Mak Med Pregled 2019; 73(2): 101-105

MMP

Original article

A NOVEL METHOD OF TREATING OVARIAN INFERTILITY: IS PLATELET-RICH PLASMA A NEW PROMISING THERAPY IN THE FUTURE?

НОВ МЕТОД ВО ЛЕКУВАЊЕ НА ОВАРИЈАЛЕН ИНФЕРТИЛИТЕТ: ДАЛИ ПЛАЗМА БОГАТА СО ТРОМБОЦИТИ Е ТЕРАПИЈА ШТО ВЕТУВА?

Snezhana Stojkovska, Gligor Dimitrov, Zoran Petanovski, Damjan Shushleski, Stefan Saltirovski, Vladimir Matevski, Makuli Hadzi Lega, Valentina Sotirovska, Daniela Stojanovska, Nikoleta Stamenkovska, Emilija Petanovska Kostova and Rina Latifi Isaki

PZU Remedika, Skopje, Republic of North Macedonia

Abstract

Introduction. In today's modern society, the treatment of patients with poor ovarian reserve presents a medical challenge of increased clinical importance. The use of platelet-rich plasma (PRP) is a new hope that improves pregnancy chances. Increased use of the PRP in a number of *in vitro* centers around the world as well as publication of thefirst experience in *in vitro* fertilization entailed the need for this systematic review.
Methods. PubMed, Cochrane and Ovid Medline were searched between 2000 and 2019 under the following strategy: [<PRP or plasma-rich with platelets> and <ovaries with reduced reserves or function> and <ovarian rejuvenation>]. Fourteen original articles published in medical scientific journals were analyzed in this study. The evidence level and quality assessment were made based on the most up-to-date, reliable, scientific evidence as well as from the number of additional relevant citations.
Results. Taking the current available proof and evidence into consideration, we can conclude that the PRP method improves the ovarian function and increases the chances of clinical pregnancy. In addition, we assume that, over time, the PRP method objectively improves the ovarian reserves. Recent studies support the theory of increasing the number of preantral follicles, followed byappropriate growth and reduction of follicular atresia.
Conclusion. The improvement of the quantity and quality of oocytes with the intra-ovarian application of PRP potentially suggests a new concept of ovarian aging, where the ovarian microenvironment plays a crucial role.

Keywords: ovarian rejuvenation, poor ovarian reserve, platelet-rich plasma

Correspondence to: Snezhana Stojkovska, PZU Remedika, Skopje, R.N. Macedonia; E-mail: sstojkovska2000@gmail.com

Абстракт

Вовед. Во денешното современо општество третманот на пациентки со намалени оваријални резерви преставува медицински предизвик со зголемено клиничко значење. Употребата на плазма збогатена со тромбоцити (PRP) е нова надеж со кој се подобруваат шансите за клиничка бременост. Зголемената употреба на PRP во поголем број на ИВФ центри, ширум светот, како и објавувањето на првоте искуства во инвитро фертилизација ја наметнува потребата од овој преглед.
Методи. PubMed, Cochrane и Ovid Medline беа пребарувани помеѓу 2000 и 2019 година според следната стратегија: [<PRP или плазма збогатена со тромбоцити> и <јајниците со намалени резерви или функцијата> и <оваријално подмладување>]. Во студијата вклучени се 14 оргинални статии објавени во медицински научни списанија. Нивото на докази и проценката на квалитетот беа направени врз основа на најсовремени, веродостојни, научни докази, како и од бројот на дополнителни релевантни цитати.
Резултати. Досегашните достапни докази покажуваат дека PRP ја подобрува функцијата на овариумите и ги зголемува шансите за клиничка бременост. Со текот на времето, исто така, се чини дека објективно ги подобрува оваријалните резерви. Неодамнешните студии ја поддржуваат теоријата за зголемување на бројот на преантрални фоликули, пратено со соодветен раст и намалување на фоликуларната атрезија.
Заклучок. Подобрувањето на квантитетот и квалитетот на ооцитите преку интраоваријална апликација на PRP, потенцијално сугерира нов концепт на стареење на јајниците, каде оваријалната микросредина има значајна улога.

