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ORIGINAL ARTICLE

A report on three live births in women with poor ovarian response following intra-ovarian injection of platelet-rich plasma (PRP)

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Abstract

The prevalence of poor response to gonadotropin stimulation is approximately 9–24% in women undergoing in vitro fertilization. Interestingly, due to containing a variety of growth factors, platelet-rich plasma (PRP) can play an important role in tissue regeneration and healing. Thus, in this research, we aimed to investigate the intra-ovarian injection of PRP in women with poor ovarian response. To this goal, 23 poor responders constituted the study population, from among whom 19 women were enrolled. These patients underwent ovarian stimulation according to the Shanghai protocol. Immediately after the first follicular puncture, 2 mL of PRP was injected into each ovary. 1 day after the first puncture and PRP injection, the second stimulation was initiated. Then, oocyte retrieval was followed. About 2–3 months after the first cycle, the patients underwent another treatment with ovarian stimulation according to the Shanghai protocol and then, follicular puncture was performed. The mean numbers of oocytes before and after PRP injection were 0.64 and 2.1, respectively. Two patients experienced spontaneous conceptions. The third case achieved clinical pregnancy and delivered a healthy baby in June 2018. The results of this study appeared to be the first report on the effects of intra-ovarian PRP injection on the increase of ovarian responses, even on the spontaneous conceptions of women with poor ovarian response.

Keywords Platelet rich plasma · Ovary · Infertility · Poor ovarian response · Pregnancy

Introduction

The prevalence of poor responses to gonadotropin stimulation is approximately 9–24% in women undergoing in vitro fertilisation (IVF) [1] .Management of this group of patients was very difficult and various therapeutic approaches had to be adopted to improve the pregnancy rates [2]. Double ovarian stimulations could produce more oocytes/embryos in a single cycle of stimulation in the short term, thus serving as a useful strategy for women with poor ovarian response [3–5].

Folliculogenesis generally consists of two phases, namely, gonadotropin-independent (preantral) and gonadotropin-dependent (antral) stages. Follicle development in the preantral stage is controlled by intra-ovarian growth factors through autocrine/paracrine mechanisms [6, 7].

Intra-ovarian growth factors include nerve growth factors, KIT ligands and their receptors, members of the transforming growth factor-β superfamily (TGF-β), such as anti-Müllerian hormone, growth differentiation factor 9 (GDF9), bone morphogenetic protein 6 (BMP6), BMP4, BMP7, and BMP15, and fibroblast growth factors (FGFs). Intra-ovarian growth factors play a potential role in the local regulation and modulation of follicular selection and development [7–9]. Platelets can release several growth factors. In particular, platelet-rich plasma (PRP) is a concentration of autologous human platelets (3–5 times higher than the plasma baseline level) containing a variety of hormones, adhesion molecules, cytokines, chemokines, coagulation factors, integrins, and growth factors, such as platelet-derived growth factors (PDGFAA, PDGF-BB, and

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PDGF-AB), TGF-β1, and TGF-β2, insulin-like growth factor 1 (IGF-I), vascular endothelial growth factor (VEGF), epithelial growth factor, and epidermal growth factor [10–12].

PRP plays an important role in promoting cell division, proliferation, differentiation, and migration, angiogenesis, extracellular matrix remodeling, tissue regeneration, and healing. Recently, clinical trials and animal studies have revealed many beneficial effects of PRP on infertility through its regenerative mechanisms [13–16].

The clinical evidence is still preliminary and the use of ovarian PRP treatment is a too new practice to be widely used since no definite clinical evidence has yet been obtained to confirm its efficacy. Therefore, this study was conducted to determine the effects of intra-ovarian PRP injection on the ovarian stimulation outcomes in the poorly responding infertile women referring to an endometrium and endometriosis research center.

Materials and methods

23 women with poor ovarian response, who had referred to the Endometrium and Endometriosis Research Center of Hamadan University of Medical Sciences for infertility treatment, were included in this clinical trial based on the sample size of a previous study [17] and the sample size calculation formula.

