IRAQI JOURNAL OF EMBRYOS AND INFERTILITY RESEARCHES (IJEIR)
Sahar et al., Vol. 13, Issue 2, Pp. 01-15 (2023) Original Research
DOI: http://doi.org/10.28969/IJEIR.v13.i2.r1.23 E-ISSN: 2616-6984 P-ISSN: 2218-0265

Leukemia Inhibitory Factor as an Indicator for Determination of the Optimal Time of Intrauterine PRP Infusion in ICSI Cases.

Sahar S. Raheem [1], Mufeeda A. Alammar [2], Muayad S. Abboud [2]

[1] College of Medicine, Al-Muthanna University, AL-Muthana, Iraq.

[2] High Institute of Infertility Diagnosis and Assisted Reproductive Technologies, Al Nahrain University, Baghdad, Iraq.

sahaharswadii1@gmail.com

ABSTRACT

The compromise of uterine receptivity is one of the main reasons for the failure of assisted reproduction techniques. Leukemia inhibitory factor is one of those molecular parameters that allow us to identify an endometrium as receptive. Platelet rich plasma (PRP) contains a range of cytokines and growth factors including leukemia inhibitory factor that encourage the migration, proliferation, and differentiation of cells. Treatments for antagonist ovarian stimulation were administered to 75 women under the age of 40, and 2-D power doppler ultrasound was used to guide the oocyte harvest. Patients were allocated into three groups according to the time of intrauterine infusion of PRP (no PRP, PRP at triggering day and PRP at ova pickup day). Power Doppler is utilized to measure endometrial thickness (ET), pulsatility index (PI), and resistance index (RI) of sub endometrial arteries, as well as serum levels of leukemia inhibitory factor (LIF). The mean endometrial thickness as well as serum level of LIF in PRP trigger group were higher, resistance index and pulsatility index were lower in a significant manner than control and PRP ova pickup group at all the days of study (triggering, ova pickup and embryo transfer days). These findings revealed that intrauterine PRP infusion on ova pick up day is associated with a significant increase in serum level of LIF that in turn enhances endometrial thickness for patients scheduled for intracytoplasmic sperm injection (ICSI).

Received: 07-Oct- 2023
Accepted: 30-Oct-2023
Published: 08-Nov-2023

How to cite:

Sahar S. Raheem, Mufeeda A. Alammar, Muayad S. abbood; Leukemia Inhibitory Factor as an Indicator for Determination of the Optimal Time of Intrauterine PRP Infusion in ICSI Cases; Iraqi Journal of Embryos and Infertility Researches (IJEIR), (2023); 13 (2): 01-15.
Doi: http://doi.org/10.28969/IJEIR.v13.i2.r1.23

KEYWORD

PRP, ICSI, ET, RI, PI, LIF.

1. Introduction

The Process of implantation is the most important limiting factor in human reproduction, which requires a competent embryo and a receptive endometrium, as well as the establishment of a process called “close dialogue: endometrium-embryo. It requires a reciprocal interaction between the blastocyst, which has its own molecular program for cell growth and differentiation, and the endometrium, a dynamic organ that exhibits a short period of receptivity called the “implantation window.” This process is governed by endocrine, paracrine, and autocrine modulatory factors, both of maternal and embryonic origin (Hernández-Valencia et al., 2014 [1]). The compromise of uterine receptivity is one of the main reasons for the failure of assisted reproduction techniques, with the phenomenon of implantation considered the most limiting factor in the establishment of pregnancy and, consequently, a major cause of infertility (Makrigiannakis,2007 [2]). Blastocysts cannot connect to an immature endometrium, which leads to failed even after the transfer of "high quality" embryos (Devesa Rodríguez, 2015 [3]) .Leukemia inhibitory factor is one of those molecular parameters that allow us to identify an endometrium as receptive. They work through a pathway common to all members of the IL6 family. LIF is key to placentation and has actions both in the pre-implantation embryo and in the uterus, its activity focusing in particular on both uterine preparations for receptivity and blastocyst anchorage. LIF's significance in implantation is further supported by the correlation between infertility and specific genotypes with reduced LIF production or activity. Based on aberrant levels of LIF in women who are infertile, the significance of LIF in human embryonic implantation has also been proven (Hoozemans et al., 2004 [4]) .

