Brain-Derived Neurotrophic Factor in The Uterine Flushing of Patients With Unexplained Infertility

Thesis

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By

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☙List of Abbreviations ❧

α1-PEG	α1-progesterone-dependent endometrial globulin
AA	Arachidonic acid
AR	Androgen receptors
BDNF	Brain derived neurotrophic factor
BIGH3	Betaig-H3
BMI	Body mass index
BMP-7	Bone morphogentic protein 7
cAM	Cell adhesion molecule
CAM family	Cellular adhesion molecule family
cAMP	Cyclic adenosine monophosphate
CNTF	Ciliary neurotrophic factor
CPE	Clostridium perfringens enterotoxin
$CPLA_2$	Cytosolic phospholipase A_2
CREB	Cyclic AMP-response element binding protein
CT-1	Cardiotrophin
ECM	Extracellular matrix
EECs	Endometrial epithelial cells
EECs	Endometrial epithelial cells
EGF	Epidermal growth factor
EPDA	Endometrial power Doppler area
ER-α	Estrogen receptor alpha
ESCs	Endometrial stromal cells
EST	Expressed sequence tags
FCS	Fetal calf serum
FZ	Frizzled
GPX3	Glutathione peroxidase 3
GPX-3	Glutathione peroxidase-3
GVBD	Germinal vesicle breakdown
HAI-I	Hepatocyte growth factor activator inhibitor type I
HB-EGF	Heparin binding-epidermal growth factor
hCG	Human chorionic gonadotropin
HES	Human uterine epithelial cell line
HOX	Homeobox Genes
HSG	Hysterosalpingography
IGF	Insulin like growth factor
IGF-βP1	Insulin-like growth factor binding protein-1
IGF-BPs	Insulin like growth factor binding proteins

☙List of Abbreviations (Cont.)❧

IGF-I	Insulin like growth factor-1
IL-1	Interleukin-1
IL-6R	IL-6 receptor
ISH	In situ hybridization
KOR	Kinase insert domain-containing-region
LAA-3	Lysophosphatidic acid receptor-3
LH	Luteinizing hormones
LIF	Leukemia inhibitory factor
MeCP2	Methyl-CPG binding protein 2
MMP-2	Metallo-proteinases-2
MMPs	Matrix metalloproteinases
MT-1G	Metallothioein-1G
Muc-1	Mucin-1
NF-KB	Nuclear factor-KB
NGF	Nerve growth factor
NT-3	Neurotrophin-3
NTs	Neutropins
OPN	Osteopontin
OSM	Oncostatin M
PBMCs	Peripheral blood monocytes
PC1	Prohormone conventase1
PG	Prostaglandin
PGES	Prostaglandin E synthase expression
PGs	PG synthase
PI	Pulsatility index
PK1	Prokineticin1
PLA2	Biosynthesis
PLC	Phospholipase C
PRL	Prolactin
RPL	Recurrent pregnancy loss
SCNT	Somatic cell nuclear transfer
SEM	Scanning electron microscope
SIL-6R	Soluble form of the IL-6R
TEM	Transmission electron microscopy
TGF-β	Transforming growth factor-β
TIMP-3	Tissue inhibitors of matrix metalloproteinases-3

List of Abbreviations (Cont.)

Trk-	Tyrosine kinases
TrK B	Tyrosine kinase B
TrK B	Tyrosine kinase B
TTMPs	Tissue inhibitors of metallo proteinases
VEGF	Vascular endothelial growth factor
VI	Vascularization index
VNTR	Variable number of identical tandem repeat

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INTRODUCTION

Unexplained infertility is a common and challenging diagnosis for both patients and physicians in 15-30% of infertile couples where a standard infertility evaluation fail to identify a likely etiology ***(Royal College of Obstetrics and Gynecology, 1998)***.

Unexplained infertility is thought to be due to defects in embryo development and its genetic control, or due to defects in molecular control of implantation window ***(Edward, 1994)***, or due to decrease in uterine receptivity ***(Ilesanmi et al., 1993 and Lessey et al., 1994)***.

Accumulating evidences indicate that a number of growth factors and cytokines act as paracrine and autocrine factors during early embryo development and implantation ***(Brisen & Schutz, 1998; Hardy & Spans, 2002 and Deyetal, 2004)***.

In addition, developing embryos produce growth factors that act in an autocrine manner to regulate their own growth and differentiation or to serve as paracrine factor by regulating endometrial receptivity for blastocyst implantation ***(Deyetal, 2004)***.

Brain derived neurotrophic factor (BDNF) is a member of neurotrophin family of proteins known to activate the high affinity tyrosine kinase B (TrK B) receptor together with the pan-neurotrophin low affinity co-receptor p75 ***(Barbacid, 1994)***.

Although neurotrophins are widely distributed in the central nervous system and are important for neuronal survival and differentiation ***(Jones et al., 1994)***, they also play an important role in non-neuronal tissue ***(Ipetal, 1993)***.

BDNF is secreted by oviductal and uterine epithelial cells and plays a role in blastocyst development and survival through paracrine as well as autocrine actions ***(Botchkarev et al., 2004)***.

The effect of BDNF to promote blastocyst development is correlated to its ability to inhibit apoptosis and increase total cell numbers in blastocyst. The apoptosis suppressing effect of BDNF is consistent with earlier studies showing the survival action of BDNF on the cells of the central nervous system ***(Hetman et al., 1999 and Han & Holtzman, 2000)***, and some peripheral tissues ***(Botchkarev et al., 2004; Raap et al., 2005 and Pyle et al., 2006)***.

