Original Article

Comparative study of influence Swin-up and Dancity-gradient on quality of different type of sperm in infertility center; ACECR

Elnaz Lak[1*], Mahmod Hashemitabar[2], Sareh Amir Zad[2], Mahsa Afrogh[2], Kamran Nasirzadh[2], Fatemh Shamolaghamsar[2]

[1] PhD: Anatomy and Embriology, Resercher of Department of Reporductive Biology, Infertility Research and Treatment Center, ACECR branch of Khozestan, Ahwaz, Iran. [2] Infertility Research and Treatment Center, ACECR branch of Khozestan, Ahwaz, Iran.

Correspondence: Elnaz Lak. PhD: Anatomy and Embriology, Resercher of Department of Reporductive Biology, Infertility Research and Treatment Center, ACECR branch of Khozestan, Ahwaz, Iran.

ABSTRACT

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. 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). The aim of the present study is to compare the effect of these two methods on the quality of semen samples in different groups, including normal samples (< 60 million and 20-60 million/ml), oligospermia, asthenospermia, teratospermia, and oligoasthenoteratospermia in patients referred to the Infertility Center (ACECR).The present experimental study was performed on 1132 these samples were collected after 3 to 5 days abstinence, the findings of pre and post-preparation motility and count parameters were studied and compared in different groups. the SU method led to the highest improvement in the sperm count in the first normal group (20-60 million/ml).). the DGC-SU method led to the best improvement in the sperm count in the teratospermia group. the sperm motility in different groups show, the highest improvement in sperm motility was observed in the asthenospermia group and the oligoasthenoteratozoospermia group in the post- preparation phase using SU and DGC-SU methods compared with other groups (P<0.01).The results showed that SU method was more effective than DGC-SU method in improving sperm count and motility.

Keywords: Swim-Up, density gradient centrifugation, normal, oligospermia, asthenospermia, teratospermia, oligoasthenoteratospermi.

Introduction

Approximately 10 to 15% of couples suffer from infertility problems with different etiologies, with male factors accounting for about half of infertility cases [1-3]. 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% [4-6]. 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]. 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 mobility 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 [8]. Despite

Access this article online	
Website: www.japer.in	**E-ISSN:** 2249-3379

How to cite this article: Elnaz Lak, Mahmod Hashemitabar, Sareh Amir Zad, Mahsa Afrogh, Kamran Nasirzadh, Fatemh Shamolaghamsar. Comparative study of influence Swin-up and Dancity-gradient on quality of different type of sperm in infertility center; ACECR. J Adv Pharm Edu Res 2020;10(S1):80-85.
Source of Support: Nil, Conflict of Interest: None declared.

studies in this field, there is little information and evidence suggesting which of these methods is a priority. Comparative studies have been carried out on the methods of sperm preparation and its results over the past years, with each of them reporting different results; so the aim of the present study is to compare the effect of these two methods on the quality of semen samples in different groups, including normal samples (< 60 million and 20-60 million/ml), oligospermia, asthenospermia, teratospermia, and oligoasthenoteratospermia in patients referred to the Infertility Center of Khuzestan University. Samples are classified as the following table:

Row	Group	Description
1	Normal (between 20-60 million/ml)	Sperm count of ≥ 20 million/ml, motility of ≥50%, morphology of ≥ 4%
2	Normal (more than 60 million/ml)	Sperm count of ≥ 60 million/ml, motility of ≥ 50%, morphology of ≥4%
3	Oligospermia	Sperm count of<20 million/ml, motility of ≥50%, morphology of ≥ 4%
4	Asthenospermia	Sperm count of ≥20 million/ml, motility of <50%, morphology of ≥4%
5	Teratosphermia	Sperm count of ≥20 million/ml, motility of ≥50%, morphology of < 4%
6	Oligoethenotrotoospermia	Sperm count of <20 million/ml, motility of <50%, morphology of <4%

Materials and Methods:

The present experimental study was performed on 1132 sperm samples taken from men over 40 years of age 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. Concentration and motility of sperm 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 6 groups based on their count, mobility, and morphology. The sperms were then randomly separated by DGC-SU and SU methods. The samples randomly preparaed with SU or DGC methods.

