



The Impact of Hyaluronic Acid Endometrial Wash on Transforming Growth Factor Serum Level and Pregnancy Outcome in ICSI

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ABSTRACT

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Fertility is a fundamental function of reproductive health that is under the control of different hormones, cytokines, and growth factors. Transforming growth factor superfamily is a group of cytokines necessary for blastocyst implantation as they regulate endometrial stromal cell proliferation, and promote embryonic adherence to the receptive endometrium. Hyaluronic acid molecule (HA) is a long, unbranched polysaccharide that plays a delicate function in reproduction. Hyaluronic acid regulates gametogenesis, folliculogenesis, and implantation, thereby was proposed to improve the rate of successful pregnancy. This study is a prospective clinical trial that includes 80 infertile Iraqi women aged (18-40 years) scheduled for fresh ICSI program. The study sample had been divided into 2 groups based on the type of intervention performed on the day of oocytes retrieval. The Hyaluronic acid group and the control group. For each participant, twice measurement of the serum level of transforming growth factor were done, the first measure was done on day of oocytes retrieval, while the second measure was performed on day of embryo transfer. A comparative statistic was made to examine the effect of hyaluronic acid intrauterine wash on the serum level of transforming growth factor and pregnancy rate. Hyaluronic acid intrauterine wash exerts a significant reduction in transforming growth factor serum level, and a significant improvement in the pregnancy outcome compared to the control group.

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KEYWORD

Hyaluronic Acid, Endometrial Wash, Transforming Growth Factor, Pregnancy Outcome

1. Introduction

Fertility is a fundamental function of reproductive health and a crucial factor determining the growth of the human population [1]. The World Health Organization (WHO) stated infertility as a global health issue, recognizing it as a medical challenge that poses a serious psychological and financial burden for infertile couples [2]. Globally, ICSI success outcomes remain unsatisfactory, with a percentage of (25-30%) live births per cycle; this low percentage is proposed to be partially attributed to the underestimation of the endometrium as an active component of the reproductive process [3]. The endometrium is a dynamic tissue that undergoes delicate cellular, biochemical, and transcriptomic modifications throughout various phases of the menstrual cycle, controlling the endometrial molecular milieu [4,5]. Endometrial cell decidualization encompasses different biological functions, including cellular proliferation and differentiation, immune system modulation, vascularization, transcription regulation, and tissue remodeling [6]. All these biological activities are governed by the maternal system via different transcription factors that facilitate the conversion of the endometrium to be receptive [7,8]. During decidualization, the delicate interplay between the decidualized endometrial cells and the maternal immune cells leads to the production of a cascade of pro-inflammatory mediators and growth factors like transforming growth factor (TGF), epidermal growth factor (EGF), tumor necrosis factors, and cytokines [5]. However, the endometrial pro-inflammatory environment during decidualization is a temporally well-controlled phase limited to the window of implantation, when the decidualization process transitions from a pro-inflammatory profile to an anti-inflammatory one to safeguard the semi-allogeneic developing embryo from the maternal immune reaction [8]. Endometrial receptivity is the period of endometrial maturation when the blastocyst adheres to the endometrial epithelium and then

invades the endometrial stroma and blood vessels [9]. Defective endometrial receptivity leads to implantation failure and impaired ICSI outcome [9]. However, endometrial receptivity and selectivity are distinct but complementary concepts introduced to define the endometrium as a biosensor of embryo quality [10]. Selectivity is an inherent, well-organized function of the endometrium that identifies and rejects the embryo with aberrant developmental potential. While receptivity enables the endometrium to offer an optimal milieu for embryonic development and placental formation [11].

