MORPHOLOGY AND PATHOMORPHOLOGY

Contribution of Ovarian Follicular Tissue Abnormalities into the Development of Ovarian Dysfunction

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Ultrastructural, *in vitro* radioautographic, and stereological study of the morphogenesis of ovarian cystic formations in women of reproductive age distinguished ovariopathy as a common pathological phenomenon, developing as a result of the ovarian follicular cell regenerative and plastic insufficiency syndrome. Degenerative dystrophic changes in the follicular compartment, reduced biosynthetic reactions in the follicular cells, and reactive sclerosis of the stroma remodulate the parenchyma-stromal relationships.

Key Words: *ovarian dysfunction; laparoscopic biopsy of ovaries; electron microscopy; in vitro radioautography*

The greater part of modern studies of ovarian dysfunction is focused on mutations of genes, involved in normal development of the ovaries and/or follicles. It was shown on experimental models that these mutations caused a wide spectrum of phenotypical restructuring of the follicles from ovarian dysgenesis to gonadotropic resistance [6,12]. Hence, modern diagnostic strategy is aimed at the search for genetic causes of ovarian dysfunction [7,11, 13,14] detected, together with other methods, by electron microscopy [11,12].

In addition to genetic factors, ovariotoxic epigenomic factors play an important role in the development of ovarian diseases [1,10]. The incidence of dystrophic degenerative processes progressively increases, which is caused by exposure to a complex of unfavorable factors, including the toxic ones. Degenerative transformation and the resultant organ dysfunction were detected in respiratory, gastrointestinal, and excretory organs and skin [3]. Follicular cell degeneration with subsequent reduction of their number can be regarded within the framework of this process.

We carried out a complex pathomorphological analysis of ovarian biopsy specimens in ovarian dysfunction in order to clear out the morphogenesis of cystic transformation.

MATERIALS AND METHODS

A complex clinical endoscopic and pathomorphological study was carried out in 82 women aged 20-37 years with cystic changes in the ovaries: 53 patients with ovarian cysts (follicular and endometrioid) and 29 with the polycystic ovarian syndrome. The main clinical syndromes were infertility, menstrual dysfunction (primarily hypomenstrual syndrome, dysmenorrhea, and secondary amenorrhea). Common clinical studies, dynamic ultrasonic examination, measurements of gonado-

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tropic and sex hormones in the blood and peritoneal fluid, endoscopic laparoscopy with biopsy of the involved and "intact" ovaries were carried out. In addition, comparative retrospective analysis of the results of surgical treatment was carried out in two groups of patients after resection of the ovaries (40 cases) and resection combined with omentoovariopexy (42 cases), regarded as a method improving innervation and vascularization of the ovaries [2].

Ovarian biopsy specimens were studied by light and electron microscopy, *in vitro* radioautography, and stereological analysis. Paraffin sections were stained with hematoxylin and eosin with Perls reaction, by the method of van Gieson with post-staining of elastic fibers by Weigert resorcin-fuchsin, and PAS reaction was carried out. Specimens for electron microscopy were treated routinely and embedded into epon-araldite mixture. Semithin sections were stained with 1% azur II solution; ultrathin sections were contrasted with uranyl acetate and lead citrate and examined under a JEM 1010 electron microscope at accelerating voltage of 80 kV.

For evaluation of the intensity of biosynthetic reactions in ovarian cell populations, *in vitro* radioautography was carried out (the samples were incubated with radioactive RNA and DNA precursors) [5]. The label density and index were evaluated in cells under a Leica DM4000B universal microscope. Microphotographs were made using Leica DFC 320 digital photocamera and Leica QWin software.

Stereological analysis of ovarian biopsy specimens was carried out using an ocular multi-purpose test system [4]. Volume densities of follicles of the first stages of maturation, collagen fibers, lumens and walls of the cortical layer perifollicular and peripheral vessels were calculated as the primary parameters, after which secondary stereological parameters were calculated.

The data were processed using Student's test; the differences were considered significant at $p<0.05$.

