Reproductive BioMedicine Online (2012) **24**, 174–179

www.sciencedirect.com
www.rbmonline.com

ARTICLE

Neurotrophins (BDNF and NGF) in follicular fluid of women with different infertility diagnoses

JC Sadeu [a], AMCM Doedée [a,b], MS Neal [a,c], EG Hughes [a,c], WG Foster [a,*]

[a] *Deptartment of Obstetrics and Gynecology, Health Sciences Centre, McMaster University, Hamilton, Ontario, Canada;* [b] *Radboud University Nijmegen, 6525 HP Nijmegen, The Netherlands;* [c] *ONE Fertility, Burlington, Ontario, Canada*
[*] Corresponding author. *E-mail address:* fosterw@mcmaster.ca (W.G. Foster).

Dr Warren Foster received his PhD in reproductive biology from McMaster University in 1991. He joined Health Canada as a reproductive toxicologist during 1990–1999 and subsequently moved to the department of obstetrics and gynaecology at Cedars Sinai Medical Center in Los Angeles as the assistant director of Women's Health and Director of Research. In 2001, he joined the department of obstetrics and gynaecology at McMaster University, where he carries out studies designed to characterize the effect of environmental and lifestyle factors on ovarian function and oestrogen-dependent target tissues.

Abstract Brain-derived neurotrophic factor (BDNF) and nerve growth factor (NGF) are intra-ovarian signalling peptides that are important in follicle development and oocyte maturation. In the ovary, neurotrophin expression is regulated by gonadotrophins. Therefore, this study postulates that aetiology of infertility will affect follicular-fluid BDNF and NGF concentrations. Follicular fluid from the first follicle aspirated from 190 infertile women attending a university-affiliated fertility programme (McMaster University and ONE Fertility, Burlington, Ontario) was collected between February 2004 and November 2010. The relationship between follicular-fluid BDNF and NGF concentration and age, day-3 FSH and peak serum oestradiol concentrations and antral follicle count was determined. Participants were aged between 24 and 44 years (mean ± SEM, 35.2 ± 0.3 years) of age. The median concentrations of BDNF and NGF in the follicular fluid was 19.4 pg/ml and 344.6 ng/ml, respectively. The concentrations of BDNF and NGF were significantly related ($P = 0.028$) but only the BDNF concentration was significantly higher ($P < 0.05$) in women with unexplained infertility compared with other causes of infertility. It is concluded that, apart from unexplained infertility, the underlying cause of infertility did not affect ovarian output of BDNF and NGF in response to ovulation induction.

KEYWORDS: brain-derived neurotrophic factor (BDNF), follicular fluid, nerve growth factor (NGF), neurotrophins, ovary

Introduction

The neurotrophins are a family of soluble polypeptide growth factors well known for their roles in neurite growth and differentiation in the central nervous system (Davies, 2000; Snider, 1994). The neurotrophins include, but are not limited to, the following: nerve growth factor (NGF), brain-derived neurotrophic factor (BDNF), neurotrophin3 (NT3) and neurotrophin 4/5 (NT4/5). Although the neurotrophin family shares a common low-affinity receptor, the tumour necrosis factor family neurotrophin receptor $p75^{NTR}$, they also signal via high-affinity tyrosine receptor kinases (TrK). NGF preferentially activates TrkA whereas BDNF and NT4/5 activate TrkB and NT3 preferentially

1472-6483/$ - see front matter
doi:10.1016/j.rbmo.2011.11.011

signals via TrkC. Several studies demonstrate that neurotrophins and their receptors are expressed in the cardiovascular, immune, endocrine and reproductive systems (Tessarollo, 1998; Yamamoto et al., 1996) including the mammalian ovary (Anderson et al., 2002; Dissen et al., 1995; Seifer et al., 2002, 2003; Tessarollo, 1998; Yamamoto et al., 1996). BDNF and NGF are thought to be autocrine and paracrine regulators of ovarian function (Kawamura et al., 2005; Paredes et al., 2004; Seifer et al., 2002; Spears et al., 2003). Collectively, the neurotrophins and their receptors play a role in mammalian ovarian function including follicular development (Dissen et al., 1995; Ojeda et al., 2000), steroidogenesis (Waraksa et al., 1995) and ovulation (Dissen et al., 1996; Mayerhofer et al., 1996). Furthermore, addition of BDNF to in-vitro maturation culture media significantly enhanced the number of metaphase II oocytes (Kawamura et al., 2005; Zhao et al., 2011).

