ORIGINAL ARTICLE

Comparative efficacy of density gradient and swim-up methods of semen preparation in intrauterine insemination cycles

Fauzia Butt,[1] Muhammad Arshad Chohan[2]

Abstract

Objective: To see the efficacy of different methods of intrauterine insemination preparation for sperm recovery and conception rate.

Methods: This prospective observational study was carried out at Chohan Reproduction and Assisted Fertility Treatment Centre, Lahore, Pakistan, from January 2014 to July 2014, and comprised infertile couples undergoing intrauterine insemination cycles. Method of sperm preparation for intrauterine insemination during super-ovulation was randomly assigned for swim-up and density gradient techniques. Total sperm count, percentage recovery of motile sperm count, conception rate and cycle fecundity (live births, miscarriages, ectopic pregnancy) were measured.

Mean percentages of sperm recovery and motility were compared. SPSS 21 was used for data analysis.

Results: Of the 220 couples who underwent 440 cycles, 138(62.7%) suffered from primary infertility and 82(37.27%) from secondary infertility. The mean age of women was 31.75±5.47 years, while that of men was 35.78±5.27 years. Mean duration of infertility in these couples was 5.50±3.51 years. Intrauterine insemination was performed at the first attempt in 180(81.8%) couples, at the second attempt in 37(16.8%) and at the third attempt in 3(1.3%) couples. Mean percentage of sperm motility in post-preparation of density gradient procedure was 83.88±15.02 (day 1) and 84.02±18.51 (day 2) while in swim-up procedure it was 91.02±11.85 (day 1) and 92.28±9.89 (day 2). Mean percentage of sperm concentration after processing in density gradient procedure was higher than swim-up (p=0.368 day 1; p=0.225 day 2) but mean percentage of sperm motility in post-preparation of density gradient procedure was less than swim-up procedure (p=0.000 day 1; p=0.000 day 2). Recovery rates in density gradient were 50.89% on day 1 and 50.13% on day 2compared to 36.69% on day 1 and 37.5%on day 2in swim-up technique (p=0.02). Conception rate was 15%.

Conclusion: Despite the difference in percentage recovery of motile sperms in different techniques, cycle fecundity remained the same.

Keywords: Density gradient, Swim-up, Intrauterine insemination, Sperm concentration, Sperm motility. (JPMA 66: 932; 2016)

Introduction

Intrauterine insemination (IUI) is the first-line assisted reproduction treatment (ART) modality for infertile couples. Since its introduction, more and more sophisticated techniques were developed for improvement in sperm recovery and separation of functionally competent spermatozoa.[1] Despite the sophistication, basic requirements for these techniques remained the same, including cost-effectiveness, simplicity of procedure, elimination of reactive oxygen species(ROS), recovery of as much motile but functionally competent spermatozoa, minimal damage to acrosome, and improved pregnancy outcome. Although none of the developed techniques follow the ideal requirement, but they are still used depending on the quality and the amount of ejaculate.[2]

The earliest technique described by Mahadevan and Baker is conventional swim-up method. This technique is based on initial sperm motility and the surface of cell pellet. There is significant reduction in chromatin condensed spermatozoa because of many layers of cells in pellet, and the production of ROS because of close cell-to-cell contact, cell debris and leucocytes. This ROS causes lipid per oxidation of sperm cell membrane thus reducing percentage recovery of motile sperm.[3]

Another method that will improve the recovery of motile sperms is density gradient method in which ejaculate is placed on the top of the density media with higher density. During this procedure, all cells reach the semen sediment. However, the highly motile spermatozoa move actively in the direction of the sediment gradient and can therefore penetrate the boundary quicker than poorly

[1]Department of Gynae and Obstetrics, Sharif Medical and Dental College/Sharif Medical City Hospital, [2]Department of Obstetrics and Gynaecology, King Edward Medical University Lahore, Lady Willingdon Hospital, Lahore, Pakistan.
Correspondence: Fauzia Butt. Email: drfauziabutt@hotmail.com

motile or immotile cells, thus highly motile sperms cells are enriched in the soft pellet at the bottom.[4]

