EFFECTIVENESS OF "SWIM-UP" AND "GRADIENT" METHODS IN SPERM PREPARATION FOR ARTIFICIAL INSEMINATION

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Abstract
Artificial insemination with sperm preparation and inseminate to the uterus is a common method of infertility treatment. Currently, two methods used for sperm washing are "swim-up" and "gradient". **Objectives and methods:** cross-sectional description in 166 cycles of artificial insemination in Hue University Hospital from April, 2012 to March, 2013 in order to compare effectiveness between "swim-up" and "gradient" methods. Samples were collected randomly into two methods. **Results:** Results of sperm preparation in both methods are equivalent in terms of the following parameters total sperm count, total number of progressive sperm, total normal morphology sperm. However, "gradient" method results in higher number of progressive sperm in case with slow motility (38.3% vs. 26.1%) or abnormal morphology (34.9% vs. 19.7%) compared with "swim-up" method. Pregnancy rates after artificial insemination were similar between two preparation methods. **Conclusion:** In case of slow motility sperm and abnormal morphology, "gradient" method should be used to increase the number of progressive sperms. Pregnancy outcome depends on many factors other than preparation methods.
Key words*: Artificial insemination, sperm preparation, inseminate*

1. INTRODUCTION

The IUI method was carried out since 1970s and has been applied widely for the infertility treatment so far. Sperm which are inseminated into the uterus have the best quality, are condensed in the minor volume, decrease harmful impacts on sperm such as acid pH of vagina or the abnormal cervix. Sperm could be from the husband or the donor.

Ovulation can be followed under the natural cycle or stimulation. IUI is a simple method, easy to implement, inexpensive and widely used in almost infertility centers. At present, two common methods of sperm washing are swim-up and gradient centrifugation (Mortimer, 2000). Each method has both advantages and disadvantages so they have different effectiveness. The research "*Effectiveness of "swim-up" and "gradient" methods in sperm preparation for artificial insemination"* aims to value the quality of sperm through the preparation methods and to find out some relevant factors between sperm analysis and the effectiveness of methods.

2. MATERIAL AND METHODS

The cross-sectional descriptive method on 166 infertile couples who were indicated for the artificial insemination at the Center of Reproduction Endocrinology and Infertility, Hue University Hospital from April, 2012 to March, 2013. The semen analysis followed the definition of World Health Organization Manual in 2010 (WHO Manual, 2010), the wife's ovaries function is still normal and with at least one normal Fallopian tube. Agreement consent was required to our research.

All ejaculated samples were collected and randomly washed by one of two methods: 83 samples for swim-up and 83 samples for gradient. The detailed steps were described as follows:

Sperm washing by swim-up: semen sample was incubated at 37°C within 15-30 minutes, mixed and splited the sample into two parts with equal volume, placed gently under the cultural media by the Pasteur pipette with two tube containing 2,5ml Ferticul Flushing of each. Tube was then placed in the incubator for 60 minutes at a 45°C angle. Gently removed pellet into a 15ml conical tube containing 3ml Ferticult Flushing. Mixed up and centrifuged at 250g for 5 minutes. Discard the supernatant to leave 300 µL at the bottom of this tube. Re-suspension is then incubated at 37°C before inseminate into the uterus.

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Density gradient preparation: prepare two 1,5ml conical tube containing 45% and 90% gradient solution (incubated at 37°C) each. Carefully overlay semen sample on the top of media. Centrifuge at 250g for 15 minutes. Discarded the supernatant and leave the remaining solution and pellet at the bottom of tube. Wash two times and centrifuge at 250g for 10 minutes with 3ml Ferticult Flushing. Re-suspend in 300 µL and incubate at 37°C before inseminate into the uterus.

Data analysis was done by SPSS-19 statistical program (SPSS Inc.Chicago, IL, USA). The percentages of the mobility sperms, concentration and the progressive recovery sperms pre- and post-washing were calculated. The comparison between the sperm quality before and after washing was checked by the student's test with two independent samples.

3. RESULTS

Table 1. Sperms parameters before washing by swim-up and gradient method

Parameters before washing	**Swim-up**	**Gradient**	**p**
Concentration (x10^6/ml)	31.1 ± 19.2	28.8 ± 20.1	0.45
Total sperm count (x10^6)	134.2 ± 106.6	115.7 ± 95.6	0.24
Progressive motile spermatozoa (x10^6)	46.7 ± 47.5	45.6 ± 58.2	0.16
Normal morphology spermatozoa (x10^6)	8.5 ± 11.9	6.6 ± 13.3	0.33

The results of semen analysis before washing by swim-up have no in statistically significant difference ($p>0.05$).

Table 2. The comparison of the sperm washing results between swim-up and gradient method.

Parameters post-washing	**Swim-up**	**Gradient**	**p**
Concentration (x10^6/ml)	30.5 ± 21.4	34.1 ± 18.9	0.25
Total sperm count (x10^6)	9.1 ± 6.4	10.2 ± 5.7	0.25
Progressive motile spermatozoa (x10^6)	7.5 ± 6.4	8.5 ± 5.6	0.31
Normal morphology spermatozoa (x10^6)	1.2 ± 1.6	0.9 ± 0.9	0.11

There was no significant difference in terms of the total sperm count, the total number of progressive motile sperm and the total number of normal morphology sperms in two washing methods.

