



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

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ABSTRACT

Swim up is the most popular technique of sperm preparation, in which sperm is selected on motility and ability to swim out the plasma of the semen. The goal of this research is to compare the influence of adding platelet rich plasma (PRP) and phosphate buffer saline in semen prepared by swim – up technique. This research included 119 semen sample evaluated according to sperms parameters stated in WHO and divided into 2 groups. The first group treated with equal quantity of phosphate buffer saline (250) μ m contain 10% human serum albumin (HSA) while second group treated with equal quantity of phosphate buffer saline (250) μ m contain 10% human serum albumin (HSA) and 2% platelet rich plasma. The samples are prepared by swim – up method. Applying swim up technique using-PBS+PRP (HAS) medium resulted in highest sperm parameters in a highly significant manner ($p < 0.001$). The values were far better in Swim -up PBS (HAS) with added 2% PRP than those produced by swim up –PBS (HAS) alone. The statistical analysis shows that the variations between Swim up-PBS (HAS) with addition 2% PRP media and swim -up PBS (HAS) were highly significant.

Received:
25-May 2021
Accepted:
23-Jun 2021
Published:
30-Jun 2021

How to cite:

Noor Ma'alla; Ali Rahim; Ula M. Al-Kawaz; Comparison of Results of Semen Parameters Prepared by Swim-up Technique Between the Effect of Adding Autologous Platelet-Rich Plasma and Phosphate Buffer Saline to the Human Semen; Iraqi Journal of Embryos and Infertility Researches (IJEIR), (2021); 11 (1): 13-24.
Doi:
<http://doi.org/10.28969/IJEIR.v11.i1.r2.21>

KEYWORDS

PBS, PRP, Swim -up, sperm preparation, HAS, Infertility.

1. Introduction:

The quality of gametes and their interactions is affected by oxidative stress (OS). Infertility is an illness which causes incapacity as a functional defect, according to (Raheem F A et al. ^[16]). It is a condition defined by an inability, after 12 months of frequent and unsafe sexual relations, to develop a clinical pregnancy. Males are participated to 50% of infertility cases in general (Borghet MV and Wyns C ^[14]). The effects of infertility are not just medical but also asocial, physical, and economic. So as to achieve enhance the outcomes of a wide range of assisted reproduction methods, considerable efforts were therefore made to improve medical care and to constantly update assisted reproductive technology (Hassan AY et al. ^[5]).

The common process for sperm is centrifugation and swim-up method, which leads to very active sperm, although it reduces the concentration (Henkel RR et al. ^[6]). Platelet-rich plasma (PRP) is a new treatment approach employed in a range of medical

fields in recent years (Lubkowska A. et al. ^[10]). It has included high platelet concentrations and growth factors than the physiological thrombocyte concentration in the entire blood (Knezevic NN et al ^[9]). Phosphate buffer saline, this product contains a protein called albumin present in the liquid blood component (the plasma) and is part of the group of therapeutic items known as plasma replacements and plasma protein divisions; each 100 mL has 20 g of total protein, with human albumin at least 95 percent. It has been found that the 2% was the best percentage for PRP preparation and yielding significant results (Bader R et al. ^[2]). Consequently, this research may well be viewed as an initial assessment and differentiated between the effects on human semen prepared with the swim-up method of adding autologous platelet plasma and PBS.

2. Materials and Methods:

One hundred and nineteenth samples were taken from men who visited a clinic for

infertility at highly institute for the diagnosis of infertility /the University of Al-Nahrain.

The investigation was accepted by the High Institute's ethical committee. Before participation in validation projects, each participant has informedly consented to utilize the remaining of the sample.

The semen sample of (18-54 years) men with 2-7 days of abstinence (normal and abnormal according to the WHO 1999) (WHO,1999^[11]). (Inclusion criteria) in this research included patients who were either normozoospermic or had mild to moderate sperm abnormalities, while (Exclusion criteria) included Patients with azoospermia or patients with severe oligoasthenoteratozoospermia. This study started in November of 2020 and ended in May of 2021.

2.1 Sample collection and processing:

Masturbation procedure was used to take samples in sterile containers. Every person took only one seminal sample. If the sample was contaminated or lose for whatever cause, the person was advised to give a new sample

in the next 3-7 days without sex. After the samples were received, they were quickly placed into the incubator 37°C for complete semen liquefaction. Then, each sample is divided into two fractions:

First group treated with an equal quantity of phosphate buffer saline (250) µm contain 10% human serum albumin (HSA), whereas the second group affected by the equal quantity of phosphate buffer saline (250) µm contain 10% human serum albumin (HSA) with added 2% platelet-rich plasma. The samples are subsequently prepared by the swim-up method for evaluation parameters of the sperm.

2.2 Preparation of Platelet Rich Plasma (PRP):

Platelet-rich plasma was made by placing (5) cc of whole blood WB in tubes comprising anticoagulants (EDTA) and softly shaking it to prevent clot formation. The blood was then placed in a (gel) tube and centrifuged for 5 minutes at 3000 rounds per minute before being divided into three layers: a top layer primarily composed of 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 testing, the upper layer (plasma) was transferred to a new, empty sterile tube (plane tube) and centrifuged for 15 minutes at a fast turn of 3500 rounds per minute. Then (the upper two-thirds of the plasma is discarded) and contains platelet-poor plasma (PPP), and the lower one-third of the plasma includes platelet-rich plasma (PRP). Later, for activation prp, 23 µl of (calcium chloride) at a percentage of 10% was added to the PRP and 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.