Modified washing-swin up method or swin up with double whashing was used for 680 semen samples received to infertility center. In this method, once the liquefaction process was carried out at 37 ° C, 1 ml of semen was poured into a 5 ml tube containing the person's full profile and 4 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. 5-0.7 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)+Swin 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. When both preparation methods were used, the semen parameters including count and motility were evaluated, and the findings of pre and post-preparation motility and count parameters were studied and compared in different groups.

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.

Findings

There was an increase in the sperm count in all groups during the post-preparation phase than the pre-preparation phase in both modified SU and DGC-SU methods, except for the second group with DGC-SU method. Sperm motility was also increased in all groups in the post-preparation phase using two methods (Diagram 1). The result of analysis on the sperm count in different groups (Tables 1 and 2) showed a significant increase in the sperm count pre and post- preparation phase using the SU method in the first normal group and in the samples of patients referred to the Infertility Center of Ahvaz ($P<0.01$); however, there was not such significant increase in the sample prepared by DGC-SU method ($P>0.05$). Comparison of two preparation methods in this group also showed a significant increase in the sperm count in the SU method as compared to the DGC-SU ($P<0.04$). A significant increase was observed in the sperm count in the sample prepared by the SU method in the second normal group, ($P <0.03$), whereas a significant decrease was observed in the sperm count of the samples prepared by DGC-SU method (P

<0.01). Results of analysis in oligospermia and asthenospermia groups also showed a significant increase in the sperm count in the post-preparation phase using SU method (P<0.01) than the pre-preparation phase; whereas no significant increase was observed using the DGC-SU method. 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 (P <0.01). The sperm count increased significantly in the teratospermia group than the post- preparation phase using the SU method (P<0.01). However, this increase was not significant in case of DGC-SU method. Results of comparing SU and DGC-SU methods also showed no significant difference in this group. The sperm count was increased significantly in the oligoteratospermia group in the post- preparation phase using SU method (P<0.01) than pre-preparation phase. DGC-SU method did not lead to a significant increase in the sperm count in this group. The results of comparing SU and DGC-SU methods showed a significant increase in the sperm count analyzed using the SU method than to the DGC-SU method in this group (P<0.01). The results of sperm motility analysis (Tables 1 and 2) showed a significant increase in this parameter in the samples of in all groups referred to Infertility Center in the post- preparation phase than the pre-prepration phase using both methods (P<0.01). There was no significant difference between of SU and DGC-SU methods in terms of sperm motility in the first and fifth groups, while in the second group (P<0.03), the third and fourth groups (P<0.01), SU method led to more significant increase in the sperm motility than the DGC-SU method.

Table 1: Mean and progression in the SU methods

Group	Initial count ($*10^6$)	Final count ($*10^6$)	enhancement	Initial motility ($*10^6$)	Final motility ($*10^6$)	enhancement
Type1	45.05±1.00	62.06±1.66	17±1.62	50.76±0.53	92.76±0.89	42±1.03
Type2	73.75±1.48	77.75±1.87	4±1.90	51.55±0.66	94.06±0.63	42.51±0.89
Type3	17.44±0.5	28.93±1.98	11.49±1.90	49.74±0.54	96.42±0.49	46.67±0.63
Type4	49.90±1.02	56.15±1.30	6.25±1.30	32.62±0.80	93.62±0.49	60.99±0.88
Type5	50.19±1.77	64.59±1.98	14.40±2.12	51.52±0.59	95.22±0.69	43.70±0.77
Type6	15.15±0.3	27.31±1.27	12.15±1.20	30.65±0.97	93.02±0.98	62.37±1.17

Table 2: Mean and progression in the DGC methods

Group	Initial count ($*10^6$)	Final count ($*10^6$)	enhancement	Initial motility ($*10^6$)	Final motility ($*10^6$)	enhancement
Type1	45.06±2.10	50.86±4.03	5.79±3.69	53.10±1.47	94.96±0.63	41.86±1.54
Type2	78.06±3.52	61.90±4.72	-16.16±4.25	56.77±2.24	92.54±1.40	35.77±2.65
Type3	14.64±1.01	20.50±4.04	5.85±3.67	55±3.27	93.28±1.40	38.28±2.78
Type4	52.76±1.80	60.81±2.53	8.04±2.08	15.65±0.38	94.08±0.74	78.43±0.85
Type5	56.90±5.49	69.04±7.40	12.14±8.29	51.19±0.83	94.09±1.42	42.90±1.63
Type6	13.17±0.94	16.79±2.76	3.62±2.25	14.13±0.87	95.65±0.86	81.51±0.89

