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USING OF AUTOLOGOUS PLATELET RICH PLASMA IN HUMAN SEMINAL FLUID CRYOPRESERVATION TECHNIQUE

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Abstract
Background: cryopreservation of semen is a technique that used to preserve sperm cells at low temperature, in liquid nitrogen at -196C. Autologous-PRP therapy is using in assisted reproductive technologies due to its well-known regenerative properties.
Objective: to investigate the effect of using autologous-PRP as a cryoprotective media on human spermato-zoa.
Materials and methods: 60 semen sample from normozoospermic male with their autologous-PRP prepared from their blood were collected at the same time of semen collection. All semen samples were tested macro and microscopically according to WHO (2010). Each sample divided into three groups (Control sample preserved with only cryomedia 0.7ml, Autologous-PRP sample 0.5ml, and mixed sample 0.5ml PRP + 0.7ml Cryomedia). Then preserve them into liquid nitrogen -196C for two months. After that, thawing in water bath 37C. then se-men parameters were evaluated and the results were compared.
Results: samples that preserved with cryomedia only, showed significantly lower concentration, total count ($p<0.001$) and progressively motile sperms percent ($p=0.010$). Also, there was significantly rise non-progressively motile sperms percent ($p=0.007$), insignificantly rise immotile sperms percent ($p=0.207$).
While samples that preserved with 0.5ml autologous-PRP alone proved there were significantly decrease concentration, total count and progressively motile sperms percent ($p< 0.001$) Moreover, there were significantly increase non-progressively motile and immotile sperms percent ($p< 0.001$).
Conversely, mixed autologous-PRP 0.5 ml with cryomedia 0.7 ml reveal better results. There was significant decrease concentration, total count ($p<0.001$), but there was no significant decrease percentage of progressively motile ($p=0.208$) or non-progressively motile sperm ($p=0.768$), and insignificant increase percentage of immotile sperm ($p=0.157$).
Conclusion: Autologous-PRP 0.5ml with cryomedia has better low significant effect on sperm parameters when used for semen cryopreservation and act as a co-factor.
Keywords: Cryopreservation; Cryoprotectant agent; Autologous-PRP.

Introduction

Cryopreservation of semen includes the freezing of sperm cells at extremely low temperatures, in most cases around -196 C using liquid nitrogen. This process is essential for preserving the viability of sperm for prolonged periods, ensuring their availability for various reproductive applications (11). There are several indications for using semen cryopreservation technique, such as assisted reproductive technologies in case of (IVF) in vitro fertilization or (ICSI) intra cytoplasmic sperm injection (3). Also, before vasectomy or sterilization (15). The process involves three types which are slow freezing; rapid freezing or vitrification, each one needs carefully con-trolled freezing and storage in liquid nitrogen to maintain sperm viability, by optimization of cryopreservation protocols, cryoprotectant concentration and freezing rates (13) ;(4). There are two types of cryoprotectant agent as penetrating which include glycerol and dimethyl sulfoxide (DMSO) (2) and the second type is non penetrating which include sugars and polyvinylpyrrolidone (PVP) (5).

platelet-rich plasma (PRP) is a component of blood containing a greater concentration of platelets than what is typically found in circulating blood. Platelets are pivotal for clotting and wound healing, and PRP is thought to encourage tissue repair and regeneration due to release various growth factors and cytokines, including platelet derived growth factor (PDGF), transforming growth factor beta (TGF-B), and vascular endothelial growth factor (VEGF) (8). PRP has been used in numerous medical fields, including orthopedics, dermatology, and dentistry, due to its regenerative properties (10). hrough the activation of several signaling pathways, including the recruitment of mesenchymal stem cells (MSCs). These cells possess multi potent differentiation capabilities and can contribute to tissue repair and regeneration (7). PRP has obtained attention for its important applications in reproductive medicine in vitro fertilization (IVF) due to the influence of PRP

on sperm parameters and fertility outcomes (9). PRP has gained notice as a pivotal cryoprotective agent due to its rich content of growth factors, proteins, and cytokines that could support cell survival during cryopreservation. However, the cytotoxic effects of PRP, especially when used in high concentrations, need to be carefully evaluated (6).

