Original Article

# Comparative Study of the influence of two sperm Preparation, on the Outcomes of Intracytoplasmic Sperm Injection (ICSI) in infertile Men Referred to Infertility Center, ACECR of Khuzestan

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## Abstract

Infertility is one of the developing problems in the most countries and it has a lot of circumstance problems, includes, emotional, social and political. About half of infertilites are due to male factor However, effective treatment for male-factor infertility was not available until 1992 ,when intracytoplasmic sperm injection (ICSI) The overall success rate of ICSI 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 In clinical practice ,the manual-visual light microscopic methods for evaluating semen quality maintain their central role in assessment of male fertility potential The semen analysis such as count,motility and morphology have been reported to be associated with a favorable outcome in ICSI methods, mainly considered as laboratory techniques for improving the quality of sperm, include 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 .so the aim of the present study is to compare the effect of these two methods on fertilized oocytes ,transfer embryo, formation of blastocyte rate(BR) ,grading of embryo and on the outcome in ICSI include of, pregnancy rate (PR), abrotion rate(AR). in different groups. This groups inaccording to samples are choosed for ICSI, including, oligospermia and asthenospermia, Teratosphermia and Oligoastenotrotoospermia. Our results revealed the effectiveness of the SU technique compared to DGC-swin up as a sperm preparation method with a favorable ICSI success, The present study not only demonstrates the greater reproducibility of the results with SU when compared with the DGC, but what stands out is the possibility to use a non-invasive method on semen treatment whit statistically better results for embryo development and pregnancy. One explanation for this could be found in the better health status of the embryo obtained from spermatozoa that have suffered less stress during the treatment.

**Keywords:** ICSI, Swim-up, Density Gradient, oligospermia, asthenospermia, tetraspermia, oligoastenotrotospermia.

## INTRODUCTION

Infertility is one of the developing problems in most countries and it has a lot of circumstance problems which include emotional, social, and political. About half of infertility are due to male factor. The introduction of in vitro fertilization (IVF) led to a great advance in treatment for infertility [1]. However, when intracytoplasmic sperm injection (ICSI) was introduced as a part of the IVF process in selected cases in 1992, the effective treatment for male-factor infertility became available [2]. The overall success rate of ICSI depends on many factors such as The age of couples, the influence of controlled ovarian hyperstimulation (COH), timing and number of insemination, and duration and cause of infertility [3]. The manual-visual light microscopic methods for evaluating semen quality maintain their central role in the assessment of male fertility potential in clinical practice. However, basic semen analysis does suggest a definitive diagnosis of male fertility. To do this, the seminal volume,

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PH, sperm concentration, motility, morphology, and vitality have to be measured [3, 4]. The semen analysis such as count, motility, and morphology have been reported to be associated with a favorable outcome in IVF [5, 6]. The World Health Organization (WHO) defines normal semen parameters, considered as a standard guide, as a semen volume of 2-5 ml, count of >15 million/ml, motility of >40%, and morphology of > 4% [7]. 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 [8, 9]. 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) [10, 11]. 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 [11, 12].

The rate of embryo growth (cleavage) was evaluated in many studies It seems obvious that a high number of blastomeres predicts the higher implantation rates. A number of cells is used as the main parameter of the highest predictive value by the majority of embryo quality classifications [13, 14]. Four or fewer blastomeres on the third day of culture indicate the extremely low developmental potential and subsequently very small chance for development after transfer. On the second day and at least 8 cells on the third day of culture, the good quality embryo has at least 4 cells. Thereafter, following the insemination or fertilization by ICSI, the embryo inspections are routinely performed in daily intervals, 40-44 hours, and 64-68 hours [13-15]. Depending on several morphological parameters that are evaluated at that time, embryos are divided into classes. The appearance of blastomeres and the presence of cytoplasm defects or fragmentation are the most often criteria used aside from the number of cells. The results of scoring are usually coded. Our laboratory has applied the following coding system:
A - symmetric blastomeres
B - Distinctly asymmetric blastomeres
C - Defects of cytoplasm

So, the present study aims to compare the effect of these two methods on fertilized oocytes transfer an embryo, the formation of blastocyte rate (BR), grading of an embryo, and on the outcome in ICSI include of, pregnancy rate (PR), the abortion rate (AR) in different groups. These groups according to samples are chosen for ICSI, including, oligospermia and asthenospermia, Teratosphermia, and Oligoastenotrotoospermia.

