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RETROSPECTIVE ANALYSIS OF SEMEN QUALITY USING SPERM ENHANCING TECHNIQUE FOR INTRAUTERINE INSEMINATION

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Abstract:

Objective: *To use sperm wash swim up technique as a diagnostic tool in Intra Uterine Insemination.*

Design: *Male semen data was recorded for 181 couples who chose to undergo IUI procedures at the Bangladesh Institute of Research & Rehabilitation in Diabetes, Endocrine & Metabolic Disorders (BIRDEM) Centre for Assisted Reproduction (CARE) from 2013-2016. Couples were selected for IUI based on initial complaints of primary or secondary subfertility. The female partner had at least patent one tube based on hysterosalphingogram or laparoscopy results. Polycystic ovarian syndrome and minimal to mild endometriosis cases were included. Male factor includes sexual dysfunction and moderate sperm abnormalities.*

Statistical Analysis: *Baseline and stratified analyses were conducted using semen data form.*

Results: *There were 181 IUI cycles done in total. 126 couples (70%) suffered from primary sub fertility while 55 couples (30%) suffered from secondary subfertility. The baseline findings show that morphology of samples is improved by nearly 28%, while rapid linear motility improves by 50% after the first wash and has less variation after the second wash. Concentration, rapid linear motility, and morphology, all have strengthened parameters with each progressive wash process (*$p < .0001$, $p < .0001$, *and* $p = .0004$, *respectively).*

Conclusions: *The result of our study provides some interesting observations in the treatment modalities for male infertility. Relatively low cost, less time consuming Swim up sperm wash technique can improve the quality of semen in a great extent.*

Kewords: Sperm enhancing technique, Intra uterine inseminating

Bangladesh J. Urol. 2017; 20(1): 36-41

Introduction:

Male factor infertility has become the single largest defined cause of infertility with an estimation of up to fifty percent. Sperm count is the most widely used parameter of semen analysis. Couples with male factor infertility as their main cause of difficulty in conceiving achieve fair results in term of pregnancy rates after undergoing treatment with IUI, IVF or ICSI cycle. Improvement in the methods of sperm preparation, which gives higher fertilization rates, would greatly improve the outcome.

The development of the intrauterine insemination (IUI) procedure has been monumental in family planning for couples that suffer from primary and secondary subfertility.[1] IUI increases pregnancy rates 2-3 times more than other insemination methods because it increases gamete density near the site of fertilization in

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comparison to intravaginal and intracervical insemination.[2,3] Therefore, IUI remains the most widely-accepted Artificial insemination (AI) technique in modern clinical practice. IUI is also easily adaptable in technology-limited medical settings thus making it a reliable assisted reproduction method in low and middle-income countries.[4]

Before insemination, semen from the male specimen must be thoroughly processed to optimize outcome. Sperm preparation methods, such as direct layering and gradient separations, were standardized in 1949, but since then adjustments to protocol have been made to improve IUI outcomes.[5] While sperm wash research has been done in developed countries since the 1990s, there has been minimal reporting on the specific technique and general IUI topics in South Asia and none in the context of Bangladesh. Indian IUI research has primarily been exploratory on establishing general protocols and feasibility of IUI in technology-limited contexts.[4,7] The only study on relevant sperm wash methods in India does not report a significant relationship between temperature control and conception and parameter outcomes.[6,8] A Pakistani study specifically on swim-up techniques shows an association between using the technique and an increase in sperm concentration, motility, and conception rates.[9] There have not been any studies done on this technique in India or any other South Asian countries. Based on regional differences in exposures that affect semen quality, it is important to contribute to this body of research to standardize IUI protocol in South Asian contexts. The research question is: Does second-wash 'swim-up' method in comparison to solely one wash cycle improve semen parameters for IUI patients? The study exposure was identified as using the 'swim-up' method during IUI wash process, and the subsequent outcome of interest is heightened semen parameters.

Different modalities have been used for couples where the main cause of subfertility is a male factor problem. Intrauterine insemination is the most widely applied treatment in infertility practice. It is considered as less stressful, less expensive and also less invasive compared to other modalities such as IVF or ICSI.

