The Egyptian Journal of Hospital Medicine (October 2025) Vol. 101, Page 5062-5072

Beyond the Ejaculate: Unlocking Fertility Potential in Patients with High-Sperm DNA Fragmentation

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ABSTRACT

Background: High sperm DNA fragmentation (SDF) significantly impairs reproductive outcomes in assisted reproductive technologies (ART), contributing to male factor infertility that affects 15% of couples globally.

Aim: This study investigated how sperm origin (testicular vs. ejaculated) and sperm selection methods (polyvinylpyrrolidone "PVP" vs. Sperm Slow) influence intracytoplasmic sperm injection (ICSI) outcomes in couples experiencing recurrent ART failures due to high SDF.

Methods: This randomized, single-blind clinical trial involved 200 couples with high SDF and prior ICSI failures. Participants were divided into two main groups for sibling oocyte injection: (1) ejaculated versus testicular aspirated sperm and (2) ejaculated sperm (prepared by density gradient) further selected using either PVP or Sperm Slow. We assessed fertilization, cleavage, blastocyst formation, and pregnancy rates.

Results: Testicular aspirated sperm yielded significantly superior outcomes compared to ejaculated sperm, showing higher fertilization rates and significantly improved pregnancy rates. Among selection methods, Sperm Slow outperformed PVP, resulting in better fertilization and pregnancy rates. Notably, aspirated sperm demonstrated higher fertilization and pregnancy rates even when compared directly to ejaculated sperm prepared with Sperm Slow.

Conclusion: Utilizing testicular sperm significantly improves ICSI outcomes for couples with high SDF and previous ART failures. When testicular sperm retrieval isn't an option, Sperm Slow appears to be a more effective sperm selection method than PVP for ejaculated sperm in this challenging patient population.

Keywords: Intracytoplasmic Sperm Injection (ICSI), Sperm DNA Fragmentation (SDF), Testicular Sperm, Ejaculated Sperm, Male Infertility.

INTRODUCTION

Male factor infertility significantly contributes to the approximately 15% of global prevalence of infertility among reproductive-aged couples, making it a major worldwide health concern [(1)]. Assisted Reproductive Technologies (ART) have revolutionized infertility treatment, offering hope to millions. Among these, Intracytoplasmic Sperm Injection (ICSI) has become a pivotal procedure, enabling fertilization even when conventional *in vitro* fertilization (IVF) methods fall short. Developed in the 1980s, Intracytoplasmic Sperm Injection "ICSI" involves the direct injection of a single sperm into an oocyte, effectively bypassing obstacles like zona pellucida abnormalities and severe sperm motility defects [(2, 3)] .

ICSI has proven particularly effective in managing male infertility stemming from conditions such as severe oligoasthenoteratozoospermia (OAT), where sperm count, motility, and morphology are severely compromised [(4)]. It's also critical for cases of azoospermia, whether obstructive or non-obstructive, by utilizing surgically retrieved sperm from the epididymis or testes. Furthermore, ICSI is often the chosen method following prior fertilization failures with conventional IVF or in situations involving sperm antibodies [(5-8)].

Despite its success, ICSI outcomes can still be compromised, especially in cases with high sperm DNA fragmentation (SDF). SDF, characterized by breaks or damage in the sperm's genetic material, is an increasing concern in male infertility. This damage can negatively impact embryo development, reduce implantation rates, and elevate the risk of miscarriage, even when fertilization is achieved via ICSI [(2, 9-11)]. Consequently, identifying strategies to mitigate the detrimental effects of high SDF on ART outcomes is crucial [(5, 12, 13)]. This study investigates two key approaches to optimize ICSI success in challenging cases marked by high SDF and recurrent ART failures: the origin of sperm (testicular versus ejaculated) and advanced sperm selection methods.

The Evolution and Efficacy of ICSI

ICSI has proven particularly effective in addressing male infertility caused by conditions such as azoospermia, the presence of antisperm antibodies, and sperm DNA damage. It has also demonstrated success in challenging scenarios, including the fertilization of cryopreserved oocytes and achieving pregnancy in couples with limited oocyte yields [(14)]. The widespread adoption of ICSI underscores its impact, now accounting for over 60% of all ART cycles globally and contributing to the birth of millions of children [(3, 15)].

Received: 08/05/2025
Accepted: 10/07/2025

The Challenge of Sperm DNA Fragmentation

Despite its remarkable success, ICSI outcomes are influenced by numerous factors, notably the source and quality of sperm. A critical concern increasingly recognized for its adverse effects on fertilization, embryo development, and pregnancy outcomes is elevated sperm DNA fragmentation (SDF), often quantified as the DNA Fragmentation Index (DFI) [9, 16, 17]. For patients exhibiting high DFI and a history of recurrent ICSI failures, testicular sperm may offer a significant advantage due to their inherently lower levels of DNA fragmentation compared to ejaculated sperm [2, 16].

Study Rationale and Objectives

Given these considerations, this study aims to evaluate the impact of sperm origin, specifically, testicular versus ejaculated sperm, on ICSI outcomes in male patients with a history of previous ICSI failures and high DFI. By meticulously comparing key parameters, including fertilization rates, blastocyst formation, and pregnancy rates, this research aims to identify the most effective strategy for enhancing ART success in this particularly challenging patient population.