Circulating oestradiol concentrations in women undergoing ovarian stimulation for IVF were positively correlated with BDNF concentrations (Monteleone et al., 2007), suggesting a role for oestradiol in the regulation of BDNF expression. Moreover, circulating BDNF concentrations vary across the menstrual cycle and fall after menopause (Begliuomini et al., 2007), supporting the proposal that oestrogens have a role in regulation of BDNF expression. Neurotrophins are produced in granulosa cells (Feng et al., 2003; Seifer et al., 2002, 2006) and output is enhanced by cAMP and gonadotrophins (Seifer et al., 2002; Zhao et al., 2011). BDNF and NGF have been quantified in the follicular fluid of women undergoing IVF (Buyuk and Seifer, 2008; Giannini et al., 2010; Seifer et al., 2003). Of note, the mean follicular-fluid BDNF concentration was lower in women with endometriosis compared with women with male factor infertility (controls) (Buyuk and Seifer, 2008; Giannini et al., 2010) whereas no differences in NGF concentrations were found (Buyuk and Seifer, 2008). In contrast, the follicular-fluid concentration of BDNF did not differ in women diagnosed with polycystic ovary syndrome (PCOS) versus women with male factor infertility, whereas NGF concentrations were lower in women with PCOS (Buyuk and Seifer, 2008). Taken together these data suggest that although gonadotrophin treatment has been shown to increase granulosa-cell BDNF and NGF expression (Seifer et al., 2002; Zhao et al., 2011), the lower concentrations of BDNF and NGF in the follicular fluid of women with endometriosis and PCOS following ovarian stimulation suggest that the underlying pathology of these gynaecological conditions attenuates granulosa-cell response to gonadotrophin stimulation through mechanisms that have yet to be defined. In view of the evidence that gonadotrophins regulate BDNF output and the positive correlation with circulating oestradiol concentrations, this study predicted that follicular-fluid BDNF and NGF concentrations following ovarian stimulation will be equivalent regardless of aetiology of infertility. Therefore, the aim of this study was to quantify BDNF and NGF in follicular fluid of women undergoing IVF treatments and assess the effect of discrete infertility diagnosis on neurotrophin concentrations.

Materials and methods

Subjects

Follicular fluid was collected from 190 women attending the ONE Fertility Clinic for assisted human reproduction treatment between February 2004 and November 2010. The mean ± SEM age of study participants was 35.2 ± 0.3 years with a range of 24—44 years. The primary diagnoses for infertility included abnormal sperm, bilateral tubal obstruction (BTO), endometriosis, PCOS, advanced maternal age, diminished ovarian reserve and unexplained infertility. In 38 of 190 participants (20%), more than one (multifactorial) cause of infertility was recorded and thus these participants were not included in any further analyses. The remaining participants were grouped into six categories exclusive of other aetiologies as follows: male factor, BTO, endometriosis, PCOS, advanced maternal age and unexplained infertility (**Table 1**). This study was approved by the ONE Fertility Clinic research committee and McMaster University Research Ethics Board.

IVF stimulation and outcomes

Ovarian stimulation was used. Briefly, pituitary down-regulation was achieved by administering subcutaneous injections (1 mg daily) of gonadotrophin-releasing hormone analogue (buserelin acetate; Superfact; Sanofi-Aventis Canada, Laval, QC, Canada) from day 21 of the previous menstrual cycle. After the onset of menses (serum oestradiol of 150 pmol/ml), patients were adminis-

Table 1 Study participant characteristics, grouped by infertility diagnosis.

Characteristic	*Male factor*	*BTO*	*Endometriosis*	*PCOS*	*AMA*	*Unexplained*
n (%)	63 (41.4)	15 (9.9)	11 (7.2)	16 (10.5)	19 (12.5)	28 (18.4)
Age (years)	34.4 ± 0.4	32.5 ± 1.2	33.6 ± 1.3	34.7 ± 0.7	40.2 ± 0.8[a]	35.8 ± 0.6
Day-3 FSH (IU)	6.6 ± 0.3	6.4 ± 0.4	7.0 ± 0.4	6.3 ± 0.4	9.1 ± 1.1	6.0 ± 0.4
AFC	8.0 ± 0.5	7.7 ± 1.1	7.4 ± 1.7	10.6 ± 1.6	6.4 ± 1.0	6.8 ± 0.8
Peak oestradiol (pg/ml)[b]	10,898 (1758—22,825)	9619 (4201—20,507)	8609 (3683—22,444)	8955 (4952—21,240)	7318 (1679—18,266)	8640 (0—16,575)

Values are mean ± SD unless otherwise stated.
[a] $P < 0.001$.
[b] Data were not normally distributed and were transformed by the square root of the raw data; values are median (minimum—maximum).
AFC = antral follicle count; AMA = advanced maternal age; BTO = bilateral tubal obstruction; PCOS = polycystic ovary syndrome.