The inclusion criteria were having a history of poor ovarian response (< 3 eggs per cycle) and abnormal ovarian reserve tests (antimullerian hormone level of < 0.5–1.1 ng/mL or antral follicle count of < 5–7), and lacking any underlying diseases causing female infertility, such as severe anemia preventing blood transfusion, cell cycle anemia, renal failure, upper and lower respiratory tract infections, neutropenia, the existence or a history of malignancy or endometriosis, submucosal myoma, Asherman syndrome, untreated hypothyroidism, untreated hyperprolactinemia, any pathologies in the fallopian tubes (e.g., hydrosalpinx, tubular obstruction, etc.) or underlying endocrine diseases, and contraindication of pregnancy. Out of 23 patients, 19 patients were enrolled since four patients declined to participate in the study. These patients accepted to undergo ovarian stimulation according to the Shanghai protocol [3] and follow-up procedures using transvaginal ultrasound. When at least one follicle above 15 mm in size was seen through ultrasound, the patients were subcutaneously administered with 0.2 IU of Diphereline and after 36 h, follicular puncture was performed. On oocyte retrieval day as previously described, PRP was prepared from their autologous blood samples at Blood Transfusion Organization in accordance with its organizational standard methodology [15, 16, 18].

Immediately after the first follicular puncture, 2 mL of PRP was injected into each ovary. One day after the first egg collection and PRP injection, the regimen was begun for the second stimulation. Monitoring was done through the serial ultrasound examination.

When at least one follicle reached the diameter of above 15 mm following the subcutaneous injection of 0.2 IU of Diphereline, the second puncture was done. The total numbers of oocytes obtained from the first and second punctures of this cycle of the Shanghai protocol were recorded. About 2–3 months after the first cycle of Shanghai, the patients underwent a treatment with ovarian stimulation according to the mentioned protocol, oocyte retrieval was conducted, and the number of oocytes obtained from this cycle was recorded. All the mature oocytes were inseminated via intracytoplasmic sperm injection (ICSI). Embryo transfer took place on the 5th day. Finally, a checklist was designed to record the patients' individual characteristics and numbers of oocytes.

It should be noted that a written informed consent was obtained from all the patiens.This research was approved by the Ethics Committee of Hamadan University of Medical Sciences, Hamedan, Iran with the registration code of IR.UMSHA.REC.1395.593. All the ethical standards of the Helsinki Declaration were observed and the study protocol was registered under the code of IRCT201703079014N150 in the Iranian Registry of Clinical Trials. All the data were analyzed using SPSS software, version 16.

Results

Of the 19 patients to whom PRP was administered in the first cycle of the Shanghai Protocol, 12 cases were referred for the second Shanghai cycle (Shanghai 2), five of whom had husbands with oligospermia in addition to poor ovarian response. None of the patients experienced post-injection complications, including fever and pelvic inflammatory disease.

Despite recommendations to perform AMH and FSH tests before and after PRP injection, a limited number of the patients did not consent to undergo the tests. six patients underwent hormonal tests before and after PRP injection. Of these, five patients showed decreased FSH levels compared to the levels recorded before PRP injection (data not shown).

In five out of these 11 patients, who underwent the second Shanghai cycle and had no oocytes in the previous cycle, the numbers of oocytes increased from 1 to 7. The mean numbers of oocytes before and after PRP injection were 0.64 and 2.1, respectively (Table 1).