Клучни зборови: оваријална рејувенација, намалени оваријални резерви, плазма збогатена со тромбоцити

Introduction

Ovarian rejuvenation thorough history

Oogenesis, the production, and development of the oocyte, is the principal role of the ovarian tissue. It was believed for a long time that women are born with certain reproductive potential which is in direct correlation withthe number of primordial follicles produced during the time of embryological and fetal period and which progressively decrease to reach a number circa 300 000 primordial follicles until the time of menarche [1]. These claims were altered by Tilly *et al.* by demonstrating the existence of germ cells in the ovarian tissue [2]. He examined new concepts for how oocytes and their precursor cells might be altered metabolically to sustain or increase ovarian function and fertility in women [2]. This has led to the development of different methods and techniques through which the scientific circles are making endeavors to find the corresponding therapeutic method for patients with decreased ovarian reserves.

The first research presented the use of dehydroepiandrosterone (DHEA) as a food supplement that can have therapeutic benefits in treatment ofpatients with decreased ovarian reserves [3]. The success of DHEA has beenclinically proven in many studies and it remains one of the first noninvasive medical treatments for ovarian rejuvenation. The use of precursors of testosterone can improve the ovarian microenvironment. DHEA reaches a maximum in people aged between 20 to 30 years and decreases approximately 2% per year [4]. A similar concept brings the use of coenzyme Q 10. The role of this molecule is not conclusive in the improvement of the mitochondrial function caused by ageing of the tissue. The androgen supplements can also have positive effect on mitochondrial function [5]. Doctorsin multiple IVF centers in the USA recommend the transvaginal trauma of ovarian tissue, i.e. piercing, as a method for ovarian rejuvenation. This method is accompanied by changes in hormonal status, local immunological response and increased vascularization in the ovarian tissue. The trauma initiates growth factor production, which promotes tissue regeneration [6]. Furthermore, Bukovsky recommended novel methods of treating premature ovarian failure and ovarian infertility. In hisstudy he has concluded that the follicular renewal is also dependent on the support of circulating blood mononuclear cells. The circulating mononuclear cells as a part of the immune system regulate the function of almost all tissues in the body, which leads to temporary rejuvenation of the endocrine and immune system in the ovarian tissue. Namely, the immune system plays a crucial role in the modulation of ovarian function, as it regulates ovarian development, follicular maturation, ovulation and formation of the corpus luteum [7, 8].

In recent years a new approach has emerged in the treatment of ovarian infertility based on the use of plasma rich with platelets (PRP) with or without stem cells as a method for ovarian rejuvenation and follicular reactivation [9,10].

Material and methods

We conducted a systematic search of PubMed, Cochrane and Ovid Medline databases from 2000 to 2019 using the following keywords: platelet-rich plasma, ovarian reserve or diminished ovarian reserve, ovarian function or diminished ovarian function, poor responders, reproductive age, IVF. This search yielded 110 studies, of which 14 original articles and reviews were included. All publications were reviewed by the authors of this manuscript, who agreed onthe analysis and interpretation of data. Some of the studies have addressed the question of whether these approaches of transvaginal intraovarian application of PRP may be beneficial in poor responders with respect of clinical pregnancy and live birth rates in patients. Studies were eligible if they met one of the following criteria: primary evidence (clinical trials) that assessed the effectiveness of a procedure correlated with an outcome measurement (pregnancy, IVF, live birth rates); meta-analyses; and relevant articles from bibliographies of identified articles. The level of evidence was evaluated using the grading system available online and wasassigned for each reference in the bibliography. The quality of evidence was evaluated using the grading system, adapted from Johns Hopkins Nursing Evidence-based Practice Grading System.

Results

The first description of PRP was introduced as a MeSH (Medical Subject Headings) term in 2007 as: “a preparation consisting of concentrated platelets in a limited plasma volume. It is used in various regeneration procedures of surgical tissues, where growth factors from platelets can affect the speeding up of healing and regeneration of the wounds” [11]. The growth factors (GFs) contained in platelet alpha granules are a major part of the PRP. They induce, through appropriate transmembrane receptors in target cells, a whole range of intracellular processes leading to proliferation, differentiation, matrix formation, osteoid production, collagen synthesis, haemostasis, and everything that leads to tissue recovery and regeneration. It is noted that the mitogen effects of PRP are only limited to augmentation of the normal healing process and is theoretically not mutagenic, as the GFs released do not enter the cell or its nucleus, but only bind to the membrane receptors and induce signal transduction mechanisms (Table 1) [12].

