Brain-derived neurotrophic factor from follicular fluid is positively associated with rate of mature oocytes collected and cleavage rate in intracytoplasmic sperm injection patients

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Abstract

Purpose The aim of the study was to evaluate the correlation between BDNF and oocyte maturation and to verify whether BDNF could predict in vitro fertilization (IVF) outcome.

Methods The follicle fluid (FF) for BDNF, E₂ and P assay were obtained from 59 patients undergoing intracytoplasmic sperm injection (ICSI). The women were divided into two groups by pregnancy outcome and their clinical and lab data were compared. And the correlation of BDNF with E₂, P, age, and IVF data were analyzed.

Results Positive correlation was observed between BDNF and E₂ concentration in FF. BDNF was positively correlated with the rate of mature oocytes collected and cleavage rate.

Conclusions The BDNF in FF could not predict IVF outcome, but BDNF in FF might play an important role in the maturation of oocyte and development of oocyte into preimplantation embryo.

Keywords Brain-derived neurotrophic factor · Oocyte · Follicle fluid · Rate of mature oocytes collected · Cleavage rate

Introduction

In recent years, IVF has become an important tool for infertility treatment. Many studies suggest it is important

to acquire high-quality oocytes for the success of IVF. The mechanism of oocyte maturation is complicated, involving not only the hormone regulation of hypothalamus-pituitary-ovary axis, but also modulation of clusters of growth factors in ovary microenvironment, such as epidermal growth factor (EGF), insulin-like growth factor (IGF-I), and vascular endothelial growth factor (VEGF) [1–3].

BDNF, a member of the neurotrophin family, is initially recognized to be important for neuronal survival and differentiation in central and peripheral nervous system, and is also found in non-neuronal tissues. Growing evidence showed BDNF was essential for the oocyte maturation [4–6]. Kawamura et al. [7] had observed that BDNF enhanced the first polar body emission and promoted oocyte development into preimplantation embryos. Treatment with BDNF during the in vitro maturation (IVM) of bovine oocytes could produce more parthenogenetic embryos, and the authors speculated BDNF could improve oocyte cytoplasm maturation [8]. This effect was also observed in porcine oocytes, which showed that BDNF could improve both nuclear and cytoplasmic maturation through autocrine and/or paracrine signal pathways [9]. One recent study demonstrated that BDNF could promote human oocyte maturation and early embryo development [10].

Seifer et al. demonstrated the presence of BDNF in human IVF, including normal cycle and IVF cycle, and found that BDNF played an important role in the progress of follicle formation and oocyte maturation [5]. As oocyte-corona-cumulus complex is directly exposed to the FF, which contains BDNF (mainly from granular cells), it is supposed that there might be a certain relationship between FF BDNF and IVF outcome. The objective of this study was to evaluate the relation between BDNF and oocyte maturation by measuring FF

Capsule BDNF in follicle fluid was assayed. BDNF could not predict IVF outcome, but was important for oocyte maturation and development of oocyte into preimplantation embryo.

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BDNF, E₂ and P levels and to verify whether BDNF could predict IVF outcome.

Materials and methods

Subjects

A total of 59 women undergoing ICSI at the Reproductive Center of the Peking Union Medical College Hospital between June 1, 2010 and March 31, 2011 were included in this study. The inclusion criteria were: [1] age ≤ 37 years; [2] the first time to undergo IVF cycle; [3] male-factor infertility; [4] basal FSH (day 3) level <10 mIU/mL; [5] both ovaries present. The exclusion criteria were: [1] any diseases that could affect ovary function, such as ovarian neoplasm, polycystic ovary syndrome, hyperprolactinemia, endometriosis and premature ovarian failure; [2] use of steroids within 3 months; [3] chronic diseases, thyroid and adrenal diseases. According to whether pregnant or not, the women were then subdivided into two groups: pregnant group and non-pregnant group. This study was approved by the institutional review board of Peking Union Medical College Hospital and written informed consent was obtained from all patients.