Materials and method:

The study was included 60 fertile men at the High Institute for Infertility Diagnosis and Assisted Reproductive Technologies /Al- Nahrain University, Harthiya International Laboratory from September 2023 until November 2023. This study had been approved by Local Ethical Committee in the High Institute. Detailed questionnaire was designed depending on history and physical examination. Particularly, cases were assorted according to certain inclusion and exclusion criteria. The inclusion criteria in this experiment were with normozoospermic criteria applied by WHO, 2010 in the 5th edition of the manual of seminal fluid analysis, and the age were ranged from 25 - 40 years old. Any other male patients with abnormal semen parameters or out of the age range were excluded.

The blood sample 10 ml from the same patient at the same time of semen collection, were collected for preparation of Autologous platelet rich plasma (PRP).

Preparation of Autologous PRP: The procedure was done as the following steps in In Vitro Fertilization (IVF) laboratory under sterile conditions:

1- Blood was sampled with 10ml drawn into PRP Tube with ACD (Anticoagulant citrate dextrose) + RA (Reju-venation Agent) + ACTIVATOR.

2- Centrifugation, PRP tube was placed in the centrifuge, spinning the blood at 3000 rpm for 10 minutes allowing the separation of red blood cells, platelet-poor plasma, and PRP.

3- 10 µL of the separation placed on the slide for platelet examination under light microscope.

4- Separation of PRP, carefully extracted and poured into falcon tube for further use.

Seminal Analysis: according to WHO (2010), Semen sample was collected after 2–7 days of sexual abstinence these semen samples were collected in a private room near the laboratory by masturbating into a clean, wide-mouth sterile container. The samples were then liquefied in an incubator (37°C) for 15-30 minutes. After complete liquefaction, the viscosity of the sample can be estimated by gently aspirating it into a wide-bore plastic, maximum 2 cm. A normal of semen sample has homogeneous, grey-opalescent appearance. The pH should be measured. A drop of semen is spread evenly onto the pH paper. After 3 seconds. The normal pH of the semen when the semen is slightly alkaline, is between 7.2 and 8. After, the macroscopic examination were done. The microscopic investigation of the sample, estimates are included the concentration, count and motility.

Each semen sample divided into three groups (Control sample with only cryomedia 0.7 ml (glycerol and sugar), Autologous PRP sample 0.5 ml, and mixed sample 0.5ml PRP+Cryomedia 0.7 ml (glycerol and sugar). Then preserve these three groups into liquid nitrogen -196 for two months. After that, thawing in water bath at 37 C. then semen parameters were evaluated and the results of the three groups were compared.

Statistical analysis:

The data were analyzed using Statistical Package for Social Sciences (SPSS) version 23.0 and Microsoft office 2010.The descriptive statistics including frequency, range, mean and standard error were measured to de-scribe the data. The groups were compared by applying paired sample t-test and analysis of variance. Post hoc tukey test of ANOVA were used to assess paired samples significance and the results were considered statistically significant when p value was equal to or less than 0.05.

Results:

Comparison of seminal analysis parameters between fresh semen samples and samples preserved with media

The comparison between fresh semen samples and sample preserved with media were presented in table 1 and figure 1, accordingly the semen sample preserved with media revealed significantly decrease sperms con-centration (44.40 ± 2.08 vs. 54.80 ± 2.27; p<0.001), total sperms count (137.78 ± 8.92 vs.174.93 ± 8.79; p<0.001) and progressively motile sperms percent (64.85 ± 1.91vs.72.35 ± 2.61; p=0.010).

There was also significantly increase non-progressively motile sperms percent (21.77 ± 1.39 vs. 17.97 ± 1.35; p=0.007) and insignificantly increase immotile sperms percent (13.13 ± 1.25 vs. 10.44 ± 1.75; p=0.207).

Table 1: Comparison of seminal analysis parameters between fresh semen samples and samples preserved with media

Parameters	Fresh semen Samples	Samples preserved with media	*p* value
Sperms concentration (10^6/ml)	54.80 ± 2.27	44.40 ± 2.08	< 0.001 Ŧ S
Total sperms count (10^6)	174.93 ± 8.79	137.78 ± 8.92	< 0.001 Ŧ S
Progressive motile sperms %	72.35 ± 2.61	64.85 ± 1.91	0.010 Ŧ S
Non progressive motile sperms %	17.97 ± 1.35	21.77 ± 1.39	0.007 Ŧ S
Immotile sperm %	10.44 ± 1.75	13.13 ± 1.25	0.207 Ŧ NS

