Dynamics of nitric oxide, altered follicular microenvironment, and oocyte quality in women with endometriosis

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Objective: To study follicular microenvironment in terms of free radical dynamics, oocyte quality, and assisted reproductive technology (ART) outcomes among women with (group A) and without (group B) endometriosis.
Design: Prospective cohort study.
Setting: University ART center.
Patient(s): Women with and without endometriosis undergoing ART (n = 28).
Intervention(s): Follicular fluid (FF), granulosa cells (GCs), immature oocytes (IOs), and ART data on sibling cohort oocytes in groups A and B were compared.
Main Outcome Measure(s): ART live birth outcomes, maturation, and aging among in vitro matured (IVM) oocytes, nitrate levels in FF, and nitrotyrosine (NT) footprints and apoptosis in the GCs.
Result(s): Clinical characteristics and ART live birth outcomes were no different between groups A and B. Women from group A had significantly lower peak serum E_2 (2,068.8 ± 244.6 pg/mL vs. 2,756.2 ± 205.0 pg/mL) and higher apoptosis (80.0% vs. 22.2%) and NT staining (70.0% vs. 22.2%) in GCs compared with group B. Fewer IOs underwent IVM to MII (0.6 ± 0.3) in group A compared with group B (1.4 ± 0.2). IVM oocytes had significantly higher incidence of cortical granule loss (83.3% vs. 24.0%) and spindle disruption (66.7% vs. 16.0%) and higher zona pellucida dissolution timing (133.8 ± 9.4 s vs. 90.5 ± 5.8 s) in group A compared with group B. FF nitrate levels were significantly higher in women who failed to conceive in group A (478.2 ± 43.1 nmol/L) compared with those that did conceive (173.3 ± 19.0 nmol/L).
Conclusion(s): Increased protein nitration, GC apoptosis, resistance to IVM, and oocyte aging indicate the involvement of oxidative dysregulation of NO in the pathophysiology of altered follicular milieu and poor oocyte quality in women with endometriosis. (Fertil Steril® 2014;102:151–9. ©2014 by American Society for Reproductive Medicine.)
Key Words: Endometriosis, oocyte aging, oocyte quality, nitric oxide, superoxide, peroxynitrite

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Received December 30, 2013; revised March 26, 2014; accepted March 27, 2014; published online May 10, 2014.
P.T.G. has nothing to disclose. A.P.G. has nothing to disclose. N.J. has nothing to disclose. E.P. is a board member of the American Institute of Ultrasound in Medicine (AIUM) and the Society for Reproductive Endocrinology and Infertility, is a medicolegal consultant for insurance, has a grant from the National Aeronautics and Space Administration, and has received payment for lectures and development of educational activities and had travel/accommodation expenses paid by the American Society for Reproductive Medicine (ASRM) and AIUM. M.P.D. is a director of and has received personal fees from Advanced Reproductive Care, has received personal fees from Auxogyn, and has a grant from AbbVie. H.M.A.-S. has nothing to disclose.
Supported by Ortho-ASRM Women's Health Grant (to P.T.G.).
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Fertility and Sterility® Vol. 102, No. 1, July 2014 0015-0282/$36.00

http://dx.doi.org/10.1016/j.fertnstert.2014.03.053

Endometriosis is an enigmatic disorder of the female reproductive tract and affects 8%–10% of women of reproductive age (1). One of the most important problems with endometriosis is its association with lowered fertility and fecundity (2, 3). This is supported by the significantly high prevalence of endometriosis in women with primary (12%–25%) as well as secondary (26%–39%)

infertility. Furthermore, an improvement in fertility may occur after laparoscopic or medical treatment of endometriosis (4, 5); however, underlying changes that actually contribute to this effect are not known (5–7). Therefore, precise directions to optimize conditions for women with endometriosis to conceive are yet to be known.

Over the past three decades, assisted reproductive technology (ART) has encompassed the management of almost all types of infertility. Despite these advances, patients with endometriosis continue to pose difficulties in achieving pregnancy (8–10). This may be due to an adverse influence of endometriosis on the processes of fertilization (11, 12), embryogenesis (13–15), and/or implantation (16, 17). Moreover, these phenomena are related to oocyte quality (18, 19). Adverse influence on the oocyte is therefore a likely central aspect in endometriosis-related infertility. This concept is strengthened by a study reporting significant improvement in the pregnancy rate in patients with endometriosis who received donated oocytes compared with their own oocytes. Conversely, the pregnancy rates were lower in subjects without endometriosis who received donor oocytes from subjects with endometriosis (20–23).

