Correlation of Seminal Plasma EMMPRIN Levels with Sperm Quality and the Efficacy of Activation Techniques

Omaima Akram Othman[1]*, Essraa Mohsen Al-Essawe[2], Mufeda Ali Jawad[2]

[1]*Department of Applied Embryology, High Institute for Infertility Diagnosis and Assisted Reproductive Technologies Al-Nahrain University, Baghdad. Email: omaima.akram@ierit.nahrainuniv.edu.iq*

[2]*Department of Physiology, High Institute for Infertility Diagnosis and Assisted Reproductive Technologies Al-Nahrain University, Baghdad*

KEYWORDS
Male infertility, EMMPRIN, DNA integrity, assisted reproductive technology, sperm preparation techniques, Sperm motility

ABSTRACT

Background: Infertility is a major global health challenge, impacting 15% to 20% of couples globally. Male infertility, which is typically associated with issues such as low sperm motility, has increased during the last two decades. Male fertility relies heavily on the development of extracellular matrix metalloproteinase inducer (EMMPRIN), a glycosylated transmembrane protein involved in sperm function and embryo implantation. Understanding how sperm preparation techniques affect sperm quality and EMMPRIN expression is crucial for achieving optimal results in assisted reproductive technologies (ART). Objective: To investigate EMMPRIN expression in human sperm and compare the impact of two in vitro sperm preparation procedures, "direct swim-up (DSU) and pellet swim-up (PSU), on various sperm characteristics. To determine the optimum technique for improving sperm quality in ART. Materials and Methods: Masturbation was employed to collect sperm samples from 44 participants (aged 21 to 45). Following liquefaction, samples were evaluated macroscopically and microscopically in accordance with WHO 2021 guidelines. The samples were separated into three aliquots for ELISA assessment of EMMPRIN levels and sperm processing with DSU and PSU techniques. Sperm chromatin immaturity (SCI) was assessed using Aniline Blue staining. Statistical investigations were carried out to determine the correlations between EMMPRIN levels, semen parameters, and the impacts of the two in vitro sperm preparation techniques. Results: Sperm concentration significantly decreased from control (40.25 ± 2.30) to DSU (15.51 ± 1.74) and PSU (9.99 ± 1.16) conditions. However, motility, morphology, and SCI significantly improved, particularly with PSU, which showed a greater reduction in SCI % compared to DSU, indicating its effectiveness in selecting higher-quality sperm. There were no significant associations detected between EMMPRIN levels with semen metrics, although PSU exhibited a slightly higher association with improved sperm parameters. Conclusion: Seminal plasma EMMPRIN levels do not appear to directly influence semen parameters or sperm chromatin maturity. However, the choice of sperm activation technique, particularly PSU, significantly impacts sperm quality. More research is needed to better understand the importance of EMMPRIN in ART outcomes and its potential as a sperm quality indicator

1. Introduction

Infertility affects a large proportion of the global population, with the World Health Organization (WHO) claiming that 15%–20% of couples face infertility challenges. Both male and female factors participate equally to infertility; but male infertility has increased dramatically over the past two

decades. Male infertility often arises from issues affecting sperm production or motility, though many cases remain unexplained (Chhikara et al., 2022). Sperm motility, a crucial aspect of male fertility, is essential for successful fertilization. Asthenozoospermia, or impaired sperm motility, is a prevalent cause of male infertility (Neamah et al., 2023). Cluster of differentiation 147 (CD147), also referred to as extracellular matrix metalloproteinase inducer (EMMPRIN) or Basigin (Bsg), is a glycosylated transmembrane protein required for sperm function and embryo implantation (Chuliá-Peris et al. 2022). CD147 consists of a cytoplasmic domain, a single transmembrane domain, and immunoglobulin domains in the extracellular region. According to studies, CD147 knockdown affects cell interactions essential for implantation and is associated with poor endometrial receptivity in women who have experienced repeated implantation failure (Asgari et al., 2023). In male mice, the loss of CD147 causes sterility due to decreased spermatogenesis, indicating its importance in maintaining the blood-testis barrier and germ cell-Sertoli cell connections (Almousa et al., 2022). Furthermore, CD147 is an important regulator of matrix metalloproteinases (MMPs), which are required for sperm function and effective implantation.