Ŧ: Paired sample t test; S: Significant (p ≤ 0.05); NS: Not significant (p > 0.05)

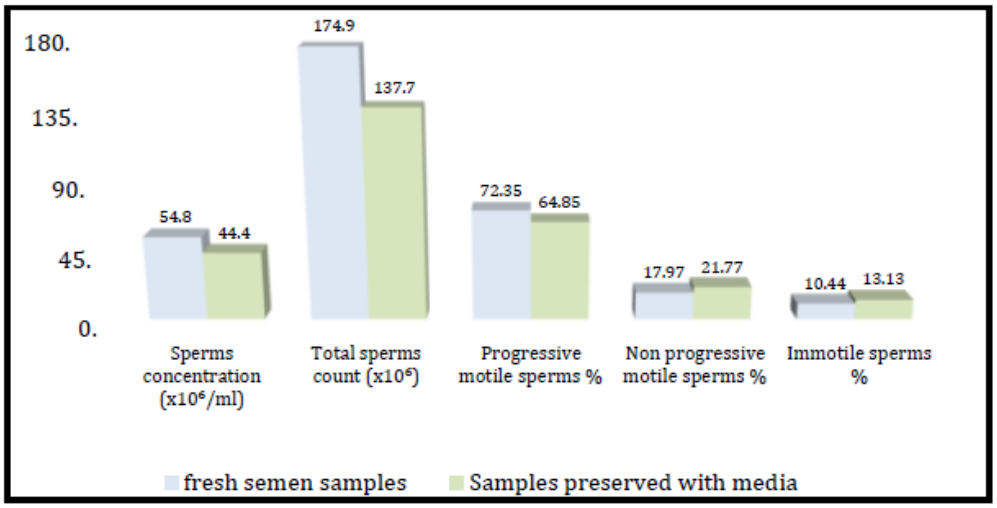

Figure 1: Comparison of seminal analysis parameters between fresh semen samples and samples preserved with media

Comparison of seminal analysis parameters between fresh semen samples and samples preserved with 0.5 ml PRP

Samples preserved with 0.5 ml PRP also had significantly decline sperms concentration (27.13 ± 2.04 vs. 54.80 ± 2.27; $p < 0.001$), total sperms count (87.02 ± 7.36 vs. 174.93 ± 8.79; $p < 0.001$) and progressively motile sperms percent (27.22 ± 2.54 vs.72.35 ± 2.61; $p < 0.001$)

On the other hand there were significantly rise non-progressively motile sperms percent (32.17 ± 3.27 vs. 17.97 ± 1.35; $p < 0.001$) and immotile sperms percent (40.60 ± 3.87 vs. 10.44 ± 1.75; $p < 0.001$) as illustrated in table 2 and figure 2

Table 2: Comparison of seminal analysis parameters between fresh semen samples and samples preserved with 0.5 ml PRP

Parameters	Fresh semen Samples	Samples preserved with 0.5 ml PRP	*p* value
Sperms concentration (10^6/ml)	54.80 ± 2.27	27.13 ± 2.04	< 0.001 Ŧ S
Total sperms count (10^6)	174.93 ± 8.79	87.02 ± 7.36	< 0.001 Ŧ S
Progressive motile sperms %	72.35 ± 2.61	27.22 ± 2.54	< 0.001 Ŧ S
Non progressive motile sperms %	17.97 ± 1.35	32.17 ± 3.27	< 0.001 Ŧ S
Immotile sperm %	10.44 ± 1.75	40.60 ± 3.87	< 0.001 Ŧ S

Ŧ: Paired sample t test; S: Significant ($p \leq 0.05$); NS: Not significant ($p > 0.05$)

