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Cerebral blood flow is increased during controlled ovarian stimulation

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Nevo O, Soustiel JF, Thaler I. Cerebral blood flow is increased during controlled ovarian stimulation. Am J Physiol Heart Circ Physiol 294: H3265–H3269, 2007. —Estrogen appears to enhance cerebral blood flow (CBF). An association between CBF and physiologically altered hormonal levels due to menstrual cycle, menopause, or exogenous manipulations such as ovariectomy or hormone replacement therapy has been demonstrated. The purpose of this study was to determine the association between ovarian stimulation and CBF in vivo by measuring blood flow in the internal carotid artery (ICA) after pituitary suppression and during controlled ovarian stimulation in women undergoing in vitro fertilization treatment cycles. ICA volume flows were measured by angle-independent dual-beam ultrasound Doppler in 12 women undergoing controlled ovarian stimulation. Measurements were performed after pituitary/ovarian suppression, in the late follicular phase, and at midluteal phase. Blood flow in the ICA increased by 22.2% and 32% in the late follicular and midluteal phases compared with the respective values obtained during ovarian suppression (r = 0.0005 and P < 0.0001, respectively). There was a significant correlation between increments in estrogen levels and increments in CBF when the late follicular phase was compared with the ovarian suppression period (r = 0.8, P < 0.001). Mean blood flow velocity significantly increased (by 15.7% and 16.9%, respectively) and cerebral vascular resistance significantly decreased (by 17.6% and 26.5%) during the late follicular and midluteal phases compared with respective measures during ovarian suppression. There was a significant correlation between an increase in estrogen levels and a decrease in cerebral vascular resistance when the late follicular phase was compared with the ovarian suppression period (r = -0.6, P < 0.05). These changes imply sex hormone-associated intracranial vasodilation leading to increased CBF during controlled ovarian stimulation.

During controlled ovarian stimulation that is utilized for in vitro fertilization (IVF) treatment cycles, there is a significant increase in serum estradiol levels during the hyperstimulated follicular phase and the luteal phase (13). The source of estrogen during an IVF cycle is the hyperstimulated follicles in the follicular phase and the corpora lutea during the luteal phase. Progesterone levels start to increase after the induction of final oocyte maturation by human chorionic gonadotropin (hCG), and supraphysiological levels are encountered during the luteal phase (11). Progesterone is secreted during the luteal phase from multiple corpora lutea that develop after trigger of oocyte maturation by hCG (11).

Shamma et al. (32) measured flow velocities in the middle cerebral artery by transcranial Doppler during controlled ovarian hyperstimulation. Lower velocities were observed during pituitary suppression compared with those measured during estradiol peak. The pulsatility index (PI) significantly increased from the period of pituitary suppression to the period of maximal estrogen level. The investigators hypothesized that the increase in velocities and PI values during ovarian stimulation reflects an increase in proximal arterial tone or a dilation of smaller-caliber vessels, implying an effect of ovarian steroids on cerebrovascular hemodynamics. However, no direct evaluation of CBF was performed during controlled ovarian stimulation cycles to substantiate this hypothesis.

The aim of our study was to determine the change in CBF by measuring volume blood flow in the internal carotid artery (ICA) during controlled ovarian stimulation and its association with serum estrogen and progesterone levels in women undergoing IVF treatment cycles.

MATERIALS AND METHODS

The study was approved by the research ethics board of Technion-Israel Institute of Technology, and written informed consents were obtained. Twelve healthy patients who registered for IVF treatment participated in the study. Patient history was documented by using a detailed form with specific questions regarding personal history of cardiac, vascular, neurological, endocrine, or metabolic disorders. Additionally, the family history of the participants was negative for the same spectrum of disorders. Exclusion criteria were cardiovascular or cerebrovascular disease, diabetes mellitus, use of medications, smoking, and anemia (hemoglobin < 10 g/dl).

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Participants did not consume caffeine or alcohol for at least 12 h before testing.