A Cochrane data-based review of 2007 of randomised control trials (RCTs) by Boomsma confirmed that no method of sperm preparation provided better cycle fecundity than others despite differences in sperm recovery.[1] The overall success rate of IUI is controversial and depends on several factors, but pregnancy rate ranges from 10-20%. Several factors increased the likelihood of pregnancy, including woman's age, duration of infertility, method of sperm preparation, unexplained infertility, anovulatory infertility and addition of controlled ovarian hyperstimulation (COH). The percentage of actual number of motile sperms in sperm preparation have positive impact while the presence of endometriosis, pelvic inflammatory disease (PID), and severe male factor have negative impact.[5]

The current study was planned to see the efficacy of different techniques for semen preparation in percentage recovery of motile sperms and conception rate in treatment cycles as well as the parameters of success in two different methods of semen preparation.

Patients and Methods

This prospective observational study was carried out at Chohan Reproduction and Assisted Fertility Treatment Centre (CRAFT-IVF) in Lahore, Pakistan, from January to July 2014, using non-probability convenient sampling technique, and comprised infertile couples. These patients underwent double insemination.

Infertile couples who already had undergone natural cycles of super-ovulation for six months or whose duration of infertility was more than one year were included. Similarly, cases where hysterosalpingography (HSG) showed patent fallopian tubes or husband semen analysis demonstrated sperm count of >5million were also included.

Cases in which the age of woman was >45 years, fallopian tubes were blocked or sperm concentration was <5 million were excluded. Also excluded were cases which showed follicular count of more than 10 in a cycle as they were associated with risk of ovarian hyperstimulation syndrome (OHSS), in which cycles were converted into natural cycles.

After informed consent, all couples were inquired about, age, duration of infertility, type of infertility, indication of IUI and the number of previous IUI attempts. Female partner was stimulated from day 2 of menstrual cycle with clomiphene citrate (CC) 150mg daily for 5 days. Injectable gonadotrophins (Menogon, Fearing) were given in a dose of 75 IU to 150 IU per day on the6th, 8th and 10th day of menstrual cycle. Transvaginal ultrasound (USG) (Toshiba Xario 100) for follicular tracking was done on the12th day of menstrual cycle. The number of follicles in both ovaries was measured and recorded. Further increment in dose of gonadotrophins was adjusted until the leading follicles reached 18-20mm. Once the diameter of the two follicles exceeded18x20mm in one or both ovaries, ovulation was induced by administering human chorionic gonadotrophin (hCG) (choriomon, IBSA). Then 10,000 IU intramuscularly (IM) and double inseminations were performed within 24 hours and 48 hours of hCG injection.

Semen samples were collected by the process of masturbation in semen production room after three days of abstinence in sterile, labelled container. Written as well as verbal instructions were given for the production of sample to get good recovery of semen sample. After production all samples were placed in an incubator at 37 degrees Celsius for 30 minutes for liquefaction. Routine semen analysis was performed using World Health Organisation's (WHO) semen analysis criteria, i.e. sperm concentration>15x10^6 spermatozoa/ml, total sperm count 39x10^6 per ejaculate or more, motility35% or more with forward progression, and morphology 14% or more with normal form. Sperm motility was assessed by microscope appraisal of 200 spermatozoa from different fields. Mecklar counting chamber (SEFI medical instruments) was used for counting of spermatozoa. Then the samples were randomly assigned to be processed either with swim-up or density-gradient procedure. Before preparation, all media were incubated at 37°C for 15 minutes.

After liquefaction, 1ml of semen was placed in 15ml labelled conical tube (BD Falcon) and 2ml of medium (GMOP-PLUS, Vitrolife) was overlaid on the semen sample. The tube was incubated at 37°C for 45-60minutes to allow progressively motile sperm to swim into the overlaid medium. Taking care not to disrupt the interface, the overlaid medium was then collected and mixed with 2-3ml of medium (GMOP-PLUS) and centrifuged at 300g for 10 minutes. Then the supernatant was removed and the pellet was re-suspended in 0.3-1ml of fresh medium (GMOP-PLUS, Vitrolife).