PATIENTS AND METHODS

Chemicals and Drugs

- Recombinant Follicle-Stimulating Hormone (rFSH): Gonal-F® (Merck Serono, Switzerland) – used for ovarian stimulation.
- Gonadotropin-Releasing Hormone (GnRH) Antagonist: (e.g., Cetrotide®, Merck Serono or Orgalutran®, Organon) – used for suppression of premature LH surge.
- Recombinant Human Chorionic Gonadotropin (rhCG): Ovitrelle® (Merck Serono, Switzerland) – used to trigger final oocyte maturation.
- Lidocaine 1%: Local anesthetic for TESA procedure (various manufacturers).
- Density Gradient Media: PureSperm® (Nidacon, Sweden) – for preparation of ejaculated sperm.
- Gamete Buffer: (e.g., Vitrolife, Sweden) – used for handling testicular sperm.
- Terminal deoxynucleotidyl transferase dUTP nick end labeling (TUNEL) Assay Kit: In Situ Cell Death Detection Kit (Roche Diagnostics, Germany) – for assessment of sperm DNA fragmentation.
- Polyvinylpyrrolidone (PVP) 10% Solution: PVP (Vitrolife, Sweden) – for sperm immobilization before ICSI.
- Sperm slow™ Medium: Origio (Denmark) – hyaluronic acid-based medium for sperm selection.
- Hyaluronidase: Hyase™ (Vitrolife, Sweden) – for enzymatic removal of cumulus cells from oocytes.
- Oocyte Handling Medium: G-MOPS™ (Vitrolife, Sweden) – used during denudation and ICSI preparation.
- Embryo Culture Medium: G-TL™ (Vitrolife) or Global Total™ (LifeGlobal, USA) – for post-ICSI embryo culture.
- Mineral Oil: (e.g., Vitrolife) – overlay for embryo culture drops.
- Progesterone Vaginal Gel: Crinone® 8% (Merck Serono, Switzerland) – for luteal phase support.

PATIENTS

The study involved 200 couples who had high sperm DNA fragmentation (DFI > 20%), primary or secondary infertility, and a history of ART failures.

Inclusion Criteria:

Couples participating in the study met the following criteria: female age ≤35 years, male age between 20 and 50 years, and high sperm DNA fragmentation (DFI) attributed to factors such as smoking, varicocele, malignancy, or renal disease.

Exclusion Criteria:

Cases were excluded if they involved azoospermia, severe male factor infertility not defined by high DFI (e.g., severe morphological defects unrelated to DFI), chromosomal abnormalities, poor ovarian reserve, endometriosis, uterine abnormalities, or an endometrial thickness of <18 mm.

Study design:

This study is a randomized, single-blind clinical trial involving 200 couples recruited from the Royal Fertility Center in Mansoura, Egypt. Participants were allocated into two main groups for Intracytoplasmic Sperm Injection (ICSI) based on sperm origin and selection methods:

Group 1: Sperm Origin Comparison:

Sibling oocytes from each female participant were randomized to be injected with either ejaculated sperm or testicular sperm from their male partner.

Group 2: Sperm Selection Method Comparison:

Sibling oocytes from each female participant were randomized to be injected with ejaculated sperm. These ejaculated sperm were first prepared using a density gradient centrifugation method, and then further selected for microinjection using either PVP (polyvinylpyrrolidone) or Sperm Slow media.

METHODS

Sperm Preparation and Analysis

Ejaculated Sperm Preparation: Ejaculated sperm samples were processed using density gradient centrifugation (DGC) for optimal sperm selection.

Testicular Sperm Retrieval and Preparation: Testicular sperm were retrieved via Testicular Sperm Aspiration (TESA) performed under local anesthesia. The retrieved tissue was then mechanically processed to isolate viable spermatozoa.

Sperm DNA Fragmentation Assessment: Sperm DNA fragmentation (SDF) was evaluated using the Terminal deoxynucleotidyl transferase dUTP nick end labeling (TUNEL) assay to determine the DNA Fragmentation Index (DFI).

Oocyte Collection and Preparation

Ovarian stimulation was achieved using a Gonadotropin-Releasing Hormone (GnRH) antagonist protocol combined with recombinant Follicle-Stimulating Hormone (FSH). Mature metaphase II (MII) oocytes were collected via ultrasound-guided transvaginal ovarian pick-up, 36–37 hours following human chorionic gonadotropin (hCG) administration. Cumulus cells surrounding the oocytes were subsequently removed to prepare for injection.

ICSI Procedure

Intracytoplasmic Sperm Injection (ICSI) was performed at 37∘°C using a hydraulic microinjection device under an inverted microscope. Following successful sperm injection, oocytes were cultured in a single-step medium.

Embryo Transfer and Pregnancy Assessment

Selected embryos were transferred into the uterine cavity 3–5 days post-oocyte retrieval, guided by ultrasound. Pregnancy was initially confirmed by serum human chorionic gonadotropin (hCG) levels measured 12–14 days post-transfer, and subsequently verified as a clinical pregnancy via transvaginal ultrasound at 4 weeks post-transfer (identifying a gestational sac with a fetal pole and heartbeat).

Outcome Measures

The primary and secondary outcome measures assessed in this study were:

Fertilization Rate:

Defined as the proportion of successfully fertilized oocytes (presence of two pronuclei and two polar bodies) out of the total number of injected oocytes.

Blastocyst Formation Rate:

Calculated as the number of blastocysts formed on Day 5 or 6, divided by the number of successfully fertilized oocytes.

Clinical Pregnancy Rate:

Defined as the presence of a gestational sac with a fetal heartbeat confirmed by ultrasound.