Pituitary desensitization and ovarian suppression were induced by a single dose of intramuscular Decapeptyl CR (3.75 mg, Ferring, Malmo, Sweden) administered on day 21 of the ovarian cycle. Once pituitary and ovarian downregulation was confirmed (after 2–2.5 wk), ovarian stimulation with human menopausal gonadotropin and urinary follicle-stimulating hormone (Pergonal and Metrodin, Teva) was commenced with a daily intramuscular dose of 225–300 IU, determined on the basis of age, weight, gonadotropin level, and response to previous treatments. Once follicular maturation was achieved (at least 3 follicles with a diameter of ≥17 mm), gonadotropin treatment was discontinued and final oocyte maturation was triggered by 5,000 IU of hCG (Chorigon, Teva) administered as a single intramuscular injection. Oocytes were collected 36 h after maturation was triggered, with transvaginal ultrasound-guided follicle aspiration. Luteal support was obtained by transvaginal administration of 600 mg/day of micronized progesterone (Utrogestan, Besins Isocesco).

Measurements of bilateral ICA blood flow, heart rate, and blood pressure were obtained three times during the IVF treatment course. Bilateral ICA flow indexes were averaged because no significant difference was found between sides throughout the study. On each occasion, blood samples were collected for serum estradiol and progesterone. Patients were studied in a semidarkened room after transvaginal ultrasound-guided follicle aspiration. Luteal support was obtained by transvaginal administration of 600 mg/day of micronized progesterone (Utrogestan, Besins Isocesco).

The first measurement was performed 2 wk after the administration of long-acting gonadotropin-releasing hormone (GnRH) agonist, once ovarian downregulation was confirmed. The second measurement was performed on the day that oocyte maturation was triggered (before hCG administration), at peak estradiol levels. The third measurement was performed 8–9 days after the administration of hCG, as previously described, during midluteal phase, when maximal progesterone levels were expected.

Statistical analysis was performed with the SAS statistical package (SAS Institute, Cary, NC). Data were analyzed for comparison between means with analysis of variance for repeated measures. Multiple comparisons to baseline were performed with the Dunnett test. Regression analysis was used to quantify the association of estrogen and CBF and to determine the Pearson correlation coefficient. Estrogen levels were expressed as the natural logarithms of the hormone concentration (ln estrogen, lnE). A value of P < 0.05 was considered statistically significant. Results are expressed as means ± SE.

RESULTS

Baseline characteristics of the patients before commencement of ovarian stimulation are presented in Table 1. Estradiol level increased from 179 ± 12 pmol/l during ovarian suppression to 6,627 ± 1,363 pmol/l in the preovulatory stage (P = 0.0006) and 2,855 ± 636 pmol/l in the midluteal phase (P = 0.001; Fig. 1). Progesterone levels were highest in the midluteal phase (224 ± 34 nmol/l) compared with preovulatory levels of 2.6 ± 0.3 and 3 ± 0.4 nmol/l (P < 0.0001; Fig. 1).

Blood flow in the ICA significantly increased from a mean of 325.3 ± 20.3 ml/min during ovarian suppression to 397.3 ± 19.8 ml/min in the preovulatory stage (P < 0.0005) and 428.9 ± 18.5 ml/min in the midluteal phase (P < 0.0001; Fig. 2). There was a significant correlation between the increase in estrogen levels (lnE) and the increase in CBF when the preovulatory stage was compared with the ovarian suppression stage (r = 0.81, P < 0.001). The association of estrogen and...
CBF is expressed in the following equation: $\Delta CBF = 53.7 \times (\Delta \ln E) - 113.7$. We did not find a significant correlation between an increase in progesterone levels and an increase in CBF when comparing the luteal phase to its preceding stages.

Mean flow velocity increased significantly from a baseline of 40.2 $\pm$ 1.47 cm/s to 46.5 $\pm$ 2.03 and 47.0 $\pm$ 1.86 cm/s during the late follicular and luteal phases ($P < 0.0017$ and $P < 0.0023$, respectively). Calculated global CBF [total volume blood flow $\times 0.108 + 10.5$; adapted from Soustiel et al. (34)], was significantly elevated in the late follicular and luteal phases compared with the baseline measurement during ovarian suppression ($96 \pm 4.2$ and 103 $\pm$ 4.0 ml·min$^{-1}$·100 g$^{-1}$, respectively, compared with 81.07 $\pm$ 4.3 ml·min$^{-1}$·100 g$^{-1}$; $P < 0.001$).

Cerebral vascular resistance, calculated by dividing mean arterial blood pressure by bilateral ICA flow (total CBF), significantly decreased from a baseline of 0.136 $\pm$ 0.009 mmHg·ml$^{-1}$·min$^{-1}$ to 0.112 $\pm$ 0.00 and 0.100 $\pm$ 0.005 mmHg·ml$^{-1}$·min$^{-1}$ during late follicular and luteal phases ($P < 0.001$ and $P < 0.0003$, respectively).

