

Observation of the dynamics of follicular development in the ovary

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Abstract

The number of ovulated oocytes is different among mammals but does not vary much within the same species. In order to sustain periodic ovulation, follicular development must be coordinated at the tissue level. Elucidating the regulatory mechanisms of follicular development is difficult because the ovary has a complicated structure and it takes a long time for primordial follicles to develop into Graafian follicles. Therefore, it is not possible to observe follicular development by conventional experiments. The authors previously developed a new ovarian tissue culture method that enabled the observation of follicular development from the early follicle stage. These findings indicated that follicular interactions are important in regulating follicular development and ovulation. This review describes the current methods of observing follicular development in the ovary and the regulatory mechanisms of follicular development.

KEYWORDS

follicle culture, follicular development, ovarian tissue culture, time-lapse image, ultrasonography

1 | INTRODUCTION

Follicle development and ovulation are controlled by follicle-stimulating hormone (FSH) and luteinizing hormone (LH), which are secreted from the pituitary gland. The secretion of FSH and LH is controlled by estradiol, progesterone, and inhibin, which are produced by the ovary. Therefore, communication between the hypothalamus, pituitary gland, and ovary is important for periodic ovulation.¹⁻⁶ In some mammals, inhibin regulates follicular development and the number of oocytes that are released during ovulation.⁷⁻¹¹ The inhibin that is secreted from growing follicles suppresses the secretion of FSH from the pituitary gland and a dominant follicle develops.¹² In humans, there are two types of inhibin: inhibin A is secreted from the corpus luteum and inhibin B is secreted from the follicles. Changes in inhibin B serum concentrations are similar in humans and other mammals.⁸ The neutralization of inhibin increases the number of growing follicles and ovulated oocytes but does not affect ovulation.¹³⁻¹⁵ This indicates that inhibin controls the number of dormant follicles in different

species. However, the mechanisms of dormant follicular selection remain to be elucidated. In addition, they are not known how the number of follicles is regulated during each wave of development and how each wave of follicular development is initiated during the estrus cycle.

In order to answer these questions, the dynamics of follicular development must be observed in the ovary. Ultrasonography has been used to observe follicular development in the ovary of domestic animals.^{12,16-18} Using this approach, it was possible to visualize follicular growth and measure the follicle sizes. However, it was not possible to observe smaller follicles with this method, including primordial, primary, and early secondary follicles. During these follicular stages, growth does not depend on gonadotrophin and is controlled by other regulatory mechanisms in the ovary. In order to define these mechanisms, new methods are required to observe the dynamics of follicular development. This review describes the current methods of observing the dynamics and regulatory mechanisms of follicular development in the ovary.

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2 | METHODS OF OBSERVING THE DYNAMICS AND REGULATORY MECHANISMS OF FOLLICULAR DEVELOPMENT IN THE OVARY

2.1 | Ultrasonography

Ultrasonography is both a diagnostic and a research tool. It was first used to study domestic animal reproduction in pregnant sheep.¹⁹ Since then, ultrasonography has been used in various fields of reproductive research, including follicular dynamics, ovulation, corpus luteum development, uterine monitoring, and fetal imaging.²⁰⁻³⁶ Tracing follicular development is important in order to elucidate regulatory mechanisms. However, it is difficult to trace the dynamics of a single follicle in the ovary of a live animal. Ultrasonography has been used to observe follicular development in the ovary of domestic animals. These studies revealed a periodic wave of development in each estrus cycle, during which a group of recruited follicles grew.^{16-18,25,37-41} A different number of developmental waves was detected in different animals: two waves were observed in cows and three waves in heifers.^{24,25} These developmental waves consisted of three events: the recruitment of growing follicles, selection of dominant follicles, and ovulation.¹² The waves of follicular development are initiated by gonadotropin.⁴² The number of follicles per developmental wave is different among species: >50 in pigs, 5-10 in cattle, and 1-4 in horses. From the recruited follicles, one dominant follicle is selected in cattle and horses, while 12 dominant follicles are selected in pigs. Generally, the follicular size is considered to be an important parameter for the selection of a dormant follicle; however, the mechanism of selection has not been fully defined.⁴² There are two possible mechanisms: (1) sensitivity to FSH selects the dominant follicle; and (2) the largest follicles inhibit the development of other follicles by an unknown factor. The selected dominant follicles then grow and mature, while the other follicles regress. During this phase, new follicles are not recruited.³⁹ Follicular dynamics can be observed during these stages by ultrasonography.