Table 1. Platelet growth factors and their specific characteristics

Platelet Growth Factor Type	Growth factor Source	Biological Actions
Platelet derived growth factor (a-b)	Platelets, osteoblasts, endothelial cells, macrophages, monocytes, smooth muscle cells	Mitogenic for mesenchymal celss and osteoblasts, stimulates chemotaxis and mitogenesis in fibroblast/glial/smooth muscle cells, regulates collagenase secretion and collagen synthesis, stimulate macrophage and neutrophil chemotaxis
Transforming growth factor TGF(alpha –beta)	Platelets, extracellular matrix of bone, cartilage matrix, activated TH1 cells and natural killer cells, macrophages/monocytes and neutrophils	Stimulates undifferented mesenchymal cell proliferation ; regulates endothelial, fibroblastic and osteoblastic mitogenesis ; regulates collagen synthesis and collagenase secretion, regulates mitogenic effects of growth factors, stimulate endothelial chemotaxix and angiogenesis, inhibits macrophage and lymphocyte proliferation
Vascular endothelial growth factor, VEGF	Platelets, endothelial cells	Increases angiogenesis and vessel permeability, stimulates mitogenesis for endothelial cells
Epidermal growth factor, EGF	Platelets, macrophages, monocytes	Stimulates endothelial chemotaxis / angiogenesis, regulates collagenase secretion, stimulates epithelial /mesenchymal mitogenesis
Fibroblast growth factor, FGF	Platelets, macrophages, mesenchymal cells, chondrocytes, osteoblasts	Promotes growth and differentiation of chondrocytes and osteoblasts, mitogenic for mesenchymal cells, chondrocytes and osteoblasts
Connective tissue growth factor CTGF	Platelets through endocytosis from extracellular environment in bone marrow	Promotes angiogenesis, cartilage regeneration, fibrosis and platelet adhesion
Insulin like growth factor – 1 IGF -1	Plasma, epithelial cells, endothelial cells, fibroblasts, smooth muscle cells, osteoblasts, bone matrix	Chemotaxis for fibroblasts and stimulates protein synthesis. enchances bone formation by proliferation and differentiation of osteoblasts

(Principles and Methods of Preparation of Platelet-Rich Plasma: a Review and Authors Perspective: 2014. Journal of Cutaneous and Aesthetic Surgery – Oct-Dec 2014, Volume 7, Issue 4)

In recent years the effect of autologous platelet-rich plasma has beenincreasingly used in the treatment of any injuries of the soft and connecting tissues as well as the bone grafts. The use of PRP is a significant part of the speed-up healing process of the injured tissue, angiogenesis and tissue remodeling - with this, the use of PRP has become a routine treatment in orthopedics, dermatology, and specific autoimmune diseases [13, 14].
Many clinicans have noticed that the use of PRP improves the function of the target organ, which resulted in an enthusiastic use of PRP in patients with ovarian insufficiency. Studies have shown that many innovative techniques find their way from theory to practice. Namely, the use of PRP in restoring both the reproductive and endocrine functions of the ovary was presentedin a study from 2017 by Ljubic. He described the first case of ahuman embryo obtained after autologous ovarian *in vitro* activation with orthotopic retransplantation [15]. The novel application of PRP in the ovarian cortex was pioneered by Pantos [9]. The idea of potential therapeutic use of autologous PRP in the renewal of egg cells and follicular reactivation was published in 2017, via the first abstract and detailed oral report on PRP as a method of renewal of egg cells atthe ESHREmedical conference.
During the following year, 2018, an increase in oocytes and embryo quality was also confirmed inScott's study [10]. This pilot study was focused on the intra-ovarian injection of the autologous plasma enriched with thrombocytes and the effect of PRP on the ovarian microenvironment and the creation of oocytes of decent/high quality. Melo's study has confirmed the efficacy of using an intracortical ovarian injection of PRP that significantly improves ovarian reserve and subsequent reproductive outcome in infertile women [16]. The effects of autologous PRP were observed in the treatment of repeated implantation failure in IVF cycles in order to improve pregnancy outcome [17]. It is considered that one of the mechanisms bywhich PRP results in changes in the ovarian reserves and the activation of the primary preantral follicles is through the synergy connection of the factors for growth contained in the PRP with the usage of gonadotropins for ovarian stimulation.
In women considered to be poor ovarian responders, there is insufficient evidence forrecommending or rejecting intraovarian injection of autologous PRP before IVF treatment.

