

Comparison of Results of Semen Parameters Prepared by Swim-up Technique Between the Effect of Adding Autologous Platelet-Rich Plasma, Ready to Use Medium, and Phosphate Buffer Saline to the Human Semen.

Noor J. Ma'alla¹, Ali I. Rahim^{1,2,3,4,*}, and Ula M. Al-Kawaz³

1.High Institute for Infertility Diagnosis and Assisted Reproductive Technologies Al- Nahrain University, Baghdad –Iraq.

2. Faculty of Medicine, University of Kufa, Najaf, Iraq.

3.Collage of medicine, University of Al-Ameed, Karbala, Iraq.

4.IVF center, Al- Kafeel Hospital Karbala, Iraq.

*Corresponding author: aliibrahim.ivf@gmail.com

Abstract

Background

The sperm preparation techniques used in intrauterine insemination (IUI) and other reproductive techniques are heavily based on the sperm parameters and their survival. Swim-up technique is the most common method of preparing sperm in this way, sperm is chosen according to their motility and ability to swim out the seminal plasma, because the plasma includes decapacitation factors that are modulate to sperm's ability to fertilize.

Aim

The objective of this study is to examine the effect of adding autologous plasma rich in platelets to the semen and comparing the results of semen preparation with ready-to-use media and phosphate buffer saline by using swim – up procedure.

Material and method

The present study includes semen samples from 119 men attending fertility clinic of High Institute for Infertility Diagnosis and Assisted Reproductive Technologies/Al-Nahrain University of normal and abnormal sperms parameters according to WHO. The semen sample divided into 3 fractions to form 3 groups:

first group influenced by the same volume of ferticult flushing medium (250) µm; second group influenced by the same volume of phosphate buffer saline (250) µm contain 10% human serum albumin (HSA) whereas third group influenced by the same volume of phosphate buffer saline (250) µm contain 10% human serum albumin (HSA) with added 2% platelet rich plasma. Samples are then prepared by swim – up procedure for assessment of main sperms parameters.

Result

Swim up-flushing media resulted in highest sperm concentration, highest total count, highest total motility, highest progressive motility (A+B), highest progressive motility (A), highest progressive motility (B), lowest non-progressive motility (C), lowest immotile sperm (D), highest concentration of active motile sperm % and highest normal morphology sperm %, followed by swim up –PBS +PRP (HAS) then by swim up –PBS (HAS), respectively in a highly significant manner (p < 0.001).

Conclusion

we conclude, the addition of PRP resulted in results that are practically very close to Swim up-flushing media with respect to sperm concentration, total count, total motility, progressive motility (A+B), progressive motility (A), progressive motility (B), non-progressive motility (C), immotile sperm (D), concentration of active motile sperm % and normal morphology sperm and the values were far better than those produced by adding swim up –PBS (HAS), nevertheless, statistical wise the minor differences between Swim up-flushing media and PRP were significant.

Key words: PBS, PRP, Swim -up, sperm preparation, IUI, HAS, Infertility.

1. Introduction

According to (Zegers-Hochschild et al., 2017) “infertility is a disease which generates disability as an impairment of function”. It is a disease characterized by the failure to establish a clinical pregnancy after 12 months of regular and unprotected sexual intercourse.

Males are found to be solely responsible for 20–30% of infertility cases but contribute to 50% of cases overall (Vander Borgh and Wyns, 2018).

Infertility is not only a medical situation but it has asocial, psychological and economic impact. So that, major effort had been used and continuously spending in order to improve the success rate in a variety of assisted conception modalities in the form of medical treatment to highly and continuously upgraded assisted reproductive technologies (Hassan, Rahim and Al-Kawaz, 2020).

Intra-Uterine Insemination (IUI) is a first method of assisted conception and it is popular modality that is easy to implement, least invasive, and cost effective with rare complications (Paula and Peter, 2003).

The usual preparation way for sperms is centrifugation and swim- up procedure resulting in very active sperms; however, it will result in lowering the concentration (Henkel and Schill, 2003).