In order to understand the mechanisms that sustain periodic ovulation, it is important to observe the successive waves of follicular development and to analyze the mechanisms that control developmental waves in the ovary. The dynamics of follicular development in the ovary of cattle have been traced by ultrasound examination.⁴³ The follicles were traced by the sectional method in order to observe the development of single follicles. Follicles with a diameter of >4 mm were identified, but follicles with a diameter of 1-3 mm could not be monitored for long periods because they grew and changed position. In order to monitor these follicles, it was necessary to change the plane of ultrasound imaging. To trace the small follicles, the researchers generated sectional maps of the ovary and compared the images that had been recorded by ultrasound. With this method, they were able to trace the development of specific follicles over a long period. They found that the dominant follicle could be identified during the early antral follicular stage when the diameter was 1 mm. This was earlier than previous reports and demonstrated that the future dominant follicle was

already larger than the other follicles and already might be selected in the early antral follicular stage.⁴⁴⁻⁴⁶ These findings demonstrated the importance of monitoring the dynamics of follicular development in the ovary in order to elucidate how ovulation is coordinated.

2.2 | New method to trace follicular development in the mouse ovary

Ultrasonography can show the development of antral follicles in the ovaries of live animals; however, it is difficult to reliably observe primordial, primary, and secondary follicles because they are smaller (<1 mm). Observing these follicles is important in order to understand their development; thus, the authors developed a new method of mouse ovarian tissue culture and time-lapse analysis (Fig. 1).⁴⁷ Under these culture conditions, follicular development was observed from the primordial (or primary) to the antral follicular stages. In addition, ovulation and follicular atresia were observed (Fig. 2). In order to analyze follicular development in the cultured ovary, the follicular area was measured in time-lapse images at 24 hours intervals with ImageJ (National Institutes of Health, Maryland, USA) and the follicular stage was identified based on the area (Fig. 3). In the bright field image, it was difficult to distinguish the primordial and primary follicles, so these follicles were described as primordial-to-primary. The follicles in the cultured ovary were classified into three groups: primordial-to-primary, secondary, and antral follicles.⁴⁷ Time-lapse images of the cultured ovarian tissues were captured at 30 minutes intervals for 4 weeks. The area of each follicle was measured to trace the follicular development. These experiments showed that the follicles grew in a developmental wave during the same period between the LH surge as one cohort and made a wave of follicular development in the tissue in the cultured mouse ovary (Fig. 3), which has not been demonstrated

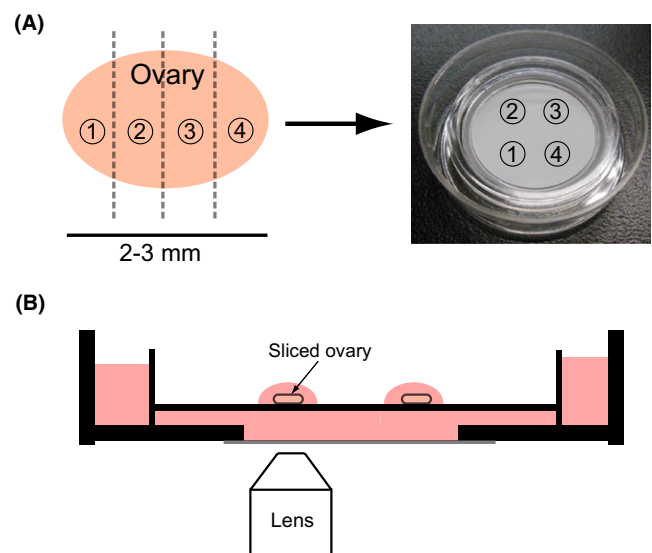


FIGURE 1 Ovarian tissue culture method. A, Slices of ovarian tissue from 4 week old mice were cultured in culture inserts. B, Time-lapse images of cultured ovarian tissue were captured by using confocal microscopy

