Annals of Medical Research
DOI: 10.5455/annalsmedres.2019.08.496 2019;26(10):2328-32

Original Article

Comparison of the effects of variables in sperm preparation techniques on pregnancy rates in intrauterine insemination

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Abstract

Aim: To investigate the effects of two sperm preparation techniques, density gradient centrifugation (DGC) and swim-up, on the pregnancy rates per cycle and per group in two intrauterine (IUI) cycles.
Material and Methods: The retrospective study reviewed 634 patients who presented to Firat University Medical School Hospital and underwent a total of 1,032 IUI cycles. Group I consisted of 306 (48.3%) patients who underwent a total of 524 IUI cycles between January 2012 and January 2016 and Group II included 328 (51.7%) patients who underwent a total of 508 IUI cycles between April 2015 and January 2019. The study investigated the effects of technical changes applied to sperm preparation techniques on pregnancy rates in both groups.
Results:A significant difference was found between the clinical pregnancy rates of the two groups with regard to the numbers of patients and IUI cycles ($p<0.001$). The technique used in Group II increased the pregnancy rate by 3.195 times compared to the technique used in Group I. A logistic regression analysis revealed that the pregnancy rate in the first cycle was 3.530 times higher than that of the second cycle in both groups.
Conclusion: The results indicated that the application of suitable alterations in sperm preparation techniques by taking into account the potential effect of all factors is likely to affect clinical pregnancy rates in IUI.

Keywords: Intrauterine insemination; centrifugation; pregnancy rate.

INTRODUCTION

Infertility is defined as inability to become pregnant with a live birth within one year of a consistent union status with no contraceptive use (1). Assisted reproductive techniques (ARTs) consist of numerous procedures that can be useful for the treatment of infertility. Studies investigating male factor in infertility over the last two decades have facilitated the advancements in the approaches and treatments used for male infertility. Of these, in-vitro fertilization (IVF) and intrauterine insemination (IUI) are the two techniques commonly used in clinical practice (2). IUI refers to the intrauterine insemination of morphologically normal and motile spermatozoa following the isolation of immotile sperm, leukocyte, and seminal plasma via in-vitro sperm preparation techniques (3). Density gradient centrifugation (DGC) is a routine ART used for testicular sperm extraction (TESE) and can isolate motile spermatozoa from immotile spermatozoa and other cells. However, DGC can also remove antioxidants from seminal plasma, thereby leading to oxidative stress (4). Oxidative stress, in turn, may induce sperm DNA fragmentation, thus affecting the success rate of ARTs (5). On the contrary, it has also been suggested that DGC may decrease oxidative stress by isolating motile spermatozoa from leukocytes and immature spermatozoa, which are a source of reactive oxygen species (ROS) (4). The primary goal at this point is to determine the optimal centrifugation speed and duration for the prevention of ROS formation during centrifugation to achieve live birth (6), mainly because

Received: 26.08.2019 **Accepted:** 22.09.2019 **Available online:**
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increased centrifugation speed and duration have been shown to increase the likelihood of sperm damage (7). The aim of this study was to investigate the effects of two sperm preparation techniques, DGC and swim-up, on the pregnancy rates per cycle and per group in two IUI cycles.

MATERIAL and METHODS

The study was approved by the local ethics committee in Firat University (Approval No.: 2019/17) and was initiated after obtaining a permission from the Head Physician's Office for the retrieval of patient records from the hospital database (Permission No.: 2019/306137). The retrospective study reviewed 634 patients who presented to Firat University Medical School Hospital and underwent a total of 1,032 IUI cycles. The patients were divided into two groups based on the sperm preparation techniques used in the patients and the study investigated the effects of technical changes applied to sperm preparation techniques on pregnancy rates in both groups.

Group I consisted of 306 (48.3%) patients who underwent a total of 524 IUI cycles between January 2012 and January 2016. DGC was performed by centrifuging the specimens at 2,500 rpm for 20 min (Eppendorf centrifugation 5804) and then the swim-up procedure was performed by centrifugation at 2,200 rpm for 10 min.

Group II included 328 (51.7%) patients who underwent a total of 508 IUI cycles between April 2015 and January 2019. Both DGC and swim-up procedures were performed by centrifugation at 1,600 rpm for 10 min.

