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Comparing Two Density Methods of Semen Preparation in Human Ejaculates

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ABSTRACT

Test tube baby is always a fascinating field of human reproductive biological science playing an important role in treating infertility. Poor semen quality is the major cause of infertility in human beings. Preparation of semen samples for intrauterine insemination (IUI) or in-vitro fertilization (IVF) is a key point in the success of test-tube embryo production. The study compared two different methods of semen preparation viz. double density gradient (DDG) and single density gradient (SDG) in patients seeking IUI / IVF treatment with their consent and permission of the hospital. Semen ejaculate from patients (n=100) was divided equally into two equal volume parts. One half of each sample was treated with DDG and another half with the SDG method of semen preparation. Results showed that sperm concentration was significantly higher (P>0.05) in SDG compared to DDG treated samples which were 58.65±181 and 49.89±180 Million/ml, respectively. Sperm motility of type-a and type-b both were significantly higher (P>0.05) in SDG compared to DDG treated samples which were 91.85± 3.15 and 68.85 ± 26.15. It is concluded that the single density gradient method is better than the double density gradient for semen ejaculates preparation during the treatment of male infertility using the in-vitro fertilization technique.

Keywords: Male infertility, IUI/IVF, Density Gradient, Semen Preparation.

I. INTRODUCTION

Semen sample preparation was done with double density gradient (DDG) and single density gradient (SDG) wash followed by a swim-up procedure with obtained sperm pellet. This randomized study was performed on different semen parameters. Density gradient centrifugation is a more sophisticated method to obtain huge motile sperms with functionally competent spermatozoa for intrauterine insemination (IUI) or in-vitro fertilization (IVF) or intracytoplasmic sperm injection (ICSI) procedures. However, we want to select the best method for semen preparation to improved sperm functions like sperm motility, morphology, good quality of sperm concentration and reduced ROS.

In this study, we aimed to give a suitable method for semen preparation and separation of poor quality, dead sperms, and other cell debris. The best semen preparation method improves their functional competence and reduces detrimental effects and increased the modern assisted reproductive technologies (ART) outcome. Last decade there has been an increasing incidence of infertility among the reproductive age group couples. Although, modifications in semen preparation techniques were help to improve pregnancy rates in a corresponding manner. However new techniques are being continuously evaluated in order to prove their value both in achieving clinical pregnancies as well as being cost-effective for those couples.

Primary treatment of infertility is IUI in case of semen quality is compromised or male sexual dysfunction or vaginal pH is higher and post-coital report is negative. The overall success rate of IUI varies with pregnancy rates ranging from 5% to 20% per cycle. In the IRCC IVF centre an IUI success rate varies between 15 – 20 %. Some predictive factors for IUI results have been projected, including the age of the women, duration of infertility, endometrial thickness, type of ovarian stimulation, number of inseminations and the total number of motile sperm inseminated. DDG & SDG processing techniques provide better sperm quality, concentration, and motility, which may lead to higher success rates in IUI, IVF/ ICSI.

Human sperm incubation at room temperature does not allow capacitation, although it does not affect human follicular fluid-induced acrosome reaction in capacitated cells. The blocking effect is overcome when spermatozoa are exposed at 37°C. We followed the World Health Organization (WHO 2010) recommended guidelines for semen analysis and washing.

II. MATERIAL AND METHODS

This retrospective study analyzed sibling's semen sample (n=100) of infertility patients, who undertake IUI at IRCC IVF Centre from the period of July 2017 to December 2017. All those patients had primary infertility with duration of 2 – 6 years. Unexplained infertility was defined as couples who had normal tubal patency, regular ovulation and no cervical factors with regular timed unprotected intercourse at least 1-year duration of infertility.

The patient ejaculates were divided into two groups, **Group A – Patients (n = 100)** semen prepared by using **DDG** (patient having sperm concentration > 20 x 106/ml and motility ≥ 32%) centrifugation and **Group B – Patients (n = 100)** being treated with SDG (patient having sperm concentration <20 x 106/ml and motility ≤ 32%) centrifugation under temperature-controlled centrifugation with SF800 centrifuge machine.