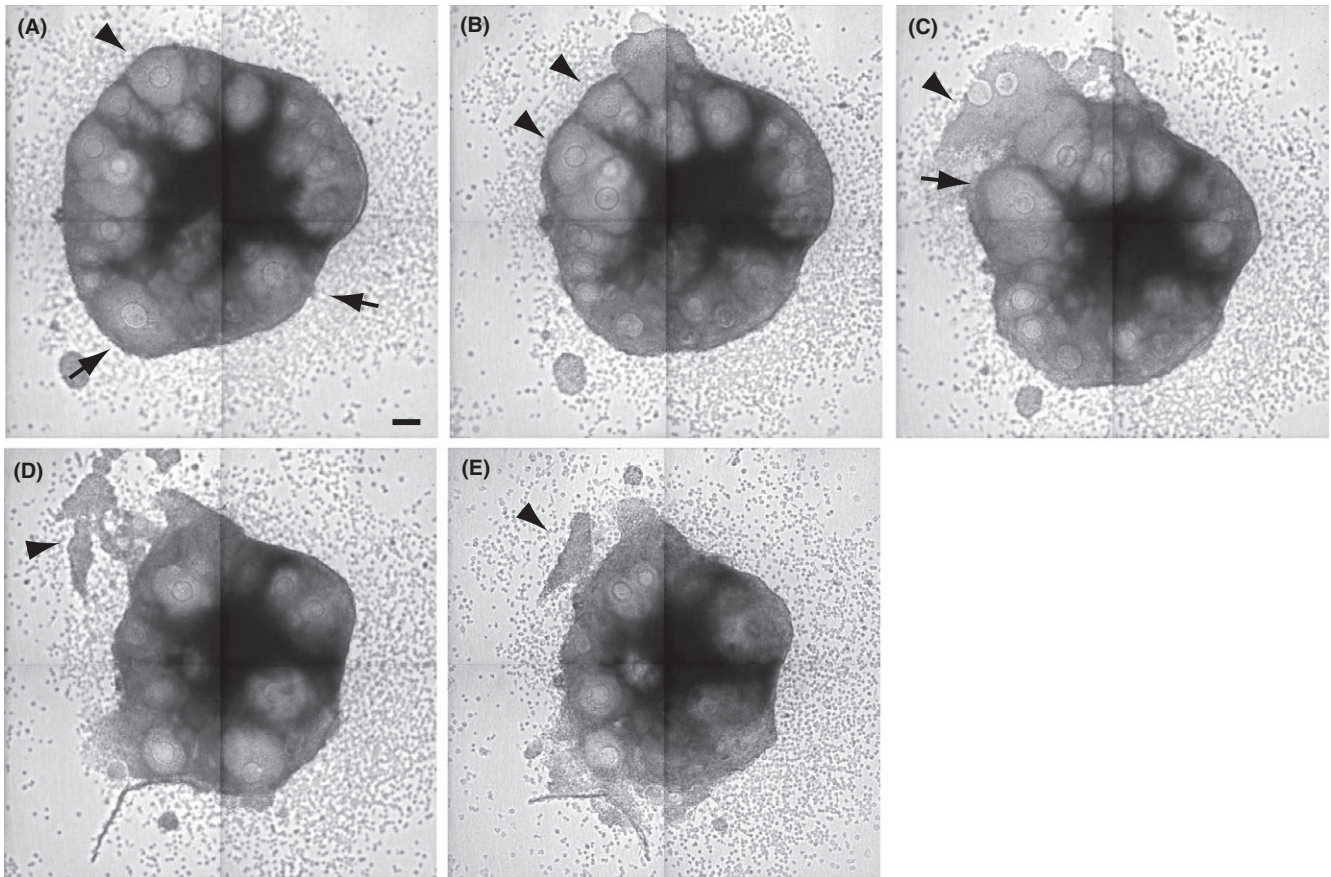


FIGURE 2 Time-lapse imaging of ovarian cultures. The culture time for each image is (A) 151 hours, (B) 169 hours, (C) 209 hours, (D) 277 hours, and (E) 325 hours. The arrowheads in (A) and (B) indicate the follicles that released oocytes after several hours and the arrowheads in (C–E) indicate the granulosa cells that returned to their original position after ovulation. The arrows indicate the regressed follicles. Scale bar = 100 μm

before in rodents. Rodents have a short estrus cycle and can be manipulated genetically, which makes them a better model than domestic animals for studying follicular development. However, rodents are too small to study follicular development by ultrasonography. Therefore, the ovarian culture and time-lapse analysis methods have made it possible to study follicular development in the mouse. The physiology of the mouse ovary was reproduced in these cultures.

