

**ORIGINAL RESEARCH****Comparative study of influence of two sperm preparation, swim up and density gradient-swim up, on the outcomes of intrauterine insemination (IUI) in different types of semen samples infertile men referred to infertility research and treatment center, ACECR branch of Khuzestan**

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**Abstract**

Infertility is one of the developing problems in most countries and it has a lot of problems, which can be emotional, social and political. Intrauterine insemination (IUI) is the first line Assisted Reproduction Treatment (ART) modality for infertile couples because this method is inexpensive and non-invasive, which is effective for the treatment of couples with unexplained infertility or patient with normal or mild male factor. Two methods, mainly considered as laboratory techniques for improving the quality of sperm, includes Swim-Up (SU) and Density Gradient Centrifugation (DGC). The SU is a common technique in IVF labs, and is mainly performed in a sample of semen having normal sperm concentration. In this technique, sperms are selected based on their motility and their capacity to leave the semen plasma. In the DGC method, sperms are selected based on the density, motile sperm are separated from dead sperms, leukocytes and other high-density semen plasmatic compounds. The aim of this method is thus to select sperms with high motility and morphology rates. Therefore, the aim of the present study is to compare the effect of these two methods on the outcome in intrauterine insemination in different groups, including normal samples (< 60 million (type1)) and 20-60 million/ml (type2), oligospermia (type3) and asthenospermia (type4), in patients referred to the Infertility Center. The present experimental study was performed on 545 couples who referred to the Infertility Research and Treatment Center, in 2016 for infertile reasons and were in a good status in terms of general health. Processing of sperm was done by two common methods, swim-up and Density Gradient Centrifugation according WHO. Our study showed the effectiveness of the Density gradient-Swim up technique compared to Swim-up as a sperm preparation method with a favourable IUI success.

**Keywords:** Infertility; Intrauterine insemination; Sperm

## Introduction

Infertility is one of the developing problems in most countries and it has a lot of problems, which can be emotional, social and political. About half of infertilities are due to male factor. Intrauterine insemination (IUI) is the first line Assisted Reproduction Treatment (ART) modality for infertile couples because this method is inexpensive and non-invasive, which is effective for the treatment of couples with unexplained infertility or patient with normal or mild male factor (1). The overall success rate of IUI depend on many factor such as, The age of couples, influence of controlled ovarian hyper stimulation (COH), timing and number of insemination, and duration and cause of infertility. On the other hand, the main parameters of sperm include concentration, motility and morphology, which have a have a key rule in the success of IUI (2,3,4). The World Health Organization (WHO) defines normal semen parameters, considered as a standard guide, as semen volume of 2-5 ml, count of >15 million/ml, motility of >40%, and morphology of > 4% (5). Although a low sperm concentration less than 20 million/ml and very little motility (less than 20%) is indicative of the risk of fertility, pregnancy sometimes occurs with these very small amounts (5,6). There are some methods by which the quality of sperm can be increased for inoculation. Two methods, mainly considered as laboratory techniques for improving the quality of sperm, include Swim-Up (SU) and Density Gradient Centrifugation (DGC) (7,8). The SU is a common technique in IVF labs, and is mainly performed in a sample of semen having normal sperm concentration. In this technique, sperms are selected based on their motility and their capacity to leave the semen plasma. In the DGC method, sperms are selected based on the density, motile sperm are separated from dead sperms, leukocytes and other high-density semen plasmatic compounds. The aim of this method is to select sperms with high motility and morphology rates (8,9). Therefore, the aim

of the present study is to compare the effect of these two methods on the outcome in Intrauterine insemination in different groups, including normal samples (< 60 million and 20-60 million/ml), oligospermia and asthenospermia, in patients referred to the Infertility Center of Khuzestan.