Discussion

It remains essential to understand the physiological basis of ovarian aging to interpret the mechanisms of action. With the use of platelet-derived growth factors (PDGFs), dysfunctional ovarian tissue is believed to be supplied with essential factors necessary for ovarian regeneration. In this context, it is necessary to mention angiogenesis and follicular vascularization and their important role in the aging of the follicles. Receptors for growth factors are present on granulose cells confirming their association with the activation process of the primordial follicles. The most important component in PRP is the transforming growth factor-beta family (TGF beta) that plays a significant role during the developmental phases of the follicle [18]. Confirmation of all the above statements is also obtained from the

Hosseini study [19]. This study evaluated the effects of platelet-rich plasma (PRP) on the growth and survival of isolated early human follicles in a three-dimensional culture system. The conclusion was that media supplementation with PRP could better support the viability and growth of isolated human preantral follicles *in vitro*.
On the other hand, the presence of OSCs on the surface of the ovarian tissue, under certain conditions, can produce *de novo* primordial follicles and thus the appearance of new antral follicles. It is noteworthy to mention that only a fraction of OSCs undergoes meiosis culture to form oocytes. It remains unknown why only a few cells express Stra8 and undergo differentiation [20]. Alternatively, it is possible that OSC aging is a result of impaired DNA double-strand break repair, a recently identified cause of aging in mammalian oocytes. Elucidating the mechanisms that cause OSCs to age could lead to new treatments that could delay ovarian aging and slow infertility. Also, several questions about the PRP's mechanism of action remain unanswered.

PRP activation and intraovarian application

According to the results in the literature different protocols which describe the optimal conditions needed for preparation of autologous PRP are detailed. The relative centrifugal force, time temperature, the use of anticoagulants and the method of activation of the PRP can influence the preparation of the autologous PRP [13, 14]. The process of the activation of α granules of the platelets is one of the key steps that could influence the availability of the released biomolecules and consequently the quality of the PRP [21]. Thrombin and/or calcium chloride is used as the most frequent activator before the administration of PRP in the damaged tissue [22]. The secretion of the active biomolecules begins 10 minutes after the activation of the platelets, and 95% of the growth factors are released in a period of 1 hour. Through the method of the activetion, the amount and the kind of the biomolecules is determined, which directly influences the tissue healing. The PRP activation strategy is determined by the type of procedure (open or laparoscopic), and the desired biological effect that is expected from the biological tissue [23].
PRP for the treatment of ovarian infertility is a lower concentration (2.5x3 times) system. The process is carried out under strict aseptic conditions as well as optimum temperature regulations i.e. 21-24^{o}C. PRP is prepared according to the manufacturer's guidelines. In the last step, the volume immediately above the erythrocyte layer is collected. Calcium gluconate is used as an activator. After activation, in a period less than 2 min, approximately 3-5 ml of the PRP isinjected into the ovaries under transvaginal ultrasound guidance. Intervention is made following a protocol set by the IVF department. The entireintervention lasts 15 to 20 minutes. The method of obtaining PRP is simple, minimally invasive and low cost. High concentration of growth factors and cytokines from PRP in the damaged tissue leads to balance between the anabolic and catabolic processes, optimizing tissue environment and favoring the process of tissue regeneration.

Predicting the effectiveness of intraovarian PRP application

FSH, estradiol and AMH levels are predictable of treatment outcomes after PRP utilization. The treatment can lead to increasing the preantral follicles and in general, may enhance ovarian function by increasing follicles recruitment.

Limitations

Even though PRP has a widespread usage in multiple medical spheres, unfortunately, there is still a lack of controlled clinical studies regarding the process. The limitation of this format should be taken into account i.e. sample size, RCT, design, etc.
The main limitation of this study was the absence of previous data attesting the safety of PRP injection into human ovaries.Future larger trials would be required to corroborate the efficacy of PRP injection for treatment ofovarian infertility, which would confirm the findings of this study.

Conclusion

The authors reviewed the best available evidence for the usage of PRP in actual medical practice. Because the diagnosis of patients with lower ovarian reserves often leaves limited time for treating, the patients need to be given a choice. There is fair evidence that the clinical and live birth rates are substantially different after the intraovarian application of PRP before IVF treatment. Further research is required to investigate whether decreasing the level of FSH and increasing the number of antral follicles following intraovarian PRP injection is sustained.
Future studies need to evaluate whether this approach may be especially beneficial in a specific subset of patients.

Conflict of interests. Not declared.

References

1. Faddy MJ, Gosden RG, Gougeon A, *et al.* Accelerated disappearance of ovarian follicles in mid-life: implications for forecasting menopause. *Hum Reprod* 1992; 7: 1342-1346.
2. Jonathan L Tilly, David A. Sinclair.Germline energetics, aging and female infertility. *Cell Metab* 2013; 17: 838-850.

