



Phosphatidylserine Levels in Seminal Plasma of Iraqi Infertile Men Using Direct and Indirect Swim Up Sperm Preparation Techniques.

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Phosphatidylserine (PS), exposed on viable sperm, is required for fertilization as it is recognized by specific receptors located on the microvilli of the oocyte to promote sperm: egg fusion. The heads of viable and motile sperm exhibit PS. With (PS) exposure progressively increasing during sperm transit through the epididymis. PS asymmetry on the leaflets of plasma membrane is arranged in a complex way, via multiple proteins functioning in opposite directions; further, PS is also essential for many intracellular trafficking events. The aim of this study was to evaluate the phosphatidylserine PS level in seminal plasma of infertile men before and after activation using Direct Swim Up (DSU) and Indirect Swim Up (IDSU) activation method and make a comparison between them. Forty infertile men were included in this study during their attendance to the infertility clinic at High Institute for Infertility Diagnosis and Assisted Reproductive Technologies, Al-Nahrain University. Normozoospermic or mild asthenozoospermic semen samples were used to choose the cases, according to WHO manual standards (1999 -2010). The collected semen samples obtained were divided into three parts: The first part prepared was as an in vitro sperm characterization before activation while the second and third parts were after activation using direct and indirect swim up techniques respectively. The level of seminal plasma phosphatidylserine was increased after the activation techniques from the baseline level before activation. Comparison of Seminal plasma phosphatidylserine levels between direct and indirect sperm activation technique were significantly higher in direct swim up activation technique than indirect one. Phosphatidylserine (PS) in semen plasma of infertile men was significantly increased after activation using Direct Swim Up DSU preparation method.

ABSTRACT

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KEYWORD

Phosphatidylserine, Direct Swim Up, Indirect Swim Up, Infertile men.

1. Introduction

The success of assisted reproduction procedures and male fertility, mostly intrauterine insemination (IUI), are associated with sperm survival and depend on the quality of both the sperm and the egg (Le MT et al., 2022 [1]). Fertilization is vital for the persistence of species. Even though Izumo1 and Juno are important for the first interaction between gametes, it is still not known what other molecules are needed on both the sperm and the egg for the sperm and egg to join together (Claudia et al., 2019 [2])

Lipids, without which cells and life would not be, play crucial functions in cellular physiology and pathology as they are the structural components of cell membranes. Furthermore, lipids serve as energy stores, sources of signaling molecules, and stand for protein conscription (Cockcroft, 2021 [3]). Phospholipids are composed of two hydrophobic fatty acyl chains and

one hydrophilic head group. Along with cholesterol, they are the major constituents of biological membranes. Most of the structural phospholipids in mammalian membranes are made up by Glycerophospholipids, like phosphatidylcholine (PC), phosphatidylethanolamine (PE), phosphatidylserine (PS), phosphatidylinositol (PI), and phosphatidic acid (PA) (Wang Bo and Peter T., 2019 [4]).

Phosphatidylserine (PS) represents about 10–15% of total phospholipids, mainly localized to the cytosolic leaflet of the plasma membrane (Cockcroft, 2021 [3])

Phosphatidylserine (PS) is the most common negatively charged phospholipid in the membranes of eukaryotic cells, and it is exposed on the head of healthy, moving sperm, and the amount of PS that is exposed grows as the sperm moves through the epididymis. Phosphatidylserine PS guides the binding of proteins with C2 or gamma-carboxy glutamic domains

and helps polycationic ligands stick to the outside of cell membranes electrostatically. PS is not spread out evenly. Instead, it is found in the inner leaflet of the plasma membrane and in the membranes of endocytic organelles. Loss of PS asymmetry is an early sign of apoptosis and tells the body to start clotting blood (Leventis, P. A., & Grinstein, S., 2010 [5]). The movement of sperm is important for fertilization, and asthenozoospermia is one of the diseases that can cause male infertility. Even so, the mechanisms that control the movement of sperm are not completely unknown Martin-Hidalgo (David M., 2020 [6])