ICA diameter did not change significantly. It was 4.98 $\pm$ 0.1 mm during baseline and 5.01 $\pm$ 0.08 and 5.13 $\pm$ 0.09 mm during late follicular and midluteal phases, respectively.

Mean heart rate increased from 70.8 $\pm$ 2.37 beats/min in the first examination to 77.4 $\pm$ 2.16 beats/min in the second and 74.3 $\pm$ 2.45 beats/min in the third examination ($P < 0.003$ and $P < 0.08$, respectively). Mean blood pressure was 84.6 $\pm$ 3.2 mmHg during ovarian suppression, and no significant change was noted in subsequent measurements (86.5 $\pm$ 2.9 and 84.4 $\pm$ 3 mmHg during follicular and luteal phases, respectively).

**DISCUSSION**

The sex hormones estrogen and progesterone are emerging as significant modulators of CBF. Previous studies have focused mainly on the cerebrovascular effect of sex hormones in animal models. Our use of angle-independent Doppler has enabled us to study CBF in a longitudinal manner, in vivo. By studying IVF-treated women, we were able to investigate the effect of exceptionally high levels of sex hormones during the treatment cycle. In this study we demonstrated a significant increase in CBF during IVF treatment that is characterized by substantially increased levels of estrogen and progesterone. We further clarified the cerebrovascular physiological effects of these exceptionally high hormonal levels by demonstrating increased flow velocities and a decreased cerebral vascular resistance.

In recent years, the effect of estrogen on CBF has been widely explored in animal models. Increased CBF has been found in animal models of estrogen replacement and when estrogen was physiologically elevated during the estrous cycle (25). During prolonged administration of a physiological dose of 17β-estradiol (E$_2$) to ovariectomized, nonpregnant ewes, brain blood flow progressively increased from day 3 to day 10 of estrogen exposure. All regions of the brain responded in a similar manner, and an overall increase of 21–29% in blood flow to the brain was observed (20). Our findings, which reflect prolonged exposure to high levels of estrogen, are consistent with the findings of Magness et al. (20). We observed a 22% increase in CBF before triggering oocyte maturation, which occurs on day 10 of the treatment cycle in most cases. The increase in CBF at this stage strongly correlated with the increase in CBF before triggering oocyte maturation, which occurs on day 10 of the treatment cycle in most cases. The increase in CBF at this stage strongly correlated with the increase in CBF before triggering oocyte maturation, which occurs on day 10 of the treatment cycle in most cases. The increase in CBF at this stage strongly correlated with the increase in CBF before triggering oocyte maturation, which occurs on day 10 of the treatment cycle in most cases. The increase in CBF at this stage strongly correlated with the increase in CBF before triggering oocyte maturation, which occurs on day 10 of the treatment cycle in most cases. The increase in CBF at this stage strongly correlated with the increase in CBF before triggering oocyte maturation, which occurs on day 10 of the treatment cycle in most cases. 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flow to reproductive (e.g., uterus, oviducts) and other nonreproductive (e.g., coronary, skin) vascular beds demonstrated acute elevation, reflecting hormone-mediated vasodilation (19, 20). Our study, which focused on women who undergo ovarian stimulation that results in gradual increase in estradiol, was not aimed at measuring the acute effect of estrogen on CBF. On the basis of previous studies, we can hypothesize that prolonged elevations in estrogen level initiate genomic pathways that include the classic estrogen receptor pathway, increased endothelial nitric oxide synthase (NOS) expression, and increased COX-1 and PG12 expression (14), substantially affecting CBF and resulting in a net increase in blood flow.

The role of estrogen in modulating CBF has not been adequately assessed in humans. Earlier investigations indicate that women have a higher mean CBF than men (10), which declines with the onset of menopause (33). Lower CBF was also noted in hypoestrogenic states (9). In early postmenopausal women, hormone replacement therapy (HRT) caused a rapid reduction of pulsatility index and an increase in mean flow velocity in the ICA (2, 7). The changes described in these studies are consistent with HRT-induced intracranial vasodilation.

