



Correlation of serum and follicular fluid EMMPRIN levels at day of ova pick up and embryo transfer to endometrial thickness and its effect on ICSI outcomes in fresh cycles.

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ABSTRACT

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EMMPRIN (Basigin) is a potent inducer of matrix metalloproteinases (MMPs). Expression of EMMPRIN in the uterus has a very important role in implantation of embryo, this study was conducted to evaluate the relationship of pregnancy rates with EMMPRIN levels in follicular fluid and serum of intracytoplasmic sperm injection patients. The study was cross sectional study analyzed the serum and follicular fluid samples of 58 patients undergo ICSI procedure. The serum and follicular fluid samples were collected from infertile women aged 20 to 42 years. However, the levels of EMMPRIN were measured in the serum and follicular fluid samples and these samples were obtained during oocytes retrieval and another serum samples were collected at the day of embryo transfer. All women have ovarian stimulation with recombinant follicle stimulating hormone (FSH) at the second or third day of the cycle. Result: The pregnancy rate was 27.6 %. The present study showed that EMMPRIN in serum and follicular fluid at day of ova pickup and its level at embryo transfer day, are significantly low in pregnant women ($p < 0.05$) compared to non-pregnant women. The statistical analysis showed no significant correlation between serum and follicular fluid EMMPRIN with endometrial thickness ($p > 0.05$). EMMPRIN level can be used as a promising parameter for the prediction of pregnancy rate in ICSI cycles.

KEYWORD

EMMPRIN, Basigin, Serum and Follicular fluid, ICSI

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1. Introduction

Intracytoplasmic sperm injection (ICSI) is a type of assisted reproductive technology (ART). It became the most common and widely used method in the treatment of infertility. At first, it was used in the management of severe male infertility, such as non-obstructive azoospermia (Esteves et al., 2018 [1]). Nowadays, ICSI is used in the treatment of infertile couples with non-male factors, although it was the costlier and more invasive procedure. The endometrial receptivity and the early interaction between mother and embryo required a functional endometrium (Governini et al., 2021 [2]). The uterine endometrial preparation that aids to support the pregnancy includes decidualization, implantation of the embryo, invasion of trophoblast, vascular reactivity, and remodeling (Fournier et al., 2021 [3]). When focusing on the morphology of the endometrium, the extracellular matrix of endometrium takes the great

importance. The extracellular matrix (ECM) represents the non-cellular part that is present in all tissues, which gives the physical support for cells of the uterus. The tissue ECM undergoes constant remodeling by enzymatic and non-enzymatic factors to reserve the homeostasis of tissue. Any disturbance in that balance leads to implantation failure that ends with infertility. Degradation of extracellular matrix can occur by several proteases' enzymes. The most important proteases are matrix metalloproteinase members (MMP) and their inhibitors (TIMPs) (Scolari et al., 2017 [4]). There are many factors that have an effect on the expression and activity of MMPs; one of these factors is extracellular MMP inducer (EMMPRIN, also known as CD147, Basigin, BSG).

2. Subject material and method

A study was analyzed serum, and follicular fluid samples of 58 infertile women undergo ICSI procedures at

the infertility centre of High Institute for Infertility Diagnosis and Assisted Reproductive Technologies/ Al-Nahrain University/ Baghdad/ Iraq from November 2021 till March 2022 .

1- Study Design:

Basal levels of serum Follicle-stimulating hormone (FSH), Luteinizing hormone (LH), and 17 estradiol (E2) and prolactin (PRL) should be done for all women on the second or third of the menstrual cycle. Another measurement of 17 estradiol (E2) levels in serum was performed on the day of HCG injection (triggering day) by an electro chemiluminescence immunoassay (ECLIA). Serum and follicular fluid samples were collected at the day of ova pick up, and another serum sample was taken at embryo transfer day and then kept in deep freezing (-20) to be utilized in the evaluation of EMMPRIN level then after. HCG was administered after three or more

follicles reaching 17 mm in diameter to achieve the full maturation of the follicle. By using transvaginal ultrasound, endometrial thickness, and oocyte quality were evaluated, and retrieval of oocytes was done 35 to 36 hours post HCG administration. When denudation and incubation of oocyte–corona complexes for two hrs. were performed, ICSI was done. Assessment of fertilization was performed after 17-18 hrs. Endometrial thickness was measured again at embryo transfer day. Levels of EMMPRIN were measured in follicular fluid at oocyte retrieval day and in serum on both days of ova pick-up and embryo transfer by human EMMPRIN ELISA kit (MyBioSource/USA). B-hCG blood levels were measured on the 14th day post embryo transfer, and biochemical pregnancy was considered positive when the level of b-hCG in blood was > five mIU/mL .