RESULTS

Reduction of the follicular system with decreased number and size of primordial and maturing follicles, or their complete absence in some cases, was seen in the majority of biopsy specimens of involved and intact ovaries. Active or ceasing corpora lutea were rare. Pronounced fibrosis of the cortical layer associated with vascular wall fibrosis was noted. The follicular system decreased at the expense of progressive degenerative dystrophic changes in the primordial and maturing follicular epithelium and cystic atresia of maturing forms.

Cystic transformation was characterized by stereotypical ultrastructural changes in follicular cells: reduction of the protein-synthesizing and mitochondrial compartments, presence of filamentous structures and formation of residual structures (Fig. 1, *a*), poorly manifest zona pellucida, and reduction of the microvilli (Fig. 1, *b*), which reflected reduced contact area between the follicular epithelium and oocyte. Oocyte ultrastructure contained no signs of intensive gamete growth stage: poor development of the protein-producing cytoplasmic organelles, few small mitochondria, reduction of Golgi complexes, and absence of cortical granules were noted (Fig. 1, *c*).

Resection in combination with omentoovariopexy led to reduction in the number of degenerative primordial and primary follicles. Cystic atresia of the follicular system was combined with compensatory theca interna hyperplasia. Restructuring of the connective tissue with predominance of the matrix over fibrous structures and intensification of the organ vascularization were seen in the cortical stroma.

In addition, changes in the intracellular organization of the somatic and gamete compartments were detected in the follicles at stage 1 of maturation. Proliferation of follicular cells (Fig. 1, *d*) was noted, filamentous structures were replaced with free ribosomes in epitheliocytes, the number of elements of the granular endoplasmic reticulum and mitochondria increased (Fig. 1, *e*), which reflects induction of intracellular regeneratory reactions and together with the findings of light microscopy could be interpreted as reduction of the follicular cell dystrophy. Hyperplasia of folliculocyte microvilli increased the area of contact between the oocyte and follicular epithelium; zona pellucida components accumulated and transosomes were visualized against its background. Improvement of the gamete nutrition caused hyperplasia of the Golgi complexes and accumulation of the cortical granules (signs of partial recovery of the gamete intense growth stage; Fig. 1, *f*).

Recovery of the menstrual cycle and reproductive function was more incident in patients with retained follicular system in comparison with the patients with significant reduction of the follicular compartment and pronounced stromal sclerosis. On the whole, the results of treatment positively correlated with the type of structural changes in the ovaries.