METHODS OF SPERM PREPARATION

After liquefaction of semen we were measure the volume and do the semen analysis. After measuring the semen volume it was divided in two equal volumes one was prepared with DDG (Group A) and second with SDG (Group B).

Group A: Double Density Gradient (DDG) Method: In the DDG, those semen samples has sperm concentration > 20 x 10^6/ml and sperm motility (type a + type b) ≥ 32% we were prepared with DDG (80% and 40 % gradient). First wash at 1500 RPM for 12 minutes. Discarded the supernatant and re-suspend the pellet in pre equilibrated human tubal fluid (HTF) medium with proper labeling of patient name and id. Second wash was done at 1500 RPM for 5 minutes and discarded the supernatant by leaving 0.5 ml HTF including pellet. We were mixed the pellet and observe the sperm count and motility. This sample is ready for IUI/IVF.

Group B: Single Density Gradient (SDG) Method: In the SDG, those semen samples has sperm concentration < 20 x 10^6/ml and sperm motility (type a + type b) < 32% we were prepared with SDG (only 80% gradient). First wash were done at 1500 RPM for 12 minutes. After that, discarded the supernatant and re-suspend the pellet in pre equilibrated human tubal fluid (HTF) medium with proper labeling of patient name and id. Second wash was done at 1500 RPM for 5 minutes and discarded the supernatant by leaving 0.5 ml HTF including pellet. We were mixed the pellet and observe the sperm count and motility. This sample is ready for IUI/IVF.

III. RESULTS

A total of 100 infertility patients semen sample were divided in two different groups of **same semen sample (1:1): Group A (*n* = 100)** had their semen samples prepared by double density gradient (DDG) and **Group B (*n* = 100)** had their semen samples prepared by single density gradient (SDG). Bothe density gradient centrifugation was performed same RPM and time. In SDG method we were observed higher sperm count and higher sperm motility compare to DDG method. The patient's demographic and semen characteristics (pre- and post-washing) of the study population between the two groups are shown in the following tables:

Basic details and Pre wash parameters		
Sr. No.		**Mean**
1	Male Age	28.52 ± 9.48
2	Female Age	27.3± 7.7
3	Active Marriage Life	2.98± 4.01
4	Duration of infertility	2.9±3.1
5	Sexual Abstinence	2.93±4.07
6	Semen volume (ml)	2.43 ±3.5
7	Semen pH	7.26 ± 0.13
8	Sperm Count	59.28 ±190
9	Sperm Motility (a+b)	30.35 ± 56.65
10	Leukocytes	0.45±0.54

	Post wash semen parameters			
	Mean			
	Double Density	**Single Density**	**t-value**	**p-value**
Post Wash Semen Volume	0.5 ml ± 0.0	0.5 ml ± 0.0	NA	NA
Post Wash Sperm Count	49.89 ±180.11	58.65 ±181.35	56.75	p>8.94*
Post Wash Sperm Motility (a+b)	68.85 ± 26.15	91.85 ± 3.15	10.39	P >1.68*

*P-value is not significant p>.05

IV. DISCUSSION

We concluded after study of 100 patient's semen sample preparation done by using DDG and SDG centrifugation depends on semen parameters. We were observed SDG centrifugation (12 minutes at 1500 rpm) on sperm concentration <20 x 10^6/ml & motility a+b <32% obtained good pellet of normal morphologically selected motile sperms and we were observed in DDG centrifugation (12 minutes at 1500 rpm) on sperm concentration >20 x 10^6/ml & motility a+b >32% obtained good pellet of normal morphologically selected motile sperms.

V. CONCLUSION

It is concluded that the SDG method is better than the DDG method for human semen ejaculates preparation during the treatment of male infertility using the in-vitro fertilization techniques.

In summary, a number of sperm preparation methods are available to process semen sample for use in IUI and ART procedures. Each infertile couple must be carefully examined to determine the best sperm preparation method is suitable. Future research should inquire to improve the worth and the safety of the sperm preparation techniques.

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ETHICAL ISSUE

The independent institutional ethical committee approved this comparable & observational study of semen preparation by DDG and SDG regarding obtaining good sperm pellet and motility in sibling's semen sample of IRCC Hospital, Panchkula, Haryana, India.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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