3. Wisser A, Gonen O, Ghetler Y, *et al*. Addition of dehydroepiandrosterone for poor responder patients before and during IVF treatment improves the pregnancy rate: a randomized prospective study. *Hum Reprod* 2010; 25: 2496-2500.
4. Walker A, Anderson D, Herndon J, Walker L. Ovarian aging in Squirrel monkeys. *Reproduction* 2009; 138: 793-799.
5. Pitteloud N, Mootha VK, Dwyer AA, *et al*. Relationship between testosterone levels, insulin sensivity, and mitochondrial function in men. *Diabetes Care* 2005; 28: 1636-1642.
6. Siristatidis C, Vogiatzi P, Bettocchi S, *et al*. Transvaginal ovarian trauma, poor responders and improvement of success rates in IVF: anecdotal data and a hypothesis. *Med Hypothesis* 2014; 83: 227-231.
7. Antonin Bukovsky. Novel methods of treating ovarian infertility in older and POF women, testicular infertility, and other human functional diseases. *Reprod Biol Endocrinol* 2015; 13: 10.
8. Haifeng Ye, Xiaoyan Li, Tuochen Zheng, *et al*. The effect of the immune system on ovrian functions and features of ovarian germline stem cells. *SpringerPlus* 2016; 5: 990.
9. Pantos K, Nitsos N, Kokkali G, *et al*. Ovarian rejuvenation and folliculogenesis reactivation in perimenopausal women after autologous platelet-rich plasma treatment. Abstracts, ESHRE 32nd Annual Meeting; 3-6 Jul 2016; Helsinki, Finland. *Hum Reprod* 2016; (Suppl. 1): i301
10. Scott Sills E, Natalie S Rickers, Xiang Li, Gianpiero D. Palermo. First data on in vitro fertilization and blastocyst formation after intraovarian injection of calcium gluconate-activated autologous platelet rich plasma. *Gynecological Endocrinology* 2018; 34: 756-760.
11. Eva Rubio-Azpeitia, Isabel Andia. Partnership between platelet-rich plasma and mesenchymal stem cells: in vitro experience. *Muscles Ligaments Tendons J* 2014; 4: 52-62.
12. Schmitz JP, Hollinger JO. The biology of platelet-rich plasma. *J Oral maxillofac Surg* 2001; 59: 1119-1121.
13. Arnoczky SP, Delos D, and Rodeo SA. "What is platelet-rich plasma?" *Operative Techniques in Sports Medicine* 2011; 19: 142-148.
14. Kellie K. Middleton, MPH, Victor Barro, *et al*. Evaluation of the Effects of Platelet-Rich Plasma (PRP) Therapy Involved in the Healing of Sports-Related Soft Tissue Injuries. *Orthop J* 2012; 32: 150-163.
15. Ljubic A, Abazovic Dz, Vucetic D, *et al*. Case Report Autologous ovarian in vitro activation with ultrasound-guided orthotopic re-transplantation. *Am J Clin Exp Obstet Gynecol* 2017; 4: 51-57.
16. Mello P, Navarro C, Jones C, *et al*. The use of autologous platelet-rich plasma versus antioxidants in women with low ovarian reserve undergoing assisted reproductive technology: a comparative controlled pilot study. Abstracts, *ESHRE 35nd Annual Meeting*; 3-6 Jul 2019; Vienna, Austria.
17. Nazari L, Salehpour S, Hoseini S, *et al*. Effects of autologous platelet-rich plasma on endometrial expansion in patients undergoing frozen-thawed embryo transfer: A double-blind RCT. *Int J Reprod Biomed* (Yazd). 2019; 17: 443-448.
18. Szafarowska M, Jerzak M. Ovarian aging and infertility. *Ginekol Pol* 2013; 84: 298-304.
19. Hosseini L, Shirazi A, Naderi MM, Shams-Esfandabadi N, *et al*. Platelet-rich plasma promotes the development of isolated human primordial and primary follicles to the preantral stage. *Reprod Biomed Online* 2017; 35: 343-350.
20. Garg N and Sinclair AD. Oogonial stem cells as a model to study age-associated infertility in women. *Reproduction, Fertility and Development* 2015; 27: 969-974.
21. Arshdeep, Kumaran MS. Platelet -rich plasma in dermatology; Bone or a bane? *Indian J Dermatol, Venereol and Leprol* 2014; 80: 5-14.
22. Roubelakis MG, Trohatou O, Roubelakis A, *et al*. Platelet-rich plasma (PRP) promotes fetal mesenchymal stem/stromal cell migration and wound healing process. *Stem cell reviews* 2014; 10: 417-428.
23. Amable PR, Teixeira MV, Carias RB, *et al*. Mesenchymal stromal cell proliferation, gene expression and protein production in human platelet-rich plasma-supplemented media. *PloS one* 2014; 9: e104662.