Two patients experienced spontaneous conceptions following PRP injection. The first case, who had delivered a healthy living girl at term (in 2017), became pregnant 2 months after PRP injection (Case 12). The second woman,

Table 1 Selected demographic and clinical features of patients before and after intraovarian injection of PRP

Cases	Age (years)	Infertility duration (years)	Male factor	Previous ovarian stimulation cycle number	Oocyte number (before PRP)	Embryo number (before PRP)	Oocyte number (after PRP)	Embryo number (after PRP)
Case 1	34	4	No	4	0	0	1	0
Case 2	34	10	Yes	4	1	0	2	1
Case 3	35	11	Yes	3	1	0	0	0
Case 4	39	7	No	5	3	1	1	0
Case 5	39	3	No	3	1	1	1	1
Case 6	38	7	Yes	5	0	0	7	5
Case 7	27	4	No	5	0	0	2	2
Case 8	36	13	Yes	5	0	0	7	5
Case 9	40	1.5	No	2	1	1	1	1
Case 10	40	8	Yes	2	0	0	0	0
Case 11	30	3	No	2	0	0	1	0
Case 12	36	10	No	3	2	1	Positive βHCG	Positive βHCG
Mean ± SD	35.57 ± 3.80	6.50 ± 3.77		4.00 ± 0.94	0.64 ± 0.92	0.27 ± 0.46	2.1 ± 2.5	1.36 ± 1.91

who conceived 4 months after PRP injection and the second ovarian stimulation, delivered a living boy at term (Case 11).

The third case of conception in our study was a 36-year-old woman with a history of ten ICSI + TESE failures, while no oocytes were obtained from her last two cycles (Case 8). This patient underwent ovarian stimulation according to the Shanghai protocol. Out of seven oocytes, two embryos were obtained in the blastocyst stage and the woman achieved clinical pregnancy after embryo transfer. She delivered a healthy baby boy weighing 3050 g in the cesarean section in June 2018.

Discussion

Increasing age is associated with declining ovarian reserves, which could influence on ovarian response to gonadotrophin. This can lead to IVF cycle cancellations, access to fewer oocytes and embryos, and ultimate decreases in pregnancy rates [2, 19].

In the present research, in about 38.5% of the patients with poor ovarian response, the numbers of oocytes increased after the intra-ovarian injection of PRP as compared to the previous cycles, while about 20% of them with fertile husbands experienced spontaneous conceptions.

Concentrations of growth factors in PRP are about 3–5 times higher than those in the plasma [20]. Various studies have shown that different growth factors, including PDGF that is effective on the activation of primordial follicles, are involved in the early stages of folliculogenesis [21–24].

GDF-9 is an oocyte-derived protein, which is essential for the growth of primary follicles [25] and is effective on all the stages of ovarian follicle growth [26]. The use of this factor leads to the increased numbers of primary and preantral follicles [27].

A novel application of transvaginal ultrasound-guided intra-ovarian PRP injection was reported in a research conducted by Pantos et al. who studied a group of eight infertile menopausal women (with amenorrhea of 12–96 months). In approximately 40% of the women, menstrual cycles were restored within 1–3 months after the injection, while 18.5% of them experienced resumption of ovulation cycles with 1–5 oocytes obtained from the IVF cycles [28]. However, the precise mechanism of PRP in ovarian rejuvenation has not yet been known [29].

Sills et al. investigated the effects of the intra-ovarian injection of activated PRP in 4 cases in 2018 and observed increased AMH and significantly decreased FSH levels with at least one embryo obtained from the IVF cycles in all the patients [29].

Although the searches included in the registry of clinical trials in 2017 suggested that our research was the first clinical trial with a registration number, Sfakianoudis et al. had previously treated three poorly responding patients with autologous PRP ovarian infusion, resulting in successful live births [30].

Various studies have revealed that appropriate ovarian environment conditions are necessary for follicular growth and oocyte quality. Oxygen and paracrine regulators, which are mainly provided by pre-follicular angiogenesis, would play an essential role in the process [31]. Primordial and preantral follicles meet their blood requirements through stromal vessels and vascular growth into ovarian thecal cells [32]. Dominant follicles have more blood vessels [33, 34] and the oocytes whose follicular cells have

appropriate blood vessels can have higher fertilization and developmental potentials [35].

