Follicular Development and Atresia in the B6.YTIR Sex-Reversed Mouse Ovary

Judy Wong, Lynette Luckers, Yuji Okawara, R.-Marc Pelletier, and Teruko Taketo

Department of Biology and Urology Research Laboratory, Department of Surgery, McGill University, Montreal, Quebec, Canada H3A 1A1
Department of Pathology and Cell Biology, University of Montreal, Montreal, Quebec, Canada H3C 3J7

ABSTRACT

The B6.YTIR mouse fails to develop normal testes despite transcription of Sry, the primary testis-determining gene on the Y chromosome. Consequently, B6.YTIR fetuses with bilateral ovaries develop into apparently normal but infertile females. This infertility can be mainly attributed to oocyte incompetence for postfertilization development. In addition, abnormality in preovulatory follicles and rapid loss of oocytes have been observed in XY ovaries. This study examined the effects of gonadotropins on follicular development and atresia in B6.YTIR prepubertal females. The results show that untreated XY females had fewer late preantral follicles and their frequency of atresia was lower. No other difference was found when they were compared with XX females. After treatment with gonadotropins for 24 h, frequency of atresia decreased in both XX and XY ovaries. After 48 h, most preovulatory follicles in XY ovaries were nonatretic, but the oocytes often were denuded. Immunocytochemical staining for connexin 43 detected punctate foci along the oocyte plasma membrane. The density of these foci changed during follicular development, which was similar in XX and XY ovaries. In conclusion, follicular development and atresia under the control of gonadotropins is not influenced by defective oocytes until the preovulatory phase.

apoptosis, follicle, follicular development, FSH, granulosa cells, hormone action, oocyte development, ovum

INTRODUCTION

Female reproductive life is limited by the number of oocytes in the fetal ovary, which continually decreases after oocytes enter meiosis. Loss of oocytes through follicular atresia during cycling also contributes to the limited oocyte pool. One possible evolutionary advantage of follicular atresia may be a form of natural selection, in which only follicles that contain healthy oocytes ovulate [1]. Recent investigations have focused on the molecular mechanisms underlying follicular atresia [1–6]. Nevertheless, we do not yet understand how follicles are selected for ovulation or atresia, and whether healthy oocytes are selected over defective oocytes. An XY sex-reversed female mouse model, named B6.YTIR, may provide an opportunity to examine the association between oocyte quality and the fate of follicles. Although the mechanism of sex-reversal in this model remains unknown, our previous studies have shown that the XY genotype makes the oocyte incompetent for ovulation and postfertilization development.

XY sex-reversal occurs when the Y chromosome of some local varieties of Mus musculus domesticus is placed onto a C57BL/6J (B6) inbred genetic background (named B6.YPOS or B6.YTIR, also previously named B6.YDOM) [7–9]. We reported that the Sry gene, the primary testis-determining gene on the Y chromosome, is present and transcribed at a normal onset time in all B6.YTIR fetal gonads [10]. Nevertheless, the B6.YTIR fetus develops ovaries or ovo-testes, but never a testis. XY offspring with bilateral ovaries develop into anatomically normal females, but none have produced litters, except in one case [7, 11].

We previously reported that reproductive functions are impaired at multiple levels in the female B6.YTIR mouse [11–13]. First, although normal oocytes and follicles appear to develop in young XY females, very few oocytes and follicles remain at 2 mo of age. Second, even while an ovary may be full of oocytes, an XY female lacks estrus cyclicity and produces few eggs at ovulation. Further studies have demonstrated all stages of follicles, except typical preovulatory follicles, in the young XY ovary. When oocytes are collected directly from a juvenile XY ovary, they may undergo efficient maturation and fertilization in vitro, but very few reach the blastocyst stage. Thus, infertility in a female B6.YTIR mouse can be attributed to the quality of its oocytes. It remains to be determined if the defective oocytes are responsible for the failure of late-stage follicular development.

In a normal ovary, a selected population of primordial follicles enter the growth phase at the same time, but the majority of them should be eliminated by atresia before ovulation. Early phase atresia is characterized by a small number of granulosa cells with pyknotic nuclei [1, 14]. This phase is followed by disintegration of the basement membrane and infiltration of leukocytes in the granulosa layer. The last phase is the collapse of follicles, hypertrophy of the thecal layer, and disappearance of follicles. In all mammals studied thus far, exposure to FSH during late-stage follicle development appears to be required for their escape from atresia and development to the ovulatory stage [14, 15].