The implantation window (WOI) is a crucial, precise time when a synchronized molecular interaction between a receptive endometrium and a competent embryo is efficiently elaborated ending to a successful pregnancy [12]. WOI is predicted to occur 5-7 days after ovulation, when a meticulously programmed morphological and molecular sequence of events enables the endometrium to be recognized as a receptive endometrium [13]. Scientific evidence highlights the importance of Cytokines in organizing successful pregnancy through regulating the maternal-embryonic interaction during implantation, controlling immune alteration, tissue remodeling, embryonic attachment to endometrial epithelium, and placental development [14]. The transforming growth factor beta (TGF- β) superfamily is a multifunctional group of cytokines involved in different biological processes, including cellular proliferation and differentiation (Wen et al., 2023). TGF- β superfamily includes various isoforms (TGF- β 1, TGF- β 2, and TGF- β 3), with TGF- β 1 being the most abundant one [15]. Scientific evidences emphasis the role of TGF- β 1 in preserving the immunological equilibrium, organizing tissue development, and in regulating vasculogenesis of the embryo, and placental [15]. Transforming growth factor β is secreted as an inactive complex, which is activated by integrin subunit beta 8 [15]. TGF- β exert their functions through

Smad and non-Smad pathways. The Smad pathway involves ligand-receptor interaction, activating type II receptors through phosphorylation and interacting with intracellular receptor-regulated SMADs, regulating gene transcription [16]. In reproduction, TGF- β 1 plays a vital role in signaling various reproductive processes, including folliculogenesis and ovulation, facilitating the maternal- embryonic crosstalk [17]. During the implantation window, the endometrial cells exhibited a highly delicate equilibration between cellular proliferation and apoptosis. Scientists proposed that TGF- β 1 isoforms serve a dual function in controlling the balance between cellular apoptosis and proliferation, highlighting TGF- β 1's significance in regulating decidualization and in promoting successful pregnancy [18]. Moreover, the effect of TGF- β isoforms on placentation has been thoroughly discussed. scientific evidences support that TGF- β 1 and TGF- β 2 control the invasion of human extravillous cytotrophoblast cells into the endometrial epithelium and into the spiral arteries to ensure a high-flow, low-resistance vascular system that maintains a steady blood supply to the placenta [19]. TGF- β is also reported to be an essential component in the regulation of immune cells and in the maintenance of immunological homeostasis necessary to modulate the maternal immune system tolerance to the semi- allogenic embryo [15]. Hyaluronic acid (HA) is a long, unbranched polysaccharide made up of repeating D-glucuronic and N-acetyl-D-glucosamine disaccharides [20]. In 1934, Karl Meyer and John Palmer first extracted HA from the vitreous body of the bovine eye; Laurent later described its structure, and Balzar introduced hyaluronan as a substitute for the word hyaluronic acid. Hyaluronic acid is a negatively charged, highly hydrophilic molecule that forms a viscous network that is distributed throughout body tissue and extracellular matrix, being most abundant in the eye vitreous body and umbilical cord Wharton's jelly [20,21]. The physicochemical properties allow HA to perform different

biological activities like hydration of the extracellular matrix [21], maintenance of tissue homeostasis, and facilitation of cellular adhesion, motility, and proliferation. Moreover, the viscoelastic properties, biocompatibility, and biological efficacy of HA make it an optimal material for medical applications, like in rheumatology and dermatology. Furthermore, the chemical alteration of HA produces biocompatible and biodegradable HA-based biomaterials widely used in regenerative medicine [22]. Hyaluronic acid is produced on the inner surface of the plasma membrane, with a half-life ranging from (12-24) hours in the skin to only a few minutes in the bloodstream. In the blood, it is catabolized inside the lymph nodes and liver, while in body tissues, it is degraded by hyaluronidase enzymes [22]. The equilibrium between HA synthesis and degradation controls its molecular weight and properties. Variant molecular sizes can exhibit opposing biological activities, such as high molecular weight HA poses anti-inflammatory effects, while low molecular weight HA exerts pro-inflammatory effects that promote angiogenesis and tissue remodeling [23]. Hyaluronic acid (HA) exerts its effect after binding to its receptors known as hyaladherins; among these receptors are CD44 and RHAMM, which are considered the primary surface membrane HA receptors[24]. After interaction with cellular surface hyaladherins, HA activates the intracellular signaling pathways associated with cellular proliferation, differentiation, and motility, in addition to degradation of the HA molecule itself [25]. Hyaluronic acid has an important and delicate function in the reproductive system; HA plays a pivotal role in gametogenesis, in folliculogenesis, and in implantation by controlling embryonic adhesion and communication with the uterine cells, thereby enhancing the rate of successful pregnancy [26]. In recent years, HA interaction with the endometrium has gained growing interest, highlighting its biological functions and potential therapeutic uses in reproductive medicine [20]. Scientific evidences stated that HA