One ml of 90% gradient (Sperm Grad, Vitrolife) was placed into 15ml conical tube (BD Falcon) with transfer pipette 3ml (BD Falcon). One ml of 45% gradient solution (Sperm Grad, Vitrolife) was overlaid on it and then 1ml of fresh semen sample was overlaid on the gradient. The specimen at 200g was centrifuged for 20 minutes. Supernatant was removed but the bottom 0.3-0.5ml and

pellet was kept. With clean transfer pipette 3ml of fresh medium was gently mixed (GMOP-PLUS, Vitrolife). Single wash was done by centrifuging the specimen at 300g for 10minutes. Supernatant was removed and the pellet was re-suspended in 0.3-1ml of fresh medium (GMOP-PLUS, Vitrolife).

Modification in density gradient procedure was made for those who wanted IUI for gender selection.

Placing 3ml of 90% sperm gradient (SpermGrad, Vitrolife) into conical tube, 3ml of 45% sperm gradient (SpermGrad, Vitrolife) was placed on it. The specimen at 300g was centrifuged for 30 minutes. For female, choice only pellet was kept as heavier X-sperms in that pellet, while for male choice only supernatant just above the pellet i.e. about 0.3ml was taken. With clean Pasteur pipette 3ml of fresh medium was mixed (GMOP-PLUS).Wash was done by centrifuging the specimen at 300g for 10 minutes. The supernatant was removed and the pellet was re-suspended in 0.3-1ml of fresh medium (GMOP-PLUS).

After the processing, all samples were assessed for sperm concentration and motility, and the specimen was incubated at 37°C till insemination was performed.

Percentage recovery of motile sperm count was determined by relative yield (RY), which is the proportion of progressively motile spermatozoa submitted to a preparative procedure that are present in final preparation. It is calculated by a method described by Mortimer:[6]

Yield (%) = (v x c xpm%)/(V x C x PM%) x100

v is the final preparation volume (0.5ml), c is the sperm concentration in the final preparation (106/ml), pm% is the prepared sperm population progressive motility, V is the volume of semen used (1ml), C is the semen sperm concentration (106/ml), and PM% is the progressive motility of spermatozoa in the semen.

After processing of semen samples, female was prepared for insemination by exposing the cervix and cleansing with distilled water and 0.5ml of sample was inseminated with Wellace catheter (SMITHS) using 1ml syringe (Normi-Ject, Germany) inside the uterine cavity as closer to the fundus as possible. Patients were allowed to rest for 15minutes. Luteal support was given in the form of vaginal micronised progesterone (Uterogestan, Galaxy Pharma) 200mg twice a day for the next 10 days.

Pregnancy test was performed after missing the periods or beta hCG level after 14 days of insemination. Clinical pregnancy was documented when transvaginal USG was performed after 4 weeks of pregnancy test once embryonic sac appeared on USG. Gender of foetus was determined at 18-week scan. Variables that determine success (pregnancy) were evaluated, including age of female patient, duration of infertility, type of infertility, number of follicles in both ovaries and ovarian side, method of sperm preparation, and cycle fecundity, i.e. live births, miscarriage, ectopic pregnancy and gender ratio.

SPSS 21 was used for data analysis. Descriptive statistics like mean and standard deviation (SD) were calculated for pre- and post-preparative sperm count and motility for both days while frequency and percentage for successful outcome and fecundity. Pre- and post-preparative sperm count were compared for statistical difference using Mann-Whitney U test for each procedure while Fisher's exact test was applied to measure the difference between conceived and non-conceived group for parameters of success. $P<0.05$ was considered statistically significant.

Results

Of the 220 couples, who underwent 440 IUI cycles, 138(62.7%) suffered from primary infertility and 82(37.27%) from secondary infertility. The mean age of women was 31.75±5.47 years (95% confidence interval [CI] 31.02-32.47), while that of men was 35.78±5.27 years (95% CI 35.085-36.48). Mean duration of infertility in these couples was 5.50±3.51 years (95%CI 5.028-5.972). Mean follicular count was 3.88±1.839.

IUI was performed at the first attempt in 180(81.8%) couples, at the second attempt in 37(16.8%) and at the third attempt in 3(1%) couples.

Mean percentage of sperm concentration after processing in density gradient procedure was 57.44±40.37 (day 1) and 53.86±38.58 (day 2), while in

Table-1: Mean comparison of density gradient and swim-up procedure.