In the present study, we examined the pattern of follicular development and atresia in the B6.YTIR ovary. One objective was to delineate how a defective oocyte influences follicular development. We were particularly interested in the response of the XY ovary to eCG. We previously reported that the XY ovary is insensitive to eCG in testosterone production [12]. Furthermore, hCG/LH binding sites, which usually appear in mural granulosa cells of preovulatory follicles, are rare in the XY ovary [13]. We hypothesized that the XY ovary may also be less sensitive to FSH, and therefore have a higher rate of follicular atresia. eCG was used in the present study for consistency with our previous studies. To assess the presence of junctions between
oocytes and cumulus cells, we performed immunocytochemical detection of connexin (Cx) 43. We have previously found out that a high proportion of oocytes are denuded at late follicular stages in the XY ovary [13]. Accordingly, we speculated that oocyte denuding may be associated with follicular atresia.

**MATERIALS AND METHODS**

**Mouse**

Male B6.YTIR mice (N30–32 backcross generations) carrying the B6 genetic background and the Y chromosome from a *M. musculus domesticus* house mouse (originally caught in Tirano, Italy) were maintained in our mouse colony and prepared as previously described [8]. B6.YTIR progeny were produced by mating B6.YTIR males with B6 females (Jackson Laboratory, Bar Harbor, ME). The day of delivery was defined as Day 0 postpartum (dpp). After weaning, the chromosomal sex of each mouse was determined by detecting the *Zfy* sequence by polymerase chain reaction amplification of DNA, using the primers and conditions designed by Nagamine et al. [16]. All experiments were conducted in accordance with the Guide to the Care and Use of Experimental Animals issued by the Canadian Council on Animal Care.

**Detection of Apoptosis**

Ovaries were dissected from B6.YTIR and XX females at 26–29 dpp. eCG (G-4877; Sigma Chemical Company, St. Louis, MO) at 5 IU/mouse (0.1 ml of 16 μg/ml solution) was i.p. injected into some females 24 and 48 h prior to their being killed. The ovaries were then fixed in 4% paraformaldehyde (or 3.7% formalin) in PBS (pH 7.4) at 4°C overnight, embedded in paraffin, and cut into 6-μm sections. Sections at 1/4, 1/2, and 3/4 depth were selected to represent each ovary. All sections were deparaffinized through toluene and ethanol, treated with 20 μg/ml proteinase K at 37°C for 15 min, and processed for TUNEL labeling (Boehringer-Mannheim, Indianapolis, IN) according to the manufacturer's protocol. After washing in PBS, the slides were rinsed in water twice, air dried under vacuum, and mounted in Antifade Prolong (Molecular Probes, Eugene, OR) containing 300 ng/ml 4′,6-diamidine-2′-phenylindole dihydrochloride (DAPI; Boehringer-Mannheim). Fluorescent signals were examined under a microscope (Axiohot; Zeiss, Don Mills, ON, Canada). The positive control was prepared by treatment of sections with 2 U/μl DNase I in Dulbeccos PBS (BRL/Gibco, Grand Island, NY) at room temperature for 10 min prior to TUNEL labeling. All positive control slides showed homogeneous staining over the sections (data not shown).

**Immunocytochemical Detection of Connexin 43**

Ovaries were dissected from XX and XY females at 27–31 dpp either with or without PMSG treatment, and fixed in Bouins fixative for 36–48 h at room temperature [17]. Immunocytochemical staining of serial sections was performed as previously described [17, 18]. The polyclonal affinity-purified antibody against the synthetic peptide of Cx43 (amino acid sequence 282–297) was a generous gift from Dr. W.J. Larsen (University of Cincinnati). Its specificity has been previously described by Hendrix et al. [19]. The negative control was prepared by replacing the Cx43-specific antibody with preimmune serum. Microphoto-

**RESULTS**

**Follicular Development and Atresia in Juvenile Ovaries**

|                | eCG     | XX (n) | XY (n) | Significance |
|----------------|---------|--------|--------|--------------|
| None           | 46.5 ± 6 | 41.3 ± 4 | 4.8 (4) | P > 0.05     |
| 24 h           | 54.5 ± 6 | 31.0 ± 3 | 8.1 (4) | P < 0.05     |
| 48 h           | 46.5 ± 6 | 39.8 ± 4 | 6.6 (6) |

eCG, Equine chorionic gonadotropin.