Ethics approval and consent to participate

This randomized, single-blind clinical trial took place at the Royal Fertility Center in Mansoura, Egypt, from January 2022 to January 2024. All participants provided written informed consent, and the study received an ethical approval from the Institutional Research Board [(18)] of the Mansoura Faculty of Medicine (Code No, MS.23.03.2321.R1.R2.R3). The ethical approval is in accordance with Helsinki.

Statistical Analysis

All collected data were analyzed using the Statistical Package for the Social Sciences (SPSS) software, version 27 (IBM Corp., Armonk, NY, USA). Categorical variables were summarized as frequencies and percentages, while continuous variables were initially assessed for normality of distribution using the Shapiro–Wilk test. Data showing a normal distribution were presented as mean ± standard deviation (SD), whereas non-normally distributed data were expressed as median and interquartile range (IQR). The Chi-square (χ^2) test was utilized to compare categorical variables between groups. For continuous variables, the independent samples t-test was applied when data met parametric assumptions; otherwise, the Mann–Whitney U test was considered for non-parametric comparisons. All statistical tests were two-tailed, and a p-value of less than 0.05 was considered indicative of statistical significance at confidence interval 95% level.

RESULTS

The mean paternal age in the study population was 35.3±6.3 years, and the mean maternal age was 28.4±3.1 years. Approximately 35% of the couples had a history of previous ICSI trials, with a median of 2 trials (ranging from 1 to 10). Additionally, about 4% of couples had experienced prior abortions, with a median of 1.5 abortions (ranging from 1 to 12) **(table 1)**.

Table (1): Demographics and obstetric history of the included patients:

Variables	Total cohort (n=100)
Paternal age (years) Mean ± SD	35.3 ± 6.3
Maternal age (years) Mean ± SD*	28.4 ± 3.1
Previous ICSI No. (%)	35 (35%)
Number of failed ICSI****:** Median (min, max)	2 (1, 10)
Previous abortion No. (%)	4 (4%)
Number of previous abortions: Median (min, max)	1.5 (1, 12)

*SD: Standard deviation. **ICSI: Intracytoplasmic sperm injection.

Ejaculated vs. Aspirated Sperm Comparison

In the comparison between ejaculated and aspirated sperm, aspirated sperm consistently demonstrated superior outcomes across all key parameters. The fertilization rate was significantly higher in the aspirated sperm group (74.67% vs. 56.33%, p<0.001), as were the numbers of top-cleaved and fertilized oocytes **(Table 2).**

Furthermore, blastocyst formation was observed in 79% of couples using aspirated sperm, compared to 45% of those using ejaculated sperm (p<0.001). Most importantly, the clinical pregnancy rate was significantly higher in the aspirated sperm group, both when analyzed by total patients (39% vs. 11%, p<0.001) and by transferred embryos (75% vs. 36.7%, p<0.001) **(Table 3).**

PVP vs. Sperm Slow Comparison

When comparing the two sperm selection methods for ejaculated sperm, the Sperm Slow method demonstrated superior outcomes for fertilization and cleavage. The number of fertilized oocytes in the Sperm Slow group was significantly higher (6.6±3.6 vs. 5.3±2.03 with PVP, p=0.004), as was the number of top-cleaved oocytes (4.7±2.2 vs. 3.6±2.3 with PVP, p=0.007). Furthermore, pregnancy rates were significantly higher with the Sperm Slow method, both when assessed by the total number of patients (25% vs. 11% for PVP, p=0.009) and by transferred embryos (50% vs. 22% for PVP, p=0.003) **(Tables 4, 5).**

Aspirated Sperm vs. Sperm Slow Comparison

Finally, in the direct comparison between aspirated sperm and the Sperm Slow method for ejaculated sperm, aspirated sperm demonstrated higher fertilization rates (74.67% vs. 67% for Sperm Slow, p=0.04) and better cleavage outcomes. When assessed by the total number of patients, aspirated sperm also led to a significantly greater clinical pregnancy rate (39% vs. 25% for Sperm Slow, p=0.03), and a higher pregnancy rate per transferred embryo (75% vs. 50% for Sperm Slow, p=0.009). However, it is noteworthy that the Sperm Slow group had a higher mean number of transferred embryos per patient **(Table 6)**.

Table (2): Comparison between ejaculated sperm group and aspirated sperm group as regards oocyte fertilization and cleavage

Variables	Aspirated sperm group (n= 100) Mean ± SD	Ejaculated sperm group (n=100) Mean ± SD	*p value**
Number of injected oocytes	**10.1 ± 4.5**	**6.3 ± 2.4**	***<0.001***
Number of fertilized oocytes	**7.5 ± 2.1**	**4.5 ± 2.1**	***<0.001***
Fertilization rate	**74.67 ± 22.1**	**56.33 ± 27.03**	***<0.001***
Number of top-cleaved oocytes	**5.8 ± 2.8**	**3.2 ± 1.4**	***<0.001***
Top-Cleavage Rate	**75.6 ± 16.3**	**72.8 ± 15.9**	***0.04***