In the present study, females were divided into two groups, pregnant and

non-pregnant, based on the presence of a positive pregnancy test or not after the ICSI procedure.

2- Statistical analysis

Data were collected, summarized, analyzed, and presented using Statistical Package for Social Sciences (SPSS) version 23 and Microsoft Office Excel 2010. Qualitative (categorical) variables were expressed as numbers and percentage, whereas quantitative (numeric) variables were first evaluated for normality distribution using the Kolmogorov-Smirnov test, and then accordingly, normally distributed numeric variables were expressed as mean (an index of central tendency) and standard deviation (an index of dispersion), while those numeric variables that are not normally distributed were expressed as median (an index of central tendency) and inter-quartile range (an index of dispersion).

3. Results

In the present study, 58 infertile women were subjected to intracytoplasmic sperm injection cycles. The pregnancy rate depending on a biochemical pregnancy test; 16 women succeeded to become pregnant, making the pregnancy rate 27.6%. The statistical analysis showed no significant association of serum and follicular fluid EMMPRIN to women's endometrial thickness ($p > 0.05$), as shown in **(Table 1)**.

EMMPRIN in serum and follicular fluid at the day of ova pickup and its level at embryo transfer day all have significant low levels in pregnant women ($p < 0.05$). The significant difference in EMMPRIN levels in serum and follicular samples suggests that there is a cutoff value for such levels for the prediction of positive pregnancy outcomes with a certain level of accuracy. Follicular fluid sample sensitivity was better than the sensitivity of serum sample, 87.5 %

versus 50.0 %, respectively, while serum sample specificity was better than follicular fluid sample specificity, 100 % versus 64.3 %, respectively. For that reason, EMMPRIN predictive power can be improved by a

combination of serum and follicular fluid levels to make a prediction of a positive pregnancy outcome since this procedure will elevate both levels of sensitivity and specificity.

Table (1): Correlations of serum and follicular fluid EMMPRIN to endometrial thickness

Characteristic	Serum EMMPRIN at the day of Ova pick up		Follicular fluid EMMPRIN at the day of Ova pick up		Serum EMMPRIN at the day of ET	
	<i>R</i>	<i>p</i>	<i>r</i>	<i>P</i>	<i>R</i>	<i>P</i>
ET at the day of Ova pick-up	-0.256	0.052	-0.230	0.083	-0.236	0.075
ET at the day of embryo transfer	-0.158	0.236	-0.228	0.086	-0.146	0.274

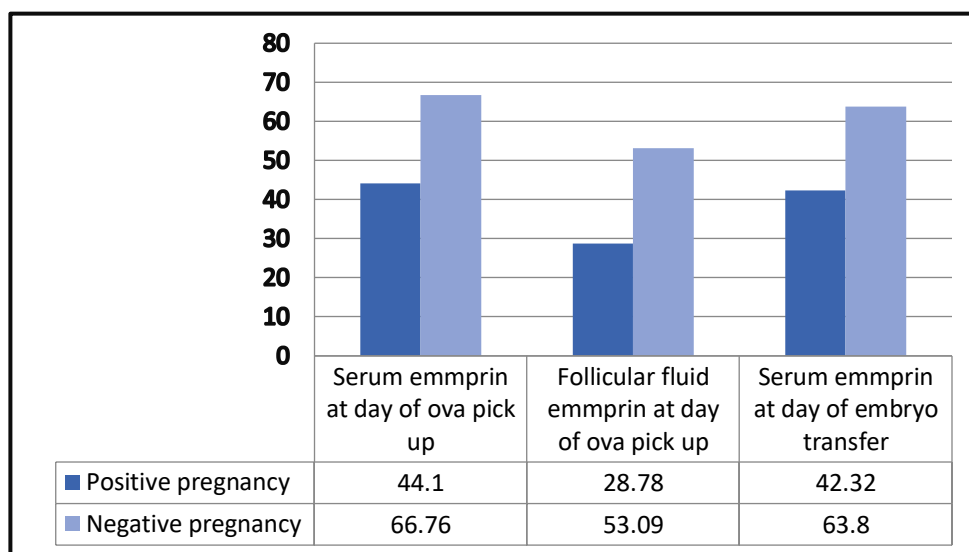


Figure (1): Serum and follicular fluid EMMPRIN

4. Discussion:

Obtaining a simple, rapid, available, and inexpensive outpatient test as a predictor for women's fertility is the main goal and aspect of the current study. In previous studies, EMMPRIN levels were evaluated in follicular fluid by examining a follicular fluid sample and by histopathological examination to mice uterine biopsy. In the current study, EMMPRIN levels in humans were estimated by taking a follicular fluid sample on day of ova pick up and a blood sample on both days of ova pick up and embryo transfer using a simple human EMMPRIN kit.