All the patients included in the study were aged between 21-38 years and had a diagnosis of unexplained infertility, with a follicle-stimulating hormone (FSH) level <10 and a body mass index (BMI) <35 kg/m2. Ejaculates were collected by masturbation, liquefied at 36.5 °C for 30 min, and then were examined for sperm parameters. The samples were respectively subjected to DGC, sperm washing, and swim-up procedures using 15-ml cone-shaped centrifuge tubes. A two-layer density gradient (80%-40%) was generated by overlayering 1 ml of gradient medium (SpermGrad, Vitrolife, Switzerland) on the lower layer of 80% and 1 of ml of gradient medium on the top layer of 40%. Subsequently, semen samples were added into the density gradient medium and then centrifuged at 2,500 rpm for 20 min in Group I and at 1,600 rpm for 10 min in Group II (Eppendorf centrifugation 5804). After the removal of the supernatant, the pellet at the bottom of the tube was pipetted into a new, clean tube and then resuspended. The resultant suspension was recentrifuged at 2,200 rpm for 10 min in Group I and at 1,600 rpm for 10 min in Group II, with the addition of an equal volume of sperm wash medium (G-IVF Plus, Vitrolife, Switzerland) in both groups. The supernatant was removed and a total of 0.5 ml sperm wash medium was added into the leftover lower seminal layer and then the tube was inclined at an angle of 45° and incubated for 1 h at 36.5 °C to permit the motile sperm to swim out of the lower seminal layer and into the upper layer of sperm wash medium. The resultant mixture was transferred transcervically into the uterine cavity.

Statistical analysis

Data were analyzed using SPSS for Windows version 22.0 (IBM SPSS Inc., Armonk, NY, USA). Pregnancy success rates were expressed as frequencies (n) and percentages (%). The effects of the ARTs administered in the two groups on pregnancy rates with regard to patient numbers were evaluated using Chi-square test. Logistic regression analysis was performed to better evaluate the ARTs administered in each group and to analyze the importance of the number of cycles by comparing the pregnancy rates with regard to the ARTs administered in each group and the number of cycles.

RESULTS

The overall pregnancy rate was 5.9% in Group I and 15.5% in Group II. The overall pregnancy rate per cycle was 3.43% in Group I and 10.03% in Group II (p<0.001). (Tables 1, 2).

In Group I, 14 (3.6%) out of 306 patients had a positive test result in the first cycle and 4 (1.8%) out of 218 patients had a positive test result in the second cycle. In Group II, 44 (11.6%) out of 328 patients had a positive test result in the first cycle and 7 (3.9%) out of 180 patients had a positive test result in the second cycle (Table 2).

As can be seen in Table 3, the model showed acceptable compliance both as a whole and with regard to the independent variables analyzed in the study (number of cycles, technical alterations) (X2=30.482; p<0.001). The logistic regression analysis indicated that these variables had an effect of 9.4% on pregnancy positivity and the

Table 1. Pregnancy rates

		Groups			
		Group I (2012-2015)		Group II (2015-2019)	
		N	%	N	%
Pregnancy	Negative	288	94.1	277	84.5
	Positive	18	5.9	51	15.5
Total		306	100.0	328	100.0
X2= 15.252; p= .000< .001					

Table 2. Comparison of pregnancy rates with regard to IUI cycles

Group	Total number of cycles	Pregnancy	Pregnancy rate %	* Cycles First N	First %	Second N	Second %
Group I (2012-2014)	524	3.43	Negative	292	96.4	214	98.2
			Positive	14	3.6	4	1.8
			Total	306	100.0	218	100.0
Group II (2015-2019)	508	10.03	Negative	284	88.4	173	96.1
			Positive	44	11.6	7	3.9
			Total	328	100.0	180	100.0

Table 3. Logistic regression analysis

		B	S.E.	Wald	df	p	Odds Ratio	95% CI for EXP(B) Lower	Upper
Step 1[a]	Technical alterations (1)	1.162	.293	15.753	1	.000	3.195	1.800	5.670
	Number of cycles (1)	1.261	.312	16.370	1	.000	3.530	1.916	6.504
	Constant	.630	.293	4.618	1	.032	1.878		

-2LL= 405.769, Nagelkerke: R2 = .094, CI: Confidence Interval
Hosmer and Lemeshow Test: X2= .155, p=0.694, S.E.: Standard Error

analysis also showed that the technique used in Group II increased the pregnancy rate by 3.195 times compared to the technique used in Group I. Additionally, it was also revealed that the pregnancy rate in the first cycle was 3.530 times higher than that of the second cycle in both groups.

DISCUSSION

Infertility often has an unexplained cause and has become an increasingly major problem around the world. Assisted reproductive techniques (ARTs) have resulted in million of births worldwide ever since their first introduction into clinical practice (1,8). The selection of high-quality sperms is highly important for the success of ARTs. An ideal sperm separation technique should (i) be rapid, easily applicable and cost-effective, (ii) isolate as many motile spermatozoa as possible, (iii) avoid sperm damage or non-physiological changes in the separated sperm cells, (iv) eliminate dead spermatozoa and other cells such as leukocytes and bacteria, (v) eliminate toxic or bioactive substances such as decapacitation factors or reactive oxygen species (ROS), and (vi) allow processing of larger ejaculate volumes (9).

There are a number of basic techniques that have long been used for successful isolation of sperms from the ejaculate and selection of highest quality sperm. The use of highest quality sperms will produce top quality embryos, thereby allowing the patients to obtain the highest benefit from ARTs (10). DGC) and swim-up are the two sperm preparation techniques most widely used in ART centers. These techniques are aimed at isolating top-quality sperm by creating in-vitro conditions that mimic in-vivo conditions (10). However, the central issue here is to choose the most ideal technique for sperm preparation.