Row	Group	Description
1	Normal (between 20-60 million/ml)	Sperm count of $\geq 20$ million/ml, motility of $\geq 50\%$ , morphology of $\geq 4\%$
2	Normal (more than 60 million/ml)	Sperm count of $\geq 60$ million/ml, motility of $\geq 50\%$ , morphology of $\geq 4\%$
3	Oligospermia	Sperm count of <20 million/ml, motility of $\geq 50\%$ , morphology of $\geq 4\%$
4	Asthenospermia	Sperm count of $\geq 20$ million/ml, motility of <50%, morphology of $\geq 4\%$

## Materials and Methods

The present experimental study was performed on 545 couples who referred to the Infertility Research and Treatment Center of Khuzestan University, ACECR, in 2016 for infertile reasons and were in a good status in terms of general health. Semen samples were collected after 3 to 5 days abstinence. The sample was taken in a sterile container and about 30-45 minutes were taken into account for the sample to liquefy. Sperm samples were evaluated in terms of semen volume, PH, liquefaction time, viscosity, count, motility, and morphology of the sperm according to WHO criteria. Sperm count and motility were evaluated using McLean chamber. A total of 100 squares were used for evaluating the sperm count and at least 200 sperms were evaluated so as to evaluate their motility and morphology and then classified into 4 groups based on their count, mobility, morphology. The sperms were then randomly separated by DGC-SU and SU methods.

Modified washing-swim up method or swim up with double whashing was used for 680 semen samples. In this method, once the liquefaction process was carried out at 37 ° C, one ml of semen was poured into a 5 ml tube containing the person's full profile and four ml of Hams F10 medium+albumin was poured on it and then mixed. It was then centrifuged at 2700 Rpm for 5 minutes. When the proper

precipitate was formed, its supernatant was discarded and 4 ml of the culture medium was again added to it. It was centrifuged again and the supernatant was discarded. 1 ml of culture medium was placed on its second precipitate for sperm swim-up in a 37 ° incubator and 0.05-0.7 ml of the supernatant containing sperm was collected for analysis after 20-30 minutes.

A total of 452 samples were prepared using Density Gradient Centrifugation(DGC)+Swim Up, which included two gradient density layers, a 40% upper layer and a 80% lower layer. The upper layer was made by adding 4 ml of the density gradient medium to 6 ml of Hams F10 medium+albumin. The lower layer was also made by adding 8 ml of density gradient medium to 2 ml of Hams F10 medium+albumin in a Conical Falcon tube No. 13. Then 1 ml of the semen sample was gradually poured from the above, placed on 40% medium, and then centrifuged at 2,700 Rpm for about 5 minutes. Afterwards, the supernatant was discarded. The resulting precipitate was removed slowly and poured in the Falcon Tube No.5 and the washing steps were carried out as similar to modified SU method. Because sperm morphology is not routinely performed on samples processed for IUI, only count and motility were evaluated, and the findings of pre and post-preparation motility and count parameters were studied and compared in different types of sperm.

All couples were inquired about age, and duration of infertility. Female partner was stimulated from day 2 of menstrual cycle with clomiphene 150mg daily for 5 days. Injectable gonadotropins were given in a dose of 75 IU to 150 IU per day from 6 day. Transvaginal Ultrasound (USG) for follicular tracking was done and the number of follicles in both ovaries was measured and recorded. Further increment in doses of gonadotropin was adjusted until the leading follicles reached 18-20mm then ovulation was induced by

administering intramuscularly 10000-5000IU hCG and 36-48 hours after injection, insemination was performed.

After processing of semen, female was prepared for insemination by exposing the cervix and cleaning with distilled water and 0.5ml of sample was inseminated. Pregnancy testing was performed after missing the periods or determining the quantitative serum  $\beta$ -Hcg level at 14 days after Hcg administration. Clinical pregnancies rate (PR) were defined by the presence of a gestational sac on transvaginal ultrasound or by histologic examination of products of conception in patients who aborted after 2 weeks of pregnancy. Live birth rate was defined of a viable fetus detected after 12 weeks of pregnancy, after that the results studied and compared in different groups of sperms.

The data analysis was later carried out using ANOVA, Tukey's method, and paired-samples T-test in SPSS Ver.19 and  $P < 0.05$  was considered as the significant level.