The number of follicles counted in three representative sections from each ovary is summarized in Table 1. In the XX ovary, the total number of growing follicles decreased after treatment with eCG. In contrast, the number of follicles in the XY ovary remained constant, with or without eCG treatment. When XX and XY ovaries were compared, there were significantly fewer follicles in the XY ovary without eCG treatment or after treatment with eCG for 24 h, whereas the numbers were comparable after treatment with eCG for 48 h.

The distribution of follicles at varying growth phases is shown in Figure 1. In the XX ovary without eCG treatment (control), the majority of follicles found were in late preantral stage (stage 5) and some were in early antral stage (stage 6). Treatment with eCG increased the proportion of follicles at preovulatory phase (stage 8) as well as those at early preantral stages (stages 3 and 4). Otherwise, the overall distribution of follicles was not very different either with or without eCG treatment. However, some of these follicles were undergoing atresia, as shown by closed columns. In the control ovary, about half of the follicles at stages 5 and 6 were atretic, and the small number of follicles at more advanced stages were mostly atretic. In contrast, after treatment with eCG for 24 h, the frequency of follicular atresia decreased at all stages, and all follicles at preovulatory stage were nonatretic. After treatment with eCG for 48 h, the frequency of atresia at late preantral and early antral stages (stages 5 and 6) increased to the control levels, while all preovulatory follicles remained nonatretic.

In the XY control ovary, the number of follicles at late preantral stage (stage 5) was less than half that in the XX ovary; however, the frequency of atresia at this stage was lower. Consequently, the number of nonatretic follicles was closer to the number observed in the XX ovary than it was to the total number of follicles. At other stages, both the number of follicles and the frequency of atresia were comparable to those observed in the XX ovary. After treatment
with eCG for 48 h, a very small proportion of follicles were atretic. The numbers of nonatretic follicles at advanced stages were larger than in the control and similar to those in the XX ovary. In contrast, the number of follicles at earlier stages were much smaller than in the XX ovary. After treatment with eCG for 48 h, the frequency of atresia increased to the control levels at stages 5–7 whereas the majority of preovulatory follicles remained nonatretic. The frequency of atresia at stage 5 was considerably lower than it was in the XX ovary. The number and distribution of nonatretic follicles were similar to those in the XX ovary.

Table 2 shows the percentage of atretic follicles at each stage that were used for statistic comparisons between different groups. When no follicle was found, it was impossible to perform calculations; therefore, the amount of available data in some stages was smaller than it was in others. Frequency of atresia decreased after treatment with eCG for 24 h with significant differences at stages 6 and 7 in both XX and XY ovaries. Frequency also decreased at stage 8 from 100% to 0%; however, limited data did not allow statistical evaluation. After treatment with eCG for 48 h, frequency of atresia returned to the control levels at stages 5 and 6 in both types of ovaries. The difference between XX and XY ovaries was significant at stage 5 in both the controls and after treatment with eCG for 48 h.