Plasma that has been fractionated from autologous blood and contains concentrated platelets is called platelet-rich plasma (Krüger et al., 2013 [5]). PRP contains a range of cytokines and growth factors that encourage the migration, proliferation, and differentiation of cells (Dhillon et al., 2017 [6]). In assisted reproductive technologies, platelet-rich plasma (PRP) is being used more frequently to increase endometrial receptivity. According to the authors, intrauterine treatment of autologous PRP increases the likelihood of pregnancy and birth, especially in individuals who have poor endometrial growth. Cytokines and growth factors are present and released by blood cells during physiological, normal endometrial growth and implantation (BosMikich et al., 2019 [7]). Similar blood mediators are released from platelet granules upon a blood vessel injury (Aghajanova et al., 2018 [8]). This study aims to determine the optimal time for PRP injection (triggering day versus ova pickup day) based on the serum LIF level and determination of their ICSI outcome.

2. Patients and Methods

A prospective comparative study was conducted in the High Institute for Infertility Diagnosis and Assisted Reproductive Technologies, Al-Nahrain University in Baghdad. Seventy-five Iraqi women were included in this study .

Exclusion criteria including all cases of endometrioses, cases of congenital anomalies of the reproductive system, patients aged more than 40 years, intake of any antiplatelet drugs, and cases with chronic systemic diseases .

A full medical, surgical, and obstetrical history was required as an assessment of height & weight to obtain BMI clinical and gynecological examinations to exclude any abnormality. For male partners, the seminal fluid analysis was assessed according to WHO 2010. Hormonal

analysis (FSH, LH, E2, Prolactin, TSH) for female partners at day 2 of the menstrual cycle.

The preparation of PRP involves a technique called differential centrifugation using the buffy coat method. All enrolled women had been undergone antagonist protocol and allocated into three groups (twenty-five women in each group):

Control group :

• Follow-up of patients done with serial vaginal ultrasound and serum level of estradiol, then accordingly ovum pick up done .

• On the day of Trigger of ovulation, 2D Power Doppler ultrasound of endometrium-zone 2, and measurement of serum level of LIF .

• On the day of oocyte retrieval, again, 2D Power Doppler transvaginal ultrasound of endometrium-zone two and serum level of LIF measured for all patients; trans vaginal ultrasound guided Oocyte retrieval done after triggering of ovulation with hCG about 35-36 hrs .

• On the day of embryo transfer, usually three days according to the number and grading of embryos, embryo transfer is done, and serum level of LIF is measured for all patients with 2D Power Doppler ultrasound of endometrium-zone 2 .

PRP Trigger group :

The same sequences of group 1, as well as all the patients, had been undergone intrauterine infusion of 0.5-1 ml of autologous PRP at the day of triggering of ovulation. 2D Power Doppler transvaginal ultrasound of endometrium-zone 2 done after 1 hour of PRP infusion .

PRP OPU group :

The same sequences of group 1, as well as all the patients, had been undergone intrauterine infusion of 0.5-1 ml of autologous PRP at the day of ova pick up. 2D Power Doppler transvaginal ultrasound of endometrium-zone 2 done after 1 hour of PRP infusion.

3. Results

There were no significant differences regarding mean demographic parameters, hormonal profile, and stimulation characteristics in all groups, as shown in (Tables 1-3).

Ultrasound findings, biophysical profile, and serum leukemia factor levels:

1- At triggering day :

The was no significant difference in mean endometrial thickness in all groups (9.53±1.56 mm in the control group, 10.2±1,21 mm in the PRP trigger group, and 10.00± 1.30 in the PRP OPU group). There was no significant difference in resistance index in all groups (0.62 ±0.05 control group, 0.55 ±0.05 in the PRP trigger group, and 0.58 ±0.04 in the PRP OPU group). There was no significant difference in the pulsatility index in all groups (1.01 ±0.15 control group, 0.95 ±0.35 in the PRP trigger group, and 0.92 ±0.25 in the PRP OPU group). Serum level of LIF was higher in the PRP trigger group in a highly significant manner than in control and PRP ova pickup day, 208.11± 6.12 versus 175.19 ± 6.97 and 163.85± 7.30, respectively.

