

A Case Series on Platelet-Rich Plasma Revolutionary Management of Poor Responder Patients

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Established Facts

- Research on poor ovarian response (POR) patients fails to provide an efficient treatment method.
- Autologous Platelet-Rich Plasma (PRP) treatment has already been widely applied for numerous medical issues without complications.

Novel Insights

- In the absence of an overall efficient treatment protocol for poor responders, PRP treatment could be successfully employed as an alternative effective and safe approach.
- Ovarian infusion of PRP could improve ovarian functionality.

Keywords

Poor responders · Platelet-rich plasma ovarian infusion · Improving ovarian functionality · Improving follicle-stimulating hormone/anti-Müllerian hormone levels

Abstract

Poor responders are described as those In Vitro Fertilization (IVF) patients who are failing to respond to controlled ovarian stimulation protocols. Extensive research has focused on

crafting the optimal treatment. However, it appears that each approach fails to be established as effective or guaranteed towards successful management. Platelet-Rich Plasma (PRP) is a novel, highly promising approach that has been successfully applied for an array of medical issues. In this case series, we present 3 poor responder patients with the common denominator of: failed IVF attempts, poor oocyte

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yield, and poor embryo quality. The option of oocyte donation was rejected. All patients were treated with autologous PRP ovarian infusion following written consent. Within a 3-month interval, follicle-stimulating hormone decreased by 67.33%, while Anti-Müllerian hormone increased by 75.18%. These impressive results on the biochemical infertility markers alone are classified as a complete biological paradox, coupled by improved embryo quality. Results report a natural conception at 24 weeks, an uncomplicated healthy pregnancy at 17 weeks and a successful live birth. To our knowledge, this is the first time such an approach and results are reported, where PRP treatment on poor responders lead to overcoming their challenging reproductive barrier.

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Introduction

One of the basic steps typically an In Vitro Fertilization (IVF) female patient is submitted to is the phase of the Controlled Ovarian Stimulation (COS) for an adequate amount of follicles to be recruited. A percentage of women under-responds to this type of stimulation and consists of a special patient group reporting poor IVF success rates called “poor responders” [1]. Despite increasing prevalence of poor responders in everyday routine practice [2], breakthrough Poor Ovarian Reserve (POR) research, which is ongoing, fails to provide an efficient approach [3]. This fact contributes to characterizing POR research as a “cold case”.

The main issue that arises when dealing with poor responders is the lack of common consensus regarding POR definition criteria, as well as the huge heterogeneity of the population of women presenting with POR. The Bologna criteria picture the first attempt of the scientific community to craft a widely accepted definition and selection of POR patients. The idea of a universal consensus was promising. However, Bologna criteria’s prognostic value presents as vague, considering various cases from patient characterization to patient management. The principal issue of this challenge is reflected by the category of true poor responders who remarkably do not fit the Bologna definition criteria [4]. Future revision of these criteria is believed to be inevitable. This case report focuses on 3 poor responder patients who are below the threshold of 40 years of age, not traditionally fitting the POR criteria. However, they do present with recurrent cancelled cycles, poor oocyte yield following stimulation, as well as high follicle-stimulating hormone (FSH) levels and low Anti-Müllerian hormone (AMH).

Based on the current trends and reports on poor responder treatment [9–14], it suffices to say that the management of poor responders is characterized by controversy and lack of standardization. The search for the “holy grail” on efficient and effective approaches still stands.

Platelet-Rich Plasma (PRP) is a novel, highly promising approach successfully employed for numerous medical issues [15–23], albeit standardization of treatment on more systems is ongoing. Preliminary results are encouraging, especially as PRP effectiveness has been evaluated on animal models studies [24] as well as on the human reproductive system, thereby promoting endometrial growth and improving IVF outcome [25]. The patients included in this report were all characterized by challenging an unsuccessful management within the scope of IVF, hitherto, having exhausted available options. In the light of the above, we sought to investigate whether autologous ovarian PRP treatment could result in successful management of this targeted group of women, namely, the extreme poor responders.

