Follicular-fluid neurotrophin levels in women undergoing assisted reproductive technology for different etiologies of infertility

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Objective: To determine follicular-fluid neurotrophin levels in women undergoing assisted reproductive techniques for different etiologies of infertility.
Design: Prospective observational study.
Setting: Academically affiliated assisted reproductive techniques unit.
Patient(s): One hundred six patients undergoing an IVF cycle for different etiologies of infertility.
Intervention(s): Assessment of follicular fluid for neurotrophins.
Main Outcome Measure(s): Follicular-fluid neurotrophin concentrations.
Result(s): Women with a history of endometriosis have significantly lower follicular-fluid brain-derived neurotrophic factor (BDNF) levels compared with women with male-factor (control) infertility. Women with diminished ovarian reserve have significantly higher levels of nerve growth factor (NGF) compared with women with male-factor infertility. Follicular-fluid BDNF levels tend to be lower in patients with endometriosis and diminished ovarian reserve and to be higher in patients with polycystic ovarian syndrome. Interestingly, NGF concentrations follow the opposite trend. Finally, follicular-fluid NT-3 concentrations are similar in women with different etiologies of infertility.
Conclusion(s): Endometriosis is associated with low follicular-fluid BDNF levels, and diminished ovarian reserve is associated with increased follicular-fluid NGF levels. (Fertil Steril® 2008;90:1611–5. ©2008 by American Society for Reproductive Medicine.)

Key Words: Neurotrophins, BDNF, NGF, infertility, endometriosis, diminished ovarian reserve, PCOS, unexplained infertility

Neurotrophins are a family of growth factors that are involved in the development of the central and peripheral nervous system (1, 2). Although they initially were thought to be restricted to the nervous system, it now is well known that they affect non-neuronal cells, as cells of the endocrine system (3). Nerve growth factor (NGF), brain-derived neurotrophic factor (BDNF), NT-3, and NT-4/5 are major members of the neurotrophin family, and together with their receptors, they are found in both rodent and mammalian (including human) ovaries (4–10). They have a wide range of functions in the ovary, from support of early survival of germ cells to control of steroidogenesis and extrusion of polar bodies, as well as ovulation (4, 5, 9–12). Moreover, it has been shown that in vitro BDNF treatment of bovine oocytes leads to the development of more parthenogenetic embryos compared with the case of controls (13). Similarly, BDNF has been shown to be important in mouse oocyte development into preimplantation embryos because it promotes the nuclear and cytoplasmic maturation of the oocyte (14). These findings demonstrate that neurotrophins are expressed in human ovaries (4, 5, 9, 10) and strongly suggest that they play a role in folliculogenesis and cytoplasmic competence of oocytes. This is further supported by a recent study showing that plasma BDNF levels change during the menstrual cycle and that concentrations fall steadily after menopause (15).

Given the apparent importance of the role that neurotrophins play in folliculogenesis, we sought in the present study to determine follicular-fluid neurotrophin levels in women undergoing IVF for infertility of different etiologies. We tested the hypothesis that women undergoing IVF for infertility of different etiologies would have varying follicular-fluid

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concentrations of neurotrophins (BDNF, NT-3, NGF) as compared with the case of women with male-factor infertility (controls).

MATERIALS AND METHODS

Subjects

Follicular fluid was collected during routine egg retrieval from 106 consecutive women undergoing controlled ovarian stimulation in preparation for IVF or ICSI. Women with male-factor infertility and no other causes constituted the control group (47 women). Polycystic ovary syndrome (PCOS) was diagnosed according to Rotterdam criteria (13 women) (16). Unexplained infertility was diagnosed when routine tests for infertility workup did not reveal any abnormality (22 women). Diminished ovarian reserve was diagnosed with a day 3 FSH level of >10 mIU/mL, with an antral follicle count of <5 in one ovary or of <10 in both ovaries, or when five or fewer eggs were retrieved after a routine controlled ovarian hyperstimulation (17 women). Patients with a history of endometriosis were diagnosed by laparoscopy (7 women). This study was approved by the institutional review board of Maimonides Medical Center.

Follicular-fluid Collection

Aspirates were obtained from the first follicle from either side in an effort to obtain clear follicular fluid. After removal of the oocyte, the fluid was processed by centrifuge at 1,000 rpm for 10 minutes, and the clear supernatant was stored at −80°C until assayed. Only clear follicular fluid associated with the presence of an oocyte, without blood or flushing solution, was collected.