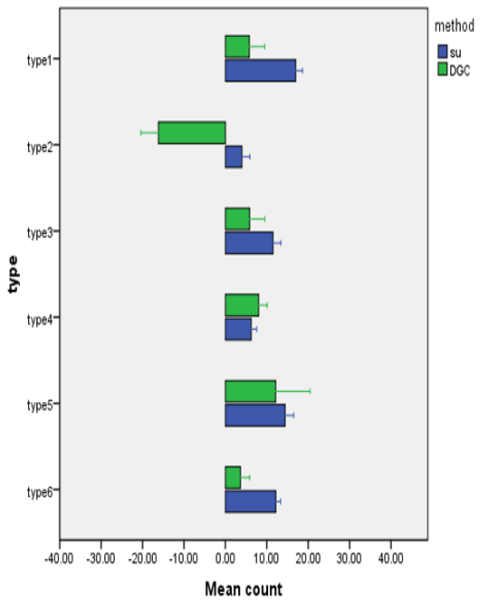

Diagram 1

Also, as Diagram 2 shows, the SU method led to the highest improvement in the sperm count in the first group (20-60 million/ml). However, significant improvement was only observed between the second normal group (<60 million/ml) and the asthenospermia group (Group 4) with the first group (P <0.01). As shown in Diagram 3, the DGC-SU method led to the least improvement in the sperm count in the second normal group (<60 million/ml) than the other groups (P<0.01).According Diagrams 4 and 5, which show the sperm motility in different groups, the highest improvement in sperm motility was observed in the asthenospermia group (Group 4) and the oligoasthenoteratozoospermia group (Group 6) in the post- preparation phase using SU and DGC-SU methods compared with other groups (P<0.01).