*Student t- test; Level of significance< 0.05

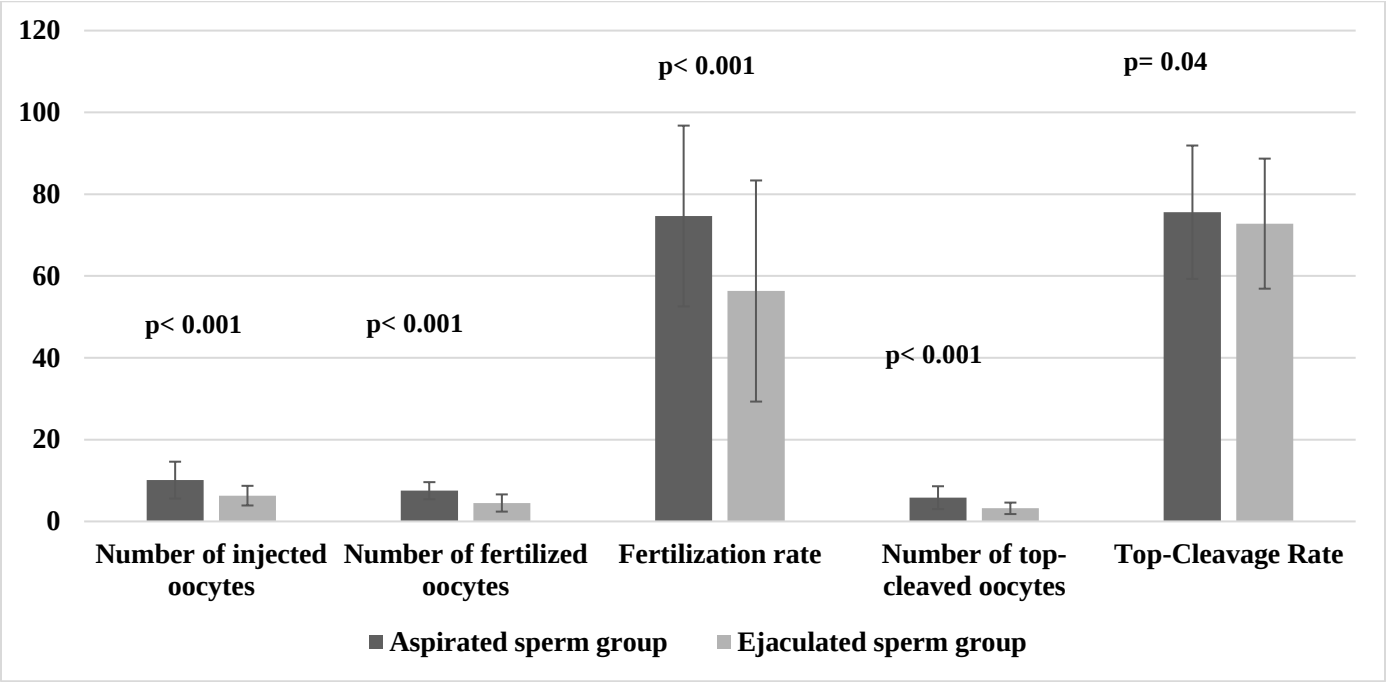

Figure (1): Comparison between ejaculated sperm group and aspirated sperm group as regards oocyte fertilization and cleavage.

Value was represented as Mean ± SD of 100 in each group; the aspirated sperms group had significantly higher numbers of injected, fertilized and top cleaved oocytes than the ejaculated sperm group. Fertilization rate and top cleavage rate was significantly higher in aspirated sperms group than ejaculated sperms group **(Figure 1)**.

Table (3): Comparison between ejaculated sperm group and aspirated sperm group as regards blastocyst formation, embryo transfer and pregnancy rate

	Aspirated sperms group (n= 100) No. (%)	**Ejaculated sperms group (n= 100) No. (%)**	**p value**
Number of blastocyst formation	4.7 ± 2.3	3.8 ± 1.5	**0.03***
Blastocyst formation rate	62.6 ± 20.2	60.17 ± 22.4	0.5*
Blastocyst formation: **Successful** **Failed**	 79 (79%) 21 (21%)	 45 (45%) 55 (55%)	**<0.001#**
Number of transferred embryos	2.4 ± 0.6	2.4 ± 0.7	0.9*
Embryo transfer: **Successful** **Failed**	 52 (52%) 17 (17%)	 30 (30%) 15 (15%)	**<0.001#**
Pregnancy rate (in comparison to total)	39/ 100 (39%)	11/ 100 (11%)	**<0.001#**
Pregnancy rate (in comparison to blastocyst formation)	39/ 79 (49.4%)	11/ 45 (24.4%)	**0.007#**
Pregnancy rate (in comparison to transferred embryo)	39/ 52 (75%)	11/ 30 (36.7%)	**<0.001#**