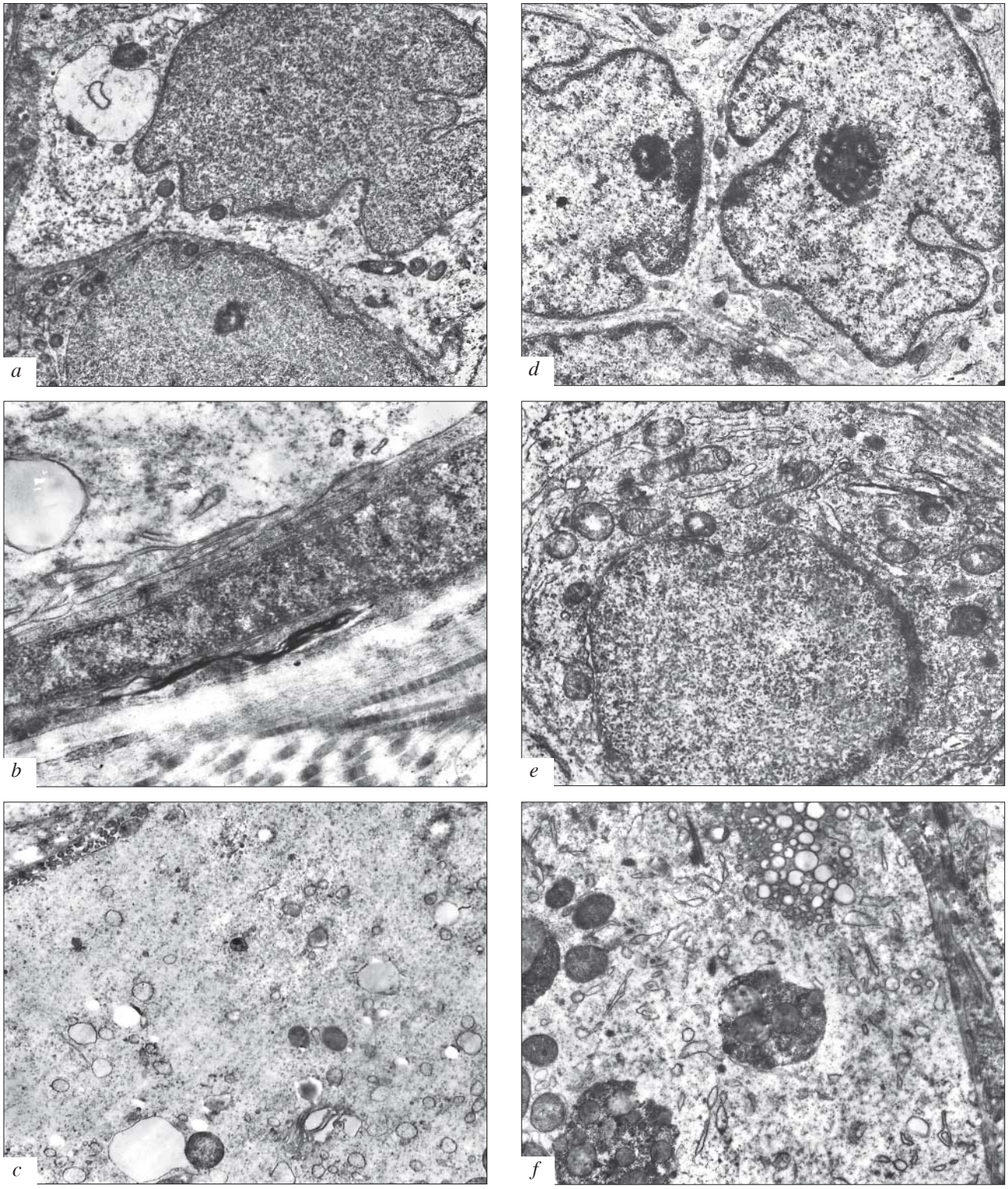

Fig. 1. Ultrastructural characteristics of the ovarian follicular system in cystic transformation. *a*) follicular cells of the primary follicle: devastated cytoplasm, autophagic vacuole, ×6000; *b*) primordial follicle epitheliocyte: reduction of microvilli, osmiophilic residual structure, ×12,000; *c*) primary oocyte: small solitary mitochondria, ×4000; *d*) proliferation of follicular epithelium, ×4000; *e*) follicular cell cytoplasm with numerous cytoplasmic organelles, ×5000; *f*) large heterogeneous cortical granules in the primary oocyte cytoplasm, widening of the zona pellucida, ×8000.