Neurotrophin Assays

Nerve growth factor and NT-3 assays Levels of NGF and NT-3 were determined by using commercially available NGF and NT-3 Emax Immunoassay Systems (Promega Corp., Madison, WI) after appropriate dilution of samples (≤1/10th). The assays were performed according to the manufacturer's protocol. Briefly, 96-well plates were coated with anti-NGF/anti-human NT-3 polyclonal antibodies, respectively, overnight at 4°C. Then the wells were blocked with blocking buffer and incubated with samples and standards for 6 hours at room temperature. Anti-NGF and anti-NT-3 monoclonal antibodies were used as reporter antibodies, respectively, and anti-mouse IgG–horseradish peroxidase conjugate was used to detect the amount of specifically bound monoclonal antibody. After incubation with the chromogenic substrate and stopping the reaction with 1 N hydrochloric acid, the absorbency was measured at 450 nm by using a microplate reader. Dilutions down to 1/16, in the case of NGF, and down to 1/128, in the case of NT-3, have been performed for some samples.

Anti-NGF was reported by the manufacturer to demonstrate <3% cross-reactivity with other neurotrophins at 10 ng/mL. The detection sensitivity of the ELISA is 7.8 pg/mL, with an intra-assay coefficient of variation of 4.2% at a mean concentration of 404 pg/mL, according to the manufacturer. The interassay coefficient of variation in our assay was 6%.

Anti NT-3 was reported by the manufacturer to demonstrate <3% cross-reactivity with other neurotrophins at 10 ng/mL. The detection sensitivity of the ELISA is 10 pg/mL, with an intra-assay coefficient of variation of 1.5% at a mean concentration of 225.1 pg/mL, according to the manufacturer. The interassay coefficient of variation in our assay was 5.3%.

Brain-derived neurotrophic factor assay Levels of BDNF were determined by using the commercially available BDNF Emax Immunoassay System (Promega). Briefly, 96-well plates were coated with anti-BDNF monoclonal antibody overnight at 4°C. Then the wells were blocked with blocking buffer and incubated with samples and standards for 2 hours at room temperature. Anti-human BDNF polyclonal antibody was used as reporter antibody, and anti-IgY– horseradish peroxidase conjugate was used to detect the amount of specifically bound polyclonal antibody. After incubation with the chromogenic substrate and stopping of the reaction with 1 N hydrochloric acid, the absorbency was measured at 450 nm by using a microplate reader.

Anti-BDNF was reported by the manufacturer to demonstrate <3% cross-reactivity with other neurotrophins at 100 ng/mL. The detection sensitivity of the ELISA is 15.6 pg/mL, with an intra-assay coefficient of variation of 2.2% at a mean concentration of 286.1 pg/mL, according to the manufacturer. The interassay coefficient of variation in our assay was 5.9%.

Statistical Analysis

Both parametric and nonparametric methods were used to analyze the data, which were highly skewed. The results were similar using either approach. Kruskal-Wallis nonparametric analysis was used to compare neurotrophin levels between groups, and analysis of variance was used to compare the log-transformed data. Tukey's Honest Difference test was used for post hoc analysis of paired comparisons. A P value of $<.05$ was considered statistically significant. We used SPSS software (version 15.0; SPSS, Chicago, IL) for computations.

RESULTS

Demographic characteristics of women in each infertility group are summarized in Table 1. There was a statistically significant difference in the mean age, day 3 FSH, and number of eggs retrieved between women with diminished ovarian reserve and other groups. Day 3 FSH level was statistically significantly low in patients with PCOS compared with other groups. Body mass index did not differ among groups.

TABLE 1

Characteristics of patients with each type of infertility.

Characteristic	MF	Endo	DOR	U	PCO	*P* value
Age (y)	32.4 ± 0.8	31.7 ± 2.1	37.4 ± 1[a]	34.2 ± 1.2	28.9 ± 1.2	.0005
BMI	24.5 ± 0.8	24.3 ± 2.6	25.7 ± 1.7	22.1 ± 0.9	25 ± 2	NS
Day 3 FSH	6.8 ± 0.3	5.6 ± 0.4	11.4 ± 0.5[a]	7 ± 0.5	4.6 ± 0.4[a]	<.0001
Total no. of oocytes	13 ± 0.8	11.4 ± 1.3	7.6 ± 0.7[a]	14.5 ± 1.3	15.3 ± 1.4	<.0005

Note: MF = male factor; Endo = endometriosis; DOR = diminished ovarian reserve; U = unexplained; PCO = polycystic ovaries.