*Student t- test; #Chi square test; Level of significance< 0.05

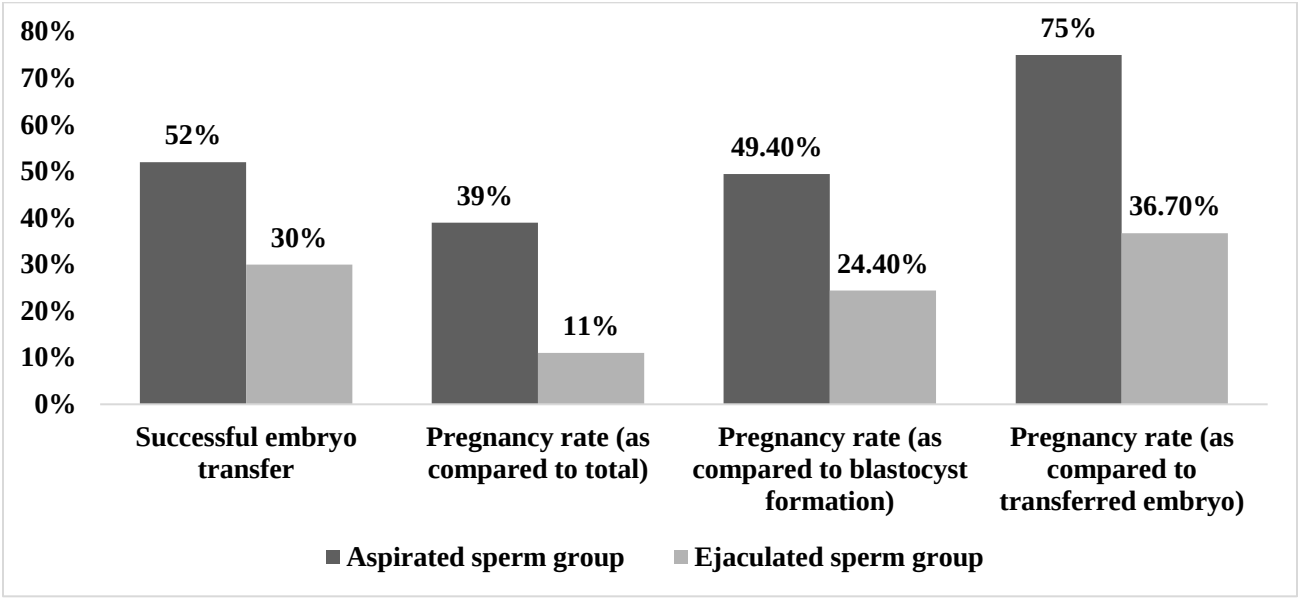

Figure (2): Comparison between ejaculated sperm group and aspirated sperm group as regards embryo transfer and pregnancy rate.

Injection of oocytes by aspirated sperms resulted in significantly higher pregnancy rates than injection of oocytes by ejaculated sperms when including all participants (39% vs. 11%; ***p< 0.001).*** in the aspirated sperms group showed significantly higher in the pregnancy rate compared than in ejaculated sperms group **(Figure 2)**.

Table (4): Comparison between ejaculated sperm group selected in PVP and sperm selected in sperm slow as regards oocyte fertilization and cleavage:

	PVP group (n= 100) Mean ± SD	**Sperm Slow group (n= 100), Mean ± SD**	***p value****
Number of injected oocytes	8.35 ± 4.1	9.5 ± 4.5	0.16
Number of fertilized oocytes	5.3 ± 2.03	6.6 ± 3.6	***0.004***
Fertilization rate	66.12 ± 22.9	67 ± 30.3	0.8
Number of top cleaved oocytes	3.6 ± 2.3	4.7 ± 2.2	***0.007***
Top Cleavage rate	60.1 ± 27.7	68.2 ± 32.8	***0.04***

*Student t- test; Level of significance< 0.05; PVP: polyvinylpyrrolidone.

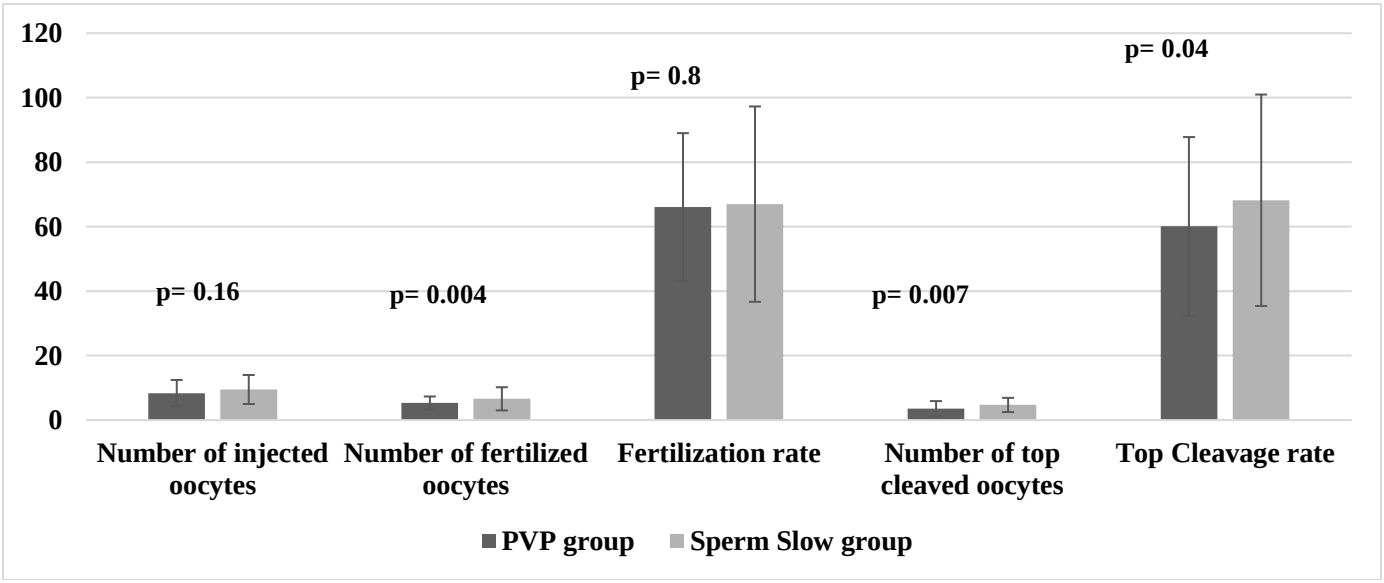

Figure (3): Comparison between ejaculated sperm group selected in PVP and sperm selected in sperm slow as regards oocyte fertilization and cleavage

Number of fertilized oocytes was significantly higher among sperm slow group than PVP group however, both groups were comparable to each other as regards fertilization rate but number of top cleaved oocytes and top cleavage rate was significantly higher among sperm slow group than PVP group **(Figure 3)**.

