

First Live Birth after 36 Spontaneous Early Abortion by Intra Uterine Injection of Zamzam Water

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Abstract: Introduction: There are many modalities of treatment of idiopathic repeated spontaneous early abortion but with no results. Zam Zam water is unique in its natural characteristics and it has special optical parameters that are different from those of bottled drinking and distilled waters. In our previous work we found that and that BDNF and Aquaporins are important for endometrial receptivity and Zam Zam water was used in the treatment of repeated implantation failure after ICSI, so the aim of this work is to introduce intrauterine injection of Zam water as a new modality for treatment of idiopathic repeated spontaneous early abortion. **Patient and method:** 39.5 years old female married for 20 years with 36 times repeated spontaneous abortion [6:8 weeks] with no result of all modalities of treatment. Serum and endometrial BDNF and aquaporins were studied using flushing technique, BDNF was measured by ELIZA and aquaporins was measured immunohistochemistry. Steril Zam Zam water was injected intrauterine through embryonal catheter at days 12 and 21 of the cycle, informed consent was taken and data analysis was analyzed by two tailed t test, chi square test was used for comparison a difference of <0.05 was considered significant. **Result:** Statistical significant increase in BDNF and Aquaporins after intrauterine injection of Zam Zam water, pregnancy occurred after the first treatment cycle, spontaneous labour pain occurs after 36 weeks resulting in delivery in a male fetus weighing 2.450 kg. **Conclusion:** Intrauterine injection of Zam Zam water is a new modality of treatment of idiopathic repeated spontaneous early abortion it acts through improvement of endometrial receptivity.

Keywords: Idiopathic repeated spontaneous abortion, uterine flushing, ZamZam water, BDNF, Aquaporins

1 INTRODUCTION:

Idiopathic repeated abortion is frustrating problem facing the gynecologist causing severe psychological upset to the parents, in the literature many modalities of treatment include immunological testing and treatment, allogenic lymphocyte therapy, intra-tubal transfer of zygotes and embryos, blastocyst transfer, sequential embryo transfer, assisted hatching, co-cultures, and preimplantation genetic screening for aneuploidy[1].

Zam Zam water is unique in its natural characteristics as it is hard carbonated water; it has been proven that there are no microbes whatsoever in the water of Zam Zam. Drinking of Zam Zam water stimulate increase lactation[2, 3]. Also it has been concluded that Zam Zam water has special optical parameters that are different than those of bottled drinking and distilled waters. Prophet Muhammad Said [the best water on the surface of the earth is that of Zam Zam, in it there is a food for the hungry and a cure for the ill]. He also Said [The water of Zam Zam is for the purpose for which it is drunk][4]

According to our previous work we found that Zam Zam water stimulate brain derived neurotrophic factor [BDNF] in uterine flushing and endometrial aquaporins in uterine flushing of repeated implantation failure after intracytoplasmic sperm injection. So the aim of this work is to introduce intra-uterine injection of Zam Zam water in idiopathic repeated early abortion[5].

2 PATIENT AND METHOD:

39.5 years old female Married for 20 years with repeated abortion for 36 times; 20 times of them at the 6th week and 16 times at the 8th week. Body weight 77 kg The following investigations appeared normal: 3D ultrasound, lipidol hysterosalpingography, all the hormonal profile, FSH, LH, E2, Prolactin, ACTH, cortisol, TSH, T3, T4, AMH, laparoscopy, hysteroscopy revealed no abnormality detected, genetic studies for both couples and the product of conception revealed no abnormality, all the immunological investigations, Preimplantation genetic diagnosis [PGD] and thrombophilia testing revealed no abnormalities. The method of treatment rank from repeated intracytoplasmic sperm injection [ICSI] sequential embryo transfer, immunological therapy, intra-uterine injection of autologous peripheral blood mononuclear cells and intra-uterine injection of autologous eye tears. 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

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clear supernatant fraction was stored at -80° until the assays. Brain-derived neurotrophic factor levels were determined [BDNF assay] using the commercially available BDNF Emax immunoassay system [Promega Corp., Madison, WI]. The ELISAs were performed according to the manufacturer's protocol. Immunohistochemistry for detection of AQP2, AQP3, AQP4, AQP7, AQP8, AQP9, AQP10, were done.