| Row | Group | Description |
|---|---|---|
| 1 | Oligospermia | The sperm count of<20 million/ml, motility of ≥50%, the morphology of ≥ 4% |
| 2 | Asthenospermia | The sperm count of ≥20 million/ml, motility of <50%, morphology of ≥4% |
| 3 | Teratosphermia | The sperm count of ≥20 million/ml, motility of ≥50%, the morphology of < 4% |
| 4 | Oligoasthenotrotoospermia | Sperm count of <20 million/ml, motility of <50%, morphology of <4% |

## Materials and Methods:

The present experimental study was performed on 330 couples who referred to the Infertility Research and Treatment Center of Khuzestan University, ACECR, from 2014 to 2016 for infertile reasons and were in good status in terms of general health. Semen samples were collected after 3 to 5 days of 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 the McLean chamber. A total of 100 squares were used for evaluating the sperm count and at least 200 sperms were assessed to evaluate their motility and morphology and then classified into 4 groups based on their count, mobility, the morphology [3, 7]. The sperms then randomly separated by DGC-SU and SU methods.

The modified washing-swim up method or swim up with double washing was used for 192 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 [8, 9].

A total of 138 samples were prepared using Density Gradient Centrifugation (DGC)+Swin Up, which included two gradient density layers, a 40% upper layer, and an 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. Afterward, 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 the modified SU method. The count, motility, and morphology were evaluated, and the findings of pre and post-preparation motility, count, and morphology parameters were studied and compared in different types of sperm [10, 11].

All couples were inquired about, age, duration of infertility. Female partners were subjected to controlled ovarian stimulation using a GnRH agonist or antagonist. When there were at least two or three follicles of over 18 mm in diameter10000-5000IU hCG was injected. Ultrasound-guided oocyte retrieval was performed 36 hours after hCG injection. The retrieved oocytes were graded on a 3-point scale for shape, size, and degree of fragmentation:1 point for poor,2 points for reasonable, and 3 points for good. The cumulus oophorus and the corona radiate of oocytes were performed with clean and sterilized glass Pasteur pipettes. After an ovarian puncture, were incubated in IVF medium. Oocytes were inseminated by using spermatozoa obtained by SU or DGC-SU. Embryos were cultured in G1 and G2 medium in relation to the culture time. Cleaving embryo was scored every day for blastomere number, cleavage plane, and degree of fragmentation [12, 16].

To define the clinical pregnancies, a gestational sac was observed with or without a fetal heartbeat on ultrasound evaluation 4 weeks after Embryo Transfer (ET). The number of sacs suggested the number of successful implantation. When a pregnancy failed to progress after an intrauterine gestational sac had been detected, a clinical miscarriage was demonstrated. Live birth data was defined as live birth per ET. The clinical pregnancy rate, miscarriage rate, and live birth rate were collected.

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 a significant level.

## FINDINGS:

In this prospective randomized study, we studied 330 couples who underwent ICSI cycles, the mean of cycles was 1/9±0/8. The mean age for women was 32/9±3/5 and for men was 33/5±6/5. The duration of infertility was 3/89±1/64 and the percentage of primary infertility was 76/1% and secondary was 23/9%.

35/4% of couples had unexplained and 52/2% had male and 14/8% had none-male infertility factor. After randomization,192 couples received the Swim-up and 138 couples had the Density gradient-swin up as a semen preparation.

Demographics and cycle characteristics of 4 types of sperm are shown in table 1. No significant differences between 4 groups in terms of age, duration of infertility, the cycle, and mature oocyte was observed.

**Table 1:** Demographics of patients in 4 type of sperm

| | Type1 | Type2 | Type3 | Type4 |
|---|---|---|---|---|
| **Male Age** | 32/3±/46 | 34/5±/48 | 32/3±/58 | 34/35±/76 |
| **Female Age** | 31/9±/39 | 32/4±/65 | 31/9±/48 | 32/5±/65 |
| **Duration of Infertility** | 4/06±/27 | 4/2±/32 | 3/9±/48 | 3/25±/65 |
| **Number of cycles** | 1/98±/18 | 1/6±/11 | 1/8±0/12 | 1/58±0/66 |
| **Primary of Infertility** | 78/8% | 79/9% | 80/6% | 77/5% |
| **Secondry of Infertility** | 21/2% | 20/1% | 19/4% | 22/5% |
| **Unexplained Infertility** | 30/7% | 33/6% | 34/6% | 32/7% |
| **Male factor Infertility** | 55/1% | 50/9% | 49/8% | 53/1% |
| **Non-male factor Infertility** | 14/2% | 15/5% | 15/6% | 14/2% |
| **Mature oocyte** | 8/87±/38 | 10/33±/43 | 8/91±/44 | 9/8±/71 |