Examples of TUNEL staining are shown in Figure 2. In controls, many follicles at late preantral and early antral stages contained abundant TUNEL-positive cells (i.e., atretic) in both XX and XY ovaries (Fig. 2. a and d). After treatment with eCG for 24 h, very few follicles were atretic (Fig. 2. b and e). After treatment with eCG for 48 h, all preovulatory follicles were TUNEL-negative, whereas some smaller follicles in the XX ovary were atretic (Fig. 2c). In contrast, in the XY ovary, some preovulatory and smaller follicles were atretic (Fig. 2f). Partly denuded oocytes were seen in both XX and XY ovaries, but more frequently in the XY ovary. Some of the follicles containing denuded oocytes were negative for TUNEL staining (Fig. 2f). Furthermore, some follicles containing abnormal oocytes (e.g., irregular in shape or enclosed in thin zona pellucida [ZP] layers) were also TUNEL-negative (data not shown). On the other hand, some morphologically normal follicles were full of TUNEL-positive cells (Fig. 2, a and d). After treatment with eCG for 48 h, very few follicles were atretic (Fig. 2c). In contrast, in the XY ovary, some preovulatory and smaller follicles were atretic (Fig. 2f). Partly denuded oocytes were seen in both XX and XY ovaries, but more frequently in the XY ovary. Some of the follicles containing denuded oocytes were negative for TUNEL staining (Fig. 2f). Furthermore, some follicles containing abnormal oocytes (e.g., irregular in shape or enclosed in thin zona pellucida [ZP] layers) were also TUNEL-negative (data not shown). On the other hand, some morphologically normal follicles were full of TUNEL-positive cells (Fig. 2, a and d); thus, no morphological features were associated with TUNEL-positive follicular atresia except for very advanced stages.

Expression of Connexin 43 in Oocyte-Cumulus Junctions

Immunocytochemical staining for Cx43 detected punctate foci along the oocyte plasma membrane as well as thick linings between granulosa cells (Figs. 3 and 4). In the XX control ovary, Cx43-positive foci were occasionally seen along the oocyte plasma membrane in the minority of preantral follicles (Fig. 3a). Before the ZP layer had thickened, it was difficult to distinguish Cx43 foci around the oocyte. The number of foci as well as the population of preantral follicles remained nonatretic. The numbers of nonatretic follicles at advanced stages were larger than in the control and similar to those in the XX ovary. In contrast, the number of follicles at earlier stages were much smaller than in the XX ovary. After treatment with eCG for 48 h, the number of Cx43 foci along oocytes increased with further follicular development (Fig. 3, b and c). However, some oocytes had no or only a few Cx43 foci despite well-developed ZP in some advanced antral follicles (Fig. 3d). After treatment with eCG for 48 h, the number of Cx43 foci along oocytes decreased at all follicular stages (Fig. 3, e and f). In particular, Cx43 foci were rare along the plasma membrane of oocytes in preovulatory follicles (Fig. 3f).

In the XY control ovary, a smaller proportion of oocytes...
appeared to contain Cx43 foci at most follicle stages compared with the XX ovary (Fig. 4, a–d). After treatment with eCG, Cx43 foci were rather abundant in the XY ovary (Fig. 4, e–h). Nonetheless, overall patterns of Cx43 staining were similar between XX and XY ovaries. In some advanced follicles, oocytes were partly denuded, yet Cx43 foci were clearly seen along the oocyte plasma membrane (Fig. 4h). No or very few Cx43 foci were seen around completely denuded oocytes (Fig. 4d).

Monolayers of granulosa cells were found beneath the ZP layer of some oocytes in both XX and XY ovaries; however, they were more frequent in the XY ovary (Fig. 3, g and h). Intense staining for Cx43 was seen along the oocyte plasma membrane as well as between invading granulosa cells.

**DISCUSSION**

Our previous studies have shown that late follicular development is impaired in the B6.YTIR sex-reversed ovary [13]. Subsequently, very few oocytes can be ovulated from the XY female and, although these eggs can be fertilized, none develop beyond the two-cell stage [13, 21]. By contrast, when as small as 5% of XX cells are present in the B6.YTIR ↔ XX chimeric female, normal fertility is observed, although the offspring are exclusively derived from XX oocytes [22]. These results suggest that the oocyte of the XY genotype is responsible for the failure in late folliculogenesis. We further studied the defect in the XY oocyte and found that the X and Y sex chromosomes remain unpaired during meiotic prophase in the fetal ovary [23]. Around half of the oocytes result in aneuploidy after the first meiotic division in vitro. These nuclear events in the oocyte alone, however, cannot explain the failure in late folliculogenesis that precedes oocyte maturation. The present study attempted to further clarify the impairment of follicular development and its association with defective oocytes in the XY ovary. It is known that most follicles undergo atresia unless rescued in the normal XX ovary, and that the early phase of follicular atresia can be detected only by biochemical methods [1]. Therefore, standard morphology alone, used in our previous studies, was not sufficient to document follicular development in the XY ovary.

