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Research Article

The impact of Heparin Binding - Epidermal growth factor-like growth factor (HB-EGF) on oocyte quality in women undergoing ICSI and its potential contribution to environmental diversity

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Abstract

Oocyte quality refers to the health and developmental potential of an egg cell (oocyte) in the context of reproductive processes. Oocyte quality is a critical factor in successful fertilization and subsequent embryo development. Heparin-binding EGF-like growth factor is known by its acronym, HB-EGF. It belongs to the protein family known as epidermal growth factor (EGF). Cell-signalling protein HB-EGF is involved in proliferation, differentiation, growth, and tissue repair among other physiological activities. In Reproductive Biology: HB-EGF has been studied in the context of reproductive biology, particularly in processes related to fertility and embryonic development. For example, it has been investigated for its role in improving blastocyst development in assisted reproductive technologies. This study aimed to analyze the correlation between follicular fluid and serum HB-EGF with oocyte quality, 44 women with Polycystic ovary syndrome (PCOS) and unexplained infertility underwent IVF cycle in the form of intra-cytoplasm sperm injection ICSI enrolled in the study, follicular fluid and serum were collected from patients at oocyte retrieval day, HB-EGF levels measured by ELISA. From 44 women, HB-EGF has no correlation with all oocyte characteristics *p-value* >0.05 except with damaged oocyte number, follicular fluid, and serum HB-EGF had significant positive correlation *p-value*= 0.428,0.357 respectively. An article found that follicular fluid and serum HB-EGF had a positive correlation with the fragile oocyte.

Keywords: HB-EGF, ICSI, oocyte quality

Introduction

Oocyte quality, which is vital to the process of fertilization and the subsequent development of the embryo, is perhaps the most significant factor limiting female fertility (Sciorio et al., 2022). This is the biochemical and molecular condition that permits a fertilized mature ovum to grow into an embryo, which, upon transfer, will allow for the embryo's healthy development to term. Accordingly, low-quality oocytes might cause spontaneous miscarriage, polyspermy, or a stop in the development of the embryo. It is also increasingly thought that oocyte developmental potential mediates the environmental elements that programmed the development of embryos and fetuses (Gilchrist et al., 2008). Aspects like the ability for progression through meiotic maturation, fertilization, appropriate embryonic development, and successful pregnancy are what determine the quality of oocyte. These characteristics are attained by the interaction between granulose cells (GCs) and theca during follicular development. (Nikbakht et al., 2021). oocyte degradation is a typical occurrence encountered by all technicians who do ICSI. ICSI technicians may lose oocytes due to degeneration, and this is a problem that has been believed to be universal throughout the field. When the oolemma is exceedingly weak, the oocyte degenerates rapidly and the membrane breaks suddenly during ICSI. A significant incidence of oocyte degradation after ICSI is seen in patients whose recovered oocytes all contain the weak oolemma (Mizobe et al., 2016), Oocyte degradation is a problem that has received little attention from researchers. Oocyte degeneration during ICSI has been linked to mechanical factors such abrupt and/or difficult oolemma rupture and the lack of persistence of the funnel following removal of the injection pipette. Viscosity of oocyte cytoplasm and oolemma fragility are two oocyte variables (Hu et al. 2021). The epidermal growth factor (EGF) family includes a protein called heparin-binding epidermal growth factor (HB-EGF), which has several roles in the body (Raab and Klagsbrun, 1997; Ozbilgin et al., 2015), There are two forms of HB-EGF: tmHB-EGF, which is an insoluble transmembrane form, and sol HB-EGF, a soluble paracrine form that is produced when tmHB-EGF is broken down by proteases(Martin et al., 1998), HB-EGF binds to and activates the transmembrane receptors HER1/ErbB-1/EGFR and HER4/ErbB-4, which are located in the human embryo, fallopian tube, and endometrium (Sun et al., 2006). HB-EGF functions in both groups as a strong cytoprotective agent, a modulator of trophoblast invasion, and a decidualization factor (Ozbilgin et al., 2015), The rat ovary showed that HB-EGF was significantly expressed in the germ cells of primordial and primary follicles, but this expression