Ovarian stimulation and the ICSI procedure

A routine long protocol was used in this study. Briefly, all patients started with an oral contraceptive pill on Day 2–4 of their cycles. Triptorelin acetate (Decapeptyl, German) was started for pituitary down-regulation after oral contraceptive had been used for 18 days, and its daily use would last till the day of HCG administration. Recombinant FSH (Gonal-F, Serono, Switzerland) was used for ovarian hyperstimulation, at a starting dose of 150–225 IU/d. The gonadotropin dosage was adjusted according to follicular growth and serum E₂. HMG (Menotrophin, Lizhu, China) was given when the largest follicle reached a diameter of 16 mm. Eventually, follicle puncture was performed under transvaginal ultrasonography 36 h after injection of 10,000 IU human chorionic gonadotropin (hCG, Serono, Switzerland).

The maturation of oocytes was investigated after removal of the cumulus cells as previously described. An oocyte with one polar body was recognized as mature. All embryos were scored using the number of blastomeres and the percentage of fragmentation 72 h after the follicle puncture. Top-quality embryos were defined as those having six or eight blastomeres with less than 10% fragments. Two or three cleavage embryos were transferred to each patient 72 h after oocyte retrieval. Luteal phase was supported by daily intramuscular injection of progesterone

starting on the day of oocyte retrieval. Biochemical pregnancy was assessed by serum β -HCG measurement 14 days after transfer. Clinical pregnancy was established by the presence of an intrauterine gestational sac with embryocardia at 7–8 weeks of gestation.

FF collection

FF of the punctured follicles was collected in each patient. After removal of the oocyte, the FF was centrifuged at $500\times g$ for 15 min to remove the cellular contents and debris. Then the supernatant was acquired and stored at -80°C for assay. FF contaminated with blood was excluded.

BDNF, E₂ and P measurements in FF

FF BDNF level was determined by enzyme-linked immunosorbent assay (ELISA) method (BDNF Emax Immunoassay System, Promega, USA) as previously described [11, 12]. Briefly, the samples were diluted with Block & Sample Buffer. 96-well flat bottom plates (Corning Costar, USA) were coated with Anti-BDNF monoclonal antibody and incubated at 4°C overnight. The plates were flushed with TBST and blocked with Block & Sample Buffer for 1 h. The control and samples were then added into the wells and the plates were incubated and shaken for 2 h at room temperature. Then the plates were washed with TBST and added with anti-human BDNF polyclonal antibody. After washing, anti-immunoglobulin Y-horse-radish peroxidase conjugate was added into the plate for the last incubation. Finally, TMB One solution was added in order to enhance the demonstrations by producing a specific colour. The absorbance was measured at 450 nm by using a microplate reader (Tecan, Switzerland) within 30 min after the reaction was stopped by 1N HCl. The BDNF antibody at 100 ng/ml demonstrated less than 3% cross-reactivity with other related neurotrophic factors (NGF, NT-3, and NT-4). The detection sensitivity of the ELISA was 15.6 pg/ml, according to the manufacturer's specification/manure.

The levels of E₂ and P were determined using electrochemiluminescence immunoassay method with Elecsys 2010 (Roche, Germany). The measurable ranges of the E₂ and P assay were 5.0–4,300 pg/ml and 0.03–60 ng/ml, respectively.

Statistical analysis

Data were analyzed by using the Statistical Program for Social Sciences (version 11.5 for Windows; SPSS Inc., Chicago, IL). The concentration of BDNF and E₂ were expressed in pg/ml and P was expressed in ng/ml. Values were expressed as mean $\pm$ standard deviation (SD). The differences between groups were analyzed using Student's

t-test if the data were abnormally distributed, or using non-parametrical Mann–Whitney *U* test if the data had no homogeneity of variance. The Spearman correlation was applied for the correlation analysis between the concentrations of BDNF in FF and other variables. $p < 0.05$ was considered statistically significant.

Results

Data of the study subjects between pregnant and non-pregnant groups

A total of 59 patients were included in the study, 2 of whom gave up fresh embryo transfer due to ovarian hyperstimulation syndrome (OHSS). The total pregnancy rate was 54.4% (31/57). The clinical and laboratory data of patients in two groups were shown in Table 1. There was no significant difference in any item between pregnant and non-pregnant groups.