**Deficit in Preantral Follicles**

The present results show that the total number of growing follicles was much smaller in the XY control ovary than in the XX ovary. This difference can be attributed mainly to the deficit in late preantral follicles in the XY ovary. The number and distribution of follicles at latter stages were comparable between the two types of ovaries. These ob-
FIG. 3. Immunocytochemical detection of connexin 43. Inset shows a part of oocyte plasma membrane at ×2. a) Preantral follicle in XX control ovary. A few Cx43 foci are seen along the plasma membrane beneath the ZP layer of oocyte. Stronger staining is seen between granulosa cells. b) Early antral follicle in XX control ovary. Several Cx43 foci are seen along the oocyte plasma membrane. The staining between granulosa cells is intensified compared with the preantral follicle in Figure 3a. c) Mid-late antral follicle in XX control ovary. The ZP layer is well developed. Numerous Cx43 foci are seen along the oocyte plasma membrane. d) Mid-late antral follicle in XX control ovary. The ZP layer is well developed. No Cx43 foci are apparent along the oocyte plasma membrane. The intense staining between granulosa cells is comparable to those seen in Figure 3, b and c. e) Early antral follicle in XX ovary after treatment with eCG for 48 h. The ZP layer is well developed. A few Cx43 foci are seen along the oocyte plasma membrane. f) Preovulatory follicle in XX ovary after treatment with eCG for 48 h. No Cx43 foci are seen along the oocyte plasma membrane. g) Antral follicle in XX ovary. Several granulosa cells are present beneath the ZP layer. Cx43 foci are seen along the oocyte plasma membrane. h) Antral follicle in XY ovary. Several granulosa cells are present beneath the ZP layer. Cx43 foci are seen along the oocyte plasma membrane as well as between the invading granulosa cells. Bar = 100 nm (a-f) or 40 nm (g and h).

FIG. 4. Immunocytochemical detection of connexin 43. Same magnification as Figure 3, a-f. a) Preantral follicle in XY control ovary. A few Cx43 foci are seen along the oocyte plasma membrane. Stronger staining is also seen between granulosa cells. b) Early antral follicle in XY control ovary. The ZP layer has irregular thickness. Fine Cx43 foci are seen along the oocyte plasma membrane. The staining between granulosa cells is comparable to that in the preantral follicle in a. c) Antral follicle in XY control ovary. The ZP layer has irregular thickness. Many Cx43 foci are seen along the oocyte plasma membrane. d) Atretic follicle in XX control ovary. Oocyte is completely denuded. Only a few Cx43 foci are seen along the oocyte plasma membrane. Intense staining remains between granulosa cells. e) Early antral follicle in XX ovary after treatment with eCG for 48 h. The ZP layer is well developed. Numerous Cx43 foci are seen along the oocyte plasma membrane. f) Mid-late antral follicle in XX ovary after treatment with eCG for 48 h. Very few Cx43 foci are seen along the oocyte plasma membrane. g) Preovulatory follicle in XX ovary after treatment with eCG for 48 h. Several Cx43 foci are seen along the oocyte plasma membrane. h) Preovulatory follicle in XX ovary after treatment with eCG for 48 h. Several Cx43 foci are seen along the plasma membrane of half-denuded oocyte.

Observations agree with the possible mechanism that adjusts the number of preantral follicles that continue through development and ovulation. This possibility is further supported by the observation that the frequency of atresia at late preantral stage is significantly lower, in favor of further follicular development, in the XY ovary.

Similar observations have been made in hemicastrated rats and aging mice [24, 25]. In these animals, fewer follicles are available in the growth phase and, as a result, fewer follicles undergo atresia during the selection process. However, in the aging mouse, the frequency of atresia is reduced at all follicular growth phases, in contrast to our results in the B6.YTIR female mouse. The difference may be attributable to cycling vs prepubertal animals [26]. In the present study, unfortunately, we could not examine cycling mice because the B6.YTIR female never establishes regular estrus cyclicity, and it rapidly loses its oocytes at young ages [11].