2.3 Preparation by Washing and Swim-Up Procedure:

The semen of the two groups is placed in a cone-shaped test tube and diluted with one volume of culture medium. The tube is softly shacked to mix the components. Then, they

centrifuged at 3000 rounds per minute (rpm) for 5 minutes. The supernatant was removed by Pasteur pipette to get the pellet, and about 0.5 ml of media was added to the final sperm pellet and incubated for about 45 minutes. After incubation, 10µl of sample from the middle of the dissolution was taken for the examination under a light microscope for main sperm parameters.

3. Statistical Analysis:

Data was analyzed using a 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. Results:

Comparison of sperm characteristics between swim-up-PBS (HSA) and swim-up-PBS+PRP (HSA) in all infertile men is shown in Table 1.

Swim-up-PBS+PRP (HSA) 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

Highest normal morphology sperm %, later swim up –PBS (HAS), approximately in a highly significant

Table (1): Comparison of sperm characteristics between swim up-PBS (HAS) and swim-up-PBS+PRP (HAS) in all infertile men:

Characteristic	swim up – PBS (HAS)	Swim up- PBS +PRP (HAS)	<i>p</i>
Concentration (m/ml)	35.90 ±22.80 B	49.21 ±28.26 A	< 0.001 P HS
Total count (m/e)	17.97 ±11.41 B	24.63 ±14.15 A	< 0.001 P HS
Total motility %	81.73 ±2.25 B	90.77 ±3.81 A	< 0.001 P HS
Progressive motility (A+B) %	37.92 ±17.20 A	59.11 ±21.03 A	< 0.001 P HS
Progressive motility (A) %	9.76 ±7.25 B	17.80 ±10.18 A	< 0.001 P HS
Progressive motility (B) %	27.95 ±11.91 B	41.16 ±14.20 A	< 0.001 P HS
Non Progressive motility (C) %	43.90 ±17.16 A	31.97 ±19.98 B	< 0.001 P HS
Immotile sperm (D) %	18.35 ±2.12 A	9.15 ±3.84 B	< 0.001 P HS
Concentration of active motile %	3.56 ±3.24 B	7.61 ±5.77 A	< 0.001 P HS
Normal morphology %	37.61 ±17.33 B	48.94 ±19.16 A	< 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**) 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**)

(C), lowest immotile sperm (D), highest manner ($p < 0.001$).
concentration of active motile sperm % and

5. Discussion

In recent years, different methods have been suggested for the treatment of infertility. Several studies have been published on the effectiveness of different semen preparation methods; there is an adequate indicator to recommend any specific sperm preparation technique (Boomsma CM et al. ^[3]). Comparative studies on sperm preparation methods have necessarily investigated outcomes such as retrieved rates and conventional semen parameters (Boomsma CM et al. ^[3], Henkel RR et al. ^[6]). Over the last decade, the effect of sperm preparation procedures on sperm quality have been assessed using new laboratory methods (Ricci G et al. ^[13]).

The most traditional sperm-processing protocols used in routine assisted reproductive techniques (ART) laboratories are those of swim-up and density gradient centrifugation (Volpes A et al. ^[15]). According to the WHO laboratory manual for the examination and processing of human semen, the swim-up method is important in choosing motile

spermatozoa as it is based on the ability of sperm to swim into the culture medium (12).

This study to contrivance of a substitute for these culture media that can perform as required as these media do with respect to sperm quality and quantity, supply 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 a thorough search in the available published articles dealing with sperm preparation techniques for ART procedures, we failed to find a study that evaluated 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 effective of adding PBS (with or without) PRP in sperm preparation and selection for ART in comparison with already available methods.

Platelet-rich plasma 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 A et al^[1]).

Experimental studies have shown that PRP has the ability to increase the number of spermatogenic stem cells, count, motility, and a tail length of the sperm (Dehghani F et al^[4]) and that PRP scaffold can reconstruct a suitable structure to the in vitro proliferation of Spermatogonial stem cells (Khadivi F et al^[8]) 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 L et al^[7]).

In the current study, a swim-up conducted using PBS +PRP (HAS) 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 (HAS) and the difference in these parameters was highly significant.

Similarly, the addition of PBS+PRP has resulted in a total sperm count that is not close to that yielded by adding PBS alone.

Subsequently 24.63 m/ejaculate versus 17.97 m/ejaculate, respectively.

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

In addition, the addition of PBS+PRP resulted in a concentration of active motile sperm that is 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 PBS+PRP produced figures that are far from that attributed to PBS alone.

In conclusion, the addition of PRP to PBS resulted in results that are practically far better than those produced by adding swim up –PBS 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, statistical wise the major differences between Swim up-PBS+PRP and PBS alone were highly significant.

6. Conclusions

We conclude that the addition of PRP resulted in results that are technically far two methods with regarding 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 in Swim -up PBS (HAS) with added 2% PRP than those produced by swim up – PBS (HAS) alone. So, statistical wise, the

important variations between Swim up-PBS (HAS) with an addition 2% PRP media and swim-up PBS (HAS) were highly significant.

Acknowledgment

We would like to acknowledge the members of the High Institute for Infertility Diagnosis and Assisted Reproductive Technologies, Al-Nahrain University (Bagdad, Iraq).

Funding

This work received no funding.

Author Contribution

Noor J. performed the study with the help and supervision of Ali I. and Ula M.

Conflict of Interest

The authors declare no conflict of Interest.

Ethical Clearance

The study was approved by the Ethical Approval Committee.

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