The Levels of Serum Periostin on the Day of Frozen-thawed Embryo Transfer Affect the Pregnancy Outcomes

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Research

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Abstract

Background: Periostin is a secretory extracellular protein that has multiple biological roles. It has been confirmed to play a significant role in embryo implantation and development,placental formation. In our study, we aimed to explore whether the levels of serum periostin on the day of frozen-thawed embryo transfer (FET) affected pregnancy outcomes.

Methods: This was a retrospective cohort study consisting of 286 frozen-thawed embryo transfer cycles(FETs). According to pregnancy outcomes, the participants were devided into the pregnant group (n=110) and non-pregnant group (n=176). The concentration of serum periostin was determined by enzyme-linked immunosorbent assay (ELISA).

Results: In the pregnant group, the level of serum periostin was dramatically higher compared with the non-pregnant group (41.25 ± 25.77 vs. 30.94 ± 18.28 ng/mL; $P<0.05$). Multivariate logistic regression analysis demonstrated that the level of serum periostin had a significant effect on the clinical pregnancy (OR=1.03, 95% CI: 1.01, 1.04). The receiver operator characteristic (ROC) curve analysis was performed to assess the predictive value of the serum periostin level (AUC 0.61; 95% CI:0.52 ,0.68).

Conclusions: In conclusion, the levels of periostin serum on the day of frozen-thawed embryo transfer affected the pregnancy outcomes for patients who underwent frozen thawed embryo transfer.

Plain Language Summary

Frozen-thawed embryo transfer (FET) has become an integral part of in-vitro fertilisation (IVF) treatment. Implantation is a vital step for successful pregnancy, and good endometrial receptivity is the determinant of achieving embryo implantation. Previous research has shown that periostin may be a new serum biomarker of endometrial receptivity and embryo-endometrial cross talk at implantation. The aim of this study was to investigate whether the levels of serum periostin on the day of frozen-thawed embryo transfer (FET) affected pregnancy outcomes. Results provided that serum periostin had higher correlation with clinical pregnancy. The levels of periostin also had certain predictive value for clinical pregnancy.

Introduction

In recent years, with the significant development of embryo culture conditions and cryopreservation technology, frozen-thawed embryo transfer (FET) has increasingly become a safer and effective approach in assisted reproductive technology (ART) [1]. FET can reduce the risk of multiple pregnancy by transfer a good-quality single embryo and the embryo and endomentrium are more synchronized compared with fresh embryo transfer. Meanwhile, cryopreservation of embryos is an option for reducing the risk of moderate and severe ovarian hyperstimulation syndrome (OHSS)[2].

However, the low pregnancy rate is a major challenge for in vitro fertilization and embryo transfer (IVF-ET). As we all know, endometrial receptivity is a crucial factor in the successful outcome in ART cycles [3].

Through the analysis of endometrial receptivity, we can effectively increase the implantation rate and pregnancy rate[4]. Therefore, it is very important to accurately determine the best condition of the endometrium receptivity. Many endometrial markers, including the presence of pinopods, immunohistochemical biomarkers, endometrial waves, and blood flow, have been adopted to assess uterine receptivity[5–8]. However, relevant studies focusing on endometrial receptivity are still limited, and more studies are needed to accurately assess it.