Implantation of the embryo is a critical process that have many complicated molecular and cellular steps between the maternal and embryonic sides. (Chen et al., 2020 [5]). This process involved extensive endometrial degradation and remodeling, particularly the stromal extracellular matrix. Window of

implantation (WOI) is a certain period in the mid-luteal phase of the menstrual cycle; the endometrium during this period has a maximum receptivity to the transferred embryo. This period starts 6-8 days after ovulation and lasts by 3-4 days. The endometrium, during the window of implantation, produces different types of cytokines, hormones, growth factors, and various types of matrix-degrading proteases, such as matrix metalloproteinases (MMPs) that affect the endometrial extracellular matrix to elevate its receptivity to the transferred embryo. Extracellular matrix metalloproteinase inducer EMMPRIN/CD147 is considered one of the factors that participated in the regulation of MMPs (Benkhalifa et al., 2018 [6]) (Figure 1) showed that serum EMMPRIN at day of ova pickup, follicular fluid EMMPRIN at day of ova pick up and serum EMMPRIN at day of embryo transfer were all significantly lower in women with

positive pregnancy in comparison to non-pregnant women ($p < 0.05$) .

Imbalance in MMP expressions, such as the hyperactivity of MMP-2 and MMP-9, are associated with the abnormal environment of the uterus, like that associated with chronic inflammation of the uterine endometrium **(Yoshii et al., 2013 [7])** .

Successful implantation is based on a balance between MMP activation and inhibition. In the current study, one of the causes that may be related to the elevation of emmprin level in non-pregnant women is hyperactivity of MMP-by-MMP inducer (EMMPRIN), which was associated with implantation failure **(Yoshii et al., 2013 [7])**. The current result agrees with a study by Takmaz et al. (2019).

In addition to that, the reason behind the low EMMPRIN level in pregnant women of the present study, the first one might be the methods used to measure the EMMPRIN vary from that utilized in the previous research that

performed on animals and revealed the highly expression of EMMPRIN in pregnant females **(Dang et al., 2013 [8])**. The present study showed that EMMPRIN in pregnant women have a low level, and secondly, the result might be related to the time of measurement of EMMPRIN oocyte retrieval day and at the day of embryo transfer while matrix metalloproteinase increased during the window of implantation period. Therefore, extracellular matrix metalloproteinase inducer level is high during this period (that starts 6 to 8 days post ovulation and persists for 3-4 days) and becomes low at both days of ova pick up and embryo transfer. On the other hand, there are different of expressions of EMMPRIN by uterine epithelial cells and stromal fibroblast in the secretory phase **(Li & Nowak, 2020 [9])**, so this different pattern of expression might affect EMMPRIN level in serum and follicular fluid .

Regarding the correlations of serum and follicular fluid EMMPRIN to

endometrial thickness is shown in **(Table1)**; there was no significant correlation ($p > 0.05$). Progesterone normally increased in the second half of the menstrual cycle, which affects the uterine endometrium and leads to proliferation and thickening. In IVF, the endometrium can be negatively affected by ovarian stimulation that may also affect the luteal phase, led to a decrease in the implantation rate and pregnancy. So, in IVF, the luteal phase is supported by taking exogenous progesterone, which differs according to several factors, like the duration of ovarian stimulation and the age of the patients **(Griesinger et al., 2020 [10])**. Braundmeier et al. (2006) and Noguchi et al. (2003) noticed that emmprin expressed widely in the secretory phase in association with high levels of progesterone (which increase the thickness of endometrium) and progesterone receptor **(Li & Nowak, 2020 [9])**. As reported by Chen et al. (2010), emmprin mRNA is detected

at all stages of development of corpora lutea and granulosa cells of the follicle. Granulosa cells and theca cells are differentiated into a corpus luteum to produce a sufficient amount of progesterone that is needed for intrauterine environment development, which is required to support the process of implantation **(Davis & LaVoie, 2019 [11]; Alzaidi, Z et al., 2021[12])**.

5. Conclusion

From results of the present study, it has confirmed that the evaluation of serum and follicular fluid EMMPRIN levels can be used as a promising parameter for the prediction of pregnancy rate in ICSI cycles. It is assumed that the pregnancy rate was affected by EMMPRIN in ICSI cycles. However, there were no correlation between serum and follicular fluid EMMPRIN to women's age, BMI, and endometrial thickness.

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Author Contribution Methaq Hassan Hashim performed the study, and Amal Abdulwahid Mohammed and Ali Ibrahim Rahim supervised the work .

Conflict of Interest

The authors declare no conflict of interest .

Ethical Clearance

The study was approved by the Ethical Approval Committee.

Financial Disclosure

There is no financial disclosure.

Ethical Clearance

The study was approved by the Ethical Approval Committee.

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