Sperm DNA integrity is a crucial factor in male fertility, with the DNA fragmentation index (DFI) serving as a measure of sperm DNA damage that may arise during sperm production, maturation, or post-ejaculation (Aziz et al., 2023). Sperm DNA fragmentation is associated with decreased fertility rates, poor embryo quality, and higher miscarriage rates (Campos et al., 2021). Obesity, stress, smoking, and old age are all potential risk factors for DNA fragmentation. Furthermore, chromatin compaction, mediated by nuclear proteins known as protamines, is critical for preserving sperm DNA on its travel to the oocyte. Inadequate chromatin compaction has been linked to decreased reproductive success, emphasizing the need of monitoring sperm chromatin state during infertility examinations (Marchiani et al., 2020). ART employs a variety of sperm techniques to obtain the most viable and motile sperm from sperm samples. These strategies aim to increase sperm quality by selecting sperm with improved motility, morphology, and chromatin integrity, all of which are required for successful fertilization (Baldini, 2021; Oseguera-López, 2019). Direct swim-ups and pellet swim-ups are two popular approaches. The direct swim-up technique allows motile spermatozoa to swim away from the seminal plasma into an above culture medium, whereas the pellet swim-up technique requires centrifugation to generate a pellet, which is then migrated into the culture medium (WHO, 2021).

The choice of sperm preparation technique can have a considerable impact on sperm quality and, as a result, ART success rates (Oseguera-López et al 2019). However, there is little understanding of how these approaches affect EMMPRIN expression in sperm, which may be an important role in conception. The goal of this study was to evaluate EMMPRIN expression in human sperm while comparing the effects of two in vitro sperm preparation methods (direct and pellet swim-up) on sperm mobility, chromatin maturity, and EMMPRIN expression in order to determine the most effective method for optimizing sperm quality in ART.

Materials and method:

Participants:

Forty-four semen samples were obtained from men aged 21 to 45 years who attended infertility clinics at Al-Nahrain University and the Ajyal Medical Complex between November 2023 and January 2024. The local institute's ethical committee (Reference code: 0702-MM-2024O35) was approved this study in November 2023. All participants gave informed consent.

Sample Collection:

Following 3 to 5 days of sexual abstinence, semen samples were collected via masturbation in an isolated room near the laboratory. Liquefaction was achieved by incubating the samples at 37°C.

Semen Analysis:

Sperm samples were assessed macroscopically and microscopically after liquefaction in accordance with World Health Organization criteria (WHO, 2021), then each sample was separated into three aliquots. The first aliquot (0.25 mL) was utilized to separate the seminal plasma for EMMPRIN level assessment; while the remaining two aliquots were processed using direct and pellet swim-up procedures.

Direct swim-up technique (DSU):

Begin by thoroughly mixing the semen sample. Then, transfer 0.25 mL of the semen into a sterile 15 mL conical centrifuge tube. Carefully layer 0.25 mL of culture medium (Ferti-Cult™ Flushing medium) over the semen. To maximize the interface between the semen and the medium, tilt the tube at a 45-degree angle and incubate it for 30 minutes at 37°C. After the incubation period, gently return the tube to an upright position. Carefully aspirate the top layer of the medium, which will contain the motile sperm cells. These collected sperm cells can then be prepared for further analysis and evaluation and sperm chromatin immaturity assesment.

Pellet swim-up technique (PSU):

First, thoroughly mix the semen sample. Dilute the liquefied semen with an equal volume of medium (Ferti-Cult™ Flushing medium). Centrifuge the mixture at 3000 rpm for 10 minutes. Carefully discard the supernatant, and then gently add 0.25 mL of fresh, pre-warmed medium over the pellet. Incubate the tube at 37°C for 30 minutes. After incubation, the prepared sample is ready for examination and further analysis. Sperm chromatin immaturity (SCI) %:

Aniline blue staining (AB), an acidic dye was used to determine sperm chromatin immaturity (SCI) percentage (Ali and Al-Essawe 2022; Shimal, et al., 2023). To summarize, a droplet (10 µL) of well-mixed semen was pipetted onto a labeled, pre-warmed microscope slide, and then spread gently and evenly with a clean, dry round-edged glass slide. Smears were air dried and fixed in 4% formal saline (paraformaldehyde in PBS) for 30 minutes at room temperature before being stained with 5% aqueous AB stain in 4% acetic acid (pH = 3.5) for 7 minutes. Excess stain was washed under running water, and the slides were wiped using filter paper. At least 200 spermatozoa were examined under magnification (X 1000) using bright field optics (by oil immersion lens); mature spermatozoa had arginine and cysteine abundant protamine, whereas immature spermatozoa have lysine rich histones. AB is an acidic dye that reacts with lysine to stain immature spermatozoa blue, leaving mature spermatozoa unstained. The stain intensity is proportional to the sperm chromatin integrity (Agarwal et al., 2019).