[a] Significantly different from other groups.

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Follicular-fluid BDNF levels for each etiologic factor are shown in Figure 1. Patients with a history of endometriosis had significantly lower mean levels of follicular-fluid BDNF compared with the control group (274 ± 96 pg/mL vs. 148 ± 42 pg/mL, $P<.005$). Patients with diminished ovarian reserve had lower levels of BDNF compared with the control group, but this difference did not achieve significance ($P=.22$). Follicular-fluid BDNF levels were not different between the control group and either women with PCOS or unexplained infertility.

Follicular-fluid NGF levels are shown in Figure 2. Patients with diminished ovarian reserve had significantly higher levels of NGF compared with male-factor patients (1,917 ± 119 pg/mL vs. 1,342 ± 188 pg/mL, $P<.022$). Patients with a history of endometriosis also had higher levels of NGF, but the difference did not achieve significance ($P<.33$). It is interesting to note that the pattern of mean NGF concentration was opposite to that of BDNF. Mainly, follicular-fluid BDNF levels were lower in endometriosis and diminished–ovarian reserve groups, whereas they were higher in the group with PCOS, compared with in controls. However, follicular-fluid NGF levels were higher in the endometriosis and diminished–ovarian reserve groups, whereas they were lower in the group with PCOS, compared with controls.

Follicular-fluid NT-3 levels are shown in Figure 3. There was no difference in follicular-fluid NT-3 concentrations among groups.

DISCUSSION

During the last few years, neurotrophins and their receptors have been localized within human ovaries, and their possible roles in folliculogenesis, ovarian steroidogenesis, and oocyte cytoplasmic competence have been elucidated (4, 5, 9, 10,

FIGURE 1

Follicular-fluid BDNF levels in women undergoing ART for different etiologies of infertility. [a]$P<.005$, comparing the two groups so marked. DOR = diminished ovarian reserve; Endo = endometriosis; MF = male factor; U = unexplained.

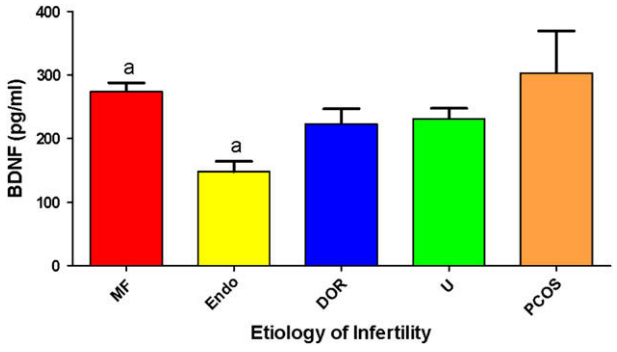

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FIGURE 2

Follicular-fluid NGF levels in women undergoing ART for different etiologies of infertility. [a]$P<.022$, comparing the two groups so marked. [b]$P<.005$, comparing the two groups so marked. DOR = diminished ovarian reserve; ENDO = endometriosis; MF = male factor; U = unexplained.