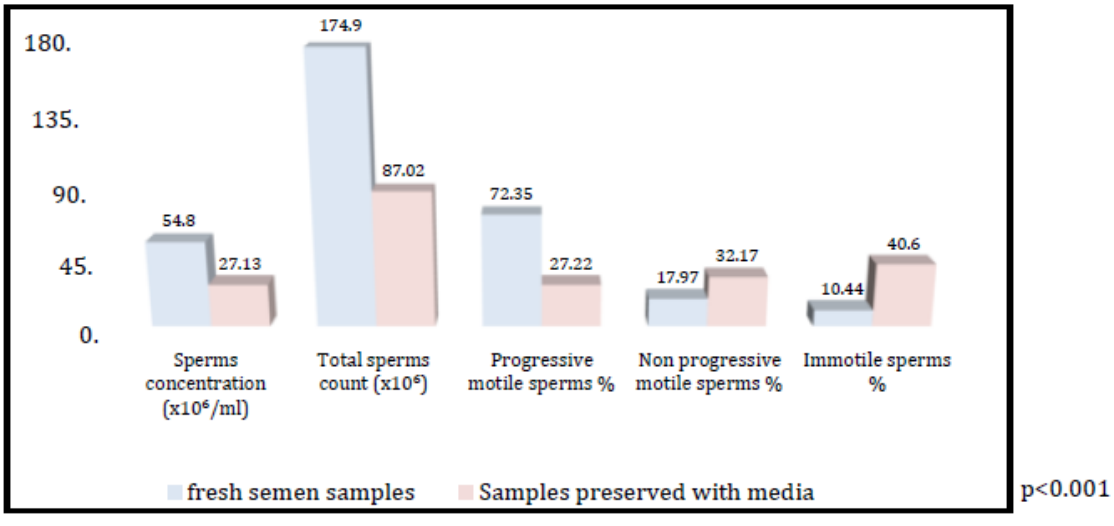

Figure 2: Comparison of seminal analysis parameters between fresh semen samples and samples preserved with 0.5 ml PRP

Comparison of seminal analysis parameters between samples preserved with media, 0.5 ml PRP and mixed samples (Media & PRP)

Comparison of seminal analysis parameters between samples preserved with media, 0.5 ml PRP and mixed samples (Media & PRP) were demonstrated in table 3 and figure 3, accordingly there were significant differences between the three samples regarding the sperms concentration (44.40 ± 2.08 vs. 27.13 ± 2.04vs. 48.73 ± 2.23; $p<0.001$), total sperms count (137.78 ± 8.92 vs. 87.02 ± 7.36 vs. 155.47 ± 8.41; $p<0.001$), progressively motile sperms percent (64.85 ± 1.91 vs. 27.22 ± 2.54 vs. 69.75 ± 2.60; $p<0.001$), non-progressively motile sperms percent (21.77 ± 1.39 vs. 32.17 ± 3.27 vs. 17.57 ± 1.38; $p<0.001$) and immotile sperms percent (13.13 ± 1.25 vs. 40.60 ± 3.87 vs. 12.67 ± 1.74; $p<0.001$).

Table 3: Comparison of seminal analysis parameters between fresh semen samples before preservation, preserved with media, 0.5 ml PRP and mixed samples (Media & PRP)

Parameters	Samples before preservation	Samples preserved with media	Samples preserved with 0.5 ml PRP	Samples preserved with media & PRP	*p* value
Sperms concentration (x10^6/ml)	54.80 ± 2.27	44.40 ± 2.08	27.13 ± 2.04	48.73 ± 2.23	< 0.001 ∀ S
Total sperms count (x10^6)	174.93 ± 8.79	137.78 ± 8.92	87.02 ± 7.36	155.47 ± 8.41	< 0.001 ∀ S
Progressive motile sperms %	72.35 ± 2.61	64.85 ± 1.91	27.22 ± 2.54	69.75 ± 2.60	< 0.001 ∀ S
Non progressive motile sperms %	17.97 ± 1.35	21.77 ± 1.39	32.17 ± 3.27	17.57 ± 1.38	< 0.001 ∀ S
Immotile sperm %	10.44 ± 1.75	13.13 ± 1.25	40.60 ± 3.87	12.67 ± 1.74	< 0.001 ∀ S

∀: ANOVA; S: Significant (p ≤ 0.05); NS: Not significant (p > 0.05)