The percentage of SCI calculated by multiplying the proportion of dark-blue stained sperm by the total number of sperm analyzed and then multiplying by 100%. An ejaculate with less than 20% stained nucleus sperm was considered normal (Auger et al., 1990). Human Extracellular matrix metalloproteinase inducer (EMMPRIN). Seminal plasma was separated by centrifuging the semen sample at 3000 RPM for approximately 20 minutes. The supernatants were carefully collected into Eppendorf tubes. If spermatozoa were detected, the centrifugation process was repeated. The samples were then stored at -20°C to preserve bioactivity until analysis. The levels of Extracellular Matrix Metalloproteinase Inducer (EMMPRIN) were measured using a sandwich enzyme-linked immunosorbent assay (ELISA) kit from Shanghai YL Biont, China (Catalog No: YLA2002HU). This method relies on a biotin double-antibody sandwich technique to accurately assay Human EMMPRIN.

Statistical Analysis:

Data collection, summarization, analysis, and presentation were conducted using IBM SPSS Statistics for Windows, version 23, and Microsoft Office Excel 2010. Initially, variables were assessed for normality distribution using the Shapiro-Walks test.

One way ANOVA followed by post hoc test (Tukey HSD) was used for comparing the mean values among groups to evaluate the effect of different sperm preparation techniques on sperm quality.

Variables were presented as mean and standard error of the mean (Mean ± Std. Error). Differences between values were considered significant when the P-value was equal to or less than 0.05. A Pearson correlation test was employed to evaluate the correlation between any two numeric variables, the results were expressed as correlation coefficient (r) and the corresponding level of significance (P).

Result

Descriptive Information and Semen Parameters:

The study included 44 semen samples, with participants having an average age of 34.76 ± 0.56 years ranging from 23 to 44 years. The descriptive information and semen parameters of all subjects are presented in Table 1, with values reported as (mean ± Std. Error). According to WHO, 2021 cut-off values, Semen parameters were within normal ranges, except for the progressive motility (A+B) 22.95 ± 2.15%, which is below the WHO reference value of 30%. The SCI percentage was 22.66 ± 0.45%, slightly above the WHO threshold of ≤ 20%. The average seminal plasma EMMPRIN level in the sample was 720.01 ± 27.53 (ng/L).

Table 1: Baseline Semen Characteristics Compared with WHO 2021 Reference Values

Parameters	mean ± Std. Error	Cut-off value (WHO 2021)
Age	34.76 ± 0.56	
Days of Abstinence	3.05 ± 0.05	3-5
Liquefaction time	30.86 ± 0.49	30-60 minutes
Semen pH	8.02 ± 0.02	7.2-8
Semen volume (mL)	3.83 ± 0.19	1.4 mL
Sperm concentration	40.25 ± 2.30	$16x10^6$/mL
Total sperm count	148.52 ± 10.11	39 x 10^6 / ejaculate
Total motility (PR+NP)	45.00 ± 3.18	42 %
Progressive motility(A+B)	22.95 ± 2.15	30 %
Non-progressive motility %	22.55 ± 1.65	
Immotile spermatozoa %	54.50 ± 3.18	
Normal sperm morphology %	16.59 ± 1.88	4 %
SCI %	22.66 ± 0.45	≤ 20 %
EMMPRIN level (ng/L)	720.01 ± 27.53	

Values are presented in (mean ± Std. Error), No. : 44 sample; SCI: Sperm chromatin immaturity

Effect of Sperm Processing Techniques:

Table 2 provides an overview of the characteristics of sperm samples subjected to different activation techniques. Sperm concentration significantly decreased from the control to DSU and PSU conditions, while motility and morphology improved. Notably, the PSU technique resulted in a significant reduction in SCI % compared to DSU, indicating its effectiveness in selecting spermatozoa with higher quality.

Sperm concentration (106/mL) showed a marked decrease across conditions, from control (40.25 ± 2.30) to DSU (15.51 ± 1.74) to PSU (9.99 ± 1.16) ($p \leq 0.05$). Total motility (PR+NP) % increased progressively from control (45.50 ± 3.18) to DSU (67.96 ± 3.59) to PSU (82.31 ± 4.32), with significant differences observed between all groups (control vs. DSU and PSU, $p \leq 0.0001$; DSU vs. PSU, $p \leq 0.024$).

Similarly, progressive motility (A+B) % followed a similar pattern, rising from control (22.95 ± 2.15) to DSU (55.29 ± 4.06) to PSU (75.04 ± 4.39), with significant differences across all groups ($p \leq 0.0001$).

Non-progressive motility % significantly decreased from control (22.55 ± 1.65) to DSU (12.66 ± 1.71) to PSU (7.27 ± 1.35) ($p \leq 0.0001$).