### Results

In this prospective randomized study we studied 554 couples who underwent 843 intrauterine insemination cycles, the mean of cycles was  $1.5 \pm 0.7$ . The mean age for women was  $29.9 \pm 2.5$  and for men was  $31.5 \pm 6.8$ . The duration of infertility was  $2.89 \pm 2.64$  and the percentage of primary infertility was 76.1% and secondary was 23.9%.

33.2% of couples had unexplained and 45.7% had male and 30.6% had none-male infertility factor. After randomization, 329 couples received the Swim up and 216 couples had the density gradient as a semen preparation.

Demographic and cycle characteristic of 4 type of sperm are shown in table 1. There were no significant differences between 4 group in terms of age, duration of infertility and, total dose of gonadotropin throughout the cycle, the number of dominant follicles and the thickness of endometrial on the day of hCG.

Table 1: Demographic and ovarian stimulation cycle characteristic of patients in 4 type of sperm

	Total	Type1	Type2	Type3	Type4
Male Age	31.5±6.86	30.6±5.2	31.6±11.2	31.8±6.1	31.9±5.57
Female Age	29.9±12.58	29.3±4.55	31.8±20.5	29.9±5.57	29.5±5
Duration of Infertility	2.89±2.53	2.95±2.42	2.36±0.84	2.67±1.23	3.14±3.28
Number of cycle	1.54±0.73	1.51±0.63	1.52±0.84	1.49±0.54	1.61±0.70
Primary of Infertility	76.1%	76.8%	84.9%	77.6%	71.5%
Secondary of Infertility	23.9%	23.2%	15.1%	22.4%	28.5%
Unexplained Infertility	33.2%	30.7%	35.6%	31.6%	34.4%
Male factor Infertility	36.2%	40.1%	30.2%	35.1%	32.1%
Non-male factor Infertility	30.6%	29.2%	34.2%	33.3%	33.5%
Total gonadotropin dose	784.10±304.61	804.65±433.20	789.62±398.65	749.51±443.61	792.65±398.27
Follicle number (≥16 mm) on the day of hCG	1.47±0.98	1.5±0.32	1.5±0.27	1.4±0.86	1.5±0.34
Endometrial thickness on the day of hCG (mm)	10.26±1.4	10.34±1.3	9.98±0.89	10.54±1.8	10.21±1.4

Studying of semen parameters, before and after preparation in deferent types of sperm are shown in Table 2 and 3. Semen parameters before and after preparation with SU (double washing) and DGC-SU and enhancement of them in different types of sperms are shown in table 2 and 3. The concentration increase after preparation with two methods whereas before preparation, except type 2, which decrease after preparation with DGC-SU. The comparison of SU and DGC-SU methods showed that the SU method led to a better improvement in the sperm count than the DGC-SU method. However, only in type 1 and 2 concentration significantly increased in the SU method in comparison DGC-SU method ( $p<0.03$ ). The motility also increase after preparation with two methods whereas before preparation. The comparison of SU and DGC-SU methods showed that the SU method led to a better improvement in the sperm motility than the DGC-SU method and in all type of sperm except type 2 enhancements were significant ( $p<0.01$ ).

Table 2: Mean and progression with SU in different groups

Group	Final motility (*10 <sup>6</sup> )	Initial motility (*10 <sup>6</sup> )	Enhancement	Final count (*10 <sup>6</sup> )	Initial count (*10 <sup>6</sup> )	Enhancement
Type1	92.40±10.7	52.01±8.6	+40.38±1.61	57.46±14.6	42.87±11.2	+14.59±1.6
Type2	93.37±12.7	52.60±7.9	+40.76±2.21	79.07±20.5	72.27±12.14	+6.8±3.09
Type3	96.94±2.9	48.60±9	+48.28±1.33	29.15±14.9	15.73±3.58	+13.4±1.8
Type4	93.58±5.1	24.04±19.2	+69.54±1.71	59.78±22.2	48.71±17.7	+11.06±2.13