Periostin is a secretory extracellular protein, and it is expressed in a wide variety of normal tissues and organs of the body, such as periodontal ligament, placenta, bone, heart, stomach, ovary, and breast [9–11]. It also plays a crucial role in embryonic development[12]. Periostin is overexpressed in most common tumors, including breast cancer, hepatocellular carcinoma and colon cancer, and it exerts an essential part in the pathogenesis of tumors, especially in the metastasis of tumors [13, 14]. Recent studies confirmed that many similarities exist between embryo implantation and the metastasis of cancer cells[15]. Based on periostin play key roles in the process of metastasis, it may influence the key step in the implantation process. Some surveys revealed that $\alpha v\beta_3$ and $\alpha v\beta_5$ have an important role in the embryo-endometrial interaction at the time of implantation[16]. Periostin is a ligand for $\alpha v\beta_3$ and $\alpha v\beta_5$ integrins, which are proteins contributing to implantation and promoting cell adhesion and motility[17, 18]. Additionally, periostin is expressed in endometrial stromal and epithelial cells, and its expression is modulated by steroid hormones. The expression of periostin is increased in the metaphase of proliferation and the early stage of secretion during the menstrual cycle[19, 20]. A previous study has pointed out that periostin may be a new serum biomarker of endometrial receptivity and embryo-endometrial cross talk at implantation [21]. Recently, study showed that low levels of serum periostin are an indicator of poor implantation, which may cause pregnancy loss in the early stages[22, 23]. However, no study has revealed whether the levels of serum periostin on the day of FET affect the pregnancy outcomes. Therefore, we aimed to explore the effects of serum periostin levels on the day of FET on the pregnancy outcomes.

Materials And Methods

Subjects

A total of 286 FETs patients from December 1st, 2020 to June 30th, 2021 at the Center for Reproductive Medicine, Affiliated Hospital of Jining Medical University (Shandong province, China) were included in the present work. The experimental protocols were authorized by the ethics committee of Affiliated Hospital of Jining Medical University and written informed consent was obtained from all participants. Study data, including age and body mass index (BMI), etc., were collected from the clinical database. Patients with embryo cryopreservation prior to IVF/ICSI cycle were included in the present study. Patients with possible confounding comorbidities (autoimmune diseases, diabetes mellitus or the intake of confounding medication, uterine malformations, and leiomyomas, adenomyoma, endometrial polyps, intrauterine

adhesions, endometrial tuberculosis history) or those with a history of recurrent abortion or implantation failure were excluded from the study.

Endometrial Preparation Protocols

The participants were assigned into different groups of endometrial preparation based on the clinician's experience and patient's characteristics. Four protocols were available for endometrial preparation as follows: 1) the natural cycle, 2) the ovulation induction cycle, 3) the hormone replacement treatment (HRT) cycle and 4) the HRT with gonadotropin-releasing hormone agonist (GnRHa) pretreatment (HRT + GnRHa).

The natural cycle was the first choice for patients with regular menstrual cycles, in which ultrasound monitoring was initiated on cycle days 10-12 to assess the dominant follicle and the endometrium. When the follicle became mature, human chorionic gonadotropin (HCG, Merck Serono, China) was administered to trigger ovulation. The timing of embryo transfer was determined based on the stage of embryonic development on the day of embryo freezing.

In the ovulation induction cycle, participants were administered with human menopausal gonadotropin (HMG, Livzon Pharmaceutical, China) at a dose of 75 IU/day from day 3 to day 5 of the menstrual cycle. The dose of HMG was adjusted according to the follicular development monitored by ultrasound and serum sex steroid measurements. Besides, once the dominant follicle reached 18 mm or more in diameter, HCG at a dose of 8,000-10,000 IU was adopted to trigger ovulation.

For the HRT cycle, the participants were orally administered with estradiol valerate tablets (Progynova, Bayer, Germany) at a dose of 3–12 mg/day from the 2nd to 5th day of the cycle, and such regimen lasted for at least 7 days. The starting dose was determined according to the patient's previous endometrium. When the endometrial thickness was≥7 mm, progesterone at a dose of 40 mg/day was given to transform the endometrium. As for HRT + GnRHa cycles, GnRH agonist was initiated on day 1 of the menstrual cycle. Moreover, on day 1 of the next menstrual cycle, estrogen stimulation was started as HRT cycles without GnRH agonist.

According to the patient's age and previous IVF cycles on the 4th (cleavage-stage embryo) or 6th day (blastocyst) after ovulation or progesterone injection, one or two thawed embryos were transferred using a soft-tipped Wallace catheter under ultrasound guidance. Luteal support with progesterone was given to all patients after embryo transfer. Such regimen was continued until 10 weeks of gestational age if a gestational sac and embryonic heartbeat at 4–6 weeks after embryo transfer was observed by transvaginal ultrasound.

