ZAMZAM WATER STIMULATE BRAIN DERIVED NEUROTROPHIC FACTOR (BDNF) IN UTERINE FLUSHING OF REPEATED IMPLANTATION FAILURE AFTER (INTRACYTOPLASMIC SPERUM INJECTION) (ICSI)

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ABSTRACT

Background: Brain derived neurotrophic factor (BDNF) is one of neurotrophins well known for their role in neuronal survival has been identified in the mammalian ovary. Zamzam water is a special type of water with a special composition.

The aim of this work is to study the role of BDNF in uterine flushing, to find a relation with repeated unexplained implantation failure after ICSI and the effect of zamzam water intake.

Materials: 20 patients experienced repeated implant-tation failure (mean ± SD- in Group I was 7.33 ± 1.5) and 20 patient subjected for tubal sterilization as a control group (Group II) were enrolled in the study. Zamzam water intake to both groups (group I, II) 500-750 c.c. for one month.

Method: uterine flushing as taken on day 26 of the cycle, BDNF assay was determined by enzyme linked immunosorbant assay (ELISA).

Results: Statistically significant decrease of BDNF in uterine flushing in unexplained repeated implantation failure in comparison to a normal control 322.2 ± 99.8 vs. 177.7 ± 68.78 $P < 0.05$, statistically significant increase in BDNF in group I after zamzam water $P<0.05$.

Conclusion: BDNF plays in important of role in endometrial receptivity and hence the repeated implantation failure. Zamzam water stimulate BDNF in uterine flashing.

Keywords: Zamzam water, Endometrial receptivity, Repeated implantation failure, BDNF, Uterine flushing, ICSI.

INTRODUCTION

Zamzam water is unique in its natural characteristic as it is hard carbonated water it has been proven that there are no microbs what so ever in zamzam water, and it has a special optical parameters **(Naeem et al., 1983, ElZaiat 2005, Ali Farid, 2008 a,b).**

Implantation is controlled primarily by locally acting growth factors and cytokines (Tabibzadeh et al 1995). It is generally assumed that approximately two thirds of implantation failures are imputable to inadequate uterine receptivity (Edwards 1994). The assumed causes of decreased endometrial receptivity are (uterine cavity abnormalities, thin endometrium, altered expression of adhesive molecules, immunological factor, thrombophilias **(Margalioth et al., 2006).**

Neurotrophins, a family of soluble polypeptide growth factors well known for their role in neural survival and neural outgrowth although other neurotrophic factors have been identified, the neurotrophins found in a variety of non-neuronal systems (cardiovascular, immune, endocrine and reproductive system. It includes nerve growth factor (NGF), brain - derived neurotrophic factor (BDNF), neurotrophin - 4/5 (NT-4/5) and neurotrophin -3 (NT3) **(Tessarolle et al., 1998, Yamamoto et al., 1996).**

To the best of our knowledge no report dealt with the relation of brain derived neurotrophin factor (BDNF) to repeated implantation failure, and the effect of zamzam water.

The aim of this work is to study BDNF in the uterine flushings of patients with repeated implantation failure and the effect of zamzam water on BDNF expression.

MATERIAL AND METHODS

Subjects:

This study was conducted at pharmacology department, Faculty of Medicine, Ain Shams University, From January 2006 till January 2007 and Heliopoles Hospital.

Group (I): A total of 20 cycle in 20 patients were included in the study all patients had experienced repeated (unexplained failures ICSI) (7.33 ± 1.5).

Group (II): Twenty patients scheduled for tubal sterilization without abdominal or pelvic pathology were subjected to uterine flushing at the 26^{th} day of the cycle were chosen as a control group.

Uterine flushing: (Ledee et al., 2002)

Uterine flushing took place on day 26 of the cycle, an embryo transfer catheter (Frydman catheter) connected to 5 ml syringe was gently inserted into the uterus. 1 ml of saline water was instilled twice and was immediately and gently aspirated. The volume of fluid after centrifugation (1000) rpm for 10 min to remove the cellular

component was assessed and the clear supernatant fraction was stored at -80° until the assays.

BDNF assay:

Brain-derived neurotrophic factor levels were determined using the commercially available DBNF Emaz immunoassay system (Promega Corp., Madison, WI). The ELISAs were performed according to the manufacturer's protocol. Zamzam water was taken to both groups (I, II) 500-750 c.c for one month.

Ethics:

The study was performed in accordance with the guidelines in the Declaration of Helsinki and has been formally approved by the local ethical committee. Informed consent was obtained from all subjects. Both groups were subjected to zamzam intake 500-750 c.c for one month and uterine flushing taken after that.

Data analyses

Uterine flushing BDNF is expressed in pg/ml. All data are reported as mean ± SD. The difference between uterine flushing BDNF of repeated failure group and control group were analyzed by the two tailed-E-test, chi square test was used for comparison between two groups. A difference of < 0.05 was considered significant.

Data analysis

Difference between BDNF before zamzam water intake and after were analyzed by two tailed t test, chi square test was used for comparison a difference of <0.05 was considered significant.

RESULTS

Table (1): Patient characteristics, and mean ± SD of BDNF in group I, group II in the uterine flushing fluid

Characteristics	Repeated failed ICSI group I (n = 20)	Control group II (n = 20)	P value
Age (years)	37.66 ±2.88	37.11 ±2.1	>0..05
BMI (kg/m^z)	22.33 ±1.88	22.55 ±1.98	>0.05
BDNF (pg/ml)	177.7 ±68.78	322.2 ±99.8	P < 0.05

The mean parity was 8.1±2.1 in the control group (II), in group (I) mean number of repeated (unexplained ICSI failures) was 7.33±1.5.