In recent years, platelet-rich plasma (PRP) is a novel therapeutic alternative that is being used in a variety of medical fields, including dermatology, orthopedics, and dentistry (Lubkowska, Dolegowska and Banfi, 2012).

Platelet-rich plasma (PRP) is the plasma fraction of autologous blood containing high concentration of platelets and growth factors than the physiologic concentration of thrombocytes in whole blood (Knezevic et al., 2016).

Ferticult flushing medium is intended for in vitro procedures involving human gametes (sperm and oocytes), including washing of gametes, sperm swim up procedures, intra uterine insemination (IUI) of the spermatozoa and sperm injection during intra cytoplasmic sperm injection (ICSI).

One the other hand, Phosphate Buffer Saline this product contains a protein called albumin found in the liquid component of the blood (the plasma) and belongs to the group of medical products called (plasma substitutes and plasma protein fractions), every 100 ml contain 20 g of total protein, of which at least 95% is human albumin.

Accordingly, thus study might be considered as a first research to investigate the effects of adding directly of PRP on human sperm parameter and compare the result of the preparation of the semen with ready to use media and phosphate buffer saline.

2. Material and methods

The study involved one hundred and nineteenth semen sample; were obtained from men attended infertility clinic in High Institute for Infertility Diagnosis and Assisted Reproductive Technologies/ Al-Nahrain University. The study was approved by the ethical committee of the High Institute. Each participant had given informed consent to use the remainder of their sample before inclusion in the validation project. The semen samples (normal and abnormal according to WHO 1999) of 119 men (18-54 year) with 2-7 abstinence days.

patients were either normozoospermic or had mild to moderate sperm abnormalities participated in this research (inclusion criteria) while (Exclusion criteria) Patients with azoospermia, or patients with severoligoasthenoteratozoospermia.. The research began in November of 2020 and ended in February of 2021.

2.1 Sample collection and processing

Sample were collected in sterile containers by masturbation method. only one seminal sample was taken each person. In case of spoiling or loss of sample for any reason, the person was asked to refer again to give another sample in next 3-7 days without performing any sexual intercourse. After getting the samples, they were rapidly put in the incubator 37°C for complete liquefaction of samples for semen analysis. Each the semen sample divided into 3 fractions to form 3 groups:

first group influenced by the same volume of ferticult flushing medium (250) μ m; second group influenced by the same volume of phosphate buffer saline (250) μ m contain 10% human serum albumin (HSA) whereas third group influenced by the same volume of phosphate buffer saline (250) μ m contain 10% human serum albumin (HSA) with added 2% platelet rich plasma. Samples are then prepared by swim – up procedure for assessment of main sperms parameters.

2.2 Preparation of Platelet Rich Plasma (PRP)

Platelet rich plasma was prepared by collected whole blood WB (5) cc in tubes containing anticoagulants (EDTA) and gently shaken to keep it from clotting. After that, blood was put in a (gel)tube and centrifuged for 5 minutes at 3000 rounds per minute, after that divide the blood into three layers: an upper layer containing mainly platelets (plasma) and white blood cells WBC, an intermediate thin layer known as the buffy coat, which is abundant in WBCs, and a lower layer containing red blood cells RBCs. For PRP processing, the upper layer (plasma) was transferred to a fresh, empty sterile tube (plane tube) and centrifuged for 15 minutes at a fast turn 3500 rounds per minute. Then (the upper 2/3 of plasma is discarded) contain platelet poor plasma (PPP) and the lower 1/3 of plasma was kept which contain the platelet rich plasma (PRP). Later, for activation prp, 23 μ l of (calcium chloride) at percentage 10% was added to the prp then incubated at 37° for 15 minutes. Finally, after incubation, the tube must be centrifuged for 10 minutes at 4000 RPM to obtain pure activation platelet rich plasma for using to activation patient semen or others uses.

It has been found that the 2% was the best percentage for PRP preparation and yielding significant results (Bader et al., 2020).