Table (5): Comparison between the ejaculated sperm group selected in PVP and the ejaculated sperm group selected in sperm slow as regards blastocyst formation, embryo transfer, and pregnancy rate:

Variables	PVP group (n= 100)	Sperm Slow group (n= 100)	*p value*
Number of blastocyst formation Mean ± SD	3.5 ± 1.7	4.1 ± 2.1	***0.02****
Blastocyst formation rate, Mean ± SD	61.8 ± 28.9	61.7 ± 18.2	0.7*
Blastocyst formation No. (%) Successful Failed	63 (63%) 37 (37%)	75 (75%) 25 (25%)	***0.04#***
Number of transferred embryos Mean ± SD	2.2 ± 0.4	2.8 ± 0.4	***<0.001****
Embryo transfer, No. (%)	50 (50%)	50 (50%)	0.8#
Pregnancy rate (in comparison to total) No. (%)	11/ 100 (11%)	25/ 100 (25%)	***0.009#***
Pregnancy rate (in comparison to transferred embryo) No. (%)	11/ 50 (22%)	25/ 50 (50%)	***0.003#***

*Student t- test; #Chi square test; Level of significance< 0.05; PVP: polyvinylpyrrolidone

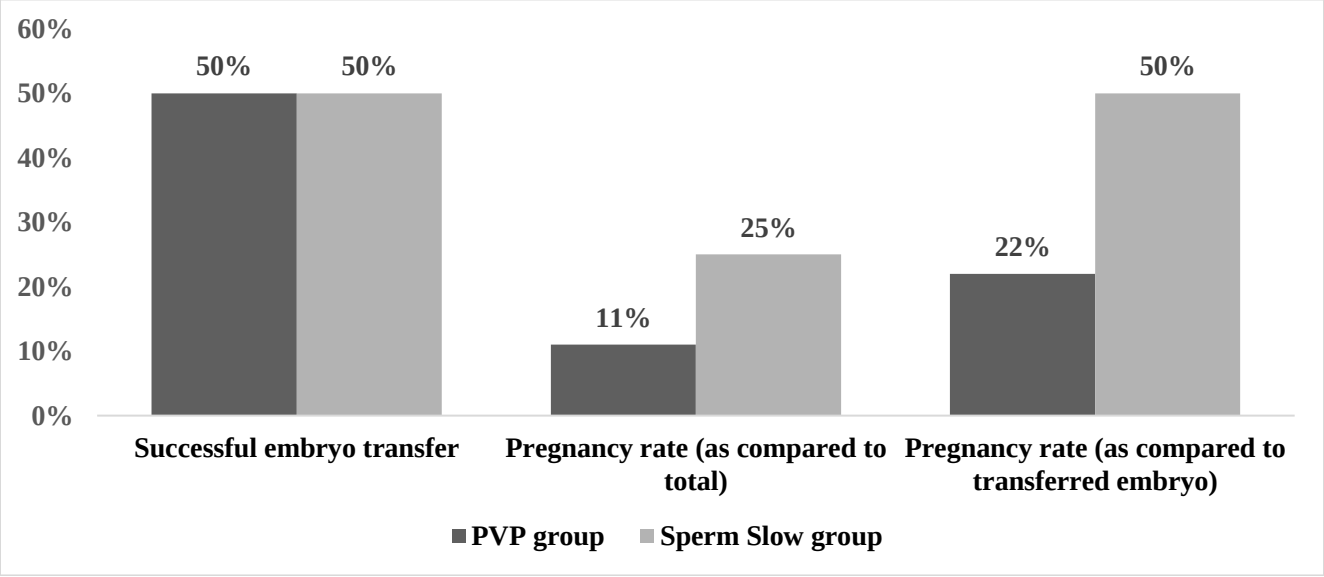

Figure (4): Comparison between ejaculated sperm group selected in PVP and sperm selected in sperm slow as regards transferred embryos and pregnancy rates

Selection of sperms in sperm slow media resulted in significantly higher pregnancy rates (50%) than selection in PVP (22%; p= 0.003) **(Figure 4)**.

Table (6): Comparison between ejaculated sperm group and aspirated sperm group as regards blastocyst formation, embryo transfer, and pregnancy rate:

	Aspirated sperm group (n=100)	**Sperm Slow group (n= 100)**	***p value***
Number of blastocyst formation Mean ± SD	4.7 ± 2.3	4.1 ± 2.1	***0.04***
Blastocyst formation rate Mean ± SD	62.6 ± 20.2	61.7 ± 18.2	0.7
Blastocyst formation No. (%) Successful Failed	79 (79%) 21 (21%)	75 (75%) 25 (25%)	0.5
Number of transferred embryos No. (%)	2.4 ± 0.6	2.8 ± 0.4	***<0.001***
Embryo transfer No. (%)	52 (52%)	50 (50%)	0.8
Pregnancy rate (in comparison to total) No. (%)	39/ 100 (39%)	25/ 100 (25%)	***0.03***
Pregnancy rate (in comparison to transferred embryo) No. (%)	39/ 52 (75%)	25/ 50 (50%)	***0.009***