Enzyme-linked immunosorbent assay (ELISA)

Samples for a fasting blood test were collected from all participants. The specimens were subjected to centrifugation at 3,000 rpm for 10 min, followed by serum collection and storage at −80°C prior to further analysis. The contents of serum periostin were measured by Human Periostin ELISA Kit (MM-13746H2), which was purchased from Jiangsu Enzyme Immune Co., Ltd, China.

Assessment of embryo quality and vitrification of embryos

Embryo morphology was assessed based on the developmental speed, degree of fragmentation, and evenness of the cleavage sphere. Cleavage-stage embryos with at least seven blastomeres and fragmentation < 20% were regarded as high-quality embryos. The blastocyst was graded by using the Gardner scoring system, and embryos graded 3BB or greater were regarded as embryos of good quality[24]. The timing of FET was determined according to the stage of embryonic development, and the embryos were vitrified and synchronized with the duration of progesterone exposure of the endometrium. The number of embryos transferred was determined according to the patient's age and previous IVF cycle, and a maximum of two embryos was allowed to be transferred.

The vitrification and thawing were carried out as previously described [25]. In brief, embryo vitrification was conducted using a Cryotop carrier system, and dimethylsulfoxide-ethylene glycol-sucrose was used as cryoprotectants. Embryos were sequentially transferred to the diluted solution to thaw (1 mol/L to 0.5 mol/L to 0 mol/L sucrose).

Definition of Clinical Outcomes

American Society for Reproductive Medicine (ASRM) 2017 consensus definitions [26] were used to assess the clinical outcomes. Clinical pregnancy was defined as a pregnancy diagnosed by ultrasonographic visualization of one or more gestational sacs or definitive clinical signs of pregnancy. The patients with the above-mentioned pregnancy were assigned to the pregnant group. When pregnancy was diagnosed only by the detection of HCG in serum (14 days after embryo transfer) or did not develop into a clinical pregnancy, it was defined as biochemical pregnancy. Consequently, the non-pregnant (blood β-HCG< 10 mIU/mL) patients or those with biochemical pregnancy were assigned to the non-pregnant group.

Statistical Analysis

Statistical analysis was performed with the statistical packages R (The R Foundation; http://www.r-project.org; version 3.4.3) and Empower (R) (www. empowerstats.com, X&Y Solutions, Inc., Boston, Massachusetts). Continuous data were presented as the means ± standard deviations, and the categorical data were expressed as N (%). P <0.05 was considered statistically significant. To assess the risk factors of pregnancy outcome, variables were analyzed by multivariate logistic regression. The

predictive accuracy of serum periostin levels on the day of FET on the pregnancy outcome was investigated according to the areas under the receiver operator characteristic (ROC) curves (AUC).

Results

Table 1
General characteristics.

	All cycles	Pregnant	Nonpregnant	P-value
N	286	110	176	
Maternal age (years)	33.53 ± 5.68	32.38 ± 4.55	34.24 ± 6.19	0.011
Male age (years)	33.90 ± 6.19	32.75 ± 5.08	34.61 ± 6.70	0.041
Body mass index(BMI, kg/m^2)	23.09 ± 3.51	23.40 ± 3.89	22.89 ± 3.25	0.511
Duration of infertility (years)	3.19 ± 2.41	3.07 ± 2.26	3.27 ± 2.51	0.695
Endometrial thickness in FET (mm)	10.13 ± 1.56	10.21 ± 1.60	10.09 ± 1.54	0.582
Gestity(n)	1.57 ± 1.58	1.37 ± 1.48	1.70 ± 1.63	0.054
Parity(n)	0.54 ± 0.56	0.45 ± 0.54	0.60 ± 0.57	0.039
Type of infertility				0.251
Primary	75	33 (30.00%)	42 (23.86%)	
Secondary	111	77 (70.00%)	134 (76.14%)	
Endometrial preparation regimen				0.130
Natural cycles	70	27 (24.55%)	43 (24.43%)	
Ovulation induction cycles	83	35 (31.82%)	48 (27.27%)	
HRT cycles	66	30 (27.27%)	36 (20.45%)	
GnRha+HRT cycles	67	18 (16.36%)	49 (27.84%)	
Embryo stage at transfer				0.329
Cleavage stage	174	63 (57.27%)	111 (63.07%)	
Blastocyst stage	112	47 (42.73%)	65 (36.93%)	
Number of embryos transferred				0.863
1	110	43(39.09%)	67 (38.07%)	
2	176	67(60.91)	109 (61.93%)	
Periostin (ng/mL)	34.91 ± 22.01	41.25 ± 25.77	30.94 ± 18.28	0.000