2- At ova pickup day:

The mean endometrial thickness was higher in the PRP trigger group in a highly significant manner than in control and PRP ova pickup day, 10.45±1.45 mm versus 9.65±1.51 mm and 10.10± 1.25, respectively. Resistance index was lower in the PRP trigger group in a highly significant manner than in the control and PRP ova pickup day, 0.42±0.04 versus 0.58±0.05 and 0.52± 0.05, respectively. Pulsatility index was lower in the PRP trigger group in a highly significant manner than the control group and PRP ova pickup day, 0.70±0.36 versus 0.95±0.19 and 0.72± 0.31, respectively. Serum level of LIF was higher in the PRP trigger group in a highly significant manner than in control and PRP ova pickup day, 225.32± 7.24

versus 168.34 ± 7.10 and 191.80± 6.20, respectively.

3- At embryo transfer day:

The mean endometrial thickness was higher in the PRP trigger group in a highly significant manner than in control and PRP ova pickup day, 10.4±1.40 mm versus 9.55±1.40 mm and 10.10± 1.30, respectively. Resistance index was lower in the PRP trigger group in a highly significant manner than the control and PRP ova pickup day, 0.45±0.06 versus 0.63±0.03 and 0.58± 0.04, respectively. Pulsatility index was lower in the PRP trigger group in a highly significant manner than the control group and PRP ova pickup day, 0.70±0.21 versus 0.97±0.33 and 0.72± 0.25, respectively. Serum level of LIF was higher in the PRP trigger group in a highly significant manner than in control and PRP ova pickup day, 215.15± 7.08 versus 181.22 ± 6.80 and 201.60± 5.20, respectively.

Table (1): Ultrasound findings, biophysical profile, and serum leukemia factor levels at the day of triggering of ovulation in all enrolled infertile women

Characteristic at triggering day Mean ±SD	**Total *n* = 75**	**control group *n* = 25**	**PRP Trigger group *n* = 25**	**PRP OPU group *n* = 25**	***p***
Endometrial thickness	9 .87 ±1.56	9.53 ±1.56	10.2 ±1.21	10.00 ±1.30	**0.205**
Resistance index	0.58 ±0.04	0.62 ±0.05	0.55 ±0.05	0.58 ±0.04	**0.316**
Pulsatility index	0.83 ±0.66	1.01 ±0.15	0.95 ±0.35	0.92 ±0.25	**0.132**
LIF (ng/l)	175.33±6. 54	175.19±6.97	208.11±6.12	163.85±7.3	< **0.001**

Table (2): Comparison of hormonal profile between all groups of patients

Characteristic Mean ±SD	Total *n = 75*	Control *n = 25*	PRP Trigger *n = 25*	PRP OPU *n = 25*	P
FSH (mIU/ml)	7.05 ±2.2	6.92 ±1.85	7.31 ±1.39	6.82 ±1.75	**0.140 NS**
LH (mIU/ml)	5.22 ±1.65	5.30 ±1.65	5.25 ±1.70	5.16 ±1.60	**0.712 NS**
Estradiol (pg/ml)	32.35 ±3.42	32.85 ±3.0	34.32 ±2.16	33.32 ±2.5	**0.744 NS**
Prolactin (ng/ml)	17.55 ±3.5	17.45 ±2.8	16.85 ±3.4	17.35 ±3.2	**0.132 NS**
TSH mIU/L	1.85 ±1.2	1.9 ±1.35	1.95 ±1.02	1.88 ±1.1	**0.789 NS**

Table (3): Stimulation characteristics

Characteristic Mean ±SD	Total *n = 75*	Control *n = 25*	PRP Trigger *n = 25*	PRP OPU *n = 25*	P
Estradiol level at day of hCG (pg/ml)	1540.30 ±810.25	1468.80 ±550.31	1495.80 ±770.22	1615.10 ±220.00	**0.059 NS**
Total gonadotropin use/cycle	1831.50 ±1043.50	1810.30 ±850.90	2005.0 ±1080.60	1680.50 ±1200.50	**0.201 NS**
Duration of stimulation (day)	9.43±1.16	9.50 ±1.11	9.35 ±1.50	9.45±0.88	**0.329 NS**

Table (4): Oocyte and embryo characteristics

Characteristic Mean ±SD	Total	Control	PRP Trigger	PRP OPU	*p*
Total oocyte count	11.70 ±5.51	12.0±4.50	11.60 ±5.11	11.50 ±5.91	0. 822 NS
MII oocyte count	7.50 ±4.00	7.32 ±5.30	8.00 ±4.50	7.25 ±4.50	0.322 NS
Number of transferred G1 embryos	2.45 ±0.70	2.23 ±0.60	2.44 ±0.60	2.52 ±0.90	0.125 NS

