ECRONICON OPEN ACCESS

EC GYNAECOLOGY
Case Report

Successful Pregnancy Following Injection of Oocyte by Thawed Frozen Sperms Using Testicular Platelet-Rich Plasma (PRP) - Case Report

Rami Alnasser, Mohammad Alghazawi* and Khalil Khalil

British-Syrian IVF Centre, ALRasheed Hospital, Damascus, Syria

***Corresponding Author:** Mohammad Alghazawi, Assisted Conception Unit, British-Syrian IVF Centre, ALRasheed Hospital, Damascus, Syria.

Received: April 27, 2023; **Published:** May 17, 2023

Abstract

This case report describes a successful pregnancy of a married couple suffering of subfertility for 13 years. The husband diagnosed of having non-obstructed azoospermia after two consecutive failed testicular biopsies. A platelet-rich plasma was injected and a second biopsy was performed after 3 months. The result was a very few immotile sperms. The immotile sperm were frozen. In the ICSI cycle, His wife managed to produce three oocytes after approximately 36 hours of triggering. It was only one mature oocyte was injected with frozen non-motile sperm by mechanical touch method. At 19 hours post injection, the single oocyte was seen fertilized, developed to four-cell embryo on day 2, and transferred at 8-cell stage and graded good on day 3. The pregnancy yielded a positive β-hCG result. The scan performed at six weeks, revealed the presence of one gestation sac with a fetal heartbeat.

Keywords: *Platelet-Rich Plasma (PRP); Pregnancy; Azoospermia*

Introduction

In vitro fertilization (IVF), entering the fifth decade of clinical practice from 1978 to the era of precision medicine is a field of dynamic research focusing on continuous improvement. Generally, the probability of an embryo to implant is 30%, and thus, in order to increase the probability of pregnancy, it is imperative that the best-quality available embryo(s) are selected for transfer. A good-quality embryo has been defined as an embryo with the number of cells corresponding to the day of its development, blastomeres of equal size with regular distribution, and less than 10% fragmentation, Male factor causes approximately 30% to 50% of infertile couples. Non-obstructive azoospermia (NOA) is the absence of sperm in the ejaculate resulting from severe deficits in spermatogenesis due to primary testicular dysfunction [1]. NOA is diagnosed in 5 - 10% of men undergoing infertility work-up. In order to retrieve sperm cells in men with NOA, a number of surgical techniques have been developed, including testicular sperm aspiration (TESA) and conventional or microdissection testicular sperm extraction (cTESE/mTESE). However, effective strategies to obtain spermatozoa in men with NOA who failed of mTESE procedures are not available [2].

In the past, males with Non-Obstructive Azoospermia (NOA) had no therapeutic options outside of assisted reproductive techniques to conceive a biological child. These patients were bound to rely on options of donor sperm or adoption [3]. Then several sources had suggested hormonal therapies and stem cell therapy for NOA [4].

Platelet-rich plasma (PRP), as a method in many medical fields, has already demonstrated its beneficial effect on tissue regeneration, angiogenesis activation, inflammation control and anabolism [5]. Unfortunately, there are still insufficient clinical data in the field of men infertility. Activating the alfa granules from the platelets is one of the most crucial steps which affects the availability of released bioactive molecules, and Ergo, the quality of the PRP. Namely, they contain more than 800 proteins, such as cytokines, hormones, and chemo-attractants of stem cells, macrophages, and neutrophils; these molecules have a fundamental role in the hemostasis and the tissue regeneration [6].

Case Presentation

A married couple for 13 years visited the British -Syrian Center at Al-Rasheed Hospital in Damascus (Syria) on 9th May 2022 to seek advice for achieving pregnancy, they had been trying to conceive for 7 years, bearing in mind that the husband's age is 37 years diagnostic of non-obstructive azoosperermiac with FSH 19 mIU/L. The lady aged 35 years, her hormonal profile showed AMH: 0.34 ng/ml and FSH: 12 mIU/L, E2: 38 pg/ml. A second fine needle testicular biopsy (Testicular Sperm Aspiration) showed spermatogenesis arrested, at primary and secondary spermatocytes stage. The husband agreed to conduct an injection of platelet-rich plasma into testicles then the third testicular biopsy performed 3 months later, we obtained very few non-motile sperm. We discussed the chance of frozen immotile sperm sample and agreed to freeze the sample for future use if needed.