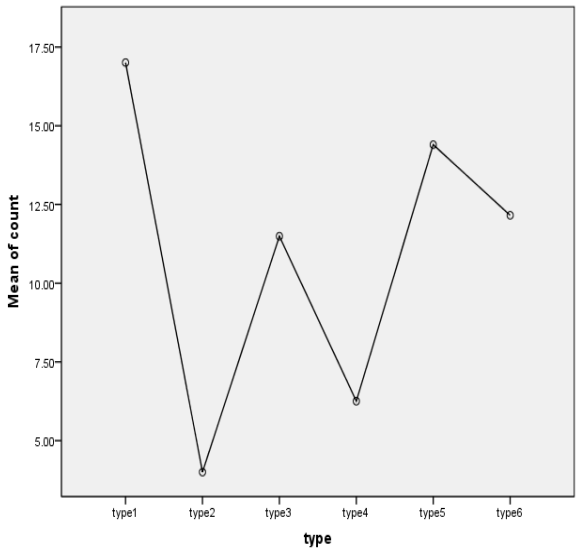

Diagram 2: Average sperm count in SU and inter-group comparison

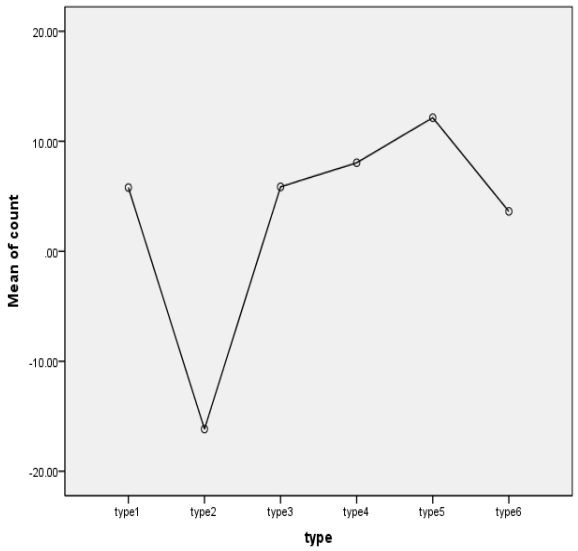

Diagram 3: Average sperm count in DGC method and inter-group comparison

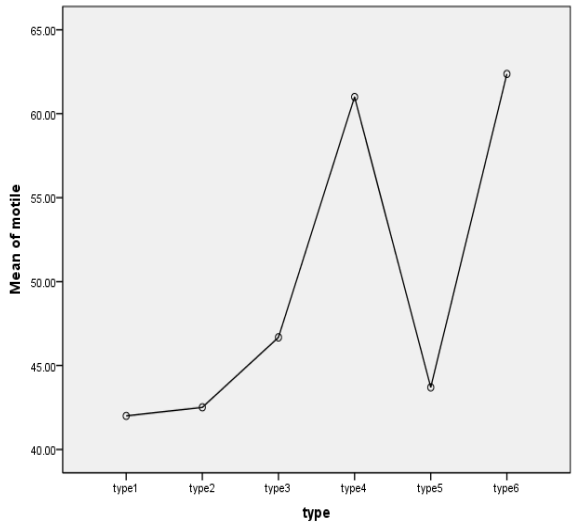

Diagram 4: Average motile sperms in SU method and inter-group comparison

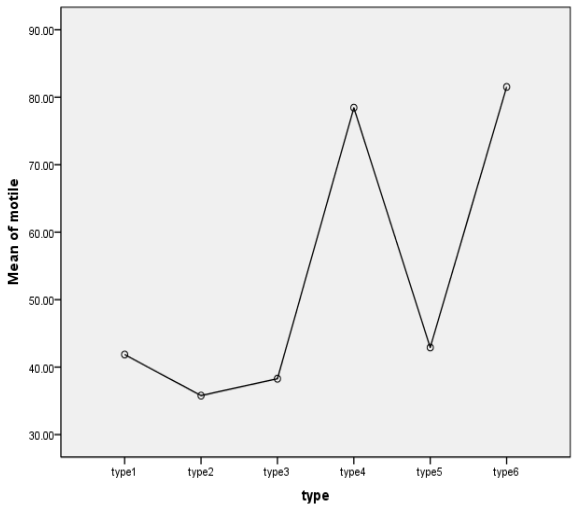

Diagram 5: Average motile sperms in DGC method and inter-group comparison

Discussion

In order to achieve a high percentage of normal sperm, the seamen preparation is done using two methods of DGC and SU in most assisted reproductive technologies (ART) centers [9]. Although extensive studies have been conducted in this field, very different result have been reported, with some of them indicating no difference in the rate of improvement in the semen parameters after preparation using the above two methods [10]. Some of the studies referred to SU method as the better method [11] and some others preferred DGC method [12]. Therefore, attempts were made in the present study to compare the effect these two methods on the quality of semen samples of patients referred to Infertility Center of Khuzestan University. The results showed that SU method was more effective than DGC-SU method in improving sperm count and motility. Both methods had the least effect in increasing the sperm count in the second normal group with >60 million sperm count. The SU method had the highest effect in increasing the sperm count in the first normal group with sperm count of 20-60 million/ml and DGC-SU method had highest effect in the teratospermia group. Also, both methods showed the best effect on the motility rate in asthenospermia and oligoasthenospermia groups. The two methods also had the least effect in increasing the sperm count in the second norm al group with sperm count of > 60 million/ml. In a study on 600 semen samples prepared using the SU method, Adiga et al. (2001) reported the highest sperm concentration and motility rate was observed in oligospermia and later in teratospermia groups, and the least rates were observed in the asthenospermia group, which were inconsistent with the results of the present study. They also reported that the SU method was much more effective in improving sperm quality in the oligoteratospermia group than other groups [12] Lannou and Blachard reported, both techniques (SU and DGC) recovery the motility of sperm and there was no statistical difference between them [13]. In a study, Yamananka et al. (2016) compared the effect of SU and DGC methods on reducing the morphological sperm abnormalities using an electron microscopy and found that the sperm motility in the DGC-treated samples was similar to non-prepared semen samples, but the progressive motility in the DGC+SU-treated samples is higher than the non-prepared samples as well as the DGC-treated samples. These researchers also showed that the SU-DGC method increased the percentage of normal morphology as compared to the DGC method using the electron microscope [14] Harris et al reported a recovery of 58% for normozoospermic men with swin-up [15] and Purvis and Egdetveit reported a reduction in sperm recovery in normozoospermic men with swin-up [16]. Giuseppe Ricci et al. (2009) also compared the effect of SU and DGC mrthods on sperm quality using an optical microscope and a flow cytometer. They found that the concentration of motile, progressive, and viable sperms increased significantly in the DGC method than the SU method. They also reported that both methods led to a reduction in the concentration of apoptotic and necrotic sperms as compared to fresh samples [17]. In a similar study, Amiri et al. (2012) reported that the concentration, motility, and morphology of sperm was increased in the DGC method than the SU, but the DNA fragmentation index (DFI) percentage was reduced. They then stated that the DGC method allows for the separation of high-DFI sperm, which results in failure of the fertility treatments [18].