Variables	Density Gradient Means	Swim-Up Means	SIG
Pre wash Sperm concentration			
DAY 1	83.6364 ±47.025	109.01±39.72	0
DAY 2	74.075±42.95	94.318±34.86	0.001
Post wash sperm count			
DAY 1	57.44±40.37	51.40±35.27	0.368
DAY 2	53.86±38.58	46.06±31.21	0.225
Pre wash sperm motility			
DAY 1	56.08±16.18	58.02±16.18	0.461
DAY 2	56.37±17.03	60.46±13.25	0.108
Post wash sperm motility			
DAY 1	83.88±15.02	91.02±11.85	0
DAY 2	84.02±18.51	92.28±9.89	0

SIG: Significance.

Table-2: Comparison of conceived versus non-conceived group for parameter of success.

Parameters of Success Significance	Concieved N=33	Non-Concieved N=187	Fisher' Exact Test
Female Age			
20-25	12 (36.36%)	22 (11.76%)	
26-30	10 (18.81%)	68 (36.36%)	0.076
31-35	6 (30.3%)	40 (21.39%)	
36-40	5 (15.15%)	57 (30.48%)	
Duration Of Infertility(Year)			
4-Jan	23 (69.7%)	87(46.52%)	
8-May	8 (24.24%)	61 (32.62)	0.0847
12-Sep	2 (3.03%)	29 (15.50%)	
>12 Year	0	10 (5.34%)	
Type of Infertilty			
Primary	14 (42.42%)	124 (66.31%)	0.02
Secondary	19 (57.57%)	63 (33.68)	
Method of Preparation			
Density Gradient	17 (51.51%)	93 (49.73%)	1
Swim Up	16 (48.48%)	94 (50.26%)	
Follicular Count			
4-Jan	15 (45.45%)	127 (67.91%)	
7-May	14 (42.42%)	53 (28.34%)	0.018
>8 <10	4(12.12%)	7 (3.74%)	
Ovarian Side			
Left	5 (15.15%)	43 (22.99%)	
Right	10 (30.30%)	58 (31.01%)	0.57
Both	18 (54.54%)	86 (45.98%)	
Previous Attempts			
First	28 (84.84%)	152 (81.28%)	
Second	5 (15.15%)	32 (17.11%)	1
Third	0	3 (1.60%)	
Indication of IUI			
Unexplained	14 (42.42%)	56 (29.94%)	
PCOD	8 (24.24%)	39 (20.85%)	
Gender	7 (21.21%)	25 (13.36%)	0.059
Male factor	2 (6.06%)	5 (2.67%)	
Multiple factors	2 (6.06%)	62 (33.1%)	

PCOD: Polycystic ovarian disease.

swim-up it was 51.40±35.27 (day 1) (p=0.36) and 46.06±31.21 (day 2) (p=0.22).

Mean percentage of sperm motility in post-preparation of density gradient procedure was 83.88±15.02 (day 1) and 84.02±18.51 (day 2), while in swim-up procedure it was 91.02±11.85 (day 1) (p=0.00) and 92.28±9.89 (day 2) (p=0.00) (Table-1).

Recovery of motile in final preparation was 50.89% (day 1) and 50.13% (day 2) in density gradient, while it was 36.69% (day 1) and 37.5% (day 2) in swim-up procedure (0.025).

No statistical difference was found in pregnancy outcome (p>0.05).Moreover, 33(15%) couples conceived; 17(51.51%) from density gradient procedure and 16(48.48%) from swim-up method (Table-2).

The probability of pregnancy was 12(36.36%) in the age group 20-25 years, 23(69.7%) with duration of infertility <5 years and 19(57.57%) among those suffering from secondary infertility. Besides, 28(84.84%) patients conceived at the first attempt. The cause of infertility was unexplained in 14(42.42%) conceived couples (Table-2).