receptors are expressed in a spatiotemporal manner according to the phases of the menstrual cycle and, more specifically, during the implantation window, proposing that the endometrium is most receptive to embryonic implantation during the peak staining of HA receptors [27]. Published literature has declared that intrauterine HA serve a vital function in regulating embryonic adhesion and in promoting maternal vessel invasion for placental development [27]. Nevertheless, ethical considerations regarding the examination of human embryos render the function of HA post-implantation in humans undefined, with existing evidence only obtained from animal models [26].

2. Patients and Methods

This study is a prospective comparative clinical trial that involved 80 randomly selected infertile Iraqi females aged 18-40 years and scheduled for a fresh ICSI program. The study was held at the High Institute for Infertility Diagnosis and Assisted Reproductive Technologies of Al-Naharin University, from September 2023 to April 2025. Informed consent was obtained from all the participants. The research was approved by the Local Medical Ethics Committee of the High Institute for Infertility Diagnosis and Assisted Reproductive Technologies/ Al-Nahrain University. Morphological assessment of the oocytes and embryos was done in the specialized ICSI laboratory of the High Institute for Infertility Diagnosis and Assisted Reproductive Technologies. TGF serum level was measured in a private laboratory. The study sample was divided into 2 groups based on the type of intervention performed on the day of oocyte retrieval. The Hyaluronic acid group (40 patients) was treated with HA endometrial wash, and the control group (40 patients) did not receive any intervention for their endometrium. Inclusion criteria include women aged (18-40) years, infertile females planned for GnRH antagonist protocol and fresh ICSI cycle, and infertile females prescribed dual stimulation (GnRH agonist plus Ovetrelle)

for ovulation triggering .While the exclusion criteria are : poor responders infertile females diagnosed according to Bologna criteria, infertile patients treated with ICSI other than GnRH antagonist protocol, infertile Females planned for frozen cycle, infertile females diagnosed with endocrine disease, infertile Females diagnosed with thrombocytopenia, infertile Females diagnosed with uterine pathology or hydrosalpingis. For each participant in the study, a comprehensive history, physical examination, and basal serum hormonal assessment were performed on the third day of the menstrual cycle (CD3). Controlled ovarian hyperstimulation (COH) was initiated using the flexible antagonist protocol, starting from CD3 with a daily dose of 150-450 IU r-FSH (Gonal F), then follicular growth was monitored using transvaginal ultrasound. When leading follicles reached 13-14 mm in size, a GnRH antagonist was administered (Cetrotide, 0.25 mg subcutaneous injection), and when three or more follicles reached 18 mm in diameter, the final oocyte maturation and ovulation triggering was performed using the dual trigger (GnRH agonist decapeptyl 0.2 mg plus HCG (Ovitrelle® 250 microgram). After 35-36 hours of ovulation trigger, oocyte retrieval was completed under transvaginal ultrasound guidance. To measure the serum level of TGF, a venous blood sample (5 ml) was withdrawn from each patient on the day of oocyte retrieval and on the day of embryo transfer; then the blood sample was kept in a dry sterile plain tube and was allowed to coagulate for 30 minutes at room temperature before being centrifuged at 5000 rpm for 10min. The supernatant serum was aspirated and put in a 0.5 mL Eppendorf tube to be stored in the deep freezer at -20 °C for subsequent analysis. Serum TGF level was measured using a commercially available human TGF kit, which uses enzyme – linked immune sorbent assay (ELISA) based on biotin double antibody sandwich technology