The percentage of immotile spermatozoa significantly decreased from control (54.50 ± 3.18) to DSU (32.04 ± 3.59) to PSU (9.74 ± 2.69) ($p < 0.0001$).

Normal sperm morphology % significantly improved, increasing from control (16.59 ± 1.88) to DSU (47.82 ± 4.66) to PSU (62.02 ± 5.49) (control vs. DSU and PSU, $p \leq 0.0001$; DSU vs. PSU, $p < 0.012$).

SCI % remained similar between control (22.66 ± 0.45) and DSU (22.30 ± 0.49), but showed a significant decrease in PSU (20.61 ± 0.53) compared to DSU ($p \leq 0.017$).

Table 2: The Effect of Direct and Pellet Swim-Up Techniques on Sperm Characteristics

		Techniques		**P value**
Characteristics	Control raw semen	Direct swim-up (DSU)	Pellet swim-up (PSU)	DSU vs. PSU
Sperm concentration 10^6/mL	40.25 ± 2.30	15.52 ± 1.74	9.99 ± 1.16	P ≤ 0.024
Total motility (PR+NP) %	45.50 ± 3.18	67.96 ± 3.59	82.31±4.32	P ≤ 0.0001
Progressive motility (A+B) %	22.95 ± 2.15	55.29 ± 4.06	75.04±4.39	P ≤ 0.0001
Non-progressive motility %	22.55 ± 1.65	12.66 ± 1.71	7.27 ± 1.35	P ≤ 0.0001
Immotile spermatozoa %	54.50 ± 3.18	32.04 ± 3.59	9.74±2.69	P ≤ 0.0001
Normal sperm morphology %	16.59± 1.88	47.82 ± 4.66	62.02±5.49	P ≤ 0.012
SCI %	22.66 ± 0.45	22.30 ± 0.49	20.61 ± 0.53	p ≤ 0.017

Values are (Mean ± Std. Error); Number of samples: 44 samples; SCI: sperm chromatin immaturity: ANOVA;

P ≤ 0.05

Correlations between Different Sperm Parameters Before Activation with Seminal Plasma EMMPRIN Levels:

The correlations between seminal plasma EMMPRIN level with seminal fluid characteristics before activation are presented in Table 3 reveals that there are no significant correlations between EMMPRIN levels and semen characteristics before or after activation, with the exception for SCI. which has a moderate positive correlation (R = 0.293) that is nearing statistical significance (P = 0.054).

Table 3: Correlation of seminal plasma EMMPRIN Levels with Semen Parameters in Unprocessed Samples

Characteristic	**EMMPRIN level**	
	R	**P**
Age (years)	0.020	0.896
Days of Abstinence (days)	0.078	0.615
Liquefaction time (Minutes)	0.118	0.447
Semen volume (mL)	-0.114	0.463
Sperm concentration 10^6/mL	0.052	0.739
Total sperm count 10^6 / ejaculate	-0.054	0.726
Total motility (PR+NP) %	-0.036	0.815
Progressive motility (A+B) %	-0.059	0.705
No progressive motility %	0.007	0.966
Immotile spermatozoa %	0.036	0.815
Total progressive motile spermatozoa %	-0.089	0.565
Normal sperm morphology %	-0.084	0.589
SCI%	0.293	0.054

R: Correlation Coefficient; P: P value; Number of samples: 44; SCI: Sperm chromatin immaturity
Correlations between Different Sperm Parameters After Activation with Seminal Plasma EMMPRIN Levels: Table 4 presents the correlation between seminal plasma EMMPRIN level and various sperm characteristics after applying two sperm preparation techniques DSU and PSU. Across both DSU and PSU methods, the correlations between EMMPRIN levels and sperm characteristics are generally weak and not statistically significant.

Table 4: Correlation of seminal plasma EMMPRIN levels with semen parameters following direct and pellet swim-up techniques.

Characteristic	EMMPRIN levels			
	Direct swim-up		Pellet swim-up	
	R	**P**	**R**	**P**
Sperm concentration 10^6/mL	-0.062	0.691	0.056	0.719
Total motility (PR+NP) %	0.214	0.164	0.052	0.740
Progressive motility (A+B) %	0.189	0.218	0.102	0.510
Non Progressive motility %	-0.002	0.990	-0.166	0.280
Immotile spermatozoa %	-0.214	0.164	-0.247	0.106
Normal sperm morphology %	0.033	0.831	-0.095	0.538
SCI %	0.106	0.494	-0.082	0.597

R: correlation coefficient P: P value; Number of samples: 44; SCI: Sperm chromatin immaturity.