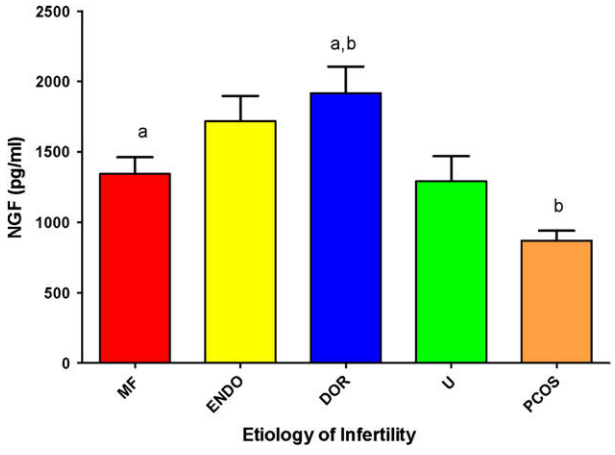

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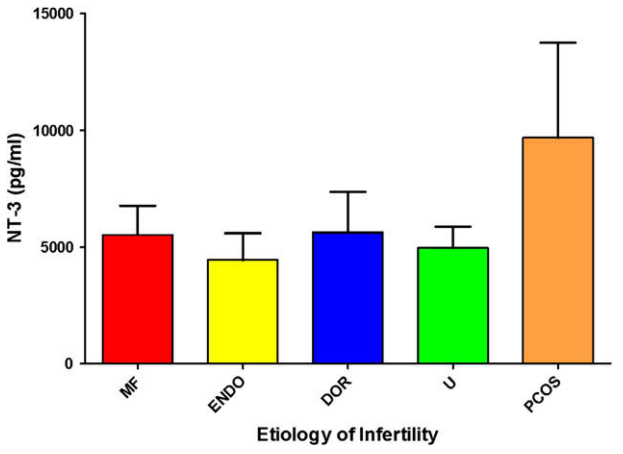
FIGURE 3

Follicular-fluid NT-3 levels in women undergoing ART for different etiologies of infertility. $P>.05$ between all groups. DOR = diminished ovarian reserve; MF = male factor; U = unexplained.



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17). The aim of this study was to examine whether there is an association between follicular-fluid neurotrophin levels and etiology of infertility.

These data demonstrate that follicular-fluid BDNF levels are significantly lower in patients with a history of endometriosis, compared with women with male infertility who served as controls. Among other pathophysiologic processes, oxidative stress is suspected to be one of the leading mechanisms in the pathogenesis of endometriosis. Studies have shown that peritoneal fluid superoxide dismutase and glutathione peroxidase concentrations are lower, whereas lipid peroxides are higher, in patients with endometriosis, compared with control patients (18). Similarly, increased peritoneal fluid nitric oxide levels are associated with endometriosis (19). Although NO is beneficial as a vasodilator in physiologic concentrations, it can be toxic in supraphysiologic concentrations because it can react with superoxide to form peroxynitrite, another highly toxic free oxygen radical. The increase in NO and other free oxygen radicals resulting from abnormal immune responses in endometriosis may stimulate macrophages to produce more NO, which may lead to a hostile peritoneal and endometrial environment, affecting ovulation, gamete transport, fertilization, embryo development, and implantation. However, a recent study showed that BDNF is induced in neuroblastoma cells that are treated with ascorbic acid after oxidative stress (20). Brain-derived neurotrophic factor is known to up-regulate superoxide dismutase, glutathione reductase, and glutathione peroxidase (21) and to activate the Ras-mitogen-activated protein (MAP) kinase pathway, promoting cell survival (22). Thus, our finding of low levels of follicular-fluid BDNF in endometriosis patients may be associated with the poor oocyte quality and poor fertility that are associated with endometriosis (23).

Begliuomini et al. (15) showed that plasma BDNF levels change during the menstrual cycle and decrease during perimenopause, with a steady decline after menopause. On the basis of those recent data, we expected to find lower levels of BDNF in the follicular fluid of patients with decreased ovarian reserve. Although levels were low compared with the case of the control group, the difference did not achieve statistical significance. We would have needed 30 patients in the group with diminished ovarian reserve to detect a difference with a power of 80%. However, the follicular fluid was collected after controlled ovarian hyperstimulation, and we have shown elsewhere that follicular-fluid BDNF levels are up-regulated with gonadotropin stimulation (17). Thus, hyperstimulation may have masked any possible difference in basal follicular-fluid BDNF level between patients with diminished ovarian reserve and the control group.

The pattern of mean follicular-fluid NGF concentration is the converse of BDNF concentration. Mainly, patients with diminished ovarian reserve and endometriosis tend to have higher levels of NGF compared to the control group while PCOS patients tend to have lower levels. This trend is the converse of what we found for BDNF, for which diminished–ovarian reserve and endometriosis patients have lower BDNF concentrations, and patients with PCOS, higher. These findings may suggest that either these neurotrophins play a contributing role in the pathogenesis of endometriosis and/or diminished ovarian reserve or they may be the by-products of the pathogenetic mechanisms leading to these conditions. In any case, they strongly point to a relation between neurotrophins and specific factors that are associated with infertility. Brain-derived neurotrophic factor and NGF have been shown to have antagonistic effects on the growth of sympathetic neurons. They are also antagonistic in the ability of these neurons to innervate their target tissues by alternative binding to their low-affinity receptor, p75 NTR (24). Further study is needed to clarify whether this is the case in ovarian folliculogenesis and to decipher the mechanisms leading to various altered patterns of neurotrophin expression that are associated with different etiologies of infertility.

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