Enhanced follicular recruitment and atresia in cortex derived from ovaries with endometriomas

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Objective: To evaluate the effects of endometriomas on the regulation of early follicular development.
Design: Histologic analysis of prospectively collected biopsy samples.
Setting: Research unit in a university hospital.
Patient(s): Women <40 years of age who have ovarian endometriomas.
Intervention(s): Biopsy of healthy cortex from ovaries affected by endometriomas (≤4 cm) and contralateral ovaries without cysts.
Main Outcome Measure(s): Histomorphological staging of early follicles, measurement of follicle, oocyte, and oocyte nucleus diameters, immunohistochemistry of proliferating cell nuclear antigen, and caspase-3.
Result(s): Thirteen cortical samples from ovaries with endometriomas and 13 samples from contralateral ovaries without endometriomas were evaluated. Cortex from ovaries with endometriomas contained significantly more morphologically atretic early follicles than cortex from contralateral ovaries without cysts. These follicles showed cleaved caspase-3 immunostaining. Diameters of primordial follicles and oocytes were decreased in cortex from ovaries with endometriomas, whereas early follicles with proliferating cell nuclear antigen-positive granulosa cells (GCs) were significantly increased in number.
Conclusion(s): Ovaries with endometriomas, which may be more prone to local pelvic inflammation, showed activated follicular recruitment and atresia of early follicles. The potential contribution of inflammation to follicle "burnout" in case of endometriomas is discussed. (Fertil Steril® 2014;101:1031–7. ©2014 by American Society for Reproductive Medicine.)
Key Words: Endometrioma, follicular development, follicular atresia, follicular recruitment, histomorphometric analysis

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Endometriosis is a common but enigmatic gynecological disorder that affects 10% of women of reproductive age (1, 2). Ovarian endometriosis (endometriomas) diagnosed in 20%–40% of women with endometriosis is one of the main manifestations of this disease (2, 3). Its pathogenesis may be different from other types of endometriosis, like pelvic implants and rectovaginal nodules (3).

Women with endometriomas are often referred to a gynecologist for subfertility problems. Although surgical intervention, such as laparoscopic cystectomy or cyst ablation, is considered the treatment of choice for endometriomas (4, 5), several lines of evidence suggest that it may have a deleterious impact on the residual ovarian reserve (5, 6). Recent reports indicate that antiMüllerian hormone levels are significantly reduced after cystectomy and that changes in (antiMüllerian hormone) serum levels may predict damage to residual healthy ovarian tissue after laparoscopic surgery (5, 7, 8).

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In addition to surgical damage, endometriomas per se could be the cause of a diminished ovarian reserve (9). Reduced follicular density associated with the presence of fibrosis was observed in the ovarian cortex of ovaries with endometriomas. It was hypothesized that fibrosis or the inflammation process triggered by endometriosis may be implicated in folliculogenesis (9), which is a tightly regulated process involving early primordial follicle recruitment and follicle selection by atresia. However, the mechanism underlying follicle loss in ovarian cortex lining endometriomas is largely unknown.

The aim of the present study was to investigate the dynamics of early folliculogenesis in women with ovarian endometriosis. To this end, follicle populations, and follicle and oocyte diameter, proliferation, apoptosis and atresia were compared between ovaries with endometriomas and their healthy contralateral counterparts.

MATERIALS AND METHODS

Use of human ovarian tissue for this study was approved by the Institutional Review Board of the Université Catholique de Louvain. Written informed consent was obtained from each patient.

Sample Collection

Between January 2010 and June 2011, ovarian cortical tissue biopsy was performed in 13 women <40 years of age undergoing laparoscopic surgery for monolocular unilateral endometriotic cysts. All subjects had regular menstrual cycles without menopausal symptoms and had not undergone previous ovarian surgery. Only young women with unilateral endometriomas (median age, 27 years; range, 22–39 years) were eligible for the study. The main indication for surgery was infertility, sometimes associated with pelvic pain. In our department, we are in favor of a surgical approach in case of infertility associated with endometriomas (3, 5). Methods of ovarian cortex sampling in women with endometriomas have been previously described in detail (9). Briefly, a small piece of macroscopically normal-looking ovarian cortical tissue was excised using scissors ≥1 cm from the site of endometriosis. Biopsies were systematically taken from macroscopically normal-looking thick ovarian cortical tissue by the same surgeon (J.D.). In ovaries with endometriomas, biopsy was taken at a distance of approximately 1.5 cm from the endometrioma bulk, at the antimesenteric level if possible. In control ovaries, biopsy was taken from normal-looking tissue at the antimesenteric level. To evaluate early-stage endometriomas and avoid the confounding effects of enlarged endometriomas on the histologic features of healthy ovarian cortex, only cortical samples from endometriomas measuring ≤4 cm were included. We thus limited possible bias due to slow growth of large endometriomas, which is a potential cause of fibrosis and thick planes of cleavage (10).

Biopsy Treatment

All biopsied tissue was fixed overnight in Bouin's solution and embedded in paraffin. For each sample, 300–360 serial sections perpendicular to the ovarian axis were performed at 5-μm intervals and stained with hematoxylin and eosin (H & E) at 50-μm intervals. In these consecutive H & E-stained sections, the 10 serial sections with the largest horizontal diameter and an intact cortical morphological appearance were selected and each section was captured as a digital image using the Mirax Midi system (Carl Zeiss). Measurements in captured sections were taken using a specific computer program (Mirax Viewer version 1.12; Carl Zeiss). This enabled us to magnify the view of each follicle in high resolution. Among the series of samples, only samples containing more than 10 follicles with a clearly visible nucleolus were selected for morphological analysis.

Histologic Evaluation of Follicular Stage and Atresia

All biopsies were analyzed by one blinded investigator (M.K.), unaware of whether samples originated from ovaries with or without endometriomas.

Only follicles with a clearly visible nucleolus were evaluated. Atresia of early follicles was diagnosed according to strict criteria (11), including eosinophilia of the ooplasm, nuclear pyknosis of granulosa cells (GCs), cytoplasmic contraction, cytoplasmic vacuoles, and dissociation of GCs and the basal membrane (11) (Fig. 1A and B). The prevalence of atretic follicles was ascertained.

The remaining nonatretic follicles were then considered as morphologically normal and classified according to the strict criteria of Gougeon and Chainy (11) as: [1] primordial follicles in which oocytes are surrounded only by flattened GCs; [2] transitional follicles in which oocytes are surrounded by a mixture of cuboidal and flattened GCs; [3] primary follicles in which oocytes are surrounded by a single layer of cuboidal GCs; and [4] secondary follicles in which oocytes are surrounded by two to three layers of cuboidal GCs (11) (Fig. 1C).

Follicle Measurements

Follicle, oocyte, and oocyte nucleus diameters were calculated according to Westergaard et al. (12) using Mirax Viewer (version 1.12) analysis software (Carl Zeiss), allowing arbitrary magnification, and the largest diameter was measured in each incorporated image. In addition, a second measurement was taken at a right angle from the midpoint of the first measurement (Fig. 1D). The two measurements were then averaged and expressed as the diameter of the structure. Follicle diameters were calculated from the outer layer of the GCs, and oocyte measurements included the zona pellucida (ZP) when present. Measurements were performed in all captured nonatretic follicles and evaluated according to their developmental stage.

Immunohistochemical Evaluation of Apoptosis and Proliferation

Sections adjacent to those selected for morphological analysis of early follicles were used for immunohistochemistry. Expression of proliferating cell nuclear antigen (PCNA) and cleaved caspase-3 were determined. For immunohistochemistry, 5-μm sections were deparaffinized and rehydrated.