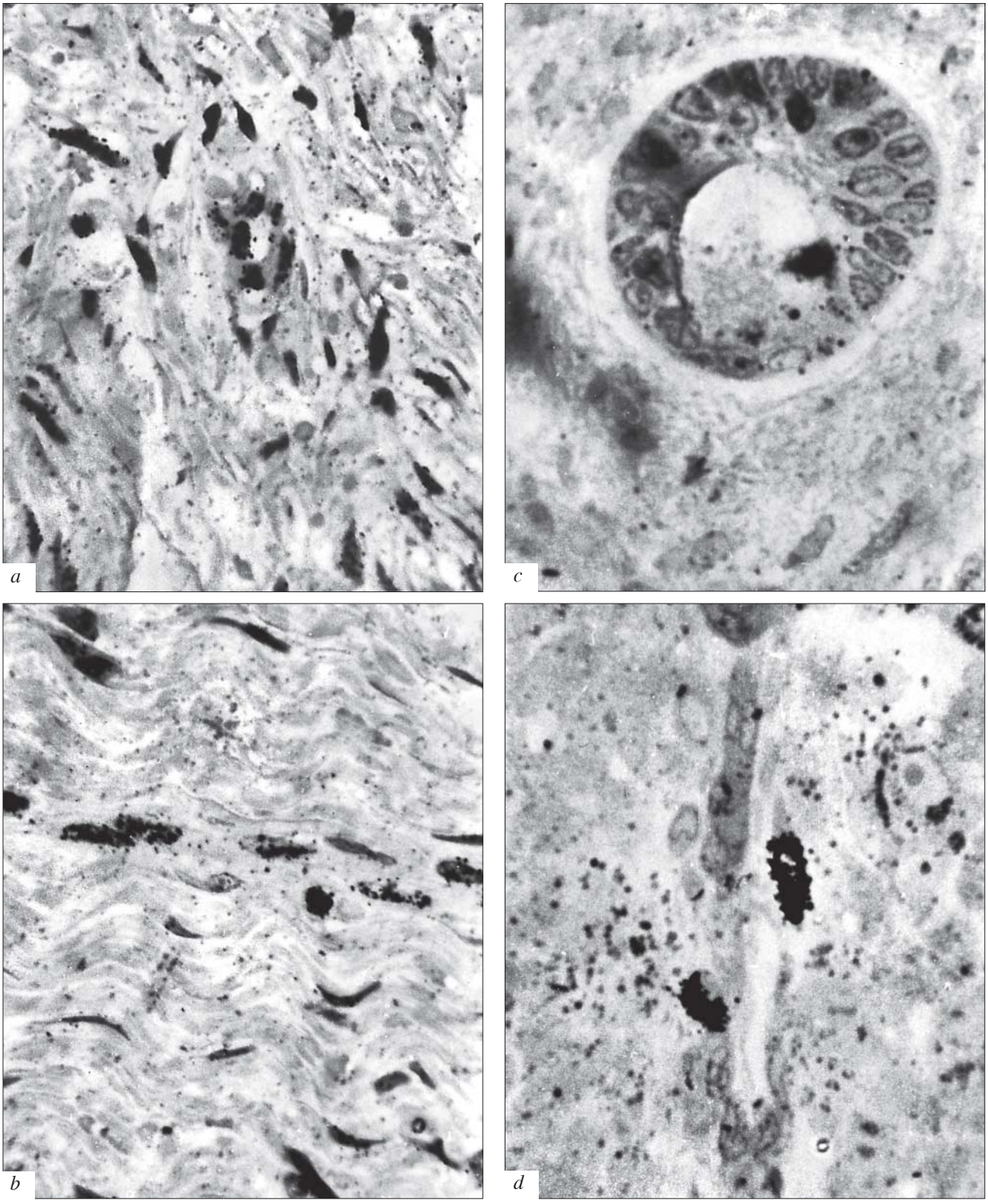

Fig. 2. Biosynthetic reactions in cell populations of ovarian biopsy specimens in ovarian dysfunction: polycystic ovarian syndrome (*a*: before treatment; *c*, *d*: after combined treatment) and endometrioid cyst (*b*). Semithin sections, azur II staining. Incubation with ^{3}H-uridine (*a*-*c*), with ^{3}H-thymidine (*d*). *a*) high radioautograph index and density in fibroblasts and vascular cells, ×250; *b*) silver grains in fibroblasts, ×300; *c*) ^{3}H-uridine radioautographs in oocyte nucleus and follicular cells, ×450; *d*) high density of ^{3}H-thymidine label in pericytes, ×650.

Biosynthetic activity of ovarian cortical layer cells was evaluated in populations of follicular cells, endotheliocytes, and fibroblasts. Radioautographs with ^{3}H-uridine were detected in 95-100% follicular cells, in 78% perifollicular vascular endotheliocytes, and 52% fibroblasts. No ^{3}H-uridine label was detected in the follicular cells of patients with the polycystic ovarian syndrome; the label index in endotheliocytes was 40-52%, in fibroblasts more than 80%. The severity of stromal fibrosis and biosynthetic activity of cells producing the matrix were higher in the polycystic ovarian syndrome than in solitary cysts (Fig. 2, *a*, *b*). On the whole, the type of biosynthetic processes reflected the severity of degenerative dystrophic changes in the follicular epithelium and organ fibrosis, most intense in the polycystic ovarian syndrome. ^{3}H-uridine label index reached 30% after combined therapy including omentoovariopexy for the polycystic ovarian syndrome, the label density in the follicular cells remaining low, but numerous silver grains were detected above the oocyte nucleus (Fig. 2, *c*); in addition, solitary ^{3}H-thymidine radioautographs appeared in the theca cells and pericytes (Fig. 2, *d*).