Often costs play an important role in deciding which treatment option to pursue in the management of infertile couples. Therefore, the choice of the treatment is frequently dictated by financial consideration.

From this descriptive analysis an attempt was undertaken: 1) to use sperm wash swim up technique as a diagnostic tool to improve the quality of semen sample. 2) by using these tool to develop appropriate guidelines for the patient selection especially in low resource setting.

Methods:

Subjects

Male semen data was recorded for 181 couples who chose to undergo IUI procedures at the Bangladesh Institute of Research & Rehabilitation in Diabetes, Endocrine & Metabolic Disorders (BIRDEM) Centre for Assisted Reproduction (CARE) from 2013-2016. Couples were selected for IUI based on initial complaints of primary or secondary subfertility. The female partner had at least one patent tube based on hysterosalphingogram or laparoscopy results.Polycystic ovarian syndrome and minimal to mild endometriosis cases were included. Male factor includes sexual dysfunction and moderate sperm abnormalities. Repeated procedures for the same couple were regarded as independent trials for study purposes.

Semen collection and Analysis: A semen sample is collected through masturbation, directing the sample into a clean container after 3-5 days of abstinence.Specimen is mixed well, taking care to avoid formation of bubbles. With the aid of a rod a small drop is placed in the centre of the lower disc of Meckler chamber. Cover glass is grasped opposite two dark points and placed on the four tips, looking again for the appearance of the color firings. This ensures the automatic spread of the drop in to a thickness of 10 microns.By using a count of x20 objective and x10 eyepiece after focusing, the stage of the microscope is moved to locate the grid in the centre of the view area. Motility evaluation is done within 3-5 minutes after application of the sample to avoid errors due to tendency of sperm to migrate from periphery. All non-motile sperms within 9 or 16 square are then counted. Then we count the motile sperms in the same area and estimate the grades of motility +1 to +4.This procedure is repeated in another area of the grid and the average is calculated. Then sperm heads within the square of the grid are counted in the same way as blood cells are counted in haemocytometer.In cases where the number of sperms is substantial, the number of the heads is counted in a strip of 10 squares. This number represents their concentration in millions per ml.

Selection of Semen Processing Techniques:

Total motile sperm in Pre wash semen:

Calculation of Total Motile Sperm –

a. Note down volume of semen after liquefaction of semen.
b. Semen count / ml.
c. Total count = Vol x Count/ml
d. Motility % = 100x non motile sperm Count/ml
e. Total Motile sperm = Total sperm count x motility % 100

Sperm preparation for IUI:

A. Sperm Wash:

The liquefied specimen is diluted with 4-5 ml of buffer media and then centrifuged at 270 g to 300 g for 10 minutes.

B. Swim –Up procedure:

0.5 ml of semen under 2 ml of medium is put in culture tubes and incubated for one hour at 37º C.At the end of this period,the upper 80% of medium containing the motile cells were withdrawn, and one or two cycles of centrifugation (500 g for 5 min) to separate the small traces of seminal plasma will result in completion of the preparation procedure. The sperm pellet is incubated for 30 min in order to allow the vigorously motile spermatozoa to capacitate.The spermatozoa are assessed following the swim- up procedure from semen followed by one cycle of centrifugation and re-suspension gives a higher quality preparation than when the spermatozoa are centrifuged twice before swim –up is performed.

IUI Procedure:

IUI is done in a well-lighted room without anesthesia.Women are placed in lithotomy position after emptying of bladder with slight Trendlenberg position.The count and motility of the processed sample is assessed with Meckler chamber.The processed sample is collected from the laboratory area after confirming the identity of the sample by cross checking the clinician's note and the laboratory technician's note as well as the label of the test tube.The clinician washes hands up to the forearms with an antiseptic soap, ideally wears cap/mask and powder free gloves for the insemination.The vulva is cleansed with normal saline. The cervix is exposed with a bi-valve specimen and the external os cleansed gently with cotton wool balls soaked in normal saline.An insemination catheter is attached to a 1ml syringe and used in draping up the prepared sperm sample. All dead spaces are removed from the catheter.Prepared sperm sample (0.5ml) is inseminated into the uterine cavity using a very thin fine flexible IUI catheter.Care is taken not to avoid bleeding during transfer.Patient is allowed to lie down for 15 – 20min and then ready to go home.Pregnancy test is performed 16 days after the IUI if menstruation has not started.