Table 3. Compare the productivity of progressive sperms recovery (%) by 2 methods

	Concentration		**Motile**		**Morphology**	
	Oligo	Normal	Abnormal	Normal	Abnormal	Normal
Swim-up	26.9 ± 12.8%	21.5 ± 16.7%	26.1 ± 17.8%	18.6 ± 13.0%	19.7 ± 12.9%	26.6 ± 19.0%
Gradient	48.7 ± 41.3%	27.5 ± 16.9%	38.3 ± 29.2%	23.4 ± 15.2%	34.9 ± 30.8%	27.5 ± 12.7%
p	0.052	0.043	0.017	0.152	0.002	0.827

Surveying the retrieval productivity in terms of motile sperm of two methods with various sperm analysis, show definite differences. Samples having the low motility (PR<32%) or abnormal morphology (<4%) post-washed by gradient had higher productivity than those by the swim-up method (38.3% vs. 26.1%; 34.9% vs. 19.7% respectively). These differences had statistical significance.

From the collected data on the total number of sperm motility pre- and post-washing by two methods, linear graphs were constructed to describe the dependence of the total motile sperm pre- and post-washing (Figure 1)

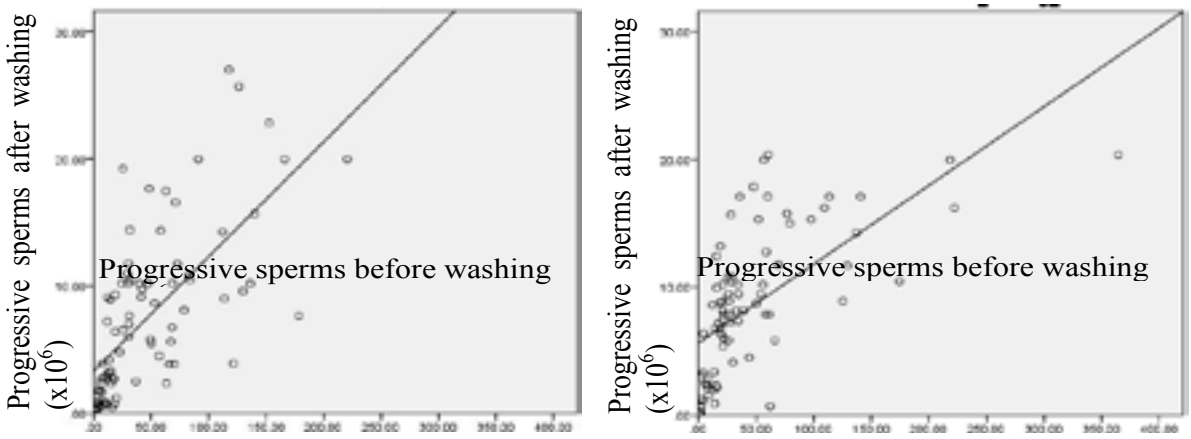

Figure 1. The linear graph describes the dependence of the total motile sperms in two preparation methods between pre- and post washing. Figure a: swim-up method. Figure b: gradient method

Table 4. The result of pregnancy in two washing methods based on progressive sperms post-preparation

The total of progressive sperms	Swim-up			Gradient		
	Pregnancy cases	Total	Rate (%)	Pregnancy cases	Total	Rate (%)
≤ 5 millions	2	37	5.4	3	24	12.5
5–15 millions	6	35	17.1	8	43	18.6
≥ 15 millions	4	11	36.4	6	16	37.5

The pregnancy rates of two methods depending on the total number of progressive sperms post-wash are similar.

4. DISCUSSION

Some previous studies demonstrated that "gradient" method results in higher number of recovery spermatozoa compared with "swim-up" method. (Sharma et al, 1997, Moohand and Lindsay, 1995; Tucker et al., 1996; Lozano et al., 2009). Our study, however, showed that both methods had no difference in recovery spermatozoa results. This result was in accordance with some previous reports by Zini et al, 2000. CAM Jansen and Tucker KE, 2002 Shamsi et al, 2008.

It is important to state that, gradient method proved to be superior to swim up method in the case of low-motility samples and abnormal morphology samples. However, there was no significant difference in the result obtained from the normal progressive samples and the normal morphology samples. In brief, gradient method was effective for samples having the total number of low progressive sperms while this result is completely contrary with swim-up method. This result was also consistent with the reports by Lozano et al publishing in 2009 (Lozano et al, 2009).

Basing on the above consideration and the linear graph about the dependence on the total number of progressive motile sperms pre- and post-washing, we could estimate the number of progressive motile sperms post-washing depending on the semen analysis results. The pregnancy rate was an important parameter to evaluate the efficacy of sperm preparation in the IUI cycle. We therefore conducted a survey to assess pregnancy rates between two methods basing on the progressively sperms recovery post-washing. Both methods had the same pregnancy rate. The number of progressive sperms was quite important for the patient's pregnancy ability assessment in performing artificial insemination. This relationship was shown in Table 4. If the number of progressive sperms was fewer than 15 millions, the pregnancy rate would go down dramatically. This result was consistent with previous reports (Berg et al , 1997; Weert et al, 2004).

In summary, there were no significant differences between two above methods in the total number of spermatozoa recovery, the total number of progressive spermatozoa or the total number of morphological normal spermatozoa. However, the recoverable productivity of motile sperms of gradient method resulted better than that of swim-up method in case of slow motility or abnormal morphological spermatozoa. The pregnancy rate between two methods was similar.

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