Fertilized oocytes were not shown a significant difference in group DGC and between different types. But in the groups SU, the best result obtained in group 2 and the least result was observed in group 3. Only Significant differences were found between the DGC and SU in group 3, and fertilized oocyte was showed significantly higher in the SU comparison to the DGC(table 2).

**Table 2:** Difference in the mean of fertilized oocyte in deferent types

| Type | SU | DGC | P-value |
|---|---|---|---|
| **Type1** | 5/09±/34 | 4/40±/48 | /230 |
| **Type2** | 9/07±/1/6 | 8/24±2/03 | /751 |
| **Type3** | 5/06±/66 | 2/87±/24 | /002 |
| **Type 4** | 7/19±/76 | 8/54±1/23 | /331 |

The results obtained embryo transfer similar to the fertilized oocyte in a different group and the best result was shown in group 2 and the least result observed in group 3. Just in group 3 the embryo transfer increased significantly in the SU compared to the DGC (table 3)

**Table 3:** Difference in the mean of transfer embryo in deferent types

| Type | SU | DGC | P-value |
|---|---|---|---|
| **Type1** | 2/96±/12 | 2/60±/25 | /063 |
| **Type2** | 2/86±/23 | 2/48±/22 | /256 |
| **Type3** | 2/93±/25 | 1/33±/11 | </001 |
| **Type 4** | 2/41±/23 | 3±/28 | /124 |

Different results between 2 methods of preparation in 4 types are shown in tables 4 and 5. No significant difference was observed between SU groups in all types of sperm concerning blastulation rate, pregnancy rate, abortion rate, however, in type1 results of BR and types 2, the results of PR and in type 4 in terms of AR increased.

**Table 4:** Difference in the blastulation rate (BR), pregnancy rate (PR), and abortion rate(AR) in SU groups:

| Type | Type1 | Type2 | Type3 | Type4 |
|---|---|---|---|---|
| **BR** | 23(57/7%) | 15(24/5%) | 14(42/4%) | 16(39%) |
| **PR** | 20(32/8%) | 21(41/2%) | 8(20/5%) | 15(36/6%) |
| **AR** | 11(18%) | 10(19/6%) | 5(12/8%) | 9(22/3%) |

But in the group DGC, type 1 was showed significantly higher in terms of BR and AR in comparison to other types in this group (P</003). In type 1 and 4 results of PR increased but it is not significant (Table 5). The best results in group SU were gotten in oligospermia type, and asthenospermia and in group DGC-SU in type oligospermia.

**Table 5:** Difference in the blastulation rate (BR), pregnancy, and abortion rate(AR) in DGC groups

| Type | Type1 | Type2 | Type3 | Type4 |
|---|---|---|---|---|
| **BR** | 18 (53/4%) | 11(26/8%) | 7(17/9%) | 10(41/7%) |
| **PR** | 19(44/5%) | 8(15/6%) | 13(29/4%) | 11(45/8%) |
| **AR** | 9(25/5%) | 2(3/4%) | 7(19/8%) | 6(20/3%) |

However, the comparison between 2 methods showed in type2 the rate of PR and AR in group SU increased in comparison with DGC (respectively P</04 and P</01). In terms of BR type 1(p</01) and 3(P</02) were increased significantly in the SU group in comparison DGC group.

About scoring embryo, in all of the grades (B, C, AB, BC) were not significantly different from all of the types in SU and DGC groups (grade A and AC diden see). However, the quality of the embryo was observed in the group SU better than the DGC group. The best result was seen in type 3 (AB 36/4%) in group SU and type 2 (AB 13/7%) in the DGC group. Grade AB in type1 (P</02) and in type 3 (P</001) increased in the SU group in comparison to the DGC group and grade C in type 2 (P</001) increased in DGC group than SU ones.