3. Statistical analysis

The study data were introduced into SPSS version 24 statistical software program. Descriptive statistics were shown using graphs as well as tables (mean $\pm$ standard deviation, frequency and percentage). The chi-square test was used to test the significance of the association between related categorical data. Independent two-sampled (T test) was used to investigate the significance of differences between means of related numerical data. A p-value < 0.05 was considered as a discrimination cutoff point of significance.

4. Results

General characteristics of the study sample are presented in Table 1. The mean age of the study participants showed no significant statistical difference between the study groups (p- value = 0.45). Similarly, no significant difference had been found in the mean of BMI and duration of infertility, with the p-value (0.069, 0.67) respectively. Table (2) showed the comparison of the basal hormonal levels performed on the third day of the menstrual cycle (CD3); no significant variation had been found between the HA and the control group, including all the measured hormones, with p- value ≥ 0.05 for all. The statistical analysis stated no significant differences in terms of the number and grading of the retrieved oocytes between the studied groups ($P \geq 0.05$). Similarly, no statistically significant difference was found in the number of embryos and the number of transferred embryos between HA and the control groups ($P \geq 0.05$).

Table (1): Sociodemographic characteristics of the study sample

Variables	Group	N	Mean	SD	P- Value
Age	Hyaluronic	40	29.40	5.777	0.276
	Control	40	30.43	5.139	
BMI	Hyaluronic	40	27.20	2.514	0.069
	Control	40	27.53	1.948	
Duration Of Infertility	Hyaluronic	40	7.13	4.636	0.67
	Control	40	6.43	3.713	

Independent t- test was used, SD: standard deviation, BMI= body mass index, p- value < 0.05 is considered significant.

Table (2): Basal Hormonal Level Of The Study Sample

Hormones Serum Level	Group	N	Mean	SD	P-Value
FSH (IU/L)	Hyaluronic	40	6.6890	2.06785	0.66
	Control	40	6.2853	1.91932	
LH (IU/L)	Hyaluronic	40	4.7755	2.10470	0.05
	Control	40	3.9273	1.28457	
PRL (µg/L)	Hyaluronic	39	18.7031	7.69501	0.64
	Control	40	15.0833	7.43023	
TSH(mIU/L)	Hyaluronic	40	2.5230	0.99088	0.68
	Control	40	2.7188	0.99479	
E2 CD3 (pg/mL)	Hyaluronic	40	33.6197	13.01699	0.38
	Control	40	28.8683	13.99955	
Cumulative FSH dose (IU)	Hyaluronic	40	1922.5000	755.70056	0.11
	Control	40	1613.1250	637.10049	
E2 on trigger day (pg/mL)	Hyaluronic	40	863.5808	519.23513	0.63
	Control	40	954.0823	420.37490	

Table (3): Clinical Parameters of The Study Sample

Variables	Group	N	Mean	SD	P- Value
Total No. of retrieved oocyte	Hyaluronic	40	10.48	5.038	0.75
	Control	40	10.58	5.542	
No. of (GV) oocytes	Hyaluronic	40	1.38	1.807	0.24
	Control	40	1.40	1.482	
No. of (MI) oocytes	Hyaluronic	40	1.78	1.860	0.85
	Control	40	2.25	1.891	
No. of mature (MII) oocytes	Hyaluronic	40	6.98	3.519	0.18
	Control	40	6.10	3.045	
No. of embryos (G1)	Hyaluronic	40	5.08	2.246	0.82
	Control	40	4.88	2.355	
No. of transferred embryos (G1)	Hyaluronic	40	2.33	0.572	0.78
	Control	40	2.30	0.564	

Independent t-test was used; SD: standard deviation, GV: germinal vesicle, G1: grade 1, MI: metaphase I, MII: metaphase II, p- value < 0.05 is considered significant.