seemed to weaken as the follicles developed and eventually disappeared in preovulatory follicles. This suggests that HB-EGF down-regulation may be necessary for the maturation of the follicles. Subsequent investigation revealed that ovarian cancer patients' blood and peritoneal fluid had higher levels of HB-EGF, and that blocking this protein inhibited the formation of tumors. (Huang et al., 2022) In this study have tried to determine if serum HB-EGF and follicular fluid contribute to the oocyte's fragility.

Material and methods

This cross-sectional study included forty-four infertile women, aged 18 to 42, who were treated with in vitro fertilisation (ICSI) at the High Institution for Infertility Diagnosis and Assisted Reproductive Techniques Al-Nahrain University in Iraq between October 2022 and April 2023. The women had either polycystic ovarian syndrome or were infertile for unknown reasons. The Local Medical Ethical Committee of Al-Nahrain University's High Institution for Infertility Diagnosis and Assisted Reproductive Techniques authorised the research, and the patients gave their consent to take part in it. The study excluded women facing infertility attributed to polycystic ovary syndrome, as well as those with unexplained infertility. Additionally, women who had undergone a unilateral oophorectomy, individuals aged 43 years and older, and those with infertility linked to thyroid disorders, autoimmune diseases, endometriosis, hydrosalpinx, or adenomyosis were not considered in the research. Ethical issues are a crucial aspect to be taken into account while doing any form of research or analysis. These considerations include the examination Informed permission was obtained from all subjects included in the research, and the study design received approval from the relevant ethical review boards. All patients provided written informed permission for their treatment and the description of their results.

Stimulating the ovaries and retrieving oocytes

Every ICSI patient followed the gynecologist's advice and underwent an ovarian hyperstimulation protocol. On the second day of the menstrual cycle, gonadotropin (r-FSH), which has 75 IU of FSH activity per ampoule, is administered. The patient's age, body mass index (BMI), and prior response to ovulation induction were taken into consideration while modifying the dose. Follicle size was assessed by ultrasonography and serum E2 levels. At 14 mm in diameter, the follicles were treated with a GnRH antagonist to downregulate the pituitary gland. It is necessary to take r-FSH and 0.25 mg of cetrelix acetate daily until the day of the final oocyte maturation trigger. Ultimately, all ICSI patients received a subcutaneous injection of 250

μg = 6500 IU of human chorionic gonadotropin to trigger ovulation after at least two follicles had grown to a size of 16–18 mm. From the day of oocyte retrieval until a pregnancy test was conducted, In the study, every participant underwent progesterone treatment to support the luteal phase, administered as cyclogest ® at a dosage of 200–400 mg transvaginally twice daily. The pregnancy test was conducted between twelve to fourteen days following the luteal phase support, which was consistently maintained throughout the initial twelve weeks of gestation. To confirm pregnancy, a vaginal ultrasound scan was performed six to seven weeks after the embryo transfer (Dashti and Eftekhar, 2021).

Serum Sample

On the day of the oocyte retrieval, five milliliters of blood were extracted from each patient via venous puncture and placed in a dry plain tube. The blood sample was then centrifuged at 3000 rpm for ten minutes after coagulating for thirty minutes at room temperature. The serum supernatant was then aspirated and placed in a micro-centrifuge tube, which was then stored at -20 c for further analysis to measure HB-EGF. Samples of hemolyzed blood were not included in the study.

Follicular fluid sample

Following oocyte pick-up, a follicular fluid sample was obtained and placed in a plain tube. The sample was centrifuged for 10 minutes at 3000 rpm; the supernatant was then separated into aliquots using micro-centrifuge tubes and kept at –20°c for the HB-EGF test. Excessive blood contamination in the sample prevented it from being examined further.