Microscopic findings were confirmed by the data of stereological analysis. Combined treatment, including omentoovariopexy, led to an increase in the structural density of the follicular compartment (0.0569±0.0035 before treatment, 0.0625±0.0036 after resection, and 0.0892±0.0025 after combined treatment) with a trend to extension of the theca interna and to a significant ($p<0.05$) increase in the volume density of perifollicular vascular lumens in comparison with the values before treatment and results of resection alone (0.1045±0.0034 *vs.* 0.0361±0.0045, and 0.0545±0.0038, respectively). Combination of resection with omentoovariopexy increased ovarian follicular/collagen index reflecting the volume ratio of follicular and fibrous tissue densities.

Hence, the most significant structural change in the ovaries in cystic transformation was reduction of the follicular compartment, caused by progressive dystrophy and degeneration of follicular epitheliocytes, reduction of the protein-producing organelles, and reduced level of biosynthetic reactions. Synchronous sclerotic changes in the stroma of the cortical and medullary layers and significant sclerotic deformation of the vascular system elements were observed. The systemic type of the pathological process is important: degenerative dystrophic changes in the follicular system were detected in the involved and "intact" ovaries, which suggests regarding this process as ovariopathy, originating from the regeneratory plastic insufficiency syndrome [3]. The morphogenesis of ovariopathy corresponds to primary dystrophic process eventuating in degeneration of the parenchymatous component and reactive stromal sclerosis in the majority of cases.

Combined correction of ovarian dysfunction brought about a pronounced clinical effect and led to positive structural changes, reflecting the induction of cellular and intracellular regeneratory reactions of the follicular epitheliocytes and restructuring of the stroma with reduction of fibrosis severity and improvement of vascularization. This is important for the choice of rational treatment strategy for rehabilitation of women of reproductive age with cystic transformation of the ovaries.

REFERENCES

1. T. G. Borovskaya, E. A. Timina, A. V. Perova, *et al.*, *Byull. Eksp. Biol. Med.*, **143**, No. 5, 551-553 (2007).
2. L. A. Leontyuk, *Biological Role of Ovariopexy* [in Russian], Minsk (1979).
3. G. I. Nepomnyashchikh, *Borderline Tissues (Mucosae and Skin) in the Morphogenesis of Common Pathological Processes* [in Russian], Novosibirsk (1996).
4. L. M. Nepomnyashchikh, E. L. Lushnikova, L. V. Kolesnikova, *et al.*, *Morphometric and Stereological Analysis of the Myocardium: Tissue and Ultrastructural Organization* [in Russian], Novosibirsk (1984).
5. L. M. Nepomnyashchikh, G. I. Nepomnyashchikh, S. V. Aidagulova, *et al.*, *Byull. Eksp. Biol. Med.*, **128**, No. 11, 591-596 (1999).
6. L. A. Allen, J. C. Achermann, P. Pakarinen, *et al.*, *Hum. Reprod.*, **18**, No. 2, 251-256 (2003).
7. K. H. Burns and M. M. Matzuk, *Endocrinology*, **143**, No. 8, 2823-2835 (2002).
8. P. G. Crosignani, M. Colombo, W. Vegetti, *et al.*, *Hum. Reprod.*, **18**, No. 9, 1928-1932 (2003).
9. J. P. de Bruin, M. Dorland, E. R. Spek, *et al.*, *Biol. Reprod.*, **70**, No. 2, 419-424 (2004).
10. P. J. Devine, C. M. Payne, M. K. McCuskey, and P. B. Hoyer, *Ibid.*, **63**, No. 5, 1245-1252 (2000).
11. K. Koyama, A. Hasegawa, N. Mochida, and G. Calongos, *Reprod. Biol.*, **5**, No. 3, 269-278 (2005).
12. N. Massin, A. Gougeon, G. Meduri, *et al.*, *Hum. Reprod.*, **19**, No. 11, 2555-2560 (2004).
13. T. L. Rankin, M. O'Brien, E. Lee, *et al.*, *Development*, **128**, No. 7, 1119-1126 (2001).
14. D. Schmidt, C. E. Ovitt, K. Anlag, *et al.*, *Ibid.*, **131**, No. 4, 933-942 (2004).