Transforming growth factor serum level showed no significant difference between HA and the control group on the day of oocyte retrieval (before the intervention). However, on the day of embryo transfer (after the intervention) TGF serum level exhibited a significant difference between the HA and the control group (p- value 0.002). Moreover, data demonstrated that TGF serum level was significantly decreased on the day of embryo transfer compared to the control group in which TGF serum level was increased (table4).

Table (4): Comparison Of Transforming Growth Factor Serum Level Among Study Groups

TGF Serum Level	Group	N	Mean	SD	P- Value
TGF At Day of Oocyte Retrieval	Hyaluronic	40	171.23	59.10	0.668
	Control	40	141.04	70.43	
TGF At Day Of Embryo Transfer	Hyaluronic	40	92.99	44.50	0.002
	Control	40	166.43	79.18	

Independent t-test was used; SD: standard deviation; TGF: transforming Growth Factor

The pregnancy rate (ICSI outcome) was significantly higher in the HA group with 50% compared to the control group with only 25% (P-value 0.001) (Table 5).

Table (5): The Association between ICSI Outcome and the Study Groups

ICSI Outcome		Group		Total	p- value
		Hyaluronic	Control		
Biochemical pregnancy	Yes	20 (50%)	10 (25%)	30	0.001
	No	20 (50%)	30 (75%)	50	

Chi- square test was used; p-value < 0.05 is considered significant.

5. Discussion

Scientific evidence recognized age, BMI, and the basal serum hormone levels as independent factors affecting infertility and thereby pregnancy outcome in ICSI [28,29]. In the present research, no significant differences were found in these parameters between HA and the control groups, thereby obliterating the bias that might affect the results of this study.

If the difference was significant. From another perspective, scientists declared that the embryos might influence the TGF- β signaling pathway via 2 mechanisms: first, the embryo has the capability to secrete TGF- β that binds to specific receptors on endometrial cells, and second, the blastocyst stimulates endometrial TGF- β activation and expression [18,30]. In the present study, data demonstrated that the difference in the total cumulative dose of rFSH, total number of retrieved oocytes, total number of embryos, and the number of transferred embryos was insignificant, reflecting that the pregnancy rate in the study groups was not affected by these parameters. The statistical analysis of the current study revealed a significant difference in the mean serum level of TGF in patients in the HA group compared to the control group. Moreover, in the HA group, the serum level of TGF was noticed to be significantly lower on the day of embryo transfer (after the intervention) than its level before the intervention, in contrast to the control group, where the TGF serum level was insignificantly higher than its level after the intervention.

Recently, interest in the correlation between TGF and pregnancy rate has been increasing. Researches emphasis TGF- β 1 role in embryonic implantation, immune tolerance control, and placental development. However, the effect of TGF- β 1 on decidualization remains controversial, with some studies hypothesizing a stimulatory effect, while other literature claims that the exposure of human endometrial stroma cells to TGF- β 1 would decrease the expression of growth factors in those cells, leading to inhibition of decidualization [15].

From that point of view, scientific evidence explains that the delicate balance between cellular apoptosis and proliferation at the site of implantation in the endometrium is paramount for normal embryonic implantation. Scientists investigated the dual role of TGF- β 1 in regulating cellular apoptosis and proliferation during implantation through three mechanisms: first, variations in TGF isoforms,