2.3 | Follicular development in mouse ovarian cultures

Ovarian tissue is dynamic during the estrus cycle. Follicular development and ovulation are followed by formation of the corpus luteum.^{48–50} Follicular development, ovulation, and follicular atresia were observed in the ovarian cultures.⁴⁷ After ovulation, the granulosa cells that were released from the ovulated follicle into the culture medium returned to their original position. However, the corpus luteum was not formed (Fig. 2).

In order to trace the development of single follicles in the cultured tissue, the follicular area was measured.⁴⁷ These analyses revealed waves of follicular development in the cultured ovarian tissues, which synchronized with the tissue pieces from the same ovary (Fig. 3). The

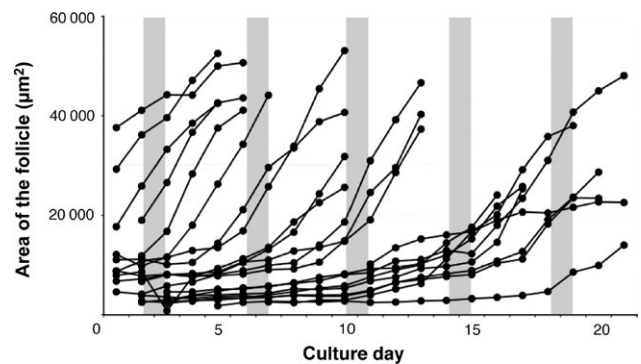


FIGURE 3 Changes in the follicular area in the cultured ovarian tissue, which was measured in time-lapse images at 24 hours intervals. The gray lines indicate the period of luteinizing hormone (LH) surges. This graph presents the follicular area measurements from one ovarian tissue slice

FSH and LH regulate follicular development after the antral follicular stage and their concentration in the blood changes during the estrus cycle.^{51–53} However, the concentration of FSH did not change under the culture conditions. Therefore, the authors' observations can be explained in two ways: (1) a LH surge coordinated the developmental

waves in the cultured ovarian tissue; or (2) the biological rhythm (e.g. the circadian clock) controlled the developmental wave and this mechanism was inherent to each cultured ovarian tissue or follicle.⁵⁴⁻⁵⁹ The timing of ovulation and the number of released oocytes were different in the ovaries from different mice. However, there were few differences between the right and left ovaries from the same mouse. Therefore, the authors believe that the inherent biological rhythm of each mouse was sustained under the culture conditions and was able to influence follicular growth and ovulation. Previous reports have shown that LH controls the expression of core oscillator elements that control the circadian clock. Therefore, the inherent biological rhythm of individual ovaries might synchronize in culture.^{47,54}

2.4 | Interaction of follicles in the ovary to coordinate follicular development

Many factors control follicular development and these have been extensively reviewed.⁶⁰⁻⁶⁸ This review focuses on follicular interactions in the ovary. Studying individual follicles is important in investigating follicular development and many researchers have cultured isolated follicles to elucidate the regulatory mechanism of follicular development.⁶⁹⁻⁷⁶ These studies revealed interactions among oocytes, granulosa cells, and theca cells and the factors that are produced by these cells.^{61,65,66} These results described the regulatory mechanisms of follicular development within a follicle, but were not able to determine the mechanism in the ovary.