150 IU daily of human gonadotropins (Menopur, Ferring, Germany) were administrated for twelve days (total gonadotrophin dose of 1800 IU) using Antagonist flexible protocol (Ctrorelix acitate "Cetrotide" Serono) with Ovitrelle 250 microgram/0.5 ml, Merck, as triggering of ovulation and Oocyte maturation. Three dominant follicle was seen on the right ovary, whereas no follicles on the left ovary, triggered by using a single injection of Ovitrelle SC injected at 10 pm, 36 hours before egg collection, Endometrium measured 9.8 mm. Transvaginal ultrasound-guided oocyte retrieval was carried out, only three oocyte were retrieved.

Preparation and injection of PRP, retrieval of sperm from testicular tissue

Platelet-rich plasma (PRP) is prepared from fresh whole blood which is collected from a peripheral vein, stored in Acid Citrate Dextrose solution A (ACD-A) anticoagulant and processed to increase platelets by separating various components of blood, hence the injected fluid prepared from autologous blood by centrifugation using Dr. PRP kits and equipment (USA), Consent form was given and signed [7].

Under sedation man had been undergone PRP intra-testicular injection with ½ ml in each testicle using fine needle. After 3 months of PRP, planned TESA was performed after semen analysis confirmed azoospermia, was performed shortly prior to second TESA. We found very few non-motile sperm in the left and right testicle upper region, we discussed the chance of frozen immotile sperm sample and agreed to freeze the sample for future use if needed.

Oocytes retrieval, ICSI embryo culture and transfer

The cumulus-oocyte-complex (COC) was isolated from follicular fluid and then rinsed in 1.0 ml G-MOPS™ plus medium (Vitrolife, Göteborg, Sweden). Following the oocytes pick-up, the oocytes was transferred to a 1.0 ml equilibrated G-IVF™ medium (Vitrolife) at 37°C, and 6% CO_2, in a Labotect incubator (Labotect, Lab C201, Germany) until the time of ICSI.

For previous frozen sample in which spermatozoa were identified in the wet preparation immediately after the TESE procedure, the biopsy specimen was frozen in one ampoule for subsequent use in therapeutic ICSI cycle. Using technique freeze and thaw short protocol of media sperm freeze solution (Vitrolife, Sweden).

Approximately 39 hours after trigger, the oocytes was treated with hyaluronidase (80 mIU/ml) for 40 seconds in order to remove the surrounding cumulus cells. At that time, only one oocyte was found to be at the MII stage, with clear extrusion of the first polar body.

Immotile sperms out of normal morphology sperm sample was selected using the mechanical touch method under an inverted microscope (Nikon Eclipse Ti-S, Japan) from frozen sample, and microinjected with the use of electrohydraulic injectors (Narishige, Japan). The oocyte was kept still by using a holding micropipette at the 9 o'clock position, and the polar body was oriented at the 12 o'clock position. The injecting pipette was then gently advanced through the zona pellucida and oolemma, until the pipette was beyond the center of the oocyte; then, the sperm was gently deposited into the oocyte's cytoplasm. The oocyte was examined for the presence of two pronuclei, and successful fertilization was confirmed at approximately 19 hours after insemination.

On day 2, the one embryo was four-cell grade 4/4 (good grade), reaching six-cells good grade on day 3, when we transferred it to the uterine cavity (Figure 1). The embryos culture was completed adopting a sequential preequilibrated medium (Vitrolife G-series) as follows. Firstly, the fertilized oocytes were placed into a 20- microliter drop of G-1™ media, covered by light paraffin oil, sterile filtered (OVOIL - Culture Oil, Vitrolife, Sweden). On the morning of day 3, the embryos were transferred from the G-1™ micro droplet to a 20-microliter droplet of G-2™ medium, and kept in culture until 3 hours, when the embryos transfer were performed.