**Table 6:** grades of an embryo in different types of SW group:

| GRADE | Type 1 | Type 2 | Type 3 | Type 4 |
|---|---|---|---|---|
| AB | 12(30.0%) | 12(29.3%) | 12(36.4%) | 7(29.2%) |
| B | 9(22.5%) | 5(12.2%) | 5(15.2%) | 3(12.5%) |
| BC | 17(42.5%) | 17(41.5%) | 11(33.3%) | 11(45.8%) |
| C | 2(5.0%) | 3(7.3%) | 3(9.1%) | 3(12.5) |

**Table 7:** grades of an embryo in different types of DGC group:

| GRADE | Type 1 | Type 2 | Type 3 | Type 4 |
|---|---|---|---|---|
| AB | 7(11.5%) | 7(13.7%) | 0(.0%) | 5(13.6%) |
| B | 12(19.7%) | 8(15.7%) | 10(25.6%) | 6(14.6%) |
| BC | 31(50.8%) | 21(41.2%) | 20(51.3%) | 23(56.1%) |
| C | 11(18.0%) | 13(25.5%) | 9(23.1%) | 6(14.6%) |

## Discussion:

Sperm preparation is the vital procedure in ICSI treatment and it strongly impacts ICSI success. In this study, a wide variety of sperm preparation protocols are currently available in IVF/ICSI treatment the most used techniques are DGC and SU in a different type of sperm. The presence of wide variability in the techniques used for semen treatment leads to a need to evaluate the potential benefits and risks of each procedure. Our results revealed the effectiveness of the SU technique compared to DGC-swin up as a sperm preparation method with a favorable ICSI success. Our study was accomplished in different types of sperm. The present study does not only demonstrate the greater reproducibility of the results with SU when compared with the DGC, but it also demonstrates what stands out is the possibility to use a non-invasive method on semen treatment whit statistically better results for embryo development and pregnancy. One explanation for this could be found in the better health status of the embryo obtained from spermatozoa that have suffered less stress during the treatment. Furthermore, the potential epigenetic modification that the serial centrifugations and ROS production can have on spermatozoa, which are at potentially greater risk because of their extended processing especially during the DGC technique must be taken into consideration [17]. Another point in favor of SU is the reduction of time and costs, although the insemination

technique (ICSI) remains expensive, it would be possible to insert the SU procedure into an IVF low-cost program, thus reducing the total cost of the treatment [18]. Our findings are similar to the study of Palini et al (2014) on the 140 couples undergoing ICSI treatment. These researchers reported the MSU (micro swim-up) had better effects on outcomes of ICSI than DGC and findings of their studying were showed procedures that minimize excessive manipulation of sperm and centrifugation be used in IVF treatment as a valid alternative to improve the outcome. This non-invasive technique enables to obtain embryo with a physiological and probably better euploid status [17]. Several reports have supported an association between sperm qualities and results of ICSI [19-21]. It is well known that the effect of the injection of an abnormal spermatozoon is not usually detected before the 4-8-cells stage when a major expression of paternal genes is initiated and activation of the embryonic genome occurs [22].

In conclusion, our result showed that SU procedure had better results than DGC in different types of sperm. This technique is suggested as an alternative method to other conventional semen preparation with different qualities of them.