Table (5): Ultrasound findings, biophysical profile, and serum leukemia factor levels at the day of triggering of ovulation in all enrolled infertile women

Characteristic at triggering day Mean ±SD	Total *n* = 75	control group *n* = 25	PRP Trigger Group *n* = 25	PRP OPU group *n* = 25	*p*
Endometrial thickness	9 .87 ±1.56	9.53 ±1.56	10.2 ±1.21	10.00 ±1.30	**0.205**
Resistance index	0.58 ±0.04	0.62 ±0.05	0.55 ±0.05	0.58 ±0.04	**0.316**
Pulsatility index	0.83 ±0.66	1.01 ±0.15	0.95 ±0.35	0.92 ±0.25	**0.132**
LIF (ng/l)	175.33±6. 54	175.19±6. 97	208.11±6.1 2	163.85±7.3 0	**< 0.001**

Table (6): Ultrasound findings, biophysical profile, and serum leukemia factor levels at Ova pick-up day in all enrolled infertile women

Characteristic at Ova pick up day Mean ±SD	**Total *n* = 75**	**control group *n* = 25**	**PRP Trigger Group *n* = 25**	**PRP OPU Group *n* = 25**	***p***
Endometrial thickness	10.06 ±1.56	9.65 ±1.51	10.45 ±1.45	10.10 ±1.25	< **0.001**
Resistance index	0.50 ±0.04	0.58 ±0.05	0.42 ±0.04	0.52 ±0.05	< **0.001**
Pulsatility index	0.79 ±0.29	0.95 ±0.19	0.70 ±0.36	0.72 ±0.31	< **0.001**
LIF (ng/l)	195.125±6.49.	168.343±7.10	225.320±7.24	191.80±6.20	< **0.001**

Table (7): Ultrasound findings, biophysical profile, and serum leukemia factor levels at embryo transfer day in all enrolled infertile women

Characteristic at embryo transfer day Mean ±SD	**Total *n* = 75**	**control group *n* = 25**	PRP Trigger **group *n* = 25**	PRP OPU **group *n* = 25**	*p*
Endometrial thickness	10.05 ±1.66	9.55 ±1.40	10.4 ±1.40	10.10 ±1.30	< **0.001**
Resistance index	0.55 ±0.04	0.63 ±0.03	0.45 ±0.06	0.58 ±0.04	< **0.001**
Pulsatility index	0.79 ±0.29	0.97 ±0.33	0.70 ±0.21	0.72 ±0.25	< **0.001**
LIF (ng/l)	199.323±6.49.	181.221±6.80	215.150±7.08	201.60±5.20	< **0.001**

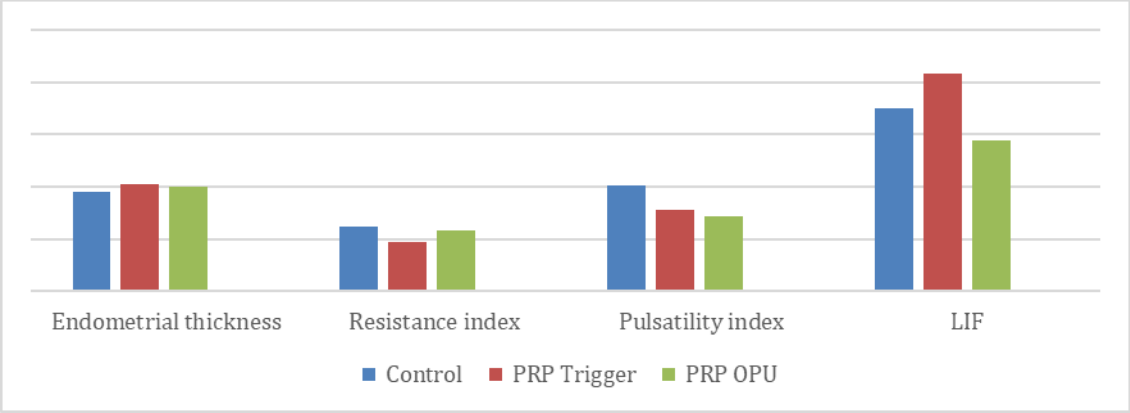

Figure (1): Comparison of mean endometrial thickness, Resistance index, Pulsatility index, and serum level of LIF in all groups at triggering day