Zam Zam well is located in the holiest mosque of the Muslims in the city of Makkah which is in the western province of the Kingdom of Saudi Arabia. The well is four thousand years old, millions of Muslims drink this water as sacred water especially during pilgrimage and Umrah each year. Sterilization of Zam Zam water was done as usual, Zam Zam water was injected twice; first injection in the 12th day of the cycle and the second injection in the mid luteal phase [day 21] by embryo transfer catheter where 5ml of Zam Zam water was injected in each time.

3 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 the patient after explaining the technique to the patient and her husband.

4 DATA ANALYSES

BDNF and Aquaporins were measured in both serum and in uterine flushing before and after the injection of Zam Zam water. Difference between BDNF before Zam Zam 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.

5 RESULT:

TABLE 1 REPRESENT SERUM BDNF BEFORE AND AFTER ZAM ZAM WATER INJECTION.

	Before injection	After injection	P value
BDNF	192.2	193.7	> .005

Statistically not significant

TABLE 2 REPRESENT BDNF IN UTERINE FLUSHING BEFORE AND AFTER ZAM ZAM WATER INJECTION.

	Before injection	After injection	P value
BDNF	177.2	369.4	< 0.0001

Statistically highly significant

TABLE 3 REPRESENT STAINING INTENSITY OF AQUAPORINS IN UTERINE FLUSHING BEFORE AND AFTER ZAM ZAM WATER INJECTION.

AQP	Before injection	After injection	P value
2	2	5	< 0.05
3	2	5	< 0.05
4	3	6	< 0.05
7	-	2	
8	4	4	> 0.05
9	-	3	
10	-	2	

In this table there was statistically significant increase in AQP 2,3 and 4 after Zam Zam water injection, Zam Zam water stimulated AQP 7, 9 and 10, there's no change in AQP 8 after Zam Zam water injection.

Pregnancy occurred in the first cycle after intra-uterine injection of Zam Zam water, Pregnancy passed smooth till 36 weeks, uterine contractions started and caesarian section was done and delivery of a male living baby weighting 2.450 kg.

6 DISCUSSION:

Idiopathic repeated abortion is frustrating problem facing the gynecologist causing severe psychological upset to the parents, although many modalities appeared in the literature yet no effect appeared to our patient.

Zam Zam well is located in the holiest mosque of the Muslims in the city of Makkah which is in the western province of the Kingdom of Saudi Arabia. The well is four thousand years old, millions of Muslims drink this water as sacred water especially during pilgrimage and Umrah each year, a total 34 elements have been found with calcium, magnesium, Sodium and chloride, fluorides in the highest concentrations [5]. Zam Zam is unique in its natural characteristics as it is hard carbonated water; it has been proven that there are no microbes whatsoever in the water of Zam Zam. Drinking of Zam Zam water stimulate increase lactation [2,3]. Also it has been concluded that Zam Zam water has special optical parameters that are different than those of bottled drinking and distilled waters. Prophet Muhammad Said [the best water on the surface of the earth is that of Zam Zam, in it there is a food for the hungry and a cure for the ill]. He also Said [The water of Zam Zam is for the purpose for which it is drunk] [4]. The effect of Zam Zam water is due to the special character of this water due to its peculiar nature which was discovered by radioimmunoassay, nanotechnology, laser femto, crystalline electromicroscopy, specific refractive index, number single oscillator, specific dispersing of optical parameters assay, Abbe number of Zam Zam water one completely different from other types of water [2,3,5,6,7].

The transmembrane ATP-binding cassette [ABC]

transporters actively efflux an array of clinically relevant compounds across biological barriers, and modulate biodistribution of many physiological and pharmacological factors. Over 48 ABC transporters have been identified and shown to be directly and indirectly involved in peri-implantation events and fetal/placental development[8]. It has been shown that ZamZam water stimulate the ABC, The most well-described ABC transporters in peri-implantation embryos and endometrium are P-gp and BCRP which ZamZam water stimulates its increase.