In one study, co-cultures of two mouse ovarian follicles were prepared in order to determine how the dominant follicle was selected.⁷⁷ Two growing follicles were co-cultured with or without physical contact. Only one of the two follicles with physical contact developed into antral follicles, while the growth of the other was suppressed. However, the two follicles without physical contact developed similarly. This indicated that contact between two follicles is important for selection of the dominant follicle. The ovary contains many follicles at various developmental stages; therefore, various regulatory mechanisms in addition to physical contact mediate follicular interactions. A regulatory mechanism was reported for primordial follicular development.⁷⁸ The focus was on the position of primordial and growing follicles and analyzing the positioning of the follicles within the ovary. The number of growing follicles that were surrounded by other primordial follicles or that were located near the edge of the ovarian surface was lower than at other locations. The researchers concluded that the primordial follicles and the ovarian epithelium inhibited the development of primordial follicles nearby. It also was reported that a distance of 10-20 μm was most effective for the inhibitory effect. These findings indicated that an inhibitory factor might be released from the primordial follicles and ovarian epithelial cells. However, the exact mechanisms remain undefined. This approach investigated systematic mechanisms in an ovary that contained follicles at various stages of development, stromal cells, ovarian epithelial cells, and others. However, only the early postnatal ovaries were used; therefore, it is not clear whether the identified mechanisms play a role during reproduction. The structure of the postnatal ovary is transient. In adult

mice, the follicles mainly grow near the ovarian surface. In early postnatal mice, the follicles start to grow in the inner part of the cortex.⁷⁹ Therefore, more primordial follicles accumulate near the ovarian epithelium in early postnatal mice, compared with adult mice. In addition to the inhibitory mechanisms of the primordial follicles and ovarian epithelial cells, there could be other regulatory mechanisms in the ovary of pubertal mice because the follicles are distributed differently than in adult mice. Furthermore, in adult mice, the corpus luteum and vascular plexus develop, which could affect follicular development.

Controlling follicular growth is important for continuous and periodic ovulation. Growth differentiation factor-9 is expressed in oocytes from the primary follicular stage and stimulates granulosa cell proliferation and androgen production by theca cells.⁸⁰⁻⁸² Bone morphogenetic protein-4 and protein-7 are present in the theca cells of the primary and secondary follicles and stimulate the transition from the primordial to the primary follicle.^{83,84} Basic fibroblast growth factor is expressed in the oocytes of primordial and primary follicles in some species and promotes primordial follicular development.⁸⁵⁻⁸⁷ These factors also can influence the neighboring follicles by diffusion. The authors previously showed that leukemia inhibitory factor (LIF) diffused from the follicles where it was produced and repressed the growth of the neighboring primary, secondary, and antral follicles.⁴⁷ In this experiment, recombinant LIF and a neutralizing anti-LIF antibody were added to the medium of ovarian tissue cultures. Neutralizing anti-LIF antibody promoted the growth of secondary and antral follicles. If LIF were to act only in the follicle where it was produced, then the antibody would not be able to neutralize LIF because the antibody would be too large to enter the follicle. These results indicated that LIF was released from the follicle into the culture medium and repressed the growth of the surrounding follicles. It is likely that other factors are released from the follicle and the concentration gradient of these factors could change during ovulation, follicular atresia, and the formation of the corpus luteum. These changes might control follicular growth at various stages of development. In cultured ovarian tissue, the growth of multiple follicles was observed by time-lapse imaging. With this approach, it is possible to analyze the regulatory mechanisms of follicular development.

3 | CONCLUSION

Leukemia inhibitory factor has been shown to promote the transition of primordial follicles to primary follicles. This was observed in the ovarian cultures of postnatal day 4 rats.⁸⁸ After 2 weeks in culture, recombinant LIF and neutralizing anti-LIF antibody were added to the medium and the numbers of primordial and growing follicles were counted. There were mainly primordial and primary follicles; therefore, it was difficult to elucidate the effect of LIF on the other stages of follicular development. The development of primordial, primary, secondary, and antral follicles must be coordinated in the ovary in order to ensure a supply of oocytes for ovulation. Therefore, it is necessary to observe the development of individual follicles simultaneously in order to elucidate the regulatory mechanism of follicular development in the ovary.

Ovarian physiology is complicated and it takes a long time for primordial follicles to become Graafian follicles. Therefore, it is difficult to determine the regulatory mechanisms of follicular development. The methods to observe follicular development in the ovary need to be improved in the future in order to enhance reproductive techniques.

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