BDNF, E₂ and P levels in FF

The concentrations of FF BDNF, E₂ and P were 242.0 ± 51.6 pg/ml, 1172644.0 ± 408260.0 pg/ml and 27658.8 ± 9922.2 ng/ml, respectively. No significant difference was found in the concentration of BDNF, E₂ and P between pregnant and non-pregnant groups, as shown in Table 2. The concentration of BDNF in FF showed a significant correlation with E₂ ($r = 0.301$, $P = 0.021$), but there was no correlation with P ($r = -0.066$, $P = 0.621$), as shown in Fig. 1.

Table 1 Comparison of the clinical and laboratory data between pregnant and non-pregnant groups

Items	Pregnant group (n=31)	Non-pregnant group (n=26)	P
Age (y)	31.52±3.40	31.00±3.51	0.469
BMI (kg/m ²)	21.85±2.47	23.58±4.60	0.184
Years of infertility (y)	3.65±2.17	4.58±2.25	0.085
Basal FSH (mIU/mL)	6.40±1.52	6.44±1.58	0.660
Dose of FSH (no. of ampoules)	24.82±4.93	24.06±6.88	0.495
Duration of FSH use before hCG (d)	8.68±0.95	8.31±1.19	0.207
Number of oocytes collected	15.4±7.09	14.31±7.01	0.480
Number of mature oocytes	12.55±5.79	12.35±5.85	0.898
Number of fertilized oocytes	10.48±4.87	9.35±5.08	0.323
Number of cleaved embryos	9.81±4.72	8.92±4.75	0.479
Number of blastula embryos	2.26±1.41	2.5±2.58	0.776
Number of transferred embryos	2.03±0.32	2.12±0.33	0.335
Rate of mature oocytes collected	0.83±0.13	0.88±0.10	0.171
Cleavage rate	0.94±0.08	0.96±0.09	0.092
Blastocyst formation rate	0.25±0.15	0.23±0.20	0.629
Ratio of good embryo transfer ^a	0.77±0.43	0.62±0.41	0.212

BMI body mass index; FSH follicle-stimulating hormone; HCG human chorionic gonadotrophin

^aRatio of good embryo transfer was defined as number of transferred good embryos/number of total transferred embryos

Table 2 The levels of BDNF, E₂ and P in pregnant and non-pregnant groups

Items	Pregnant group (n=31)	Non-pregnant group (n=26)	P
BDNF(pg/ml)	244.48±51.83	240.10±52.74	0.949
E2(pg/ml)	1139648±365990	123006±464613	0.391
P(ng/ml)	27993.7±9721.8	26331.1±9897.6	0.511

BDNF brain-derived neurotrophic factor; E₂ estradiol; P progesterone

Correlations between BDNF and age and IVF data

The Spearman correlation analysis between age, rate of mature oocytes collected, cleavage rate, blastocyst formation rate and FF BDNF were shown in Fig. 2. There was no correlation between age, blastocyst formation rate and BDNF in FF ($r = 0.071$, $P = 0.593$ and $r = -0.001$, $P = 0.993$ respectively). However, the rate of mature oocytes collected and cleavage rate were strongly correlated with FF BDNF ($r = 0.41$, $P = 0.001$ and $r = 0.312$, $P = 0.016$ respectively).

Discussion

The study evaluated the correlation between BDNF in FF and IVF outcome, and tried to find whether BDNF could predict IVF outcome. It seemed that BDNF in FF was positively correlated with the rate of mature oocytes collected and cleavage rate but not with blastocyst formation rate. It could be speculated that BDNF in FF was important for the development of oocytes because it was positively associated with oocyte development. This

Fig. 1 The correlation between BDNF in FF and E₂, P

study did not find any difference of BDNF in pregnant between that in non-pregnant groups. Therefore, the IVF outcome could not be predicted by BDNF value in FF.