Table 3: Mean and progression with DGC-SU in different groups

Group	Final motility (*10 <sup>6</sup> )	Initial motility (*10 <sup>6</sup> )	Enhancement	Final count (*10 <sup>6</sup> )	Initial count (*10 <sup>6</sup> )	Enhancement
Type1	86.96±16.3	60.1±17	+26.82±4.63	50.18±20.6	43.87±13.1	+6.38±3.07
Type2	93.10±8.1	55.09±10.2	+38.09±1.91	68.02±27.8	76.33±19.3	8.3±4.04
Type3	92.70±4.4	50.20±12	+42.50±2.17	23.50±13.5	14.04±5.36	+9.4±2.4
Type4	95.87±11	15.9±4.7	+79.92±1.25	62.92±21.2	53.63±21.1	+9.29±2.25

The percentage of pregnancy rate (PR) was 19.7% and abortion rate (AR) was 11.8% and live birth rate was 7.9%. Data from the different type of sperm are shown in table 4. The pregnancy rate was significantly higher in the density gradient in all groups referred to Infertility Center of Ahvaz in comparison to the swim up. No significant difference in abortion rate was observed between swim-up and gradient groups in all type of sperm. The live birth rate in the type 1 and 2 were significantly higher in the density gradient in comparison to the swim up groups but in the type 3 and 4, however the percentage of live birth were higher in the density gradient in comparison to the swim up but, this increase was not significant. No significant difference was observed between gradient groups in all type of sperm with regard to pregnancy rate, abortion and live birth rate. In the swim up method also didn't show no difference.

Table 4: Demographic of pregnancy rate, abortion rate and live birth rate of patients in 4 type of sperm preparation with Swim-up

	Type1	Type2	Type3	Type4
Pregnancy rate	20.9%	23.5%	13.4%	19.8%
Abortion rate	12.3%	12.7%	7.6	13.2%
Live birth rate	8.5%	11.7%	5.7%	6.6%

## Discussion

Sperm preparation is a vital procedure in IUI treatment and it strongly impact on IUI success. In this study, we compared the effectiveness of two mostly used sperm preparation techniques, Swim-up and Density gradient-Swim up, on IUI success in different types of sperm. The best outcomes, pregnancy rate and live birth rate, received in type 2 (more than 60 million.ml), after two methods, however these aren't significant when compared with other types, and the most abortion rate obtained in the type 4 after two methods (it s not significant) the lowest rate of pregnancy, abortion and live birth obtained in type 3 after preparation of two methods. Our

study showed the effectiveness of the density gradient-Swim up technique compared to Swim-up as a sperm preparation method with a favorable IUI success. Our findings are similar to study of Karamahmutoglu et al. (2014) on the 223 couples were randomized into swim up or density gradient technique for sperm preparation, the clinical and on going pregnancy rates were evaluated and they reported pregnancy rates of density group were significantly higher in comparison with the swim up group (10) Also Tugnait et al., (2013) found that pregnancy rate for swim up 28% and for density gradient 31% and the miscarriage rate for swim up was 6.78% and the rate for density gradient was 16.95%. And they concluded the density gradient method of sperm preparation has better outcome in term of live birth rate and pregnancy rate in couples who have been subjected to IUI treatment (3). In a study conducted by Morshedi et al., including 311 couples comparing the simple washing and the gradient method no significant difference was observed in pregnancy rates. However, in the subgroup including patient with a low sperm count (sperm concentration < 22 million/ml) the gradient technique yielded greater pregnancy rates that. (11) Posada et al., 2005 studied 82 couples who underwent IUI. In their study in DGC group pregnancy rate per couple was

13.33% and in swim-up group it was 38.5%. Miscarriage rate per couple in DGC group was 3.33% and in the swim-up group was 0.00% (12). In the study of Butt and Chohan, 33 (15%) couples conceived; 17 (51.51%) from density gradient procedure and 16 (48.48%) from swim-up method but no statistical difference was found in pregnancy outcome with two procedure(13). Carral et al., (1998) evaluated the association sperm preparation methods and IUI outcome involved 5 different sperm preparation techniques in a group of 363 women and reported the pregnancy rate for the swim-up and percoll gradient significantly greater than swim-down and wash (14).

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#### **Conflict of interest**

Authors declare no conflict of interest.

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