Factors assay Heparin Binding-Epidermal Growth Factor (HB-EGF)

The concentrations of Heparin-binding EGF-like growth factor (HB-EGF) in both serum and follicular fluid were assessed using a commercially available enzyme immunoassay kit specifically designed for human HB-EGF (Human HB-EGF-ELISA KIT). The ELISA kit, identified by the unique number ELK 1090, was procured from ELK Biotechnology CO., Ltd.

ELISA Test principle

This test kit uses sandwich enzyme immunoassay as its test principle. An antibody specific to HB-EGF has already been pre-coated on the micro-titer plate included in this kit. The appropriate microtiter plate wells coated with a biotin-conjugated antibody specific to HB-EGF were filled with standards or samples. Subsequently, each microplate well was filled with Avidin coupled with Horseradish Peroxidase (HRP) and allowed to incubate. Following this, a solution

of Tetramethylbenzidine (TMB) substrate was added. The only wells that changed color were those that included HB-EGF, biotin-conjugated antibody, and enzyme-conjugated Avidin. A sulphuric acid solution was added to stop the enzyme-substrate reaction, and the color change was measured spectrophotometrically at 450 nm ± 10 nm in wavelength. Ultimately, by comparing the samples' Optical Density (OD) to the standard curve, the quantities of HB-EGF in the samples were ascertained.

Statistical analysis

This cross-sectional study's statistical analysis was carried out using Microsoft Excel 2016 and GraphPad Prism version 7. The median and 95% percentile were used to characterise numerical data. Count and percentage were used to characterise categorical data. Whitney-Mann the U test is used to compare two groups. Bellow or equal to 0.05 is the lower limit of a statistically significant difference that is accepted. The correlation between each variable is estimated using the Spearman correlation method (Elliott &Woodward, 2007).

Results

Demographic parameters of the research population's descriptive distribution

The current findings indicate that within the sample of patients, 81.82% (36 out of 44) had primary infertility, while the remaining 18.18% (8 out of 44) experienced secondary infertility, as seen in Figure 1. According to the data shown in Figure 2, it can be seen that out of the total 44 patients, 31 (70.45%) had a negative pregnancy result, while the remaining 13 patients (29.55%) had a good pregnancy outcome.