So far, the relationship between some markers and IVF outcome had been studied. For example, Inhibin B was positively correlated with ovary response, quality of oocyte and pregnancy rate [13]. Anti-mullerian hormone in FF could predict oocyte fertilization outcome [14]. However, there were little data about the correlation between BDNF and IVF outcome. This study indicated that there was no statistical significance in BDNF between pregnant and non-pregnant groups, which was in line with another study that discovered the BDNF plasma concentration could not be

used to predict IVF outcome [15]. Recently, Buyuk et al. [12] reported that the concentration of BDNF in FF was different in women undergoing assisted reproductive technology for different etiologies of infertility. They found the BDNF levels in FF were lowest in patients with a history of endometriosis, whereas there were no significant difference between other groups and the control group (male factor). They presumed that low levels of BDNF in FF might result in poor-quality oocytes in endometriosis patients. Therefore, it was thought that BDNF in FF was closely associated with oocyte maturation. In our study, the concentration of BDNF in FF was significantly correlated with the rate of mature oocytes collected and cleavage rate

Fig. 2 The correlation between BDNF in FF and age, IVF data. Note: *Rate of mature oocytes collected* was defined as number of mature oocytes/number of oocytes collected; *Cleavage rate* was defined as number of cleavage embryos/number of fertilized oocytes; *Blastocyst formation rate* was defined as number of blastula embryos/number of fertilized oocytes

(Fig. 2), indicating that BDNF in FF played an important role in the maturation of oocytes and promoted the development of oocyte into preimplantation embryo.

As we know, oocyte maturation is an extremely complicated process, which needs not only the regulation of gonadotrophins, but also the modulation of large numbers of different microenvironmental cytokines secreted from ovary. However, the underlying mechanism remains unclear how BDNF promoted the maturation of oocyte and the development of oocyte into preimplantation embryo. Seifer et al. [6] proved the presence of BDNF and its specific tyrosine kinase receptors (TrkB) inside the cumulus and mural cells, and TrkB in oocytes. They assumed that BDNF could promote the maturation of oocytes through an autocrine and paracrine way. Kawamura et al. [16] found BDNF could promote blastocyst development by increasing total cell numbers in blastocysts and inhibiting trophoblast cells apoptosis. In another study, they demonstrated that BDNF could improve fetal growth by increasing trophoblast cell growth and survival during implantation and subsequent placental development through a certain autocrine or paracrine mechanism of modulation [17].

This study showed that the BDNF was positively correlated with E_2 , a finding consistent with previous studies [15, 18]. Increasing evidence showed the synthesis of BDNF was positively correlated with the synthesis of estrogen. It seemed estrogen could induce an increase of BDNF production and release [19]. In the study, there was no relationship between BDNF and P in FF. However, Begliuomini et al. [18] demonstrated that the level of BDNF was positively correlated with P in fertile women. Chen et al. [20] found BDNF significantly inhibited the production of P in a dose-dependent manner when they cultured human granulosa-lutein cells in medium without or with different dosage of BDNF. They considered BDNF could suppress P synthesis by inhibiting the activity of some enzymes or reducing protein production during P synthesis.

Growing evidences have demonstrated that serum BDNF level decreased with increasing age. Begliuomini et al. [18] found serum BDNF level in menopausal women was significantly lower than that in fertile women, and found that BDNF level in postmenopausal women was negatively correlated with menopausal age. Ziegenhorn et al. [21] drew the similar conclusion that the serum BDNF concentration was negatively correlated with age. But in our study, the results showed no correlation between BDNF in FF and age. This might be in part correlated with some drawbacks in the study. Firstly, subjects included in the study were less than 37 years old, and this could contribute to non-correlation between age and BDNF. Secondly, only 59 patients' data were analyzed and the small sample could

also lead to bias. Finally, The BDNF concentration was determined using serum samples in previous study but FF samples in our study. A study revealed the BDNF concentration was significantly higher in women undergoing IVF than that in normal cycling women. It seemed the BDNF concentration could be up-regulated by gonadotropin [5]. Therefore, it is necessary to evaluate the relationship between BDNF in FF and age in further studies.

In conclusion, the present study indicated BDNF in FF could not be used to predict the IVF outcome. The results showed BDNF was positively correlated with the rate of mature oocytes collected and cleavage rate and suggested BDNF in FF might play an important role in the maturation of oocyte and development of oocyte into preimplantation embryo.

Conflicts of interest no

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