In this study, we present our experience on PRP treatment on 3 challenging poor responder cases promising a refreshing groundbreaking approach. All 3 patients were evaluated and approved, in regard to their eligibility for undergoing PRP treatment taking into consideration contraindications and their thorough medical history.

Case Series Description

PRP Treatment and IVF Procedure

A detailed medical history was obtained from all patients during the first consultation appointment during which time their reproductive history was recorded. The hormonal profile of the patients was analyzed on 2 checkpoints: before and after the PRP treatment. FSH was measured on the 3rd day of the menstrual cycle. Values for AMH were recorded on the same day. The hormonal values that existed prior to PRP treatment refer to the menstrual cycle immediately before treatment, while the recordings done after PRP treatment refer to the directly subsequent menstrual cycle. FSH levels were determined by the chemiluminescent microparticle immunoassay on an Abbott-Architect Immunoanalyser (Abbott Laboratories, Abbott Park, IL). The inter-assay coefficient of variation as indicated by manufactures was <4.6%. AMH levels were determined at the same chronic instants using the AMH Gen II ELISA kit (Beckman Coulter, Nyon, Switzerland) with <5.6% inter-assay coefficient of variation.

The autologous PRP was prepared using the RegenACR®-C Kit (Regen Laboratory, Le Mont-sur-Lausanne, Switzerland) according to manufacturer’s instructions. The PRP was infused into the ovaries using a non-surgical, transvaginal ultrasound-guided multifocal intramedullary injection and diffusion in the subcortical layers. The volume of PRP employed and instilled in the ovaries

was approximately 5 mL for each ovary. The volume of peripheral blood required to yield approximately 10 mL of prepared PRP for infusion per patient was 70 mL.

Following PRP treatment, all 3 patients were monitored by ultrasound and following their upcoming menstrual cycle, natural IVF cycles were performed subsequent to PRP ovarian infusion. Ovarian monitoring was performed every 2 days until a follicle of >16 mm was observed. Ovulation triggering was achieved with 5000 IU of rec-human chorionic gonadotropin (hCG), while oocyte retrieval (OR) was performed 36 hours following hCG administration by follicle aspiration through the transvaginal route under ultrasound guidance (Toshiba Capasee SSA-220A Diagnostic Ultrasound, Toshiba Medical System Europe). All mature oocytes were inseminated by Intracytoplasmic Sperm Injection and vitrified in order to be collectively thawed in a future cycle employing cryopreserved embryos. The embryos were cryopreserved at the pronuclei stage. They were subsequently devitrified and further cultured to blastocyst stage employing sequential media, while embryo transfer took place on day 5. The scoring system for cleavage stage embryos Grade 1-Grade 5 was employed as described by Veeck [26]. For the blastocyst stage embryos, grading was performed according to Gardner's criteria [27–29]. An hCG blood test was performed 10 days following embryo transfer to confirm a biochemical pregnancy.

The Hospital Ethics Board along with Independent Committee approved the study protocol in accordance to the Helsinki declaration and all participants gave their informed consent to be part of this study.

Case 1

Patient 1 aged 40 was referred to our clinic; she reported 5 previous failed IVF attempts fitting the POR criteria at another Assisted Conception Unit. A detailed reproductive examination was performed. FSH levels were recorded at 27.8 mIU/mL and AMH at 0.65 ng/mL. Following consultation, the patient opted for the “embryo banking” approach following natural cycles. Subsequently 4 natural cycles followed leading to 1 empty follicle, 2 immature oocytes and a poor quality oocyte, which failed to be fertilized. The poor outcome coupled by the previous numerous failed IVF attempts, and the exceptionally high FSH levels and low AMH prompted the discussion of exploring the PRP treatment protocol. The patient responded confidently, as egg donation was an option offered and discussed in counselling; however, the couple rejected this option as non-acceptable. Following written consent, PRP treatment was performed.