Table 2
Multivariate logistic regression analysis

	OR (95% CI)	P value
Maternal age (year)	0.98(0.91-1.06)	0.67
Male age (year)	0.96(0,090-1.03)	0.29
BMI (kg/m2)	1.05(0.97-1.13)	0.24
Duration of infertility (year)	0.97 (0.86-1.09)	0.60
Endometrial thickness in FET (mm)	1.02 (0.86-1.21)	0.81
Gestity	0.97(076-1.23)	0.78
Parity	0.58(0.31-1.11)	0.10
Type of infertility		
Primary	Reference	Reference
Secondary	0.69(0.31-1.58)	0.38
Endometrial preparation regimen		
Natural cycles	Reference	Reference
Ovulation induction cycles	2.01(0.93-4.56)	0.07
HRT cycles	2.38 (1.11-5.08)	0.03
GnRha+HRT cycles	2.81(1.27-6.23)	0.01
Embryo stage at transfer		
Cleavage stage	Reference	Reference
Blastocyst stage	0.47 (0.20-1.11)	0.08
Number of embryos transferred		
1	Reference	Reference
2	0.53 (0.23-1.23)	0.14
Periostin (ng/mL)	1.03 (1.01-1.04)	0.00

Table 3
Predictive value of serum periostin levels for pregnancy outcome

	Sensitivity (%)	Specificity (%)	AUC (95%CI)
Periostin	36.4	85.2	0.61(0.54-0.68)

A total of 286 FET cycles were investigated in this study, of which 110 were clinically pregnant and 176 were not pregnant. Table 1 summarizes the demographic characteristics of both groups. Two groups had comparable baseline characteristics, such as maternal age, male age, BMI, infertility cause and duration, gravidity, parity, and so on. The maternal age, male age, and parity were significantly higher in the non-pregnant group compared with the pregnant group ($P<0.05$). The type of endometrial preparation and the stage and number of embryos at transfer was not statistically different between the two groups ($P>0.05$). The levels of serum periostin measured on the day of FET were significantly higher in patients who had a clinical pregnancy compared with those who did not achieve the pregnancy (41.25 ± 25.77 vs. 30.94 ± 18.28 ng/mL; $P < 0.001$) (Figure 1). Multivariate logistic regression revealed that the type of endometrial preparation (HRT cycles and GnRh+HRT cycles) and periostin level were independently and significantly associated with the pregnancy outcome (Table 2). ROC was used to access the level of periostin in predicting clinical pregnancy. The AUC of periostin in predicting clinical pregnancy was 0.61 [95% (CI), 0.52 to 0.68]. The cut-off point of the serum periostin level in FET was 46.21 ng/mL (sensitivity of 85.2% and specificity of 36.4%) for pregnancy (Figure 2 and Table 3).

Discussion

In the present study, we investigated the effects of serum periostin levels on the day of FET on pregnancy outcomes. The results demonstrated that the levels of serum periostin were significantly higher in the pregnant group compared with the non-pregnant group. The multivariate logistic regression showed that the level of periostin was significantly associated with pregnancy outcomes. The ROC curves showed that the levels of serum periostin had a certain predictive value for the pregnancy outcomes of FET.