Discussion

The era of ART brings revolution in the lives of infertile couples by bringing new treatment modalities. IUI is a first step towards this revolution. Percentage recovery of morphological normal motile spermatozoa is of paramount importance in the success of IUI. This results in variations in techniques of IUI procedure which helps in selection of good motile sperm concentrates that increase the likelihood of pregnancy.[7]

Swim-up and density gradient methods are variably used depending on the initial sperm count and motility and both the techniques proved equally effective in percentage recovery of sperm motile sperm count. In this study we used swim-up and density gradient methods and compared them for percentage recovery of motile density.[8]

When we compared mean percentage of sperm concentration after processing in both methods it was comparable in both methods but percentage motility was significantly higher in the swim-up method; it was 91.02±11.85 (day 1) and 92.28±9.89 (day 2) (p=0.000 on both days).

The success or applicability of a sperm-washing method can be considered in terms of either the absolute or RY of motile spermatozoa that one obtains at the end of the technique. Usually, progressive motility is used for this purpose because non-progressive spermatozoa are unlikely to be potentially functional.[9]

In this study RY was 50.89% in density gradient and 36.69% in swim-up method on day 1 and 50.13% and 37.5% on day 2 (p=0.025). It showed that percentage recovery of motile sperm was higher in density gradient method as compared to swim-up method. Our results were comparable to a study[10] which showed that in both normal and abnormal groups sperm concentration in density gradient was significantly greater than at swim-up and the percentage of progressive motility was greater in swim-up samples than in density gradient. The number of motile sperm reflecting the percentage recovery was still

greater in density gradient samples.

Combined data from two large studies involving 446 cycles in 443 couples yielded a borderline benefits (odds ratio [OR] 1.7; CI 1.0-2.9) for density gradient procedure. However, larger randomised trials are needed to prove it till such time method may be chosen depending on initial sperm count and motility on individual basis.[11]

Reported effectiveness of IUI in terms of pregnancy rate in literature is around 10-14% when CC and gonadotrophins were used in combination therapy and the cumulative conception rate reaches >40% after three to four treatment cycles.[12]

In this study, 220 patients underwent 440 cycles of treatment and the cumulative pregnancy rate was 33(15%) - 17 from density gradient and 16 from swim-up method. Most of the patients, i.e. 84.45%, conceived in the first attempt of treatment (95%CI 72.62-97.08), particularly women who suffered from anovulatory disorders like Polycystic Ovary Syndrome (PCOS) and unexplained infertility. Comparative studies using these methods showed a pregnancy rate of 15% which is comparable to our study.[3] The number of treatment cycles was considered good prognostic indicators in success of IUI as the chances of pregnancies are higher in the first two treatment cycles. These results are comparable to one study.[13] The age of female partner is a well-known indicator of oocyte quality as judged from ART cycles. Evidence from different studies also indicates an important determinant in IUI success. In literature, reported success rate is upto 20% in 30years age group followed by persistent decline as age advances: 10% in 35 years and 5% in> 35 years.[13] In this study, 84.84% of the women who conceived were <35 years.

Another important parameter in success of IUI is the duration of infertility. In this study, more patients conceived with shorter duration of infertility less than four years (69.7%). In reported meta-analysis, in logistic regression of 240 cycles the conception rate was10% if duration of infertility exceeds more than 72 months and 20% if shorter duration of infertility.[14]

A recent retrospective analysis of 2,473 cycles and logistic regression analysis predicts that unexplained infertility and anovulatory disorder increase the likelihood of pregnancy.[15] The results are comparable to our study as most of our patients conceived in this group with unexplained (42.42%) and in PCOS group (24.24 %).

Regarding the impact of insemination frequency on pregnancy outcome, a recent prospective study indicated an increase in pregnancy outcome (OR 2.3; 95% CI 1.4-3.9) in double versus single insemination.[16,17] In this study, we performed double insemination techniques on 24 and 48 hours of hCG injection. Despite the production of samples on two days without abstinence, no difference in sperm concentration and motility was found.

The mean follicular count in conceived group was five (0.018) and the ovarian side did not make a difference in cycle outcome (0.570). Looking at cycle fecundity, out of 33 patients who conceived, 2(6.06%) ended in abortion,1(3.03%) in ectopic pregnancy and live births were in 30(90.90%) pregnancies, of which 22(73.33%) were males and 7(23.33%) females, reflecting the fact that IUI is an effective tool for gender selection methods by just modifying the techniques of IUI particularly for those couples who cannot go for higher and costly ARTs like preimplantation genetic diagnosis (PGD).[18]

Conclusion

Density gradient method of sperm preparation appeared to be more efficacious in percentage recovery of motile sperm concentration compared to swim-up method. However, fecundity remained the same in both groups and the success was largely dependent on age of female partner, duration of infertility, indication of IUI, number of treatment cycles and the type of infertility.