FSH levels were recorded on the first menstrual cycle following PRP and the decrease was impressive at 11.1 mIU/mL and an impressive increase was noted for AMH respectively at 1.1 ng/mL. The decrease of FSH corresponded to an impressive percentage drop on FSH of 60.07% and an increase on AMH was noted at 69.23%. Six natural cycles followed the PRP application and resulted in collecting 1 immature and 5 mature oocytes with a fertilization rate of 100%, leading to cryopreserving 5 good quality zygotes. In preparation of a cycle employing the cryopreserved embryos, 3 of the embryos stored for this patient (3 zygotes) were devitrified and cultured to the blastocyst stage. Three embryos survived the devitrification process, all of which entered the cleavage stage and were morphologically classified on day 3 of development as excellent quality embryos graded as follows: 8c¹, 8c¹, 7c². Further culture to blastocyst stage provided us with 3 embryos graded

as per Gardner's blastocyst grading criteria as follows 5AA, 5AA, 5AA describing 3 excellent quality blastocysts. All 3 blastocysts were transferred, and a positive biochemical pregnancy test was performed, which led to an uncomplicated healthy pregnancy. This resulted in a successful live-birth following a caesarean section delivery of a healthy baby boy weighing 2330 g on the 37th week of gestation.

Case 2

Patient 2 aged 37 was referred to Genesis Athens Hospital with a history of 4 previous failed attempts as a poor responder in another IVF centre. Infertility investigation was performed by our physicians as protocol dictates for every patient arriving in our hospital and in the lack of evidence suggesting otherwise, IVF procedure was initiated. FSH and AMH hormones were recorded to contribute to the clinician's evaluation (FSH = 18.3 mIU/mL and AMH = 0.54 ng/mL). This patient was submitted to 12 IVF cycles following mild ovarian stimulation protocols and 3 natural cycles. In the course of the above procedure, ovarian response of this patient was reported as extremely poor. The subsequent approach resulted in embryo freezing at the pronuclei stage due to an inadequate number of oocytes retrieved in each attempt employing the “embryo banking” approach. The maximum number of embryos developed per cycle was limited to 2 for 4 cycles. The 12 mild stimulation cycles resulted in one cancelled cycle and 15 oocytes of which 10 were mature and 5 immature. Application of Intracytoplasmic Sperm Injection resulted in 10 embryos that were cryopreserved as zygotes at the pronuclei stage. The 3 natural cycles resulted in 1 immature oocyte. In preparation of a cycle employing both fresh (current cycle) and the cryopreserved embryos stored, all the embryos stored for this patient (10 zygotes) were devitrified and cultured to the blastocyst stage along with 2 fresh embryos retrieved from this fresh cycle. All of the embryos survived the devitrification process. Of the 12 embryos, only 7 entered the cleavage stage and on day 3 were classified as follows: 8C², 8C², 6C⁴, 5C⁴, 4C⁴, 4C⁴, 4C⁴. It is important to highlight the nearly 50% arrested development of the 4 cell stage embryos on day 3. Good quality characterized only 2 of the embryos with the remaining categorized as poor quality cleavage stage embryos. By day 5, only 2 reached the blastocyst stage graded according to the Gardner's blastocyst grading scale as 4BB and 3BB describing the first blastocyst as expanded with average quality Inner Cell Mass (ICM) and trophectoderm (TE), and the second as expanding blastocyst with an ICM and TE of average quality. The remaining 5 embryos were classified on D5 as poor quality compacting morulas, while 3 were degenerated. The poor prognosis anticipated due to the particularly reduced embryo yield was further burdened by the detrimentally poor embryo quality condition for the majority of the embryos characterizing this poor responder. This was clearly reflected by the strikingly low blastocyst formation rate for this cycle at a noted 16.7%. These embryos were transferred, yet no pregnancy ensued. Following this, 3 stimulation cycles were performed with equally poor OR yield as described resulting in one immature oocyte. The patient's distress and agony were noted. Counselling to this couple was offered throughout their management to assess and aid their psychological well-being. Reaching what the physician as well as the couple realized as a dead-end, the alternative option of donor eggs was recommended, but the idea was immediately rejected by the couple. As a final resort, PRP treatment was suggested. The fact that PRP employ-

ment is an autologous treatment prepared and administered in the Hospital's premises was reassuring and the patient eagerly consented to treatment.