In this study BDNF is studied in uterine flushing of patients with unexplained infertility as a factor affecting endometrial receptivity.

AIM OF THE WORK

The aim of this study is to demonstrate a relationship between the presence of brain derived neurotropic factor (BDNF) in the endometrial fluid during the luteal phase as an important factor in implantation and being a cause of infertility in patients with unexplained infertility.

I – Unexplained Infertility

Recent medical literature has quite extensively addressed the use of various terminologies within the field of reproductive medicine. This, discussion has, however, so far overlooked the fact that one of the most frequently made diagnosis, the so-called unexplained infertility. It may, indeed, represent the most frequent female infertility diagnosis, with a reported evidence of prevalence of approximately 25-30% of all infertility ***(Evers, 2002; Smith et al., 2003 and Gleicher & Barad, 2006)***.

The diagnosis of unexplained infertility is highly subjective. It is dependent on which diagnostic tests have been performed (or have been omitted) and at what level of quality, with the diagnosis of unexplained infertility being more frequently reached if the diagnostic work-up is incomplete or of poor quality ***(Gleicher and Barad, 2006)***.

Authoritative European sources have previously noted that the diagnosis of unexplained infertility is one of exclusion and it is therefore important to seek agreement on which diagnostic tests are required to be done before concluding that a couple have unexplained infertility. However, currently, there are no universally accepted methods for diagnosing unexplained infertility, which is based on exclusion of the other recognized causes of infertility ***(Crosignani et al., 1993)***.

Reaching a specific infertility diagnosis is really difficult because one can never be sure that even a well-documented diagnosis, represents the only cause of a couple's infertility as other factors may be unknown to the treating physician or, when known, be past of so-called multi-factorial infertility. The presumptive diagnosis of unexplained infertility is reached when whatever diagnostic work-up has been chosen reveals no obvious cause for a couple's infertility. The didactic term unexplained infertility, therefore does not describe a clinical situation, characterized by specific diagnostic findings, but is used to describe a negative, i.e., the absence of specific diagnostic findings. Negatives are, however, practically impossible to prove. To quote Carl Sagan: "Absence of evidence is not evidence of absence" ***(Gleicher and Barad, 2006)***.

Such a diagnosis should then only be reached if all appropriate diagnostic tests were performed and have failed to detect one or more presumed causes for a couple infertility and here is much of the current debate "What are the appropriate diagnostic tests?" The appropriate diagnostic tests has, however, remained undefined, as the literature is quite unanimous in suggesting that there is no agreement as to what constitutes a complete infertility work-up ***(ESHRE Gapri Workshop Group, 1996 & 2000 and American Society for Reproductive Medicine "ASRM", 2000)***.

What constitutes an appropriate infertility work-up may, in addition, also vary based on clinical circumstances. For example, age of females will, of course, greatly affect the decision-making process ***(ASRM, 2002)***. In addition, it seems obvious that the quality of performance of diagnostic procedures can vary and, therefore, with it, their reliability. Finally, the diagnostic relevance of tests may be interpreted differently by different clinicians. For example, the evaluation of anti-phospholipid antibodies in infertile patients has been strongly discouraged by some ***(ASRM, 1999)***.

Yet, others disagree, based on reports which have demonstrated that sub-clinical autoimmune diseases, have been associated with decreased fecundity ***(Nelson et al., 1993; Sicman & Black, 1998 and Gleicher, 1999)***. Abnormal autoimmune function is also found at increased prevalence in infertile women ***(Gleicher et al., 1989 and Geva et al., 1997)***.

Another example for high degree of observer-to-observer variation in the interpretation of diagnostic testing has been well documented in women with endometriosis. Endometriosis is frequently misdiagnosed as unexplained infertility ***(Cook and Rock, 1995)***, because a diagnosis of endometriosis even with laparoscopy, can be easily missed ***(Olive and Schwartz, 1993)***. Similarly, the diagnostic accuracy of hysterosalpingography (HSG), a mainstay of infertility diagnosis, has been questioned ***(Gleicher et al., 1992)***.

Also from the causes that is frequently misdiagnosed as unexplained infertility is the prematurely aging ovaries. A number of investigators have shown that some women follow a premature aging curve ***(Beckens et al., 2002; Nikoloaou & Templeton, 2003 and Gleicher, 2005)***.

This concept of prematurely aging ovaries is based on a number of observations:

First, the process of ovarian aging appears to be statistically correlated with the total number of follicles within the ovary ***(Te Velde et al., 1998 and Faddy, 2000)***. The original supply of follicles continues to decline from birth and a state of sub-fertility is reached at approximate age 30-31 years, when the remaining follicles have become a fraction of their original number ***(Te Velde et al., 1998)***.

By age 37-38 years, a critical point is reached, with approximately 25,000 follicles remaining in the ovaries. At that point, follicular depletion accelerates towards menopause, which is reached at approximate follicular count 1000, at an average age 51 years ***(Guzick et al., 1994; Te Velde et al., 1998; Faddy, 2000 and Nikolaou & Templeton, 2003)***.

A second, very important observation suggests that the time period between accelerated decline in fertility (i.e., age 37-38 years and 25,000 follicles) is fixed approximately at 13.5 years ***(Faddy, 2000 and Nikolaou & Templeton, 2003)***.