Berman et al. (4) demonstrated that the hormonal milieu modulates regional CBF in young women by measuring regional CBF with positron emission tomography. Increased concentrations of endogenous estrogen during normal menstrual cycles were associated with a 15% increase in end-diastolic velocities in the ICAs (15). This change was subsequently shown to be related to a decreased resistance index in the ICAs, signifying decreased cerebrovascular impedance (16). There was no significant change in ICA diameter or in systolic blood pressure throughout the menstrual cycle. We have similarly observed a significant decline in cerebrovascular resistance and an increase in blood flow velocity in the ICA during the follicular and luteal phases of ovarian stimulated cycles. CBF is mostly controlled at the level of small vessels, which are essentially affected by vasodilators such as nitric oxide (NO), although NOS can also be found in large basal vessels. Prolonged exposure to estrogen affects cerebral arterial and arteriolar tone by endothelium-dependent NO-dependent dilation, as shown previously (8). Endothelial NOS (21, 26) and neuronal NOS (23, 27) play an important role in modulating cerebral arterial tone, and both are induced by estrogen. Current literature suggests that both small and large vessels are responsive to estrogen (14, 28). Our findings of a decreased resistance and an increased velocity in the ICA during ovarian stimulation, which may reflect the reported effect of estrogen on both small and large vessels, could explain the substantial effect of estrogen on CBF.

The role of progesterone in modulating CBF is even less clear. In contrast to estrogens, progesterone may not result in direct CBF augmentation but rather modulate cerebral auto-regulation (37) or CBF response to cortical activation (4). In the present study, the influence of progesterone levels on CBF is suggested by higher CBF values in the midluteal phase compared with the follicular phase. Moreover, a greater increase in blood flow velocities was also observed in the ICA, while cerebral vascular resistance was lower during the midluteal phase compared with the preovulatory phase. Unlike the relationship between estrogen and CBF, we did not find a significant correlation between an increase in progesterone levels and an increase in CBF when comparing the luteal phase to its preceding stages.

In this study, we explored the effect of controlled ovarian stimulation, which is characterized by supraphysiological levels of sex hormones, on CBF. The largest mean estrogen level in our study group was eight times higher compared with mean natural cycle levels (6,627 pmol/l vs. 750 pmol/l), and the midluteal progesterone level was six times higher compared with physiological midluteal levels (224 nmol/l vs. 35 nmol/l). The effect of supraphysiological levels of estrogen and progesterone on cerebral circulation has not been consistently studied in women. A micromolar concentration of estradiol was found to induce cerebral vessel vasorelaxation in vitro (30), supporting our finding of decreased cerebral vascular resistance. However, this concentration is much higher than circulating estradiol levels even during ovarian stimulation.

The effect of supraphysiological level of progesterone on CBF is even less explored, and there are no data regarding its dose-response effect on CBF. Our patients were also treated by GnRH agonist to suppress endogenous secretion of gonadotropins. The effect of GnRH agonists on cerebral hemodynamics is probably mediated via reduced estrogen levels. Women who were treated with GnRH agonist were found to have stable flow indexes in ICA and middle cerebral artery compared with the pretreatment values, implying that the decreased estrogen levels do not have a significant effect on cerebral hemodynamics (29).

During controlled ovarian stimulation, the ovaries secrete multiple factors in addition to estrogen and progesterone, among them vascular relaxants that may affect CBF. Vascular endothelial growth factor (VEGF) is known to be secreted from the ovaries, and its levels are increased during controlled ovarian stimulation (18). VEGF was reported to increase local CBF in a rat model (38). Relaxin is a second factor that was reported to affect renal and cardiovascular hemodynamics (6) and is secreted by the stimulated ovaries during the luteal phase (17). Those factors, and possibly others, may act in concert with estrogen to promote increase in CBF during the luteal phase. We did not measure the levels of those nonsteroidal factors in the present study; however, it is known that there is a correlation between the serum level of VEGF and relaxin and the number of oocytes/corpora lutea and steroidal hormones during natural or stimulated cycles (1, 17, 18).

In summary, our findings suggest that elevated levels of endogenous estrogen or a combination of estrogen and progesterone during ovarian stimulation are associated with a significant increase in volume flow rates in the ICA that closely correlates with CBF. The association between sex hormone levels and cerebral hemodynamics suggests a potential role in the management of patients with compromised cerebral perfusion. It may also account, at least in part, for the neuroprotective effect of estrogen and progesterone in various models of brain injury (12, 36). According to our study, sex hormone level in human subjects, even of endogenous origin, is an additional factor that affects CBF and should be accounted for when measuring CBF in patients who have increased circulating levels of estrogen or progesterone.

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