Expressions of all the members of the PDGF family have been reported in the rat, mouse, pig, and human ovaries. This factor, which exists in the oocyte and granulosa cells with receptors located in granulosa cells, plays an important role in activating primordial follicles [28]. During follicular growth, the follicle fulfills its own blood supply via angiogenic modulating factors, such as VEGF [8].

The ovary is one of the rare organs, in which neovascularization can occurr. VEGF whose mRNA is expressed in cumulus oophorus, mural granulosa, and thecal cells controls ovarian angiogenesis [36].

An increase in the blood flow of a follicle enhances its ability to respond to gonadotropins, receive more oxygen and nutrients, and growth factors to increase its numbers. This follicular behavior plays an important role in follicle selection [37]. Besides this, VEGF regulates folliculogenesis and is effective at all stages of follicular growth and viability [38], thus preventing follicular atresia by inducing expressions of anti-apoptotic proteins in endothelial cells [39].

Regarding the findings of the current study, as well as the study of Pantos et al. on postmenopausal women, the hypothesis that the cell growth factors present in PRP may stimulate the remaining stem cells in the ovaries and thus provide necessary conditions for the differentiation of those cells is strengthened [28].

On the other hand, PRP injection may activate postnatal oogenesis in the ovary, leading to the formations of new primordial follicles (even about 1000 follicles in menopausal women's ovaries) via the stimulation and activation of GnRH receptors [28].

Additionally, the activities of endothelial and vascular growth factors in PRP augment angiogenesis and ameliorate blood flow. These factors can also induce differentiations of ovarian stem cells to develop de novo oocytes and promote ovarian regeneration [29].

The probable presence of ovarian stem cells (OSCs) and neo-oogenesis after birth were first proposed by Tilly et al. [40].

Edessy et al. [41] transplanted autologous bone marrow mesenchymal stem cells into the ovaries of women with ovarian failure, resulting in the births of living, healthy babies. Studies have shown that instead of rejuvenating old oocytes, healthy oocytes can be obtained from the old ones by manipulating the ovarian micro-environment and stimulating the intra-ovarian stem cells [41].

The evaluated patients displayed already unsatisfactory results with the conventional protocol at our clinic; yet, the double-stimulating opportunity in the same cycle with intra-ovarian PRP administration aiming to increase the number of oocytes could be a novel therapeutic approach to restoring ovarian reserve quality.

Nonetheless, this investigation had some limitations, the main ones of which could be mentioned as follows: (1) missing actual data due to incomplete lab test assessments; (2) psychological burdens of treatments, repeated cycles, and poor prognoses probably playing a very significant role in the majority of poor-responding patients' unwillingness to continue the follow-up periods and subsequent decisions to drop out from serial hormonal measurements despite motivating consultations and recommendations; and (3) having limited follow-up terms.

Some other limitations were related to having a small patient population with no patients over the age of 40 and lacking a placebo group. Hence, any confirmative claims of PRP effective and beneficial impacts would have been more persuasive with a larger sample including older women and longer follow-up terms.

Furthermore, the present research was an uncontrolled longitudinal study with all the patients receiving the same pre- and post-interventions without a control group. Therefore, for the best valid comparison homogeneity, a control group can be included in future studies.

Even with the above-mentioned limitations, we can conclude that the results of this investigation appear to be the first report on spontaneous conceptions and three live births in women with poor ovarian response following intra-ovarian PRP injection.

The evidence on the clinical application of intra-ovarian PRP injection is very novel and has not yet been sufficiently elucidated. Thus, this issue should be further studied with larger sample sizes. So far, new horizons in ovarian rejuvenation have been opened considering the presence of ovarian and germline stem cells in the ovarian surface epithelium with their abilities to differentiate into oocytes under certain conditions, as well as the existence of endothelial, and vascular cell growth factors in PRP. PRP can be utilized to create primary ovarian follicles and consequently antral follicles in advanced age, especially in women with poor ovarian response, premature ovarian failure, and chemotherapy-induced ovarian dysfunction before and after menopause [28].

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

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