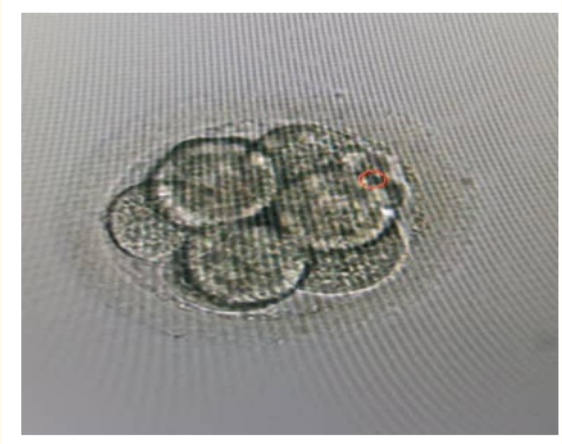

Figure 1: *Embryo transferred on day 3.*

We hereby report a compelling case in which an ongoing pregnancy with fetal heartbeat was obtained from one oocyte with immotile frozen sperm after testicular PRP. The embryos replacement were completed under transabdominal ultrasound guidance using a soft transfer catheter (Wallace TM Classic, Cooper Surgical, USA). We started the luteal phase support with 90 mg progesterone vaginal gel twice a day (Crinone 8%, Merck) on the evening of egg collection day and continued till the 8 gestational weeks, a tablet of baby Aspirin 75 mg daily and folic acid one tablet a day was added.

Hormone measurement

Serum progesterone and estradiol level was measured on the day of hCG administration. Sample was tested with a microparticle enzyme immunoassay (Axsym System, Advia Centaur; Sie- mens). The results showed progesterone and estradiol (1.2 ng/mL, 750 pg/mL), respectively.

Pregnancy outcome

First ultrasound scan was performed six weeks from the last day of menstrual, which revealed the presence of one gestational sac with fetal heartbeat.

Discussion

The use of assisted reproduction technology (ART) to overcome infertility has increased steadily in the worldwide. *In-vitro* fertilization has been highly successful using both conventional insemination (IVF) and intracytoplasmic sperm injection (ICSI) methods for fertilization. ICSI bypasses both the ZP barrier and sperm defects in the male gamete that compromise its ability to fertilize. The ability of ICSI to achieve higher fertilization and pregnancy rates regardless of sperm characteristics makes it the most powerful micromanipulation [8].

Platelet-Rich Plasma (PRP) is a novel therapeutic agent used in multiple medical fields and one of these fields is the reproduction for best spermatozoa preparation and activation for upgrading activity and motility of the spermatozoa. According to Bir., *et al*, Sanchez-Gonzalez., *et al*. the regenerative potential of platelet rich plasma comes from the growth factors released by the platelets [9,10]. The PRP was significantly effective in improving the sperm activity and upgrading sperm motility [11]. PRP could support the maintenance of proliferation and differentiation spermatogonia stem cells SSCs *in vitro* [12].

Study found in infertile men with NOA and a history of one failed mTESE, testicular PRP injection resulted in identification of sperm cells in 21.8% (12/55), and sustained implantation/livebirth rate of 10.9% (6/55). In men with NOA who failed 2 or more prior mTESE, PRP was less effective with identification of sperm cells in 8.3% (3/36), and sustained implantation/livebirth rate of 2.8% (1/36) [2]. In addition, Oraiopoulou., *et al*. found the quality of testicular tissue influences both fertilization rate and embryo development [13]. Moreover, cryopreservation of low-quality testicular samples has a negative impact on the number of available embryos for transfer. However, Amer., *et al*. pointed out that use of frozen testicular sperm from men with NOA results in fertilization and clinical pregnancy rates similar to those of fresh sperm [14].

Although fertilization, top-quality embryo rates, cumulative CPR and LBR decreased when using immotile spermatozoa, ICSI is still valid; therefore, it should be considered and offered to couples before embarking on a donor sperm insemination cycle, or cryopreserving oocytes for future additional testicular sperm retrieval [15].