According to our previous work that Zam Zam water stimulate BDNF and Aquaporins in uterine flushing in repeated implantation failure this stimulate us to use ZamZam water in this work. In this work we found no difference in the serum BDNF and Aquaporins before and after treatment, but we found statistically highly significant increase in BDNF in uterine flushing after using Zam Zam water, regarding Aquaporins we found increase in AQP 2,3 and 4 after Zam Zam water injection, Zam Zam water stimulated AQP 7, 9 and 10, there's no change in AQP 8 after Zam Zam water injection. Brain derived neurotrophic 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 [9] 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[10] they also play important roles in non-neuronal tissues [11] 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 oocyte [12] and in endometrial flushing in unexplained infertility. This means that Zam Zam water improved endometrial receptivity through local production of BDNF from the endometrium. Again we demonstrated in previous work decreased BDNF in the follicular fluid in an unexplained infertility.

BDNF was found to play an important autocrine/ paracrine role during implantation and during placental growth and development as well as fetal growth by increase trophoblastic cells growth and survival, also BDNF promoted blastocyst outgrowth and as the outgrowth of the trophoblastic cells from blastocyst is believed to reflect the proper differentiation of the embryo important for trophoblastic invasion of endometrial stroma during implantation, meanwhile BDNF suppresses embryo apoptosis, BDNF 30mg/ml during in vitro maturation [IVM] in porcine oocyte significantly increase first polar

body extrusion and glutathione level in the oocyte, this will lead to increase developmental competence of oocyte to the blastocyst stage and somatic nuclear transfer [SCNT]

BDNF acts on its receptor TrkB in the oocyte to enhance first polar body exclusion and to increase the competence of oocytes 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 [13,14]. 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[15] 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 incomparable 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.

Aquaporins are water selective membrane proteins active in tissues with high water transport. The first water channel was identified in human erythrocytes in 1992 [AQP1], the AQPs are a family of small 25-34 kDa hydrophobic integral membrane channel protein that facilitate, rapid passive movement of water. AQPs may play an important role in reabsorption of luminal fluid and the antimesometrial positioning of the blastocyst [16]. It was found that AQP2 expressed at midsecretory phase suggesting that AQP2 might play a physiological role in the receptivities of human uterus[17]. AQP 1, 4, 5 are significantly expressed in the peri-implantation uterus and AQP8-9, mRNA were expressed in the implanting blastocysts in a mouse pregnancy model, previous study by microarray analysis detected a decreased expression of AQP gene in the endometrial implantation window suggesting a possible role of AQPs in implantation window [18,19]. It has been shown that estrogen is involved in the up regulation of AQP2 in the mouse uterus [20], in human the role of progesterone also included [16], but the continuous increase in the AQP2 during the luteal phase [21] suggests that other factors may be involved in this regulation so we can reach to the point that aquaporin in the endometrium involved in the endometrial receptivity and it should be one of the measurement of this receptivity [22,23,24]. In our previous work we demonstrated for the first time in the literature that ZamZam water had an effect on endometrial AQPs, we demonstrated that aquaporins 2,3 and 4 expression were increased after ZamZam water injection. This increase was statistically significant. There was stimulation of expression of other types of AQP 7, 9, 10. No change regarding

AQP8. These data showed the effect of ZamZam water in the endometrial receptivity [25].

Added to this according to our previous work The supposed mechanism by which Zam Zam water is used in the treatment of implantation failure [26,27,28] are [stimulation of: endometrial prolactin, Alpha and beta defensin, LIF, endometrial VEGF, an angioprotein receptors, IL8, proteases, Aquaporine 2, expression of monoamine oxidase A in the endometrium, L-Selectin Ligand MECA – 79], stimulate of inflammation of endometrium, evoking favourable inflammatory reaction in the uterine cavity. In addition Zam Zam water cause upregulation of gap junction inter cellular communication and connexin 43 in endometrium. Recently, we demonstrated that it stimulate stem cells differentiation in the endometrium, again the high calcium and Magnesium content is important for many biochemical processes in the endometrium, and acting as coenzyme. And formation of immunoglobulin,] also Zam Zam stimulate the formation of implantation windows through stimulation of aquaporine its high content of fluorides contributed to its strong antimicrobial action. We should not forget the psychological impact. And reassurance and self-satisfaction of the patient while using Zam Zam water [29,30,31].

7 CONCLUSION:

ZamZam water is a new modality of treatment of idiopathic early repeated abortion through many mechanisms. It has no side effect and it stimulates other biological endometrial function but, this is a case report and many cases and randomization are needed before the elucidation of this line of treatment in idiopathic repeated abortion, again the cost benefit ratio is very satisfactory.

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