It is known that periostin is an extracellular matrix secreted protein that promotes osteoblast cell adhesion and proliferation [27]. Moreover, periostin greatly contributes to cell adhesion, migration, proliferation, and gene regulation, particularly in the development and metastasis of tumors. In tumor metastasis, periostin can enhance the Wnt signaling pathway, which is also involved in the development of the preimplantation embryo and embryo implantation[28, 29]. It is also a ligand for $\alpha v\beta_3$- and $\alpha v\beta_5$-integrins to promote cell movement [30]. Liu et al. have pointed that $\alpha v\beta_3$ integrin can predict good endometrial receptivity, and it plays a fundamental role in blastocyst implantation [31]. We speculated that periostin was related to embryo implantation. Previous studies have identified that both stromal and epithelial cells express periostin, and its expression at the protein level can be elevated during pregnancy and in the early secretory phase. Ahn et al. have found that the expression of periostin is higher in the endometrium treated with progesterone, inducing the retention and migration of trophoblast cells [19]. Based on these above-mentioned results, we speculated that periostin was associated with conception in

early pregnancy. Morelli et al. have compared the serum and endometrial periostin levels between the two groups of spontaneous abortion and voluntary termination of pregnancy and found that women in the group with spontaneous abortion have significantly lower levels of periostin in the serum, trophoblast, and decidual tissues [21]. Eroglu et al. have found that the levels of serum periostin are remarkably lower in the spontaneous abortion group, while they do not detect differences in tissue expression [23]. Similar findings have been reported by Freist et al. that when the pregnancy loss occurs spontaneously rather than the induced one, the level of periostin is significantly reduced[22]. They speculate that periostin can be used as a marker of endometrial receptivity and as a predictive indicator for the success of endometrial implantation. Moreover, periostin has a fundamental fundtion in gestation. However, studies and data in this area are limited, and more further research is required.

In the light of these studies, we investigated the effects of serum periostin levels on the day of FET on pregnancy outcomes. We found that the level of serum periostin was closely correlated with the pregnancy outcome. The contents of serum periostin were dramatically lower in the non-pregnant group. Therefore, we speculated that a low level of serum periostin predicted poor implantation, causing implantation failure.

In conclusion, serum periostin may be serum biomarker of endometrial receptivity and higher concentrations of serum periostin on the day of FET might predict a better pregnancy outcome. Moreover, periostin could be adopted as a potential indicator to estimate pregnancy outcomes after transplantation.

Limitations and Future Directions

There is no doubt that our study has some limitations. First, the sample size was relatively small in this study. This, however, might bias our results toward the null. Studies with a larger sample size are necessary. Additionally, we included only serum periostin levels on the day of FET. We did not dynamically observe the changes of serum periostin concentrations, such as serum periostin levels at days 14 and 21 after embryo transfer. This aspect needs to be considered in subsequent studies to more accurately evaluate the effect of serum periostin on pregnancy outcomes. In order to seek better guidance on frozen embryo transfers and increase pregnancy rates.

Abbreviations

FET, frozen-thawed embryo transfer; ELISA, enzyme-linked immunosorbent assay; ART, assisted reproductive technology; IVF-ET, in vitro fertilization and embryo transfer; ICSI, Intracytoplasmic sperm injection; HRT, hormone replacement treatment; BMI, body mass index

Declarations

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Authors' contributions

YG and YS conceived the original idea, designed the study, and wrote the manuscript. XL, YL and YC performed data analysis. ZL and AY helped supervise the study and revised the paper. All authors read and approved the final manuscript.

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Availability of data and materials

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Ethics approval

All patients provided their informed written consent. The study was approved by the Ethics Committee of Affiliated Hospital of Jining Medical University.

Consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interest.