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## References

1. Jain T, Missmer SA, Hornstein MD. Trends in embryo-transfer practice and in outcomes of the use of assisted reproductive technology in the United States. New England Journal of Medicine. 2004 Apr 15;350(16):1639-45.
2. Palermo G, Joris H, Devroey P, Van Steirteghem AC. Pregnancies after intracytoplasmic injection of single spermatozoon into an oocyte. The lancet. 1992 Jul 4;340(8810):17-8.
3. Centola, GM., Ginsburg, KA. Evaluation and treatment of infertile males. Cambridge University Press, 1996.
4. Sakkas, D., Tomlinson, M. Assessment of sperm competence. Semin Reprod Med; 2000; 18.133-39
5. Mai XC, Ding L, Xu YF, Ceng P, Tao D. Effects of Sperm Morphology and Total Motile Spermatozoa Number on the Rate of Pregnancy Through Artificial Insemination. Acta Medica Mediterranea. 2018 Jan 1;34(3):883-7.
6. Khalili MA, Vahidi S, Aflatounian A, Karimzadeh Ma, Amir-Arjmand Hu. Intracytoplasmic Sperm Injection For The Tre Atment Of Male Fa Ctor Infertility-The First Prelimina Ry Report From Iran. Medical Journal of The Islamic Republic of Iran (MJIRI). 1997 Nov 10;11(3):181-5.
7. Cooper TG, Noonan E, Von Eckardstein S, Auger J, Baker HW, Behre HM, Haugen TB, Kruger T, Wang C, Mbizvo MT, Vogelsong KM. World Health Organization reference values for human semen characteristics. Human reproduction update. 2010 Jan 1;16(3):231-45.
8. Ghumman S, Adiga SK, Upadhya D, Kalthur G, Jayaraman V, Rao SB, Kumar P. Combination of swim-up and density gradient separation methods effectively eliminate DNA damaged sperm. Journal of the Turkish German Gynecological Association. 2011;12(3):148.
9. Natali, II. Artifical Insemination in Farm Animals. Capter 2011; 7.21:116-22
10. Smith S, Hosid S, Scott L. Use of postseparation sperm parameters to determine the method of choice for sperm preparation for assisted reproductive technology. Fertility and sterility. 1995 Mar 1;63(3):591-7.
11. Ding DC, Liou SM, Huang LY, Liu JY, Wu GJ. Effects of four methods of sperm preparation on motion characteristics and nitric oxide concentration in laboratory-prepared oligospermia. Zhonghua yi xue za zhi= Chinese medical journal; Free China ed. 2000 Nov;63(11):822.
12. Ou Z, Yang L, Chen Z, Deng Y, Wang H, Sun L. Comparison of the outcomes of different human spermatozoa selection methods in assisted reproduction. Biomedical Research. 2018; 29(12):2615-19
13. Fisch JD, Rodriguez H, Ross R, Overby G, Sher G. The Graduated Embryo Score (GES) predicts blastocyst formation and pregnancy rate from cleavage-stage embryos. Human Reproduction. 2001 Sep 1;16(9):1970-5.
14. Shen S, Wong C, Ho K, Telles TL, Fujimoto VY, Cedars MI. The morphology of 2 pronuclear (2PN) embryos is related to the quality of day 3 embryos. Fertility and Sterility. 2002 Sep 1;78:S52-3.
15. Terriou P, Sapin C, Giorgetti C, Hans E, Spach JL, Roulier R. Embryo score is a better predictor of pregnancy than the number of transferred embryos or female age. Fertility and sterility. 2001 Mar 1;75(3):525-31.
16. Palini S, De Stefani S, Primiterra M, Benedetti S, Barone S, Carli L, Vaccari E, Murat U, Feichtinger W. Comparison of in vitro fertilization outcomes in ICSI cycles after human sperm preparation by density gradient centrifugation and direct micro swim-up without centrifugation. JBRA assisted reproduction. 2017 Apr;21(2):89.
17. Palini S, De Stefani S, Primiterra M, Benedetti S, Barone S, Carli L, Vaccari E, Murat U, Feichtinger W. Comparison of in vitro fertilization outcomes in ICSI cycles after human sperm preparation by density gradient centrifugation and direct micro swim-up without centrifugation. JBRA assisted reproduction. 2017 Apr;21(2):89.
18. Teoh PJ, Maheshwari A. Low-cost in vitro fertilization: current insights. International journal of women's health. 2014;6:817.
19. Chaichian S, Tamannaie Z, Rohani H, Ahmadi M, Nasr MH, Pazouki A, Mehdizadehkashi A. Relationship between sperm parameters and intracytoplasmic sperm injection outcome. Middle East Fertility Society Journal. 2015 Dec 1;20(4):251-4.
20. Garolla A, Fortini D, Menegazzo M, De Toni L, Nicoletti V, Moretti A, Selice R, Engl B, Foresta C. High-power microscopy for selecting spermatozoa for ICSI by physiological status. Reproductive biomedicine online. 2008 Jan 1;17(5):610-6.
21. Braga DP, Setti AS, Figueira RC, Nichi M, Martinhago CD, Iaconelli Jr A, Borges Jr E. Sperm organelle morphologic abnormalities: contributing factors and effects on intracytoplasmic sperm injection cycles outcomes. Urology. 2011 Oct 1;78(4):786-91.
22. Vanderzwalmen P, Hiemer A, Rubner P, Bach M, Neyer A, Stecher A, Uher P, Zintz M, Lejeune B, Vanderzwalmen S, Cassuto G. Blastocyst development after sperm selection at high magnification is associated with size and number of nuclear vacuoles. Reproductive biomedicine online. 2008 Jan 1;17(5):617-27.