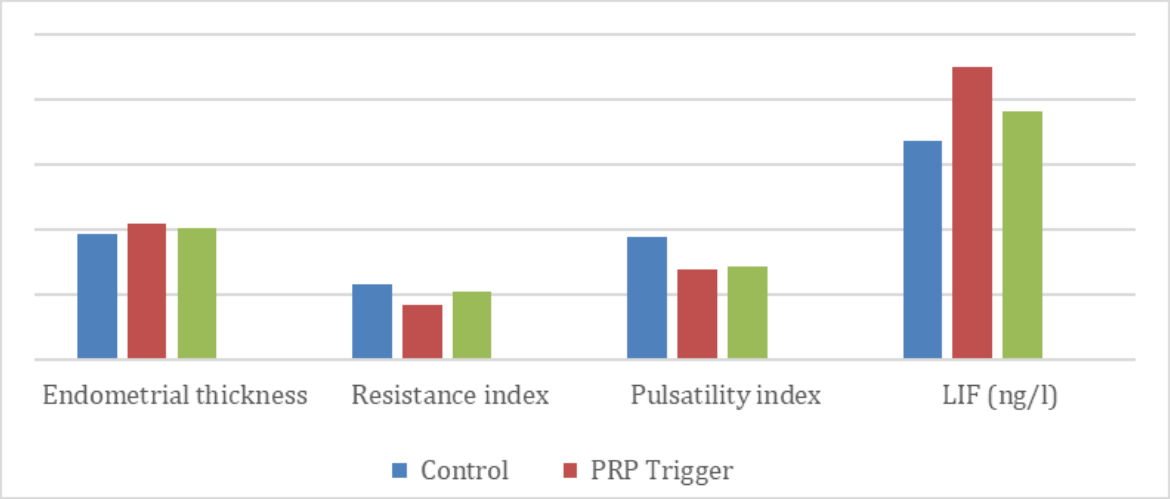

Figure (2): Comparison of mean endometrial thickness, Resistance index, Pulsatility index and serum level of LIF in all groups at ova pick up day.

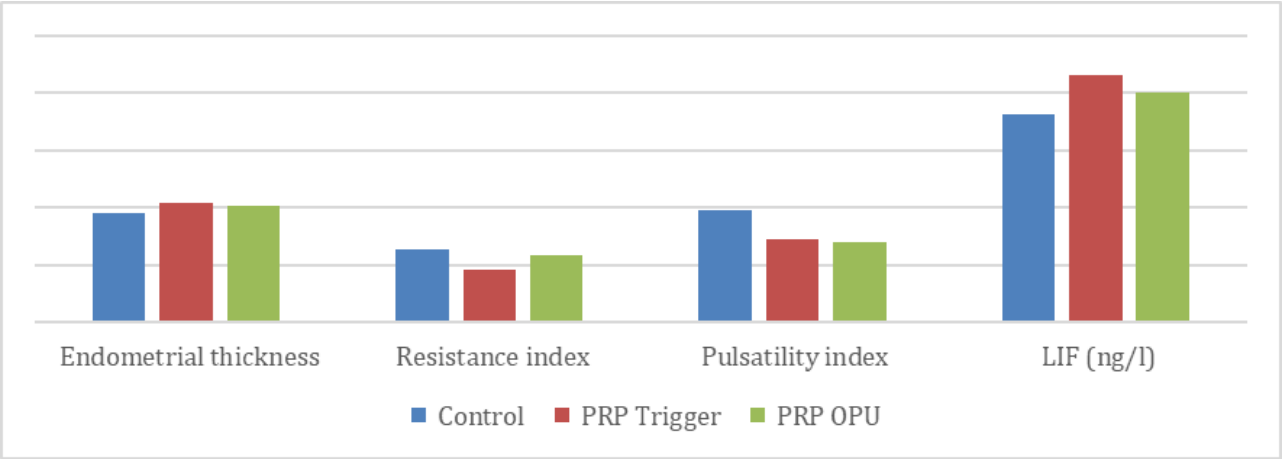

Figure (3): Comparison of mean endometrial thickness, Resistance index, Pulsatility index, and serum level of LIF in all groups at embryo transfer day.