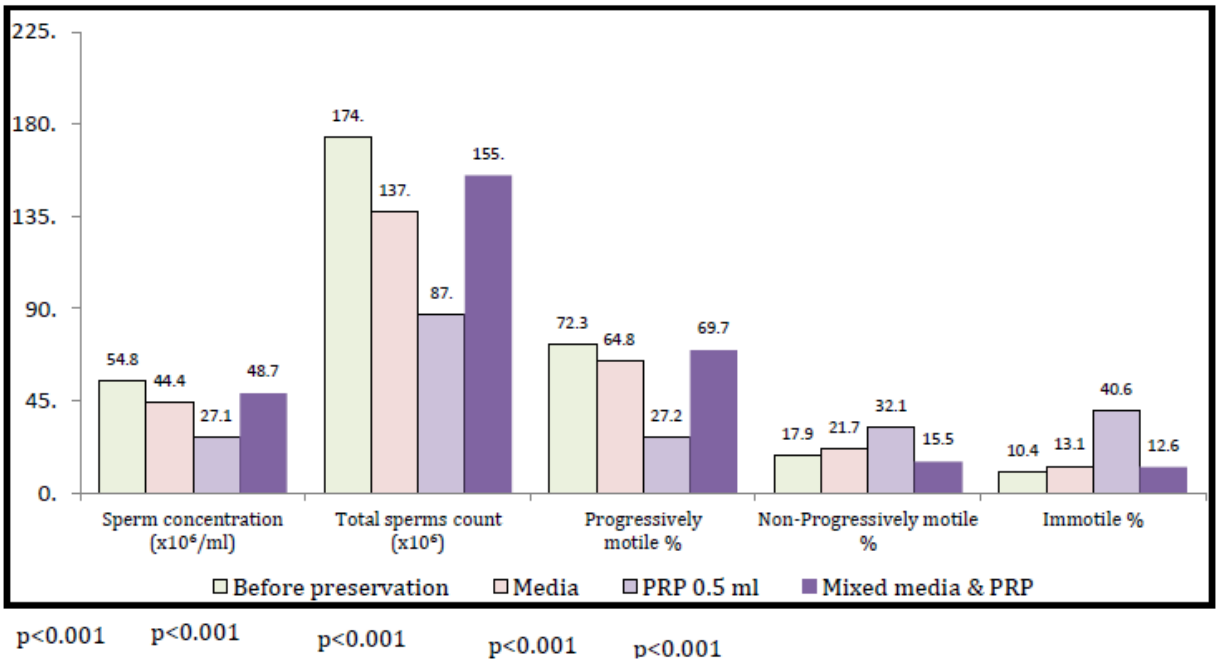

Figure 3: Comparison of seminal analysis parameters between fresh semen samples before preservation, preserved with media, 0.5 ml PRP and mixed samples (Media & PRP)

Discussion:

In the present study the results in table (1) revealed there were noticeable differences in various semen parameters. The findings suggest that semen preservation with media has a significant influence on sperm concentration, total sperm count, and sperm motility. the significantly lower sperm concentration and total sperm count in preserved samples compared to fresh semen samples indicate potential harmful effects of the preservation media on sperm viability and functionality. This observation aligns with previous studies indicating that certain media components or preservation techniques may affect sperm quality (14).

On the other hand, the results of utilizing of 0.5 ml Autologous PRP as a cryoprotective medium alone showed significant alterations in sperm parameters in table (2), indicating potential consequences for sperm quality and fertility outcomes. The findings illustrate a considerable decline in sperm concentration, total sperm count, and percentage of progressively motile sperm may be due to various cytotoxic and oxidative stress inducing factors found in PRP composition and its interaction with sperm cells. Several studies have concentrated on the detrimental effects of PRP on sperm quality and functionality, One study conducted by (1) inspected the effects of PRP on sperm parameters and investigated a significant lowering in sperm motility and viability after exposure to PRP. The cytotoxic effects of PRP components, involving leukocytes and cytokines,

were found to compromise sperm integrity and functionality, as a result of that leading to the reduction in semen parameters. However, the results in table (3) emphasized the preserving of semen samples in a combination of cryoprotective media (glycerol and sugar) 0.7 ml with Autologous PRP 0.5 ml obtain best results on semen parameter than using cryoprotective media or Autologous PRP alone, as proved by the study of (1) the synergistic effects of glycerol, sugar, and PRP in cryoprotective media for semen preservation. showed improved sperm parameters and reproductive tract cytokine profiles after using the combination of cryoprotective media mixed with PRP. In addition, the combination of glycerol, sugar, and PRP has been shown to increase post thaw sperm viability, motility, and DNA integrity, compared to conventional cryoprotective media formulations (12).

Conclusion:

In summary, due to the results of this study on the semen parameters, these conclusions were made, the addition of cryopreservation media only (glycerol and sugar) 0.7 ml to the semen shows low positive results, while the addition of Autologous PRP 0.5 ml as a cryoprotective agent shows negative results. On the other hand, the adding both of media (glycerol and sugar) 0.7 ml with Autologous PRP 0.5 ml reveal better results of semen parameters than cryomedia alone.

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