Density gradient centrifugation (DGC) is a technique that

separates sperm cells based on the density of normal and abnormal spermatozoa. Following centrifugation, normal sperms are deposited in the bottom layer. Other components and abnormal sperms are deposited in the upper layer, thereby leading to the isolation of healthy sperms from the semen (9). However, DGC can also remove antioxidants from seminal plasma, leading to oxidative stress (5). Additionally, repeated centrifugation, resuspension, and vortexing may lead to excessive production of ROS in the motile sperm population of the washed specimen and may also cause harm to motile sperm, thereby reducing the success of ART being administered (11). Of particular interest, as DNA now can be measured in most patients with unexplained, idiopathic infertility, the importance of ROS-induced DNA damage is now better understood (9,10). Increased ROS levels are attributed to loss of sperm motility and disruption of membrane fluidity. Moreover, increased ROS levels can reduce sperm quality and function via lipid peroxidation (6,13). In seminal fluid, sperm cells and leukocytes constitute the intrinsic and extrinsic sources of ROS, respectively, and their most critical effect on fertility is reflected as DNA damage (14). Increased DNA damage has been shown to have a positive correlation with the percentage of atypical sperm cells and a negative correlation with sperm concentration and sperm motility (15). Male germ cells are susceptible to DNA damage mainly due to the lack of DNA repair mechanisms and the loss of apoptosis ability of the normal cells in the late stages of spermatogenesis (16,17). The presence of increased sperm DNA fragmentation in infertile patients has been shown to cause abnormal sperm parameters and to have a negative impact on pregnancy rates in IUI treatments (11,18-20).

Low amounts of ROS are needed for fertilization, acrosome reaction, hyperactivation, motility, and capacitation (21). However, the central issue here is to determine the optimal centrifugation speed and duration for the prevention of ROS formation during centrifugation. Therefore, a well understanding of the fundamentals of sperm preparation techniques and an evaluation of the differences among the techniques from a cause-and-effect perspective are highly essential (7). Moreover, standardization of sperm selection techniques and centrifugation protocols will facilitate the isolation of motile sperms, thereby increasing the likelihood of pregnancy (11). Accumulating evidence suggests that sperm selection techniques, particularly DGC, reduce the percentage of DNA-damaged sperms while increasing the percentage of live, progressive, and motile sperms and are also highly effective in the isolation of morphologically normal sperms (22). Moreover, the culture media used in these methods are includes on simple balanced salt solutions, optimized to mimic in-vivo conditions, and also allow completion of sperm capacitation and acrosome reaction while causing no adverse effect on sperm motility and providing optimum pH balance due to adequate buffering (10). İn addition, a previous study suggested that the lower layer enriched in motile sperm shows lower ROS levels compared to unwashed specimen and this layer allows the use of sperm preparation techniques in IUI and classic IVF (5).

In our study, the reduced centrifugation speed and duration were found to have a positive effect on pregnancy rates. Accordingly, the technique used in the second cycle increased the pregnancy rate by 3.195 times compared to the technique used in the first cycle. Increased centrifugation speed and duration have been shown to increase ROS production, leading to an increased risk of sperm DNA fragmentation. Accordingly, the findings of our study appear consistent with those reported in the literature (11). On the other hand, it was also revealed that the pregnancy rate in the first cycle was 3.530 times higher than that of second cycle in both groups, suggesting that the repeated cycle had no effect in the improvement of the pregnancy rate. Similarly, previous study also indicated that the pregnancy rate was higher in the first cycle compared to second and third cycles, implicating that the repeated cycles had no effect on the pregnancy rate (23).

The key issue in sperm preparation techniques is sperm heterogeneity as it is highly likely that every individual will have different sperm parameters and urinary system pathologies. Therefore, each ejaculate may include normal sperm cells as well as sperm cells with various pathologies. Accordingly, it is tempting to consider that the IUI outcomes may vary across clinics due to a number of factors including differentiation of patient populations (due to different IUI indications), ovulation induction protocols, sperm preparation techniques, number of cycles, and semen parameters that could affect pregnancy rates (24).

CONCLUSION

The results indicated that the application of suitable alterations in sperm preparation techniques that would lead to minimal damage to sperm and would aim at isolating as many functional sperms as possible is likely to have a positive effect on pregnancy rate. It was also revealed that the reduction of centrifugation speed and duration also increased the pregnancy rate, and the pregnancy rate in the first cycle was higher than that of second cycle. Further large-scale and comparative studies are needed to substantiate our findings.

Competing interests: The authors declare that they have no competing interest.
Financial Disclosure: There are no financial supports
Ethical approval: The study was approved by the local ethics committee in Firat University (Approval No.: 2019/17)

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