4. Discussion

A viable embryo, a receptive endometrium, suitable embryo endometrial cross-talk, and sufficient mother immune protection are all necessary for successful implantation . While in the present study, the comparison was between the control and both PRP groups revealed proper endometrial receptivity after intrauterine infusion, so PRP enhancing receptivity of endometrium by increasing serum LIF concentration in the face of comparable levels of estradiol at the day of trigger.

To prevent bias in the current study, all cases were selected with nearly identical embryo characteristics. The mean SD of mature metaphase II (M II), oocyte count, and the number of transferred grade one embryos for all enrolled women showed no significant differences for any of these variables. This is said to be at odds with other study (Lazzaroni-Tealdi et al., 2015 [9]), which found that the overall oocyte scoring is a reliable indicator of embryo quality. It seems that better oocytes produce better day-3 cell counts and embryo grades, maybe because these outcomes rely on high

levels of scoring systems. Six criteria were used in their study to evaluate each individual oocyte: morphology, cytoplasm, perivitelline space (PVS), zona pellucida (ZP), polar body (PB), and oocyte size, each assigned a value of -1 (worst), 0 (average) or +1 (best), so establishing a rigorous total oocyte score system (TOS) while in our center they used the most straightforward one based on the stage of division, they created a strict scoring system (GV, MI, MII) .

An easy, non-invasive technique that has been investigated as a sign of endometrial receptivity is the measurement of endometrial thickness by ultrasound and a possible predictor of the potential success of IVF-ET treatment. The goal is to provide a more precise estimate of implantation potential (Tiitinen 2015, [10]). In the present study, there was a highly significant difference between all groups at the triggering day and day of OPU (the mean ± SD of endometrial thicknesses is higher with the PRP trigger group). This is supported by other study.

(Chang, et al., 2015. [11]). Who diligently investigated the use of PRP for the treatment of infertile women with a thin endometrium (< 7 mm). Five women who were undergoing in vitro fertilization (IVF) had poor endometrial responses to thin endometrium (< 7 mm), and they had to postpone the embryo transfer cycle because of this. HRT and intrauterine injection of PRP from autologous blood were both used. In each cycle, PRP infusions were performed 1-2 times. When the endometrial thickness reached > 7 mm, embryos were transferred. After PRP infusion, all patients experienced effective endometrial growth and pregnancies. Intrauterine PRP infusion represented a new method for the thin endometrium with poor response at that time.

In addition, other study (Gupta, J., Jain, B. and Jain, K., 2020. [12]) agreed that the endometrial thickness showed a good response to PRP therapy. PRP

therapy significantly changed the endometrial

Pattern from thin linear to triple line. PRP was instrumental in improving the sub-endometrial flow and uterine artery resistance, more so in a subgroup of patients suffering from intrauterine adhesions. About 43.3% of their cases showed positive β-hCG. A clinical pregnancy rate of 30% was seen. They endorsed that PRP has promising results in patients with suboptimal endometrium by increasing endometrial thickness, ameliorating pattern, and improving vascularity with reduced cancellation rate .

Other studies (Stewart et al., 2022. [13]; Zhao et al., 2014. [14]; Motwani and Sharma, 2020. [15]; Alzaidi, Z,2021 [16]) agreed that PRP should be regarded as a front-line, non-invasive treatment for individuals with repeated implantation failure in order to increase endometrial thickness and implantation. When compared to the results of earlier endometrial thickness tests and embryo transfers, they saw a substantial improvement in

Endometrial thickness of 1.2 0.21 mm and a clinical pregnancy rate of 37%.

6- Conclusion

In all enrolled man, there was a positive association was observed between SCI% and smoking habit. There was a negative, non-significant correlation between smoking status and fertilization rate. No significant association between smoking status and embryo grade 1, indicating. Similarly, there were weak and non-significant correlations between smoking status and embryo grade 2, as well as embryo grade 3.

Acknowledgment

We would like to acknowledge the High Institute of Infertility Diagnosis and Assisted Reproductive Technologies/ Al Nahrain University, Baghdad, Iraq.

Funding

This work received no funding .