There was no significant statistical difference between mean age and body mass index (BMI) between the 2 groups.

There was statistically significant decrease in BDNF in uterine flushing in the group I in comparison to group II.

Table (2): BDNF in uterine flushing in group I before and after zamzam water

	Before zamzam intake	**After zamzam intake**	**P value**
Mean±SD	177.7±68.78	299.1±63.14	<0.05

Table (3): BDNF in uterine flushing in group II before and after zamzam water

	Before zamzam intake	**After zamzam intake**	**P value**
Mean±SD	322.2±99.8	389±91.0	>0.05

There was statistically significant increase of BDNF in group I P <0.05 and no statistically significant increase in group II P>0.05.

DISCUSSION

The activation of BDNF by zamzam water due to peculiar nature of zamzam water which was discovered by radioimmunoassay, nano-technology, laser femto, crystaline electromicroscopy, specific refractive index, number single oscillator, specific dispersing of optical parametersal assay, Abbe number of zamzam water was completely different from other types of water **(Ali Farid et al., 2008).**

Successful implantation requires a receptive endometrium, a normal and functional embryo at the blastocyst developmental stage and a synchronized dialogue between maternal and embryonic tissues **(Simon et al., 2000).**

Endometrial receptivity consists of the acquisition of adhesion ligands together with the loss of inhibitory components that may act as a barrier to an attaching embryo **(Aplin, 2000)**; implantation failure remains an unsolved problem in reproduction medicine.

Inadequate uterine receptivity is responsible for approximately two third of implantation failures **(Ledee Bataille et al., 2002).** In this work the mean number of repeated failure ICSI was 7.33 ± 1.5. The definition of repeated implantation failure of Tan et al 2005) which is failure to achieve pregnancy following 2-6 IVF cycles in which more than 10 high grade embryos were transferred to the uterus was used.

A large number of molecular mediators have been identified to date, including: adhesion molecules, cytokines, growth factors, lipids and others to be associated with uterine receptivity. To the best of our knowledge there is no report in literature to deal with brain derived neurotrophic factor BDNF in uterine fluid of human with repeated implantation failure.

Brain derived nerurotrophic factor (BDNF) is a member of the neurotrophin family of proteins known to activate the high affinity tyrosine kinase B (TrKB) receptor together with the pan neurotrophin low affinity co-receptor p 75 **(Barbacid, 1994)** in addition to BDNF other factors included nerve growth factor (NGF) neurotrophin 4/5 (NT-4/5) and neurotrophin 3 (NT-3). Although neurotrophins are widely expressed in the central nervous system and are important for neuronal survival and differentiation **(Jones et al., 1994),** they also play important roles in non neuronal tissues **(Ip et al., 1993)** it has been identified in the mammalian ovary and have been shown to play a role in ovulation, steroid secretion and follicular development in the rodent. Recently there is presumptive evidence for the secretion of neurotrophins and the presence of their receptors in human cumulus cells consistent with identification of TrKB receptors in the occyte **(Seifer et al., 2006).**

Kawamura et al. (2005) demonstrated that BDNF acts on its receptor TrKB in the occyte to enhance first polar body exclusion and to increase the competence of occytes for development into early embryo in the light of the very particular role of this neurotrophin and of the fact that BDNF easily crosses the blood brain barrier and can be measured in plasma **(Pan, 1998).** For the first time in the literature we developed a non invasive technique to demonstrate BDNF production by the endometrium i.e of uterine flushing. The non invasive technique eliminated the stress. It is known that BDNF expression increase under stress. **Ledee et al. (2002)** used uterine flushing fluid method to study concentration of leukemia inhibiting factor (LIF) and found that it is highly predictive of embryo implantation. A statistically significant decrease of BDNF in uterine flushing of repeated (unexplained failures ICSI) was presented in comparable to control $P < 0.05$. This means that there is production of BDNF from the endometrium and it is one of the factors that contribute to the receptivity of the endometrium. All cases in (group I) were subjected to preimplantation diagnosis.

In unpublished work we demonstrated statistically significant decrease of BDNF in the endometrium of repeated implantation failure compared to control. **Kawammura et al. (2007)** demonstrated the expression of BDNF and TrKB in preimplantation embryo and pregnant oviducts and uteri of mice and they demonstrated paracrine and potential autocrine effect of the BDNF / TrKB signaling system in the development of implantation embryos. **Kawamura (2007)** found that the development of cultured preimplantation embryos from the two cell stage to expanded and hatched blastocyst stages was promoted by treatment with BDNF.

The effect of BDNF to promote blastocyst development is correlated with its ability to inhibit apoptosis and increase total cell numbers in blastocepts **(Raap et al., 2005).** Kawamura et al, 2007 found that both the BDNF promotion of blastocyst development

and the suppression of embryonic cell apoptosis were blocked by cotreatment with K252a.

Zamzam water stimulate BDNF in group I (mean ±SD before zamzam intake was 177.7±68.78 comparable to 249.1±63.14 after zamzam intake $P<0.05$ this means that zamzam water is a trophic to BDNF, but in group II there was an increase but this increase was not statistically significant. To the best of our knowledge this is the first report in the world literature to explore the effect of zamzam on BDNF.

CONCLUSION

We demonstrated for the first time the production of BDNF from the endometrium and its reduced production led to implantation failure demonstrating again a role in endometrial receptivity. The current findings open a new era for endometrial receptivity and treatment of infertility.

Also, we demonstrate if the first time that zamzam water stimulate BDNF in uterine flashing.

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