*Student t- test; #Chi square test; Level of significance< 0.05

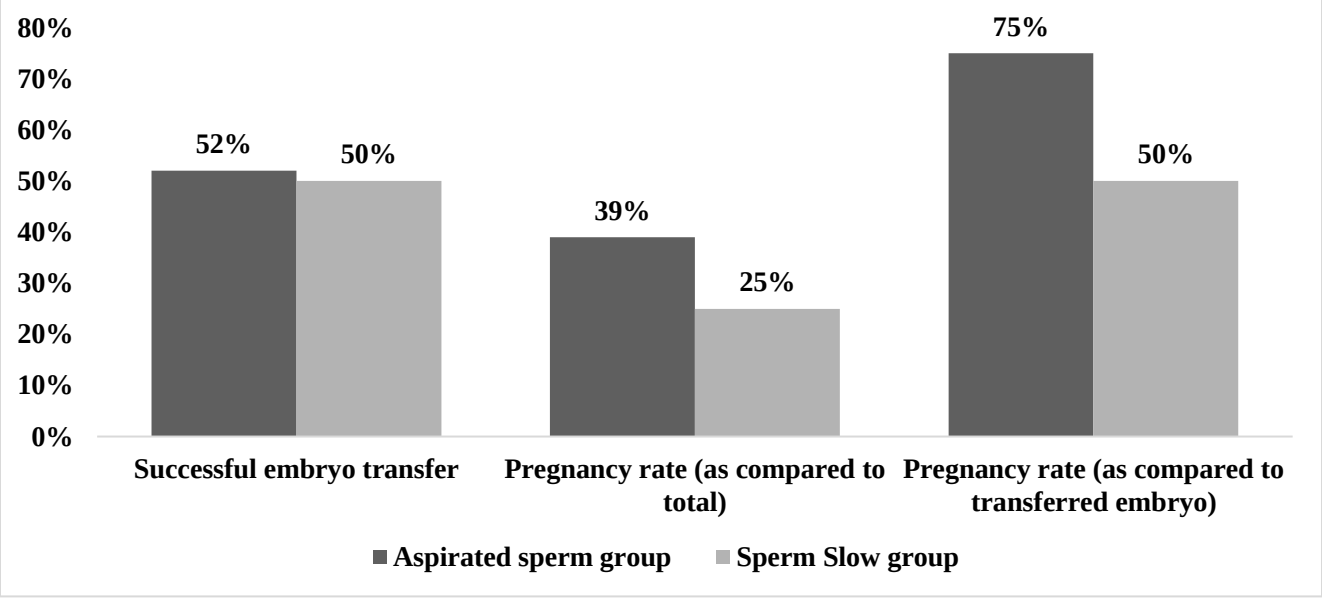

Figure (5): Comparison between aspirated sperm group and sperm selected in sperm slow as regards transferred embryos and pregnancy rates

Transferred embryo and pregnancy rate higher in aspirated sperm group than sperm slow group **(Figure 5)**.

DISCUSSION

Sperm DNA fragmentation (SDF) poses a significant challenge in assisted reproductive technologies, as elevated levels have consistently been linked to impaired embryonic development, suboptimal fertilization rates, and reduced pregnancy success in couples undergoing Intracytoplasmic Sperm Injection (ICSI) [19]. Addressing these challenges is paramount to improving reproductive outcomes. This study contributes to the growing body of evidence exploring effective strategies, specifically focusing on testicular sperm retrieval (aspirated sperm) and hyaluronan-based sperm selection (Sperm Slow), to mitigate the adverse effects of high SDF.

Our findings strongly support the superiority of testicular sperm over ejaculated sperm in improving ICSI outcomes for couples with high SDF. The aspirated sperm group demonstrated significantly enhanced fertility rates (74.67% vs. 56.33%, $p < 0.001$), a greater proportion of high-quality embryos, and notably higher pregnancy rates (39% vs. 11%, $p < 0.001$). These results align with recent comprehensive reviews and meta-analyses, such as that by **Cano-Extremera *et al.*** [10], which reported superior live birth rates, reduced SDF, and lower miscarriage rates when using testicular sperm in ICSI cycles for couples with high ejaculated SDF. Similarly, **Esteves *et al.*** [20] and **Abdelbary *et al.*** [21] have consistently highlighted the benefits of lower DNA fragmentation observed in testicular sperm. The increased number of good-quality embryos in the aspirated sperm group is also in line with the observations of **Arafa *et al.*** [22] , further emphasizing the potential for improved embryonic development. The biological rationale for this advantage lies in the fact that testicular sperm bypass the post-testicular environment, where much of the oxidative stress and DNA damage can occur during epididymal transit and storage [23].

However, it is crucial to acknowledge the existing inconsistencies in the literature. Some studies, including those by **Rauchfuss *et al.*** [24] and ***Zhou et al.*** [25], have reported no significant differences between testicular and ejaculated sperm outcomes. Such discrepancies may be attributed to various factors, including differences in study populations, varying inclusion criteria for SDF levels, the assays used for SDF assessment, and diverse procedural protocols. The invasiveness of testicular sperm retrieval also remains a point of consideration, prompting a continuous search for less invasive yet effective strategies.