Recently, the role played by free radicals has attained central stage in the pathophysiology of endometriosis (24–28). Accordingly, endometriosis subjects may have increased oxidative stress due to increased oxidant free radical production, compromise of the antioxidant defenses, or both (29). Similarly, endometriosis also involves significant disarray in the production and metabolism of nitric oxide (NO) (30). Nitric oxide is a ubiquitous free radical in the oocyte microenvironment that plays a vital role in virtually every step of oocyte development, including meiotic maturation, fertilization, embryonic cleavage, and implantation (31–38). Furthermore, we have demonstrated a significant role of NO in delaying oocyte aging and maintaining the integrity of the spindle apparatus (39, 40). Decreased bioavailability of NO under certain pathologic conditions could therefore result in abnormalities in oocyte viability and developmental capacity (40, 41). Although endometriosis could affect NO production as well as metabolism, one of the main mechanisms affecting NO bioavailability could be its consumption by superoxide (42), with resultant formation of highly reactive peroxynitrite ($ONOO^-$). Peroxynitrite promotes nitration of tyrosine residues, depletes lipid soluble antioxidants, and initiates lipid peroxidation (42–45).

In the present study, we aimed to study the quality of oocytes from endometriosis subjects in terms of clinical parameters pertaining to ART data and outcomes. We also studied follicular fluid (FF) and follicular cells for dynamics of NO and apoptosis; and unique cellular markers were used to assess the occurrence of preatretic aging among in vitro matured (IVM) oocytes from women with and without endometriosis (groups A and B, respectively).

MATERIALS AND METHODS

Study Design

This study was approved by the Institutional Review Board of Wayne State University. The subjects in the study included patients seeking ART who consented to donate their FF, follicular cells, and immature oocytes for research. Design of the study included collection of data regarding patient demographics, infertility factor, history of endometriosis, and clinical findings, as well as imaging or laparoscopic data for confirmation of endometriosis (Supplemental Fig. 1, available online at www.fertstert.org). Subjects with endometriosis were identified only after confirmation on imaging and/or direct visualization with laparoscopy, without regard to severity and stage of endometriosis. All women without endometriosis, regardless of their infertility factor, were included as control subjects. The diagnosis of endometriosis had been ruled out among the control subjects. A review of the clinical history and examination was aimed at identifying the factor contributing to infertility. The laboratory investigation involved standard tests for preconception testing, as well as day 3 FSH, E_2, PRL, and TSH levels. A complete semen analysis of the male partner was performed, including sperm morphology evaluation according to the standard Kruger criteria (46). Abnormalities in the sperm parameters led to the diagnosis of a male factor.

Controlled Ovarian Stimulation

All subjects underwent ovarian stimulation with gonadotropins (Gonal-F; EMD Serono; and Repronex, Ferring Pharmaceuticals) and ganirelix acetate (Antagon; Organon) for pituitary down-regulation. Patients began receiving oral contraceptives 1 month before stimulation. Gonadotropins were administered from stimulation day 1 until the day of the hCG trigger according to a step-up protocol. The GnRH antagonist (0.25 mg daily) was added from the day when at least one follicle reached 14 mm in mean diameter and continued until hCG administration. Supplemental LH and FSH (Repronex) in the form of one ampule each per day were added to the gonadotropin regimen when GnRH antagonist administration was started. This regimen was continued until and including the day of hCG trigger when at least two follicles were >18 mm in diameter. Retrieval of the oocytes was performed 36 hours after the hCG trigger by ultrasound-guided transvaginal follicular aspiration. Transfer of the embryos to the uterine cavity was performed on day 3 after oocyte retrieval for all of the patients. Luteal phase support was given by daily injections of progesterone in oil (100 mg intramuscularly) until a negative pregnancy result or until 10 weeks of pregnancy. Initial pregnancy was defined by a rise in serum hCG levels from days 14 to 16 after oocyte retrieval. Biochemical pregnancy was defined as a positive serum β-hCG test result with no identifiable gestational sac on transvaginal ultrasound (TVUS). Clinical pregnancy was defined when a gestational sac and embryonal heart rate were observed on TVUS at 6 weeks of gestation. Miscarriage was defined if absence or discontinuation of embryonal heart rate was noted subsequently. Ongoing pregnancy was defined as pregnancy continuing beyond 28 weeks' gestation. None of the cycles was cancelled.

Collection, Transportation, and Processing of Follicular Fluid and Oocytes

Follicular fluid was aspirated from the follicles and collected in sterile polypropylene tubes (Falcon), which were examined