Phosphatidylserine (PS) is a biomarker that can be used to find fertile sperm. Phosphatidylserine (PS) receptors on the oocytes are expressed in pairs. Blocking or getting rid of some of these P'S receptors makes it hard to get pregnant, and sperm can join with myoblast cells in a PS-

dependent way (Smith Ryan P. and Jeffrey,2022 [7])

Phosphatidylserine (PS) is an amphiphilic phospholipid that is found inside the cell membranes of both prokaryotic and eukaryotic cells. There are two different ways to make PS in mammalian cells, which are different from how bacteria do it. The movement of sperm PS from the inner leaflet of the plasma membrane to the outer leaflet is thought to be linked to sperm death and male infertility. The amount of PS that sticks out of human sperm is used as a measure of how good the sperm is. Flow cytometry or magnetic-activated cell sorting can be used to quickly separate PS-externalized sperm at the molecular level. This can improve the quality of sperm and the success rate of assisted reproductive technology (Zhong and Lin, 2013 [8])

Apoptosis is a key biological process that has been shown to be important in a number of other biological

processes. During programmed cell death, chromatin breaks apart during a process called apoptosis. This is a separate process that leads to DNA fragmentation. This biological process is important for making germ cells and keeping the ratio of germ cells to Sertoli in the testis at the right level (Latchoumycandane et al., 2020 [9]; Bejarano et al., 2018 [10])

There are two different ways for apoptosis to start: the extrinsic pathway, also called receptor apoptosis, and the endogenous pathway, also called mitochondrial apoptosis. The perforin-granzyme A and B pathway and the P53 pathway, which causes apoptosis, are two examples of specific mechanisms (Asadi Asadollah et al., 2021 [11])

Aim of Study: To measure the phosphatidylserine (PS) level by Enzyme-Linked Sorbent Assay (ELISA) in seminal plasma of infertile men before and after activation using Direct Swim Up (DSU) and Indirect

Swim Up (IDSU) activation method and make a comparison between them.

2. Patients and Methods

Forty (40) infertile men were included in this study during their attendance to the infertility clinic at High Institute for Infertility Diagnosis and Assisted Reproductive Technologies, Al-Nahrain University. Normozoospermic or mild asthenozoospermic semen samples were used to choose the cases, according to WHO manual standards (1999 -2010). The collected semen samples obtained were divided into three parts: The first part prepared was in vitro sperm characterization before activation as a baseline, the second part was after activation using a direct swim-up technique, while the third part was after activation using an indirect swim-up technique. Seminal plasma of the three aliquots was frozen at -20°C to measure the phosphatidylserine levels using ELISA .

1- Direct Swim Up technique (DSU):

The semen sample is added beneath the pre-warmed culture medium, was called FertiCult Flushing (semen layered down a flushing medium), then incubate at 37 degrees for 30 minutes. A drop of 10 μ L was aspirated from the test tube, put it on the slide with a coverslip, and tested under the microscope (400X) for assessment of the parameters of spermatozoa (Ren SS.et al., 2018 [12])

2- Indirect Swim Up technique (IDSU):

It is done by adding (1 ml) of liquefied semen to the test tube have (1 ml) of media was called FertiCult, flushing with mixing them, then centrifuge at 3000 rpm for 3 minutes, after that, the supernatant will be discarded then 0.5ml of media will be added to the pellet, then incubate at 37 degrees for (30minutes). A drop of 10 μ L was aspirated from the test tube, put it on the slide with a coverslip, and tested under the microscope (400X) for assessment of the parameters of spermatozoa (Ren SS.et al.,2018 [12])

3-Enzyme-Linked ImmunoSorbent Assay for Evaluation of Phosphatidyl Serine:

Semen plasma samples for all patients were carried out for the measurement of PS pre and post-activation with the aid of commercially available ELISA kits, SunLong Biotech laboratory, China. According to the manufacture leaflet, the procedure was performed.