Beyond sperm source, the method of sperm selection for ICSI also plays a critical role. Our study's comparison between Polyvinylpyrrolidone (PVP) and hyaluronan-based sperm selection (Sperm Slow) revealed the latter's clear superiority in several key outcomes. Sperm Slow significantly increased the number of fertilized oocytes (6.6 ± 3.6 vs. 5.3 ± 2.03, $p = 0.004$) and pregnancy rates (50% vs. 22%, $p = 0.003$). This is consistent with the understanding that hyaluronan-based selection mimics a more physiological selection process, as mature sperm with good DNA integrity tend to bind to hyaluronan [26, 27]. Recent meta-analyses, while sometimes showing inconsistent benefits for fertilization and pregnancy rates across all studies, have often highlighted a reduction in miscarriage rates with hyaluronan-based selection [5, 28]**.** This suggests that hyaluronan binding effectively identifies sperm with lower levels of DNA damage and aneuploidy, ultimately contributing to more viable pregnancies.

Our findings align with previous research by **Erberelli *et al.*** [29] and **Kim *et al.*** [30], which highlights hyaluronan's crucial role in ICSI success. These studies, consistent with our own, demonstrate hyaluronan's capacity to bind to mature sperm, specifically those exhibiting superior DNA integrity. This selective binding is believed to be a key factor in improving ICSI outcomes.

Furthermore, our study observed a notable increase in the number of top-cleaved oocytes and formed blastocysts within the Sperm Slow group. This supports the hypothesis that using Sperm Slow, which leverages hyaluronan's properties, contributes positively to the optimization of embryonic development.

However, it's important to acknowledge the contrasting results reported by **Craciunas *et al.*** [18] and **Miller *et al.*** [31]. Their studies found no significant differences in fertilization or pregnancy rates when comparing PVP and Sperm Slow. These discrepancies underscore the significant influence of patient variability and methodological differences across various research studies. Such variations can lead to diverse outcomes, making it crucial to consider these factors when interpreting and applying research findings in clinical practice.

Our study offers a novel contribution to the existing literature by directly comparing outcomes between aspirated sperm and ejaculated sperm selected by Sperm Slow in ICSI cycles. The observed superior fertilization (74.67% vs. 67%, $p = 0.04$) and pregnancy rates (75% vs. 50%, $p = 0.009$) associated with aspirated sperm provide compelling evidence for its clinical utility. These findings strongly suggest a biological advantage of testicular sperm, likely attributable to their reduced exposure to oxidative stress within the ejaculatory pathway.

The seminal fluid and ejaculatory tract are known sources of reactive oxygen species (ROS), which can induce DNA damage and compromise sperm function[23, 32]. By bypassing this environment, testicular sperm may retain greater integrity, leading to improved reproductive outcomes. This aligns with a growing body of evidence supporting the notion that sperm extracted directly from the testis exhibits lower DNA fragmentation compared to ejaculated sperm, particularly in men with high sperm DNA fragmentation (SDF) [20].

While direct randomized controlled trials comparing aspirated and Sperm Slow-selected ejaculated sperm are limited, our results underscore the clinical relevance of considering aspirated sperm, when feasible, for patients presenting with high SDF. This approach may offer a valuable strategy to enhance ICSI success in selected cases. Finally, despite efforts to standardize conditions, inherent variations in maternal age, oocyte quality, and environmental factors can influence ICSI outcomes. Maternal age, in particular, is a well-established prognostic factor for ICSI success, with advanced maternal age often correlating with reduced oocyte quality and embryo developmental potential [33, 34].

Strength of the study:

The robust methodology employed in this study, characterized by its randomized and single-blinded design, significantly minimizes bias and enhances the reliability of our findings. The inclusion of detailed subgroup analyses further strengthens the conclusions, providing nuanced insights applicable to diverse patient scenarios. This meticulous approach to study design is crucial for generating high-quality evidence in reproductive medicine.

Limitation of the study:

Nevertheless, it is essential to acknowledge certain limitations that warrant consideration for future research and interpretation. The single-center design of our study, while allowing for rigorous control over protocols, may inherently limit the generalizability of these findings to broader populations and varied clinical settings. Furthermore, while the sample size of 200 couples was substantial, the statistical power for certain subgroup analyses was reduced, potentially obscuring more subtle effects. Future studies with larger sample sizes and adequately powered subgroup analyses would be beneficial to explore these nuances more comprehensively. Although our study attempted to account for these variables, their residual influence cannot be entirely excluded. Moreover, the exclusion of azoospermia and low-SDF patients restricts the direct applicability of our findings to these specific infertility cases.

CONCLUSION

This study provides compelling evidence for the superior efficacy of aspirated sperm in improving ICSI outcomes, particularly for patients challenged by high sperm DNA fragmentation (SDF). Our findings strongly suggest that aspirated sperm may be the preferred retrieval method when clinically feasible, offering a significant advantage in fertilization and pregnancy rates. Furthermore, our research underscores the benefits of using Sperm Slow over PVP. When testicular sperm retrieval isn't an option, Sperm Slow emerges as a valuable alternative, demonstrating clear advantages in enhancing fertilization and pregnancy outcomes. Ultimately, these results lay a robust foundation for optimizing ICSI protocols and advancing reproductive care. By highlighting the distinct benefits of both aspirated sperm and Sperm Slow, we aim to provide clinicians with clearer guidance for couples navigating high SDF-related challenges. Future research, ideally through larger, multi-center studies encompassing more diverse patient populations, will be crucial to further refine these findings and broaden their applicability in clinical practice.

RECOMMENDATION

Future research should aim to include a broader spectrum of male factor infertility to provide more comprehensive insights into the optimal sperm source and selection methods for various patient profiles. Multi-center studies are crucial to confirm these observations across diverse demographics and practice patterns.

Conflicting interests: None.
Funding: None.

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