Following PRP treatment, an impressive decline of FSH (4.1 mIU/mL) and an impressive increase of AMH (0.93 ng/mL) were detected on the first menstrual cycle following PRP. The decrease of FSH corresponded to an impressive percentage drop of 77.6% accompanied by an equally impressive increase on AMH of 72.22%. As a next step, 2 natural cycles were performed, resulting in 2 good quality mature oocytes and 2 subsequent zygotes, which were cryopreserved in order to be employed at a future cycle. Subsequently the patient contacted the hospital to inform the physicians of a natural conception on the third menstrual cycle following PRP. The patient is currently 24 weeks pregnant with a good follow-up and a complication-free pregnancy.

Case 3

Patient 3 aged 37 was referred to our clinic with no previous IVF attempts in her reproductive medical history. Extremely high levels of FSH and low levels of AMH were recorded as follows: FSH = 24.1 mIU/mL and AMH = 0.44 ng/mL. Prior to PRP treatment, this patient was submitted to 4 COS cycles and 4 subsequent natural cycles. In the course of the above process, ovarian response of this patient was reported as poor. The patient's oocyte yield was 3 oocytes for each of the cycles and all were classified as mature. However, a low fertilization rate managed to secure only one zygote corresponding to each cycle reflecting the poor oocyte quality and the low collective fertilization rate of 33.3%. The approach of "embryo banking" was employed here as well. Four zygotes were cryopreserved. The 4 natural cycles resulted in 2 empty follicles and 2 mature poor quality oocytes, which failed to fertilize. All of the patient's cryopreserved embryos were devitrified aiming for a blastocyst stage culture and transfer. However, on Day 3, embryos presented as follows: 1C (arrested), 2C⁵, 4C⁵, 5C⁴. In spite of the small number of extracted oocytes, the true major issue regarding this patient's oocytes was their extremely poor quality and arrested development at 75%. The embryo transfer of embryos 4C⁵ and 5C⁴ was performed with negative outcome. The exceptional poor embryo quality and consecutive failed attempts on this POR couple led to exploring the option of PRP infusion, which was performed following written consent. Following PRP treatment, an impressive decline of FSH (8.6 mIU/mL) accompanied by an impressive increase of AMH (0.81 ng/mL) was detected on the third menstrual cycle following PRP. The decrease of FSH corresponded to an impressive percentage drop of 64.32% accompanied by an astonishing increase on AMH at 84.09%. Six natural cycles followed, accompanied by one mature oocyte yield per OR leading to 100% fertilization and subsequent cryopreservation of 6 resulting zygotes. In preparation of a cycle employing the cryopreserved embryos, 3 of the embryos stored for this patient (3 zygotes) were devitrified and cultured to the blastocyst stage. Three embryos survived the devitrification process, all of which entered the cleavage stage and were morphologically classified on day 3 of development as excellent quality embryos graded as follows: 8c¹, 8c¹, 8C¹. Further culture to blastocyst stage provided us with 1 arrested embryo at the 10-cell stage and 2 embryos graded as per Gardner's blastocyst grading criteria as follows: 4AA and 3AA describing 2 excellent quality blastocysts. The 2 blastocysts were transferred, and a positive biochemical pregnancy test was en-

sued leading to a singleton healthy pregnancy. Clinical pregnancy was confirmed 6 months following PRP treatment and the patient is currently 17 weeks pregnant.

Discussion

Current approaches regarding the efficient management of poor responders present with a wide range of options. However, the huge heterogeneity regarding these patients exhibits great difficulties in proposing just a single optimal treatment strategy or just a single optimal ovarian stimulation protocol suitable for all the disputed categories of diagnosed patients.