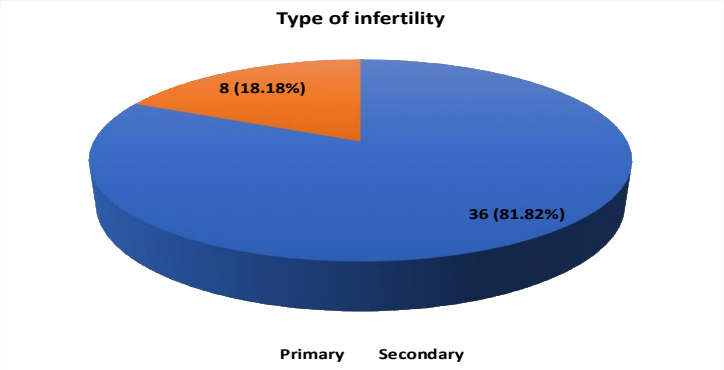

Figure 1. Distribution of study samples by different types of infertility

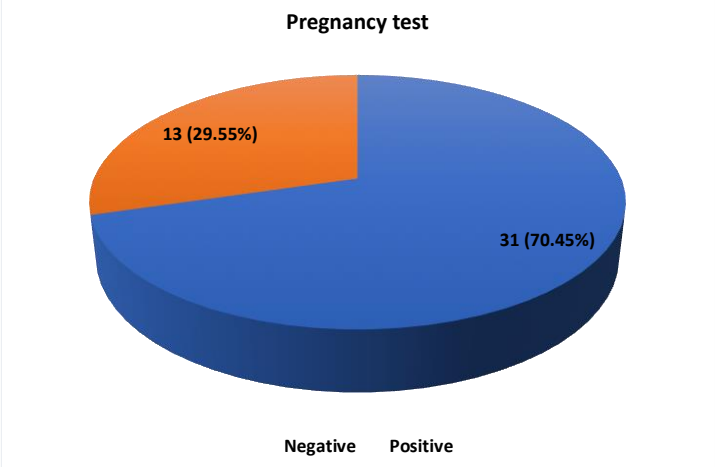

Figure 2. Distribution of study samples by different kinds of pregnancy outcome

Relationship between the oocyte and embryo features in pregnant and non-pregnant women with levels of HB-EGF in the blood and follicular fluid

Table 1 indicates that there is no statistically significant correlation between HB-EGF concentrations in follicular fluid and serum and most oocyte characteristics in both groups. However, there is a notable exception for fragile oocytes, where a significant positive correlation is observed with both follicular fluid and serum HB-EGF in women has negative pregnancy test ($r = 0.428$, p-value 0.016; $r = 0.357$, p-value 0.048) respectively. Additionally, the same table reveals a significant positive correlation ($r = 0.589$, p-value 0.034, $r = 0.678$, p-value 0.011) between the number of transferred embryos and follicular fluid HB-EGF in pregnant women, as well as between embryo grade III and serum HB-EGF level.

Table 1. Correlation between the amounts of HB-EGF in follicular fluid and serum with oocytes and embryo features.

Oocyte & embryo characteristic		Positive pregnancy outcome		Negative pregnancy outcome	
		Follicular fluid HBGF (pg/ml)	Serum HBGF (pg/ml)	Follicular fluid HBGF (pg/ml)	Serum HBGF (pg/ml)
oocyte number	r	-0.126	0.282	0.052	0.022
	p	0.682	0.35	0.78	0.905
Germinal vesicle oocyte	r	0.102	-0.032	-0.237	-0.116
	p	0.74	0.917	0.199	0.534
Metaphase I oocyte	r	-0.334	-0.198	-0.077	-0.166
	p	0.265	0.516	0.682	0.372
Metaphase II oocyte	r	0.029	0.283	-0.012	0.007
	p	0.924	0.349	0.949	0.969
Abnormal oocyte	r	-0.118	-0.171	0.263	0.085
	p	0.701	0.577	0.152	0.651
Damaged oocyte	r	-0.207	-0.247	.428*	.357*
	p	0.498	0.417	0.016	0.048

Discussion

The current study found a significant positive correlation between the number of fragile oocytes in cases of negative pregnancy outcomes with the levels of HB-EGF in serum and follicular fluid *p-values*= 0.428 and 0.357 respectively. Additionally, a significant positive correlation was observed between serum HB-EGF levels and embryo grade III *p-value*= 0.678, as well as between follicular fluid HB-EGF levels and the number of embryo transfers in cases of positive pregnancy outcomes *p-value*=0.589. Previous research has shown a correlation between HB-EGF and many physiological and pathological states, such as tumor proliferation and the formation of new blood vessels (angiogenesis) (Edwards et al., 2009). Furthermore, prior studies have supported the observation that the expression of HBEGF is elevated in both the glandular and luminal epithelium during the late secretory phase of the menstrual cycle, specifically on cycle days 20-24 (Hammadeh et al., 2005). In contrast, research done by Al-Dujaily et al. (2017)

showed that the levels of HB-EGF were found to be higher in women who had been diagnosed with polycystic ovarian syndrome (PCOS) in comparison to those who did not have PCOS (Al-Dujaily et al., 2017), Consequently, in the course of infertility therapy, HBEGF could serve as a biomarker for predicting endometrial receptivity and the likelihood of successful embryo implantation (Sutaji et al., 2023), These investigations lead us to infer that HB-EGF is not consistently present during typical oocyte growth and maturation. Consequently, it is plausible to hypothesize that the presence of HB-EGF may have deleterious effects on oocytes.

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