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Figures

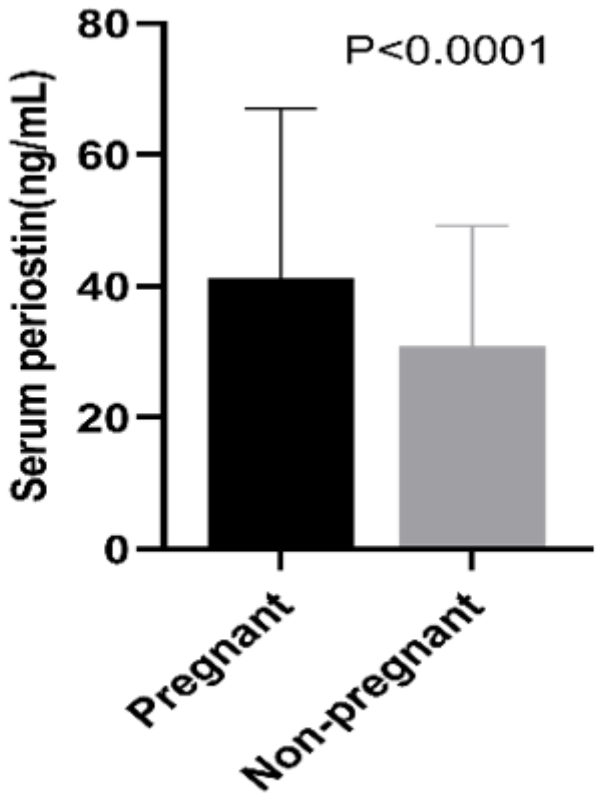

Figure 1

The association between serum periostin concentrations measured on the day of FET and the outcomes of embryo transfer

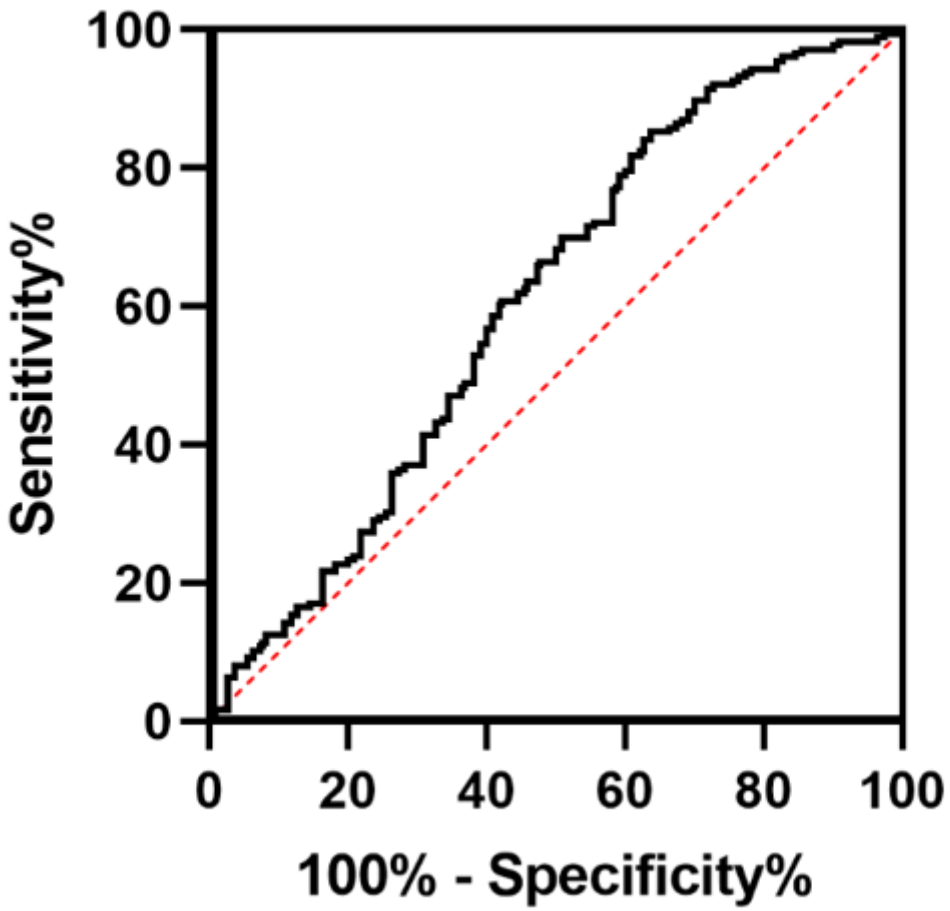

Figure 2

Values of serum periostin levels in predicting clinical pregnancy in patients with frozen-thawed embryo transfer