FAuthor Contribution

Sahar S. Raheem performed the study, and Mufeeda A. Alammar, Muayad S. abbood, 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

References

[1] Hernández-Valencia M, Valencia-Ortega J, Ríos-Castillo B, Cruz-Cruz PR, Vélez-Sánchez D. Elements of implantation and placentation, clinical and molecular aspects. Playback 2014; 7: 102-116.

[2] Makrigiannakis A, Minas V. Mechanisms of implantation. Reproductive BioMedicine Online 2007; 14 (1): 102-109 .

[3] Devesa Rodríguez de la Rua M. Factors associated with implantation failure: embryonic factor in advanced maternal age. [Doctoral Thesis]. Barcelona: Autonomous University of Barcelona; 2015.

[4] Hoozemans, D.A., Schats, R., Lambalk, C.B., Homburg, R. and Hompes, P.G. (2004) Human embryo implantation: current knowledge and clinical implications in assisted reproductive technology. Reprod Biomed Online 9:692-715

[5] Krüger, J., Freymann, U., Vetterlein, S., Neumann, K., Endres, M. and Kaps C., 2013. Bioactive Factors in Platelet-Rich Plasma Obtained by

Apheresis. Transfusion Medicine and Hemotherapy, 40 (6), pp.4-4.

[6] Dhillon, M.S., Patel, S. and John, R., 2017. PRP in OA knee–update, current confusions, and future options. Sicot-j, 3.

[7] Bos-Mikich, A., Ferreira, M.O., de Oliveira, R. and Frantz, N., 2019. Platelet-rich plasma or blood-derived products to improve endometrial receptivity? Journal of assisted reproduction and genetics, 36 (4), pp.613-620.

[8] Aghajanova, L., Houshdaran, S., Balayan, S., Manvelyan, E., Irwin, J.C., Huddleston, H.G. and Giudice, L.C., 2018. In vitro evidence that platelet-rich plasma stimulates cellular processes involved in endometrial regeneration. Journal of assisted reproduction and genetics, 35 (5), pp.757-770

[9] Lazzaroni-Tealdi, E., Barad, D.H., Albertini, D.F., Yu, Y., Kushnir, V.A., Russell, H., Wu, Y.G. and Gleicher, N., 2015. Oocyte scoring enhances embryo scoring in predicting pregnancy chances with IVF, where it counts most. PLoS One, 10 (12), p.e0143632.

[10] Tiitinen, A., 2019. Single embryo transfer: why and how to identify the embryo with the best developmental potential. Best Practice & Research Clinical Endocrinology & Metabolism, 33 (1), pp.77-88.

[11] Chang, Y., Li, J., Chen, Y., Wei, L., Yang, X., Shi, Y. and Liang, X., 2015. Autologous platelet-rich plasma promotes endometrial growth and improves pregnancy outcome during in vitro fertilization. International Journal of Clinical and Experimental Medicine, 8 (1), p.1286.

[12] Gupta, J., Jain, B. and Jain, K., 2020. Effect of autologous platelet-rich plasma on clinical outcome in patients with suboptimal endometrium in fresh ICSI/FET cycle− an interventional prospective study. Fertility Science and Research, 7 (1), p.70.

[13] Stewart J. Russell, Yat Sze Sheila Kwok, Tina Tu-Thu Ngoc Nguyen & Clifford Librach. Autologous platelet-rich plasma improves the endometrial thickness and live birth rate in patients with recurrent implantation failure and thin endometrium. Journal of Assisted Reproduction and Genetics. 39, pages1305–1312 (2022)

[14] Zhao, J., Zhang, Q., Wang, Y. and Li, Y., 2014. Endometrial pattern, thickness, and growth in predicting pregnancy outcome following 3319 IVF cycle. Reproductive biomedicine online, 29 (3), pp.291-298.

[15] Motwani, Y. and Sharma, R.K., 2020. To study the effect of autologous platelet-rich plasma instillation on suboptimal endometrium and pregnancy outcomes in infertile women undergoing frozen embryo transfer cycle. Fertility Science and Research, 7 (2), p.141.

[16] Alzaidi, Z., Yildiz, Ş.M., Saatçi, Ç. et al. The effect of cytokine leukemia-inhibitory factor (LIF) and interleukin-11 (IL-11) gene expression on the primary infertility related to polycystic ovary syndrome, Tubal factor, and Unexplained infertility in Turkish women. Egypt J Med Hum Genet 22, 85 (2021). https://doi.org/10.1186/s43042-021-00201-9