This case is very important, particularly in view of sperm donation is prohibited in some counties, this technique represents a new approach for couples with non-obstructive azoospermia. It gives real hope of biological parenthood. Equally important is to share such cases within the medically assisted reproduction (MAR) community.

Conclusion

We report here a compelling case in which a 37-year-old man suffering from azoospermia managed to obtain very few non-motile sperms after 3 months of injecting platelet-rich plasma into the testes.

The lady managed to produce three oocytes after approximately 36 hours of triggering. It was only one mature oocyte was injected with frozen non-motile sperm by mechanical touch method, fertilized confirmed. Our case-report stimulates the discussion on the above aspects particularly in view of egg or sperm donation is not an option according to local authority. The case reported achieving successful pregnancy.

Bibliography

1. Kumar R. "Medical management of non-obstructive azoospermia". *Clinics* 68.1(2013): 75-79.

2. Gudelci T., *et al*. "The effect of intratesticular injection of autologous platelet rich plasma (prp) on sperm retrieval rates and ivf outcomes in men with non-obstructive azoospermia and history of failed testicular sperm extraction". *Fertility and Sterility* 116.3 (2021): e331.

3. Vij SC., *et al*. "Biological therapy for non-obstructive azoospermia". *Expert Opinion on Biological Therapy* 18.1 (2018): 19-23.

4. Hendriks S., *et al*. "Perspectives of infertile men on future stem cell treatments for nonobstructive azoospermia". *Reproductive Biomedicine Online* 28.5 (2014): 650-657.

5. Schmitz JP and Hollinger JO. "The biology of platelet-rich plasma". *Journal of Oral and Maxillofacial Surgery: Official Journal of the American Association of Oral and Maxillofacial Surgeons* 59.9 (2001): 1119-1121.

6. Tilly JL and Sinclair DA. "Germline energetics, aging, and female infertility". *Cell Metabolism* 17.6 (2013): 838-850.

7. Al-Nasser R., *et al*. "The Effectiveness of autologous platelet-rich plasma (prp) in the therapy of infertile men with non-abstractive azoospermia". *Journal of Reproductive Medicine Gynaecology and Obstetrics* 3.11 (2018): 2223100.

8. Al Nasser R and Al Ghazawi M. "A Live Birth Following ICSI Using Frozen Immotile Sperm of Testicular Biopsy and Single Oocyte of Poor Responder Women". *MedPress Case Reports and Clinical Images* 1.2 (2022).

9. Bir SC., *et al*. "Angiogenic properties of sustained release platelet-rich plasma: characterization in-vitro and in the ischemic hind limb of the mouse". *Journal of Vascular Surgery* 50.4 (2009): 870-879.

10. Sánchez-González DJ., *et al*. "Platelet-rich plasma peptides: key for regeneration". *International Journal of Peptides* (2012).

11. Abduljabar WA., *et al*. "Beneficial Role of Platelet–Rich Plasma (PRP) combined with Glass Wool Filtration for Best Recovery of Spermatozoa in Infertile Men". *European Journal of Clinical Medicine* 2.5 (2021): 11-13.

12. Lestari SW., *et al*. "Human spermatogonia stem cells (SSCS) in a culture system with platelet rich plasma and correlations with spermatogenesis level". *Journal of Medical Pharmaceutical and Allied Sciences* 11.1 (2022): 4409-4416.

13. Oraiopoulou C., *et al*. "The impact of fresh and frozen testicular tissue quality on embryological and clinical outcomes". *Andrologia* 53.5 (2021): e14040.

14. Amer M and Fakhry E. "Fresh vs frozen testicular sperm for assisted reproductive technology in patients with non-obstructive azoospermia: A systematic review". *Arab Journal of Urology* 19.3 (2021): 247-254.

15. Aizer A., *et al*. "Cumulative IVF outcomes after retrieval of testicular spermatozoa: should we use immotile spermatozoa for ICSI?" *Reproductive Bio Medicine Online* 43.2 (2021): 269-277.

Volume 12 Issue 6 June 2023