2.3 Preparation by Swim-Up Procedure

The three volumes of semen of the 3 groups are placed in a conical test tube and diluted with one volume of culture medium. The tube is gently shaken to mix the components. Then, they

centrifuged at 3000 round per minute (rpm) for 5 minutes. The supernatant is removed by Pasteur pipette to obtain the pellet, and about 0.5 ml of media was added to the final sperm pellet and incubated for about 45 minutes. After incubation, 10 μ l of sample from the middle of the suspension was taken for the examination under light microscope for main sperms parameters.

3. Statistical Analysis:

Data was analyzed using statistical package for the social sciences (SPSS version 23) computer software program.

The P-value below or equal to 0.05 was considered to be statistically significant.

4. Result

Comparison of sperm characteristics among three methods, swim up-flushing media, swim up-PBS (HAS) and swim-up-PBS+PRP (HAS) in all infertile men is shown in table 4.3. Swim up-flushing media resulted in highest sperm concentration, highest total count, highest total motility, highest progressive motility (A+B), highest progressive motility (A), highest progressive motility (B), lowest non-progressive motility (C), lowest immotile sperm (D), highest concentration of active motile sperm % and highest normal morphology sperm %, followed by swim up –PBS +PRP (HAS) then by swim up –PBS (HAS), respectively in a highly significant manner ($p < 0.001$).

Table 4.3: Comparison of sperm characteristics among three methods, swim up-flushing media, swim up-PBS and swim-up-PBS+PRP (HAS) in all infertile men

Characteristic	Swim up – flushing media	swim up – PBS	Swim up- PBS +PRP (HAS)	<i>p</i>
Concentration (m/ml)	52.77 ±28.60 A	35.90 ±22.80 C	49.21 ±28.26 B	< 0.001 P HS
Total count (m/e)	26.39 ±14.30 A	17.97 ±11.41 C	24.63 ±14.15 B	< 0.001 P HS
Total motility %	93.51 ±3.53 A	81.73 ±2.25 C	90.77 ±3.81 B	< 0.001 P HS
Progressive motility (A+B) %	64.29 ±22.02 A	37.92 ±17.20 C	59.11 ±21.03 B	< 0.001 P HS
Progressive motility (A) %	21.14 ±11.16 A	9.76 ±7.25 C	17.80 ±10.18 B	< 0.001 P HS
Progressive motility (B) %	43.20 ±14.10 A	27.95 ±11.91 C	41.16 ±14.20 B	< 0.001 P HS
Non Progressive motility (C) %	29.29 ±20.75 C	43.90 ±17.16 A	31.97 ±19.98 B	< 0.001 P HS
Immotile sperm (D) %	6.49 ±3.53 C	18.35 ±2.12 A	9.15 ±3.84 B	< 0.001 P HS
Concentration of active motile %	8.76 ±6.17 A	3.56 ±3.24 C	7.61 ±5.77 B	< 0.001 P HS
Normal morphology %	56.00 ±19.15 A	37.61 ±17.33 C	48.94 ±19.16 B	< 0.001 P HS

Data were expressed as mean ±standard deviation; **P**: Pillai's Trace multiple repetition test; **HS**: highly significant at $p \leq 0.01$; Capital letters (A, B and C) were used to indicate the level of significance following **post hoc LSD** test so that similar letters indicate no significant difference whereas different letters indicate significant difference and letter (A) takes the highest value followed by letter (B) and then by (C)

5. Discussion

In recent years, different methods have been proposed for the treatment of infertility. Several studies have been published on the effectiveness of different semen preparation methods, there is insufficient evidence to recommend any specific sperm preparation technique (Boomsma et al., 2004). Comparative studies on sperm preparation methods have essentially investigated outcomes such as recovery rates and conventional semen parameters (Boomsma et al., 2004; Henkel and Schill, 2003). Over the last decade, the effects of sperm preparation procedures on sperm quality have been evaluated using new laboratory methods (Ricci et al., 2009).