second, cell regulation of receptor types, and third, the presence of antagonist molecules [15]. Marhemati et al. (2020)³¹ explained that functional polymorphisms in the TGF- β 1 gene reduce its expression, which subsequently decreases the secretion of proteolytic enzymes necessary for penetration of the trophoblast into the receptive endometrium. Similarly, Fang et al. (2020)¹⁹ stated that abnormalities in BMP signaling components, including the TGF- β 1 signaling pathway, lead to defective decidualization ending with female infertility. From another point of view, TGF- β 1 was proposed to be a key regulator of endometrial vascular maturation. Lu et al. (2021)³² correlated TGF- β 1 with certain gynecological disorders, as the level of TGF- β 1 was noticed to increase in the endometrium of females with a history of recurrent pregnancy loss, while TGF- β 1 level was reduced in patients complaining of heavy menstrual bleeding. In line with the earlier study, Martin et al. (2021)³³ declared an increased level of TGF- β 1 with pre-eclampsia and a decreased level in female cases presenting with a history of repeated miscarriage, referring to these conditions to an assumed defective immune tolerance caused by disruption of normal TGF- β 1.

Nevertheless, scientist highlighted the importance of HA use as a scaffold to increase the delivery of growth factors, enhancing their action [34]. Martin et al. (2021)³³ studied the effect of an HA scaffold that assists the migration of TGF, planned to augment cartilage repair; results displayed a promising augmentation in TGF competence in tissue regeneration. Taskan et al. (2021)³⁵ conducted an experimental study on an animal model, revealing that applying HA gel to a wound treated with a full-thickness flap improved its healing process. The optimistic role of HA on the pregnancy rate in ICSI cycles, as observed in this study, might be attributed to its function as a scaffold for attracting and enhancing the function of growth factors essential in optimizing endometrial receptivity, embryonic development and facilitating the fruitful endometrial- embryonic cross-talk.

However, arguments have been raised about the side effects of HA therapy on cellular growth. Santo et al. (2019)³⁶ argued that the stimulatory effect of HA on the growth factors might lead to endometrial hyperplasia. Lin et al. (2022)³⁷ defended that issue as they declared that the uterine HA natural half-life is estimated to be (1–2) days, and that the natural secretion of endometrial hyaluronidase enzyme makes HA a safe therapy for the human endometrium. Additionally, De-Maria and colleagues (2025)³⁸ stated that the use of HA as a prodrug in therapeutic angiogenesis was considered a safe and effective treatment, as it solely activated endothelial cell proliferation without affecting other cellular metabolic processes, e.g., collagen synthesis and cellular proliferation. Moreover, Abudukeyoumu et al. (2020)³⁴ explained that the endometrial TGF- β isoforms play a vital role in regulating the function of immune cells, which is considered the cornerstone in modulating tissue remodeling and ensuring a balance between immune tolerance and protection throughout pregnancy. High intra-uterine HA concentrations during implantation support embryonic adherence to endometrial epithelial cells, according to studies done by Ruane et al. (2020)³⁹ and Rashki et al. (2024)²⁶, facilitating receptor interaction on both surfaces. Additionally, the use of HA as an embryo glue was stated to increase the pregnancy rate in ICSI, as rendering it a promoting factor for embryonic development and as a stimulating molecule for successful embryonic-endometrial crosstalk [40]. The integral function of HA in reproductive biology is gaining significant importance for optimizing assisted reproductive technology outcomes. Scientists highlighted the vital impact of HA in addressing a wide range of reproductive disorders that cause infertility. Nonetheless, the function of HA in reproduction necessitates more elucidation to provide innovative prospects for addressing female and male infertility.

6. Conclusion

This study demonstrated that the administration of monosodium glutamate at a dose of 0.5 g during pregnancy resulted in a significant reduction in birth rate, offspring number, and body weight. Additionally, significant decreases were observed in the weights of the reproductive organs, as well as in the diameters of the ovaries and oviducts. Furthermore, MSG exposure showed a suppressive effect on reproductive hormones, including FSH, LH, and E2.

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Author Contribution

Ghada Bassim Raaf and Manal Taha Al-Obaidi performed the study; Lubna A. Al-Anbari supervised the work.

Conflict of Interest

The authors declare no conflict of interest .

Ethical Clearance

The Ethical Approval Committee approved the study.

Financial Disclosure

There is no financial disclosure.

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