3- 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 deviation, were measured to describe the data.

The groups were compared by applying an independent sample t-test (Unpaired t-test between two subjects in different two groups)

3. Results

Seminal plasma phosphatidylserine levels before activation were summarized or illustrated in Table 1. Seminal plasma phosphatidylserine levels after activation with swim-up methods show increased levels and a comparison between Direct Swim Up

(DSU) and Indirect Swim Up (IDSU) were shown in Table 2 and Figure 1. There were also significantly higher Phosphatidylserine levels in the seminal plasma of the direct swim-up technique in comparison with before activation (235.16 ± 63.04 vs 124.76 ± 37.58).

(Table 1): Baseline seminal plasma phosphatidylserine levels before activation

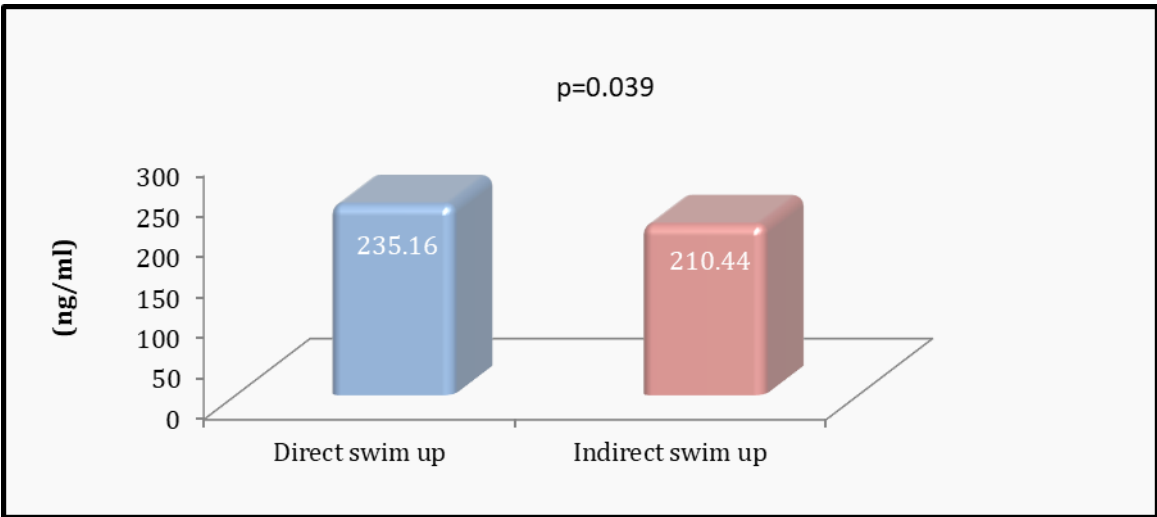
Parameters	Range	Mean \pm SD
Seminal plasma phosphatidylserine (ng/ml)	53.53 – 192.18	124.76 \pm 37.58

SD: Standard deviation

(Table 2): Comparison of seminal plasma phosphatidylserine levels between direct and indirect swim-up techniques

Parameters	Direct swim up (Mean \pm SD)	Indirect swim up (Mean \pm SD)	p-value
Seminal plasma phosphatidylserine (ng/ml)	235.16 – 63.04	210.44 \pm 39.66	0.039 F S

SD: Standard deviation; S: Significant ($p \leq 0.05$); F: Independent sample t test



(Figure1): Comparison of seminal plasma phosphatidylserine levels between direct and indirect swim-up techniques

4. Discussion

In vitro sperm activation, in this study, was done using the direct and indirect swim-up techniques. The swim-up is known as the most basic, simple, easy and low cost method for sperm cell preparation (Baldini et al., 2021[13]). The swim-up was more commonly used than other methods of sperm preparation in Al-Nahrain University's High Institute for Infertility Diagnosis and Assisted Reproductive Technologies. It is less expensive and more useful for the worker (Jameel, 2018 [14]).