The smaller number of preantral follicles may reflect the limited oocyte pool in the B6.YTIR ovary. We have previously found that all oocytes in the medullary region degenerate prenatally in the XY ovary [11]. The oocytes in...
FOLLICULAR DEVELOPMENT AND ATRESIA IN THE XY OVARY

the same region are destined to degenerate even in the normal XX ovary, but usually after follicles begin to develop around them in neonatal life. Consequently, secondary androgen-producing cells are deposited after follicular atresia in the XX ovary [27]. Therefore, it was conceivable that an excess number of oocytes in the cortical region may be recruited for follicular growth and atresia to compensate for the deficiency in androgen-producing cells in the XY ovary; however, the present results did not show any excessive frequency of follicular atresia in the XY ovary at the pre-pubertal ages examined. Accordingly, the oocyte pool must have been reduced in earlier postnatal life. Alternatively, the early phase of follicular development may be inefficient in the XY ovary.

Prevention of Follicular Atresia by eCG

After treatment with eCG for 24 h, the frequency of atresia decreased significantly in both XX and XY ovaries. These results suggest that FSH, the major component of eCG, can function efficiently to prevent follicular atresia in the XY ovary. It must be noted that the data in Figure 1 and Table 2 underestimate late follicular stages because only the follicles containing oocytes were counted. In fact, the ovary after treatment with eCG for 48 h was occupied by many preovulatory follicles, but only a few contained oocytes in selected sections.

Antral stage is the most crucial branching point in follicular development, and this is the stage during which gonadotropins exert their major regulatory actions [28, 29]. In addition to FSH, LH plays a key role in the growth of small antral follicles to the preovulatory phase [29]. Because we have observed a similar transition from antral to preovulatory stages in XX and XY ovaries after eCG treatment, not only FSH but also LH appears to be effective in the XY ovary. This finding may contradict our previous finding that eCG fails to increase testosterone production in the XY ovary [12]; on the other hand, we have demonstrated normal distribution of LH/hCG binding sites in theca cells in the XY ovary [13]. The mechanism of LH insensitivity in steroid production in the XY ovary needs further investigation.

Development of Antral Follicles into the Preovulatory Phase

After treatment with eCG for 48 h, the overall distribution of follicles was similar between XX and XY ovaries. However, much higher proportions of follicles at antral stages were atretic in the XY ovary. Unexpectedly, most of the follicles at preovulatory stage were TUNEL-negative, although they often contained denuded oocytes. These observations suggest that denudation of oocytes is neither an immediate cause nor a consequence of follicular atresia. Our previous studies have shown that most preovulatory follicles in the XY ovary fail to express LH receptors in mural granulosa cells [13]. These follicles often contain, instead, thickening of thecal layers with abundant hCG binding. We believe that defective oocytes render late antral follicles to undergo precocious luteinization instead of entering the normal preovulatory phase in the XY ovary. Denudation of oocytes may reflect an improper follicular environment.

Oocyte-Cumulus Cell Junctions

The oocyte develops an intimate and interdependent association with its surrounding granulosa cells during folliculogenesis [30–32]. Cx37 is the predominant component of the oocyte-cumulus junction and plays an essential role in ovulation [33]. Cx43 is also expressed in granulosa cells in the ovary, and its expression is critically coordinated with follicular growth and atresia [34–37]. However, expression of Cx43 in the oocyte-cumulus gap junction had not been conclusively demonstrated [33, 34]. In the present study, we have detected Cx43 in punctate foci along the oocyte plasma membrane when the ZP layer began to develop at early preantral stage. Moreover, the number of Cx43 foci in oocytes increased with advancement of follicular development. After eCG treatment, Cx43 foci decreased in most follicles, particularly preovulatory follicles, in the normal XX ovary. Therefore, distribution of Cx43 along the oocyte plasma membrane appears to be tightly associated with follicular development. Previous studies using fluorescent immunocytochemistry might have been unable to detect Cx43 staining in the oocyte-cumulus junction because these foci are much finer than those between granulosa cells. The high specificity and affinity of the antibody used, combined with histochemical staining for peroxidase, probably allowed us to detect the fine Cx43 foci in oocytes.