The most common sperm-processing protocols used in routine ART laboratories are those of swim-up and density gradient centrifugation (Volpes et al., 2016). According to the WHO laboratory manual for the examination and processing of human semen, the swim-up method is useful in selecting motile spermatozoa as it is based on the ability of sperm to swim into the culture medium (World Health Organization, 2010).

Ready to use culture media are often used to improve sperm quality including both motility and morphology; however, these media are costly and often need to be exported. The invention of a substitute for these culture media that can perform as required as these media do with respect to sperm quality and quantity, provided that these materials are available locally and

be produced with low cost, there will be no more need for these expensive culture media. To the best of our knowledge and after thorough search in the available published articles dealing with sperm preparation techniques for ART procedures, we failed to find a study evaluating the clinical use of PRP in sperm preparation in comparison with culture media. Therefore, the point of originality in this article is the evaluation of the effectiveness of adding PRP in sperm preparation and selection for ART in comparison with already available methods.

Platelet-rich plasma (PRP) is collected from the autologous blood samples of patients and is 4–5 times richer in platelets than circulating blood. Moreover, in PRP, cytokines and growth factors have more activity. These factors include the vascular endothelial growth factor (VEGF), transforming growth factor (TGF), platelet-derived growth factor (PDGF), and epidermal growth factor (EGF) (Allahveisi et al., 2020).

Experimental studies have shown that PRP has the ability to increase the number of spermatogenic stem cell, count, motility and tail length of the sperm (Dehghani et al., 2019) and that PRP scaffold can reconstruct a suitable structure to the *in vitro* proliferation of Spermatogonial stem cells (Khadivi et al., 2020) and in a recent experimental study on animal semen, the incubation of semen with PRP resulted in significant improvement in sperm motility and morphology (Hernández-Corredor et al., 2020).

In the current study, swim up-flushing media resulted in highest sperm concentration, highest total count, highest total motility, highest progressive motility (A+B), highest progressive motility (A), highest progressive motility (B), lowest non-progressive motility (C), lowest immotile sperm (D), highest concentration of active motile sperm % and highest normal morphology sperm %, followed by swim up –PBS +PRP (HAS) then by swim up –PBS (HAS), and the difference in these parameters was highly significant.

Similarly, the addition of PRP has resulted in a total sperm count that is very close to that yielded by adding flushing media, and substantially better than that provided by adding PBS alone, 24.63 m/ejaculate versus 26.39 m/ejaculate versus 17.97 m/ejaculate, respectively. Despite, the existence of significant statistical significance, the addition of locally derived autologous PRP markedly improved the sperm total count when compared to PBS alone.

The same can be applied to sperm motility parameters, since adding PRP resulted a total motility, progressive grade A motility, progressive grade B motility, non-progressive grade C motility and immotile sperm proportions that are numerically very close to that yielded by adding flushing media and far from that produced by adding PBS alone.

In addition, the addition of PRP, resulted in a concentration of active motile sperm that is very close numerically to that produced by adding flushing media and far from that produced by adding PBS alone. And the same can be said with respect to normal morphology sperm proportion since the addition of PRP produced figures that are very close to that produced by adding flushing media and substantially better than that attributed to PBS alone.

In conclusion, the addition of PRP resulted in results that are practically very close to Swim up-flushing media with respect to sperm concentration, total count, total motility, progressive motility (A+B), progressive motility (A), progressive motility (B), non-progressive motility (C), immotile sperm (D), concentration of active motile sperm % and normal morphology sperm and the values were far better than those produced by adding swim up –PBS, nevertheless, statistical wise the minor differences between Swim up-flushing media and PRP were significant.

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Conflict of Interest

The authors declare no conflict of interest.

Ethical Clearance

The study was approved by the Ethical Approval Committee. High Institute for Infertility Diagnosis and Assisted Reproductive Technologies/ Al-Nahrain University.

