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# The serum brain-derived neurotrophic factor concentration prior to initiation of an in vitro fertilization cycle predicts outcome

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ABSTRACT

Our objective was to determine if the concentration of circulating brain-derived neurotrophic factor (BDNF) prior to cycle initiation predicts outcome in women undergoing in vitro fertilization (IVF). Stored serum samples from 226 women – 54 with a live birth, 45 with a spontaneous abortion, 38 with a biochemical pregnancy, 54 who did not become pregnant and 35 with an ectopic pregnancy- were retrospectively blindly tested for BDNF by ELISA. The median serum concentration of BDNF was highest in women with an extrauterine ectopic pregnancy (7.3 ng/ml), intermediate in women whose embryos did not implant (5.5 ng/ml) and lowest in women with a spontaneous abortion (4.2 ng/ml), biochemical pregnancy (3.8 ng/ml) or a live birth (3.6 ng/ml) ($P < 0.0001$). Among women with a positive pregnancy test an elevated BDNF level predicted an ectopic pregnancy with a sensitivity of 0.853 (0.689, 0.950) and a specificity of 0.949 (0.897, 0.979). We conclude that elevated BDNF in serum obtained before IVF cycle initiation is predictive of an extrauterine pregnancy.



## 1. Introduction

Brain-derived neurotrophic factor (BDNF) is a member of the neurotrophin family necessary for central nervous system development and differentiation (Huang and Reichardt, 2001). More recent studies have identified BDNF in the female reproductive tract. It is present in human and animal ovary, endometrium and myometrium (Krizsan-Agbas et al., 2003; Russo et al., 2012; Wessels et al., 2014) and is involved in pre- and post-implantation embryo development (Kawamura et al., 2007; Kawamura et al., 2009). BDNF is present in the circulation and levels are elevated in the luteal phase as compared to the follicular phase and higher in fertile as opposed to amenorrheic women (Begliuomini et al., 2007).

The majority of BDNF in the circulation is bound to platelets and is released following platelet activation; activated platelets release more BDNF than any other mediator (Fujimura et al., 2002). Recent data have established a central role for platelet activation in the initiation of innate and acquired immune system activation in response to non-physiological conditions (Semple et al., 2011). Platelets also express Toll-like receptors and complement and immunoglobulin receptors and become activated when infectious microorganisms bind to these receptors (Hamzeh-Cognasse et al., 2015). There are one trillion platelets in the human circulation at any one time (Semple et al., 2011), suggesting that measurement of BDNF release by platelet activation may be a sensitive early warning signal of physiological stress.

Given the involvement of BDNF in reproductive tract functions as well as its potential as a biomarker of physiological stress, our study objective was to test the hypothesis that serum BDNF levels would be a predictive biomarker of adverse IVF outcome.

## 2. Materials and methods

### 2.1. Subjects

The patient cohort in this retrospective study were women who underwent an IVF-embryo transfer cycle at the Center for Reproductive Medicine and Infertility at Weill Cornell Medicine. Sera from 226 women were collected on day 2 of their menstrual cycle before initiation of any stimulation mediations to induce ovula-

*Abbreviations:* BDNF, brain-derived neurotrophic factor; IVF, in vitro fertilization; hCG, human chorionic gonadotropin; ROC, receiver operator curve; AUC, area under the curve.

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**Table 1**
Characteristics of the study population.

| Parameter | Live birth N = 54 | Biochem N = 38 | Spont abort N = 45 | Not preg N = 54 | Ectopic N = 35 |
|---|---|---|---|---|---|
| Age (years) | 36.0 +/− 0.6 | 37.4 +/− 0.7 | 38.6 +/−0.6[a] | 39.4 +/− 0.4[a] | 37.0 +/−0.7 |
| BMI (kg/m$^2$) | 23.0 +/− 0.6 | 24.0 +/− 0.7 | 23.9 +/− 0.4 | 24.3 +/−0.7 | 23.6 +/− 1.0 |
| Oocytes retrieved | 13.4 +/− 0.8 | 11.5 +/−1.1 | 9.9 +/− 0.8 | 9.7 +/− 0.8 | 11.3 +/−1.0 |
| Mature oocytes | 10.1 +/−0.6 | 9.7 +/−0.9 | 8.0 +/− 0.6 | 7.9 +/− 0.7 | 9.1 +/−0.8 |
| Embryo grade | 2.6 +/− 0.3 | 3.0 +/− 0.3 | 2.6 +/− 0.3 | 2.5 +/− 0.2 | 2.7 +/− 0.3 |
| No. fertilized | 7.7 +/− 0.5 | 7.6 +/− 0.8 | 6.5 +/− 0.5 | 6.0 +/− 0.6 | 6.8 +/− 0.7 |
| No. transferred | 3.0 +/− 0.2 | 2.9 +/−0.2 | 3.1 +/− 0.2 | 2.8 +/− 0.2 | 3.0 +/− 0.2 |