Both the DSU and PSU techniques effectively isolate spermatozoa with higher motility and better morphology compared to the control group. However, PSU demonstrates greater efficacy in selecting spermatozoa with superior motility and morphology. The quality of the ejaculate significantly influences the choice of sperm separation method. The PSU technique, which typically recovers a

highly motile sperm fraction with a low yield (Henkel & Schill, 2003; Baldini, 2021). This procedure is widely used in both in vitro fertilization (IVF) and intracytoplasmic sperm injection (ICSI), and it is approved by WHO (2021) in cases of low sperm motility. Despite the fact that it involves spinning, the swim-up technique is mild, producing low reactive oxygen species (ROS) and selectively recruiting mature, activated sperm cells (Bui et al. 2018). Sperm motility is significantly associated with IVF success and pregnancy outcomes (Dcunha et al., 2022). Improvements in motility and morphology are thus critical for increasing ART success rates, as these properties are important drivers of fertilization potential (Oseguera-López et al., 2019). The study also looked at the association between EMMPRIN levels and several sperm parameters. The lack of significant associations between EMMPRIN expression and sperm concentration, motility, and morphology using both DSU and PSU approaches shows that EMMPRIN may not have a direct influence on these elements of sperm quality. This is remarkable given EMMPRIN's recognized roles in cell adhesion, invasion, and matrix metalloproteinase (MMP) modulation, all of which are important for sperm function and implantation. Chen et al. (2021) discovered lower CD147 levels (an EMMPRIN marker) in sperm from men with asthenozoospermia, which was associated with impaired sperm motility. EMMPRIN is essential for spermatogenesis, and alterations cause spermatogenesis failure (Chen et al., 2011).

In reproductive situations, EMMPRIN is necessary for normal fertility, with studies revealing a link between its levels in seminal plasma and IVF success rates (Almousa et al., 2022). However, in this investigation, EMMPRIN levels did not exhibit significant relationships with seminal fluid features, implying that other factors may outweigh their effects. One possible explanation is that EMMPRIN's role in sperm is more complex, involving pathways or variables that were not investigated in this work. Furthermore, individual variability in EMMPRIN expression could explain the limited relationships reported. The study also looked at the effect of sperm preparation techniques on sperm chromatin integrity (SCI), which is important for retaining paternal genetic material during fertilization. Asmarinah et al. (2016) demonstrated that poor sperm chromatin maturity and integrity can impair zygote development following ICSI treatment, implying that these characteristics should be included in standard sperm analysis for ICSI. Sperm DNA abnormalities cause up to one-third of miscarriages (West et al., 2022), while sperm DNA damage reduces conception rates and slows fetal development following ICSI (Ribas-Maynou et al., 2022).

The PSU conduct resulted in an average drop in SCI%, indicating improved selection of sperm with intact chromatin. Both DSU and PSU techniques demonstrated the ability to recover spermatozoa with low DNA fragmentation rates. Given its low cost and reduced time, PSU appears to be the best option for treating semen samples during IVF/ICSI (Volpes et al., 2016). However, the weak correlation between EMMPRIN levels and SCI suggests that EMMPRIN may not play a significant role in maintaining chromatin integrity. Instead, factors such as protamine content, oxidative stress, or DNA repair mechanisms may be more critical. To our knowledge, this is the first study comparing EMMPRIN levels and sperm parameters across different sperm preparation techniques. Our conclusions on the differentiation between pre- and post-activation semen samples in improving EMMPRIN assessment and DNA fragmentation need validation through additional research. Further studies are necessary to fully understand the mechanisms behind these observations and explore the potential benefits of these techniques in ART. Addressing the study's limitations, such as sample size, in future research will be crucial to confirm these findings and further investigate the molecular pathways involved.

Acknowledgment

The authors would like to express their gratitude to the Andrology Lab staff at the institute for their support in handling the semen samples. We are also appreciative to all subjects who provided samples.

Funding

No funding.

Conflict of Interest

None

Ethical Clearance

The study was approved by the institutional ethical committee of the High Institute for Infertility Diagnosis and Assisted Reproductive Technologies at Al-Nahrain University in Baghdad, Iraq in November 2023 (Ref. code 0702-MM-2024O35). Every participant provided informed consent.

Authors' Contributions

Omaima Akram Othman, Essraa Mohsen Al-Essawe, and Mufeda Ali Jawad designed the experiment and planned the work. Omaima Akram Othman collected the samples and conducted laboratory work under the supervision of Essraa Mohsen Al-Essawe and Mufeda Ali Jawad. Essraa Mohsen Al-Essawe performed the statistical analysis. Omaima Akram Othman prepared the manuscript. All authors read and approved the final version of the manuscript.

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