Acknowledgement

The authors wish to thanks Dr. Serajia; Dr. Nekbakht and Dr. Debavand and all individuals for their contribution.

Funding

This study was supported by grant from Infertility research and treatment center,ACECR branch of Ahwaz,Iran.

References

1. Berek. JS, Infertility. In:Yao M, Schust D. (2002). Novak Gynecology. 13 Edition, Williams & Wilkins Company: p; 973-1046
2. Speroff. L, Fritz, MA (2005). Clinical Gynecologic Endocrinology and infrttility. Lippincott Williams & Wilkins Company:p;1143
3. Khatun. M, Rahman. Md and Pang, MG (2018). Clinical assessment of the male fertility. Obset Gynecol. 6192):179-91
4. Bo.LI, Ma. Y and et al. (2014). The effect of human normal sperm morphology rate of cycle outcome and assisted reproductive methods selection.Plos One. 9(11):45-50
5. Cooper, TG., Noonan, E., Von, ES et al. (2010). World Health Organization reference values for human semen characteristics.Hum Repord Update.16:231-45
6. Ghumman. S., Adiga. SK et al. (2011). Combination of swin-up and density grasient separation methods effectively eliminate DNA damaged sperm. J Turkish-German Gynecol Assoc. 12:148-52
7. Natali, II. (2011). Artifical Insemination in Farm Animals.Capter 7.21:116-22
8. Bartoov, B., Berkovitz, A., Eltes, F., Kogosowski, A., Menezo, Y. and Barak, y. (2002). Real time fine morphology of motile human sperm cells is associated with IVF-ICSI outcome. J Androl.23:1-8
9. Smith, S., Hosid, S and Scott, L. (1997). Use of postsparation sperm parameters to determine the method of choice for sperm preparation for assisted reproductive technology. Fertil Steril. 63:591-97

10. Palomo, M., Izquierdo, D., Mogas, T and Paramio, M. (1999). Effect of semen preparation on IVF of preoubertal goat oocytes. Theriogenology. 51:927-40
11. Ding, D., Liou, S., Huang, L., Liu, J and Wu,G. (2000). Effect of four methods of sperm preparation on motion characteristics and nitric oxide concentration in laboratory-prepared oligospermia. Zhonghua Yi Xue, ZZ. 63:822-27
12. Adiga, SK and Kumar, PR. (2001). Influence of Swim-Up Method on the Recovery of spermatozoa from different types of semen samples. J of assisted Reprodction and Genetics. 18(3):160-64
13. Lannou, DL and Blanchord, Y. (1998). Nuclear maturity and morphology of human spermatozoa selected by percoll density gradient centrifugation or swin up procedure. J. Repord. Fert.84:551-56
14. Yamanka, M. et al. (2016).Combination of density gradient centrifugation and swim up methods effectively decreases morphologically abnormal sperms. J of Reprodct and Develop. 62(6):600-06
15. Harris, SJ., Milligan, MP., Masson, GM and Dennis, KJ. (1981).Improved separation of motile sperm in asthenospermia and its application to artificial insemination homologous (AIH). Fertil Steril. 36(2):219-21
16. Purvis, K and Egdetveit, I. (1993).Factor affecting yield during swim-up. J Assist Reprod Genet; 10(2):145-50
17. Giuseppe Ricci, MD., Sandra Perticararic, BC et al. (2009). Semen preparation methods and sperm apoptosis, Swin up versus Gradient density centrifugantion technique. Fertile & Steril. 91(2): 632-38
18. Amiri, IR., Ghorbani, MA and Heshmati, SA. (2012). Comparison of the DNA fragmentation and the Sperm parameters after processing by the Density Gradient and the Swim UP methods. J of clinical and diagnostic Recerch. 6(9):1451-53.