The more gentle approach of the natural cycle over high doses of hormonal treatment is also stated in the literature [35] and is claimed to be successfully adopted by a respected amount of clinicians in everyday practice as an alternative worthy and effective option [36]. This model is complete and practiced by repeated natural cycles along followed by vitrification of the respective embryos at the zygote stage. This may be described as the "embryo banking" approach. In our clinic, the approach of repeated natural cycles and subsequent cryopreservation of zygotes is successfully adopted. The model of "embryo banking" through consecutive natural cycles and collective devitrification toward blastocyst embryo transfer has been employed and our experience on the general pool of poor responder patients treated in our Assisted Conception Unit shows that it may represent a mild, and yet effective approach.

In light of the above – at times conflicting – approaches, PRP ovarian infusion may be an efficient model in ascertaining the successful management of poor responder patients. PRP consists of high platelet concentrations following peripheral blood's centrifugation [37]. Hormones, macrophages, neutrophils, chemo-attractants of stem cells, cytokines, and a variety of growth factors are the main components of PRP, which contribute to tissue healing and regeneration, anabolism enhancement, differentiation and proliferation, angiogenesis activation, inflammation control as well as in cell migration [38, 39]. Given the highly angiogenic structure of the ovary and the critical role of various platelet-derived factors on the vascular activation and stabilization [40], PRP treatment could probably enrich the dysfunctional ovarian tissue of our patients with essential factors for neoangiogenesis leading to tissue regeneration and reactivation.

Regarding the reproduction system, there have been reports stating its treating potential, especially intrauter-

ine PRP treatment has been shown to promote the endometrial growth in patients with thin endometrium, improving the assisted reproduction outcome [25, 41–43]. In humans, PRP has been employed in an autologous ovarian transplantation in order to improve the vascularization and quality of the implant. The successful transplantation resulted in a live birth following COS [44].

Applying such a novel treatment one could not help but ponder on the possible side effects and complications that might arise from its application. There are possible scenarios discussed in contemporary literature raising anticipated questions regarding its safe use. It should be highlighted that hypotheses and conclusions on complications rely on data from PRP application regarding other systems and not the female reproductive system. Certain similarities stand and could be extrapolated; however, this novel approach deserves a delineating investigation. To our knowledge until today, there are no studies reporting any specific side effects following PRP treatment. The application of PRP is performed under aseptic conditions, limiting the incidence of infections. While plasma itself has been found to hold antimicrobial characteristics [45]. Hence, PRP therapy might hold control of the local inflammatory response [46]. PRP is autologous and should be intrinsically safe, minimizing the incidence of an allergic reaction. On a theoretical model, patients with hereditary platelet function disorders, acquired platelet function disorders, or certain platelet disorders should be of absolute contraindication in the usage of PRP, as these dysfunctions are expected to affect the adhesion, the aggregation, or the secretion process of the platelets. Moreover, patients who are on pharmaceutical regimes such as non-steroidal anti-inflammatory drugs, or Aspirin and other anticoagulants might not be expected to experience improvement following PRP treatment, since the platelet function is compromised. Relative contraindications might include intense tobacco use, systemic use of corticosteroids, hematopoietic or bone cancer, and thrombocytopenia.

All 3 patients presented had experienced numerous failed IVF attempts. Poor oocyte yield <3, poor oocyte quality (dark, vacuolated), and poor fertilization rate could have collectively or specifically contributed to cancelled ETs. The “embryo banking” approach was employed for all 3. The corresponding embryos presented a considerable extent of poor quality, degeneration, and arrested development. Cancelled ORs due to poor response as well as cancelled ET cycles due to lack of embryos on the grounds of the above-mentioned circumstances were described. In the cases where ET could be performed, the employment of poor quality embryos resulted in the total

implantation failure. It is possible that the hyperbolic approach of high dosage protocols employed in the past on these patients as a last resort – while pursuing the management of low response – could have contributed to diminishing their last chances of successful IVF treatment by the time they were referred. It is of importance to highlight the fact that these patients could be classified as extreme poor responders. Such characterization is attributed to low ovarian reserve based on AMH and FSH levels, as well as the poor oocyte yield corresponding to previous IVF attempts.

