equine species have a higher concentration of follicles in the region close to the ovulatory fossa and the inner portion of the ovary (medullary region; Gonzalez et al., 2017).

In the ovarian follicles population, there are preantral or non-cavitary follicles (primordial, primary, and secondary) and antral or cavitary follicles (tertiary and pre-ovulatory). It is suggested, for the initial phase of follicular growth, a predominantly local action, and several growth factors were identified in the first follicular changes. Among the most studied, are the Kit Ligand, bFGF (Fibroblast growth factor), LIF (Leukemia inhibitory factor), and GDF-9 (Growth differentiation factor-9). Thus, according to the degree of follicular evolution, the ovarian population is divided into preantral or non-cavitary follicles (primordial, primary and secondary) and antral or cavitary (tertiary and pre-ovulatory follicles).

The preantral or non-cavitary follicles are differentiated by the shape and number of layers of the cells that surround the immature oocyte. The primordial, primary and secondary follicles are characterized differently according to morphology. The primordial follicles have an oocyte surrounded by a layer of 4 to 8 cells of the flattened or paved granulosa; the primary obtains an oocyte surrounded by a layer of 11 to 12 cells of the cuboid granulosa and the secondary ones have from two layers of cells of the cuboid granulosa. Also, these follicles are classified according to their integrity.

The class of antral or cavitary follicles comprises tertiary follicles and De Graaf follicles, also known as mature, preovulatory, or dominant. With the intense proliferation of granulosa cells, the follicular antrum appears, an area filled with follicular fluid, characterizing the antral follicle. In cattle, the antral cavity can develop in follicles whose diameters vary from 0.14 to 0.28 mm. The diameter of the primordial follicles increases from 0.020-0.040 mm (bovine) to more than 10 mm before ovulation. Two estrous cycles are required for the follicle to grow from the beginning of antrum formation (0.13 mm) to preovulatory size. The tertiary follicles are made up of an oocyte surrounded by the pellucid zone, several layers of granulosa cells, a small antral cavity, basal membrane, and two layers of theca cells (internal teak and external teak). They are characterized by the presence of numerous microvilli within the pellucid zone and numerous rounded and elongated lipid particles and mitochondria. De Graaf's follicles represent the terminal stage of follicular development. There is a predominance of rounded mitochondria, and the smooth and rough endoplasmic reticulum is observed in large quantities. Cortical granules and microtubules can also be identified in the ooplasm of the oocyte. The perivitelline space is formed at this stage of development, and there is an increase in the number of vesicles and Golgi complexes. At the end of this phase, there is an indication of the marginalization of the fibrillar centers, causing the inactivation of the nucleolus function. Thus, at the same time, there is a decrease in the transcriptional activity of the oocyte.

In this context, FSH has been considered an essential factor due to its endocrine and paracrine roles. For example, FSH plays a modulation on FGF (fibroblast growth factor) family, as the FGF-8. After that occurs the antrum formation, which represents a remarkable step of follicular growth. Deviation and dominance are crucial modifications at this time, and essential aspects about LH and its receptor have been described in this stage. Furthermore, the presence of follicular fluid allows ultrasonographic evaluations, an essential method for *in vivo* studies until the final fate of the follicle: atresia or ovulation.

Follicular atresia

Among all ovarian follicles, only a small portion culminates in ovulation; the rest of these follicles undergo an apoptotic process called atresia. Follicular atresia does not occur equally during follicular development. It refers to a physiological process of unknown duration, suggesting that it is one of the elements that controls the number of selected follicles until ovulation. The precise duration and stage of development at which the ovarian follicles culminate in atresia are not known. The atresia process differs between preantral follicles (these being the primordial, as well as the primary and secondary) and antral follicles.

For preantral follicles, the first signs indicative of atresia occurs in the oocyte, such as nuclear chromatin retraction and oocyte fragmentation, which triggers the process of irreversible elimination of ovarian follicles at this stage of development. In the granulosa cells of the preantral follicles, alterations are rarely observed. It should be noted that after the formation of the antrum, there is a change in the sensitivity of the granulosa cells and also in the oocytes. The oocyte becomes highly resistant from this stage, and the first changes indicative of atresia are observed in the granulosa cells. The appearance of granulosa cells with pycnotic nuclei, chromatin condensation, and nuclear retraction can be considered the first morphological signs of atresia, which is observed predominantly in granulosa cells vicinity of the antral cavity. Subsequently, fragments of pycnotic nuclei of apoptotic bodies are observed in the antral cavity.

With the progression of atresia, there is a reduction in the number of layers belonging to the granulosa cells, as well as the invasion of the follicle by fibroblasts and also macrophages. After these drastic changes in the granular layer, the oocyte frequently undergoes pseudo-maturation, fragmented, and, finally, is eliminated during the final stages of atresia. Despite being a physiological phenomenon, atresia significantly reduces the number of potentially valuable oocytes, consequently decreasing the production of viable oocytes during an animal's reproductive life (Veitia, 2020).

Conclusion

The regulation and all aspects of folliculogenesis remain a universe to be studied and explored. The extraordinary progress of the last few years is notable, in which many researches contribute so that the gaps are filled, especially in the preantral phase. The elucidation of the mechanisms that regulate folliculogenesis, including the atresia process, is important for the better use of follicles in improving the reproductive efficiency of production animals.

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