The distribution of Cx43 foci in oocytes in the XY ovary was similar to that in the XX ovary. This observation suggests that the communication between the oocyte and cumulus cells was maintained despite the defect in oocytes in the XY ovary. Furthermore, Cx43 foci remained in half-denuded oocytes. Therefore, the high frequency of oocyte denudation at late follicular stages was unlikely a consequence of loss of oocyte-cumulus cell junctions. In our future studies, we would like to examine the expression of Cx37 and its association with Cx43 on the oocyte plasma membrane during follicular development.

Summary

The present study shows that follicular development and atresia are properly regulated by gonadotropins, despite defective oocytes in the XY ovary. However, at the last stage of folliculogenesis, most oocytes become denuded and their follicles appear to undergo precocious luteinization instead of ovulation. Neither atresia nor loss of Cx43 junctions precedes this transition. The mechanism that blocks the antral follicles to proceed into normal preovulatory phase in the XY ovary remains to be investigated.

ACKNOWLEDGMENT

We are grateful to Dr. W.J. Larsen (University of Cincinnati) for his generous gift of antibody against connexin 43.

REFERENCES

1. Hsueh AJW, Billig H, Tsafirri A. Ovarian follicle atresia: a hormonally controlled apoptotic process. Endocrinology 1994; 145:707–724.
2. Tilly JL, Tilly KI, Kenton ML, Johnson AL. Expression of members of the Bcl-2 gene family in the immature rat ovary: equine chorionic gonadotropin-mediated inhibition of granulosa cell apoptosis is associated with decreased Bax and constitutive Bcl-2 and Bcl-x long messenger ribonucleic acid levels. Endocrinology 1995; 136:232–241.
3. Hsu SY, Lai RJ, Finegold M, Hsueh AJW. Targeted overexpression of Bcl-2 in ovaries of transgenic mice leads to decreased follicle apoptosis, enhanced folliculogenesis, and increased germ cell tumorigenesis. Endocrinology 1996; 137:4837–4843.
4. Bergeron L, Perez GI, Macdonald G, Shi L, Sun Y, Jurisicova A, Varmuzsa S, Latham KE, Flaws JA, Salter JCM, Hara H, Moskowitz MA, Li E, Greenberg A, Tilly JL, Yuan J. Defects in regulation of apoptosis in caspase-2-deficient mice. Genes Dev 1998; 12:1304–1314.
5. Kim J, Yoon Y, Tsang BK. Involvement of the Fas/Fas ligand system
in p53-mediated granulosa cell apoptosis during follicular development and atresia. Endocrinology 1999; 140:2307–2317.
6. Robles R, Tao X-J, Trbovich AM, Maravei DV, Nahum R, Perez GI, Tilly KI, Tilly JL. Localization, regulation and possible consequences of apoptotic protease-activating factor-1 (Apaf-1) expression in granulosa cells of the mouse ovary. Endocrinology 1999; 140:2641–2644.
7. Eicher EM, Washburn LL, Whitney III JB, Morrow KE. Mas poshivirus Y chromosome in the C57BL/6J murine genome causes sex reversal. Science 1982; 217:535–537.
8. Nagamine CM, Taketo T, Koo GC. Studies on the genetics of tda-l XY sex reversal in the mouse. Differentiation 1987; 33:223–231.
9. Carlisle C, Winking H, Weichenhan D, Nagamine CM. Absence of correlation between Sry polymorphisms and XY sex reversal caused by the M. m. domesticus Y chromosome. Genomics 1996; 33:32–45.
10. Lee C-H, Taketo T. Normal onset, but prolonged expression, of Sry gene in the B6.YDOM sex-reversed mouse gonad. Dev Biol 1994; 165:442–452.
11. Taketo-Hosotani T, Nishioka Y, Nagamine C, Villalpando I, Merchant-Larios H. Development and fertility of ovaries in the B6.YDOM sex-reversed female mouse. Development 1989; 197:95–105.
12. Villalpando I, Nishioka Y, Taketo T. Endocrine differentiation of the XY sex-reversed mouse ovary during postnatal development. J Steroid Biochem Mol Biol 1993; 45:265–273.