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References

1. Boomsma CM, Heineman MJ, Cohlen BJ, Farquhar C. Semen preparation techniques for intrauterine insemination (Review).Cochrane Database Syst Rev. 2007;(4):CD004507.
2. Henkel RR, Schill WB. Sperm preparation for ART. Repro Bio & Endo. 2003; 1:108.
3. Adiga SK, Kumar P. Influence of swim-up method on the recovery of spermatozoa from different types of semen samples. J Assist Reprod Genet. 2001; 18:160-4.
4. Dodson WC, Moessner J, Miller J, Legro RS, Gnatuk CL. A randomized comparison of the methods of sperm preparation for intrauterine insemination. FertilSteril. 1998; 70:574-5.
5. Haken E Duran, MahmoodMorshedi, Thinus Kruger, Sergio Oehninger. Intrauterine insemination: a systemic review of determinants of success. Human reproduction updates. 2002; 8:374-84.
6. Mortimer D. Practical Laboratory Andrology. Oxford University Press, 1994.

7. Allamaneni SS, Agarwal A, Rama S, Ranganathan P, Sharma RK. Comparative study on density gradients and swim-up preparation techniques utilizing neat and cryopreserved spermatozoa. Asian J Androl. 2005; 7:86-92.
8. Ren SS, Sun GH, Ku CH, Chen DC, Wu GJ. Comparison of four methods for sperm preparation for IUI. Arch androl. 2004; 50:139-43.
9. Sushil K, Hemangi A, Awasthi Rt, Anupam K, Srinivas S. Role of intrauterine insemination and sperm preparation techniques in treatment of unexplained infertility. MJAFI. 2000; 56:209-12.
10. Chen SU, Ho HN, Chen HF, Chao KH, Lin HR, Haung SC, et al. Comparison between a two layer discontinuous Percoll gradient and swim-up for sperm preparationfor normal and abnormal semen samples. J Assist Reprod Genet. 1995; 12:698-703.
11. Inaudi P, Petrilli S, Joghtapour A, Trasusso P, F Petraglia. Reduction of steps in the preparation of motile sperms for intrauterine insemination does not reduce efficacy of the procedure: simplified one step swim-up method versus classic swim up. Hum Repro. 2002; 17:1288-91.
12. Streda R, Mardesic T, Sobotka V, Koryntova D, Hybnerova L, Jindra M. Comparison of different starting doses (50, 75 and 100iu daily) for ovulation induction combined with intrauterine insemination.Arch Gynecol Obstet. 2012; 286:1055-9.
13. Pramila Koli, Anil M, N R Ramya, Kamal Patil, MK Swamy. Intrauterine insemination: a retrospective review on determinants of success. Int J Reprod contracept Obstet Gyecol. 2013; 2:311-4.
14. Kamath MS, Bhave P, Aleyamma T, Nair R, Chandy A, Mangalaraj AM, et al. Predictive factors for pregnancy after intrauterine insemination. A prospective study of factors affecting outcome. J Hum Reprod Sci. 2010; 3:129-34.
15. Farimani M , Amiri L. Analysis of prognostic factor for successful outcome in patients undergoing intrauterine insemoination. Acta Medica Iranica. 2007; 45:101-6.
16. Lan XU, ren-Kang LU, Ling CHEN, Yan-Luan ZEHENG. Comparative study on efficacy of three sperm separation techniques. Asian J Androl. 2000; 2:131-4.
17. Check JH, Katsoff D. A prospective study to evaluate the efficacy of modified swim-up preparation for male sex selection. Hum Reprod. 1993; 8:211-4.
18. Han TL, Flaherty SP, Ford JH, Matthews CD. Detection of X and Y bearing spermatozoa after motile sperm isolation by swim up. Fertil Steril. 1993; 60:1046-51.