The values given are mean +/− standard error.
[a] $p \leq 0.009$ vs. live birth; Biochem, biochemical pregnancy; spont abort, spontaneous abortion; Not preg, not pregnant; BMI, body mass index.

tion and stored at −80 °C for later analysis. The study population consisted of 54 women with a subsequent live birth (delivery of a live-born infant), 38 with a biochemical pregnancy (defined as a transient rise and fall of human chorionic gonadotropin (hCG) without documentation of a gestational sac or ectopic pregnancy), 45 with a spontaneous abortion (non-viable fetus following observation of a uterine gestational sac), 54 who did not become pregnancy (negative hCG two weeks after embryo transfer), and 35 with an ectopic pregnancy (ultrasound documentation of a gestational sac or fetal pole in adnexa or surgical/pathological confirmation). Availability of serum was the only criterion used for sample analysis. Selection was totally random and based solely on the quantity of blood initially obtained and the amount frozen. Exclusion criteria were women with multiple gestations, preterm delivery, oocyte donation, preimplantation genetic diagnosis, known genetic abnormalities and the utilization of frozen oocytes or embryos. A complete blood count including platelet count was routinely obtained from each subject. The Institutional Review Board at Weill Cornell Medicine approved this study and all subjects gave informed written consent, including the use of their sera in subsequent blinded analyses.

### 2.2. IVF protocol

All patients followed a fresh embryo transfer protocol (stimulated IVF cycle). Women were treated with standard stimulation protocols, which began on day 2 of their treatment cycle and consisted of a combination of gonadotropins (human menopausal gonadotropin) and/or follicle-stimulating hormone. Some women received estrogen priming or a Lupron microflare and GnRH antagonists were administered when deemed appropriate by the woman's attending physician. When at least two follicles reached 17 mm diameter as measured by transvaginal ultrasound, the trigger injection of hCG was administered (3300–10,000 IU). Oocyte retrieval was conducted by transvaginal ultrasound-guided follicular puncture 35–36 h after hCG administration. A minority of the women in our study had an endometrial biopsy prior to their IVF cycle since in vitro co-culture of embryos with autologous endometrial cells has been shown to improve IVF outcome in women with multiple failed cycles of IVF (Spandorfer et al., 1998).

Following fertilization, embryo grade was determined by evaluating the number of blastomeres, blastomere symmetry and the number of anucleated fragments. Embryo transfer occurred on either day 3 or day 5 after retrieval, as clinically indicated. The great majority of transfers occurred on day 3. The number of embryos transferred was dependent on maternal age and history, but mostly 1–3 embryos were transferred. Progesterone supplementation was initiated on the third day after hCG administration (50 mg intramuscular/day) and was continued until the sonographic documentation of a fetal heart beat at approximately 7 weeks of gestation.

### 2.3. BDNF assay

Peripheral blood was collected just prior to the initiation of the IVF cycle and centrifuged to obtain the serum fraction which was stored at −80 °C until assayed. The sera were assayed in duplicate for BDNF using a commercially available ELISA kit (Millipore Corp, Billerica, MA) by laboratory personnel who were blinded to all clinical data. The lower limit of sensitivity was 15 pg/ml. Intra- and inter-assay variability was <10%.

### 2.4. Statistical analysis

GraphPad (San Diego, California) was used for statistical analysis. Data were analyzed by the non-parametric Kruskal-Wallis test with a Dunn's post-hoc analysis and by the Mann-Whitney test since the data did not follow a normal distribution. A p value of <0.05 was considered statistically significant. Receiver Operator Curve (ROC) analysis was employed to investigate the strength of the correlations between BDNF level and ectopic pregnancy, expressed as Area Under the Curve (AUC), and to determine which BDNF concentration was the best predictor. The cut off (c) was determined using the Youden index (J); "c" was defined as the value that maximizes the equation: $J_c$ = "Sensitivity$_c$ + Specificity$_c$ − 1". Sensitivity, specificity, and positive and negative predictive values with 95% confidence intervals were determined.

## 3. Results

### 3.1. Subject characteristics

Characteristics of the study population are shown in Table 1. There were no differences between groups in body mass index, number of oocytes and mature oocytes retrieved, number of oocytes fertilized, embryo grade and the number of embryos transferred.

The women who did not become pregnant or who had a spontaneous abortion were older (median age 39.4 and 38.6 years, respectively) than women who had a live birth (median age 36.0 years) ($p \leq 0.009$). The cause of infertility – male factor, tubal occlusion, low ovarian reserve/advanced maternal age, anovulation/polycystic ovaries, endometriosis, uterine anomaly, idiopathic – did not vary significantly between women with different outcomes. Tubal occlusion contributed to infertility in 10% of women with a live birth, 16% with a biochemical pregnancy, 18% with a spontaneous abortion, 15% not pregnant and 15% with an ectopic pregnancy. The day of embryo transfer did not affect the outcomes or findings. There were an equal number of day 3 and day 5 transfers among women who did or did not become pregnant.