13. Amleh A, Ledee N, Saeed J, Taketo T. Competence of oocytes from the B6.YDOM sex-reversed female mouse for maturation, fertilization, and embryonic development in vitro. Dev Biol 1996; 178:263–275.
14. Hirshfield AN. Rescue of atretic follicles in vitro and in vivo. Biol Reprod 1989; 40:181–190.
15. Chun S-Y, Eisenhauer KM, Minami S, Billig H, Perlas E, Hsueh AJW. Hormonal regulation of apoptosis in early atrial follicles: follicle-stimulating hormone as a major survival factor. Endocrinology 1996; 137:1447–1456.
16. Nagamine CM, Chan K, Kozak CA, Lau Y-F. Chromosome mapping and expression of a putative testis-determining gene in mouse. Science 1989; 243:80–83.
17. Pelletier R-M, Trifano J-M, Carbajal ME, Okawara Y, Vitale ML. Calcium-dependent actin filament-severing protein scinderin levels and localization in bovine testis, epididymis, and spermatozoa. Biol Reprod 1999; 60:1128–1136.
18. Hendrix EM, Mao SJT, Everson W, Larsen WJ. Myometrial connexin 43 trafficking and gap junction assembly at term and in preterm labor. Mol Reprod Dev 1992; 33:27–38.
19. Peters H. The development of the mouse ovary from birth to maturity. Acta Endocrinol 1969; 62:98–116.
20. Merchant-Larios H, Clarke HJ, Taketo T. Developmental arrest of fertilized eggs from the B6.YDOM sex-reversed female mouse. Dev Genet 1994; 15:435–442.
21. Amleh A, Taketo T. Live-borns from XX but not XY oocytes in the chimeric mouse ovary composed of B6.YTIR and XX cells. Biol Reprod 1998; 58:574–582.
22. Amleh A, Smith L, Chen H-Y, Taketo T. Both nuclear and cytoplasmic components are defective in oocytes of the B6.YTIR sex-reversed female mouse. Dev Biol 2000; 219:277–286.
23. Gosden RG, Laing SC, Felicio LS, Nelson JF, Finch CE. Imminent oocyte exhaustion and reduced follicular recruitment mark the transition to acyclicity in aging C57BL/6J mice. Biol Reprod 1983; 28:255–260.
24. Hirshfield AN. Compensatory ovarian hypertrophy in the long-term hemicastrate rat: size distribution of growing and atretic follicles. Biol Reprod 1983; 28:271–278.
25. Hirshfield AN. Patterns of [1H]thymidine incorporation differ in immature rats and mature, cycling rats. Biol Reprod 1986; 34:229–235.
26. Erickson GF, Magoffin DA, Dyer CA, Hofeditz C. The ovarian androgen producing cells: a review of structure/function relationships. Endocr Rev 1985; 6:371–399.
27. Hirshfield AN. Size-frequency analysis of atresia in cycling rats. Biol Reprod 1988; 38:1181–1188.
28. Richards JS. Hormonal control of gene expression in the ovary. Endocr Rev 1994; 15:725–751.
29. Anderson E, Albertini DF. Gap junctions between the oocyte and companion follicle cells in the mammalian ovary. J Cell Biol 1976; 71:680–686.
30. Gilula NB, Epstein ML, Beers WH. Cell-to-cell communication and ovulation. A study of the cumulus-oocyte complex. J Cell Biol 1978; 78:58–75.
31. Buccione R, Schroeder AC, Eppig JJ. Interactions between somatic cells and germ cells throughout mammalian oogenesis. Biol Reprod 1990; 43:543–547.
32. Simon AM, Goodenough DA, Li E, Paul DL. Female infertility in mice lacking connexin 37. Nature 1997; 385:525–529.
33. Valdimarsson G, De Sousa PA, Kidder GM. Coexpression of gap junction proteins in the cumulus-oocyte complex. Mol Reprod Dev 1993; 36:7–15.
34. Wiesen JF, Midgley AR Jr. Changes in expression of connexin 43 gap junction messenger ribonucleic acid and protein during ovarian follicular growth. Endocrinology 1993; 133:741–746.
35. Wiesen JF, Midgley AR Jr. Expression of connexin 43 gap junction messenger ribonucleic acid and protein during follicular atresia. Biol Reprod 1994; 50:336–348.
36. Juneja SC, Bar KJ, Enders GC, Kidder GM. Defects in the germ line and gonads of mice lacking connexin 43. Biol Reprod 1999; 60:1263–1270.