References

1. Allahveisi, A, Seyedoshohadaei, F, Rezaei, M, Bazrafshan, N and Rahimi, K (2020). The effect of platelet-rich plasma on the achievement of pregnancy during frozen embryo transfer in women with a history of failed implantation. *Heliyon* 6, e03577.
2. Bader, R. et al. (2020) 'In vitro effect of autologous platelet-rich plasma on H₂O₂-induced oxidative stress in human spermatozoa', *Andrology*, 8(1), pp. 191–200.
3. Boomsma CM, Heineman MJ, Cohlen BJ, Farquhar C. Semen preparation techniques for intrauterine insemination. *Cochrane Database Syst Rev* 2004;3:CD004507.
4. Dehghani F, Sotoude N, Bordbar H, Panjeshahin MR, Karbalay-Doust S. The use of platelet-rich plasma (PRP) to improve structural impairment of rat testis induced by busulfan. *Platelets*. 2019;30(4):513-520.
5. Hassan, A. Y., Rahim, A. I. and Al-Kawaz, U. M. (2020) 'Twenty-four hours sperm survival test after sperms preparation with and without Pentoxifylline adding', *EurAsian Journal of BioSciences*, 14, pp. 6503–6506.
6. Henkel RR, Schill WB. Sperm preparation for ART. *Reprod Biol Endocrinol* 2003; 14:108–29.
7. Hernández-Corredor L, León-Restrepo S, Bustamante-Cano J, Báez-Sandoval G, Jaramillo X. Effect of the incorporation of plasma rich of platelets on the spermatozoa physiology of ram semen. *Journal of Dairy, Veterinary & Animal Research*. 2020. 9 (1): 34-38.
8. Khadivi F, Koruji M, Akbari M, Jabari A, Talebi A, Ashouri Movassagh S, Panahi Boroujeni A, Feizollahi N, Nikmahzar A, Pourahmadi M, Abbasi M. Application of platelet-rich plasma (PRP) improves self-renewal of human spermatogonial stem cells in two-dimensional and three-dimensional culture systems. *Acta Histochem*. 2020 Dec;122(8):151627. doi: 10.1016/j.acthis.2020.151627. Epub 2020 Sep 28. PMID: 33002788.
9. Knezevic, N. N. et al. (2016) 'Is platelet-rich plasma a future therapy in pain management?', *Medical Clinics*, 100(1), pp. 199–217.
10. Lubkowska, A., Dolegowska, B. and Banfi, G. (2012) 'Growth factor content in PRP and their applicability in medicine', *J Biol Regul Homeost Agents*, 26(2 Suppl 1), pp. 3S-22S.
11. Organisation, W. H. (1999) WHO laboratory manual for the examination of human semen and sperm-cervical mucus interaction. Cambridge university press.

12. Organization, W. H. (2010) 'WHO laboratory manual for the examination and processing of human semen'.
13. Paula, R. and Peter, B. (2003) 'Assisted conception. I--General principles', *BMJ (Clinical research ed.)*, 327(7418), pp. 799–801.
14. Ricci G, Perticarari S, Boscolo R, Montico M, Guaschino S, Presani G. Semen preparation methods and sperm apoptosis: swim-up versus gradient-density centrifugation technique. *Fertil Steril.* 2009 Feb;91(2):632-8. doi: 10.1016/j.fertnstert.2007.11.068. Epub 2008 Jan 18. PMID: 18206147.
15. Vander Borgh, M. and Wyns, C. (2018) 'Fertility and infertility: Definition and epidemiology', *Clinical biochemistry*, 62, pp. 2–10.
16. Volpes A, Sammartano F, Rizzari S, Gullo S, Marino A, Allegra A. The pellet swim-up is the best technique for sperm preparation during in vitro fertilization procedures. *J Assist Reprod Genet.* 2016;33(6):765-770. doi:10.1007/s10815-016-0696-2.
17. Zegers-Hochschild, F. et al. (2017) 'The international glossary on infertility and fertility care, 2017', *Human reproduction*, 32(9), pp. 1786–1801.