For our knowledge, this is the first study that compared the level of seminal phosphatidylserine PS as a biomarker post-activation with direct and indirect swim-up techniques .

During capacitation, there is an AC/cAMP/PKA-dependent lipid remodeling of the sperm plasma membrane due to phospholipids translocation that led to externalization of phosphatidylserine (PS) and phosphatidylethanolamine and to an albumin-mediated efflux of cholesterol resulting in an increase in membrane fluidity (Flesch et al., 2001 [15];

Salicioni et al., 2007 [16]). According to the results of our study, the rise in seminal plasma phosphatidylserine levels might go with the mechanism that Externalization of phosphatidylserine (EPS) was related to sperm capacitation process due to a bicarbonate-activated outward translocation of PS as studies by (Smith, Ryan & Jeffrey, 2022 [7]; de Vantéry et al., 2009 [17]) found. In contrast, other reports found correlations between EPS and apoptotic events such as DNA fragmentation, caspase activities, and/or loss of MMP integrity (Lachaud et al., 2004 [18]; Moustafa et al., 2004 [19]) Taken together, EPS spermatozoa could be survivors of a testis abortive apoptotic process or the result of oxidative stresses initiated during the transit or storage in the male genital tract or be linked to a physiological event in the post-ejaculation period. They also predict that spermatozoa might enter an

apoptotic-like pathway, giving a physiological role to EPS during capacitation/acrosome reaction for the acquisition of fertilizing ability in a natural mechanistic economy. Such a mechanism would be dependent on the right platform for successful fertilization, or otherwise, it would be converted in the apoptotic signal for elimination by phagocytic cells (de Vantéry, 2009 [17]). Integrity of the sperm membrane, of which phosphatidylserine (PS) plays a central role, is essential for fertilization. The externalization of PS (EPS) occurs during capacitation and the acrosome reaction. EPS, from the inner to the outer membrane, is considered as a sign of early apoptosis (Tavalaee et al., 2014 [20]). Therefore, EPS may have a dual function in sperm. Comparison of seminal plasma phosphatidylserine levels between direct and indirect swim-up techniques showed that there were significantly higher Phosphatidylserine levels in the seminal plasma of direct

swim-up than the indirect swim-up technique (235.16 ± 63.04 vs 210.44 ± 39.66). These findings are consistent with the following studies about the Direct swim-up (DSU) and its preferable more than Indirect Swim Up (IDSU) :The World Health Organization manual indicated a swim-up procedure without initial centrifugation (DSU) because possible oxidative damage of the sperm membrane after initial centrifugation (WHO 2010, [21])

Researches has shown that the Swim Up method, but not the Density Gradient Centrifugation DGC technique, produces a significantly lower ratio of DNA fragmentation than when using fresh samples Le MT, Dang et al., 2022 [1]. According to other studies, Swim Up SU is the best method because it can significantly reduce sperm DNA damage and may have a predictive value in IUI (Muratori et al.,2019 [22]).

Other studies as (Taha et al., 2022 [23]

; Kadhim et al., 2017 [24]) showed that Indirect Swim Up (IDSU) is the best option in the treatment of semen samples during sperm preparation as spermatozoa with intact DNA after Indirect Swim Up IDSU and Density Gradient Centrifugation DGC were significantly higher (Taha et al., 2022 [23]; Kadhim et al., 2017 [24]).

5- Conclusion

Phosphatidylserine levels increased significantly after activation with the swim-up method, mainly with Direct Swim Up (DSU), and can be used as a biomarker for the capability of sperm for fertilization.

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Author Contribution

Dhamyaa A. Neamah, performed the study, Hayder A. L. Mossa, Muayad S., supervised the work .

Conflict of Interest

The authors declare no conflict of interest .

Ethical Clearance

The study was approved by the Ethical Approval Committee.

Financial Disclosure

There is no financial disclosure.

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