As demonstrated in this case series report, an approximate 67.33% decrease was noted in FSH levels comparing the prior and following PRP treatment levels for all 3 cases within the first 3 months of treatment and respectively an increase of 75.18% for AMH. Undoubtedly, this is a strikingly strong indication of the improvement brought by PRP regarding ovarian functionality. However, AMH levels following PRP treatment still corresponded to low AMH levels anticipated in cases of a poor responder patient. This indicates that besides the impressive FSH improvement possibly depicting an improvement in the overall reproductive potential of these patients, they still remained classified as poor responders according to AMH. Nonetheless, it is a complete biological paradox for FSH levels to drop and AMH levels to increase to such an extent. It is not possible though to extrapolate and hypothesize that a drop on FSH levels is an absolute marker of PRP therapy mode of action. In fact, it is equally important to highlight that due to the difficulty of patient follow-up, serial measurements were not possible to be provided. This fact undoubtedly accounts for the limitation in this case series. It is well documented in literature that the stability of AMH levels – even during the menstrual cycle – is debatable and may result in conflicting interpretation [47]. Possible fluctuation may not be related to different AMH assays and hence may account for patient variability [48], hence the variability of AMH in the infertile population merits yet delineation [49]. The possible variability on AMH levels has been reported to be heightened for the “younger” in comparison to the “aging” ovary [50]. This is something to ponder on, especially in light of our patient’s age at 40, 37 and 37 respectively. These are all concerns accounted for in the interpretation of these preliminary results of this high increase especially regarding the AMH levels. The possible sample instability along with the biological variability should be taken into consideration prior to suggesting robust conclusions. It is our opinion that prospective well-controlled clinical trials on the effect of ovarian PRP

treatment particularly on AMH levels are required to ascertain validity.

On another note of observation, the persistent pattern of natural cycles followed by empty follicles, cancelled cycles and poor embryo quality was clearly reversed when PRP infusion was applied in these patients. The improvement of oocyte quality leading to enhanced embryo quality may be the underlying key factor securing all 3 pregnancies reported. All patients were presented and counselled towards egg donation although this option was not welcomed. Our 3 patients faced the dilemma of carrying on with their own poor prognosis or resorting to egg donation, which was rejected due to their psychological distress. With respect to patient 2, it may be important to raise the point that PRP may not equal IVF treatment, but perhaps offer these particularly reproductively challenged patients the possibility of natural conception where male factor is not involved.

Despite the promising nature of the latest proposed treatment regimens for POR patients, the common denominator is the idea that the key solution to this profound problem lies in the stimulation phase. The huge variety of treatments available originates from the lack of total efficiency and the reality is that while every treatment method presents with some advantages, it is also a liability. This report highlights the novel approach of PRP infusion on a specific group of patients, the poor responders. The results are promising and it is of substance to report the reverse mode of a declining FSH being a biological paradox, along with the successful management of these patients achieving a pregnancy within 6 months of the treatment. All 3 pregnancies were described as uncomplicated and healthy, while thus far one has resulted in a live birth of a healthy male baby. It is in our belief that these facts hold power to cement PRP treatment in the sphere of unsolved infertility mysteries. The next step is validation of its application through a prospective regis-

tered clinical trial on a larger sample of a higher scale. Plausible disadvantages of this regime should be examined, mainly due to its invasive nature despite its autologous provenance, the in-house preparation and administration. PRP treatment needs to be cemented and included as an acceptable and efficient approach to the management of poor responders and perhaps other subgroups of patients facing various infertility issues mainly related to the declining fertility associated with older age, POR, and recurrent implantation failure.

The main question raised is in regard to the bioethical aspect of this innovative technique's clinical application. If PRP does in fact reverse aging, who should have access to this treatment-perhaps considered a privilege, and to what extent? Could PRP treatment reprogram and restart folliculogenesis as indicated on in vitro studies on animal models [51]? Should the age limit of patients' receiving IVF following PRP treatment and the relevant legislation and Code of Practice be revisited and reconsidered? Experience and history have undoubtedly proved that the scientific community benefits the most from innovative approaches when the margins and work-frame are challenged in a safe, productive and controlled fashion promoting the good Code of Practice.

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Disclosure Statement

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