Statistical Methods

SAS 9.4 statistical software was used for all data analysis. Baseline data was found for pre-wash, post-wash, and final IUI semen characteristics and later stratified by primary and secondary couple subfertility. Median and standard deviation (SD) were expressed for normal variables, while median and interquartile ranges (IQR) were computed for non-normal variables. Data was then grouped by wash procedures and subfertility status for stratified analysis. One-way ANOVA parametric tests analyzed normal semen parameters, while non-parametric Kruskal-Wallis tests analyzed the non-normal parameters. *P*-values <.05 were considered significant associations.

Results:

There were 181 IUI cycles done in total. 126 couples (70%) suffered from primary subfertility while 55 couples (30%) suffered from secondary subfertility. The baseline findings show that morphology of samples is improved by nearly 28%, while rapid linear motility improves by 50% after the first wash and has less variation after the second wash. Concentration, rapid linear motility, and morphology all have strengthened parameters with each progressive wash process ($p < .0001$, $p < .0001$, and $p = .0004$, respectively). The volume used in the final IUI sample was about half of the initial volume obtained via masturbation. It is interesting to note that concentration and standard deviation actually decreased in all the samples after the initial wash step, but increased substantially in the final sample. These trends were also observed after stratifying the data by primary and secondary subfertility. There are progressive changes in quality of semen parameters after the first wash. (figure –I)

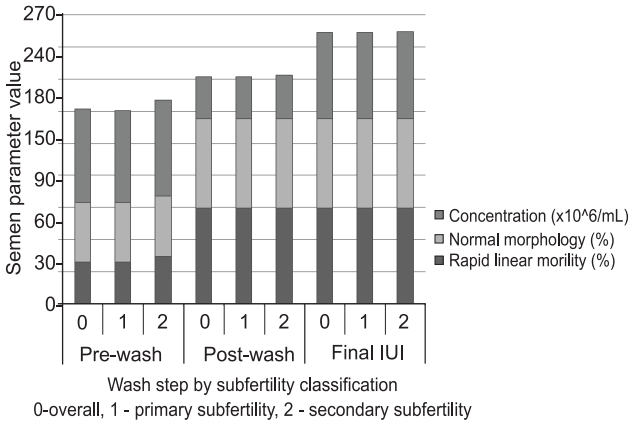

Fig.-1. *Quality of semen parameters in each progressive wash cycle for overall study population and within stratified subfertility groups*

Table-I

Baseline characteristics of semen pre- and post-wash (n = 181) during sperm preparation for IUI, BIRDEM Centre for Assisted Reproduction, Dhaka, Bangladesh, 2013-2016

Parameters*	Pre-Wash	Post-Wash 1	Final IUI Sample?
Volume (mL), mean ± SD	2.4 ± 1.4	–	1.1 ± 0.7
pH, median (IQR)	7.2	–	–
Concentration (, n			
86.8 ±	39.4 ±	80.9 ± 51.1	
(%), mean ± SD	48.7	21.4	
Rapid linear motility (%), median (IQR)	40 (25)	90 (10)	90 (7.5)
Normal morphology (%), mean ± SD	55.5 ± 5.7	83.5 ± 5.6	83.5 ± 5.6

*Dilution factor was 1:1 consistently for all samples
?Synonymous to Post-Wash 2 semen characteristics

A reduction in IQR is observed from wash 1 to wash 2 (-2.5) while the median value for rapid linear motility remains the same, which suggests that there is less variation in the median outcomes. This same variation was not observed after stratifying the data by primary and secondary subfertility.

Table-II

Stratified analysis of semen pre- and post-wash by couples diagnosed with primary and secondary subfertility

	PRIMARY SUBFERTILITY n = 126			SECONDARY SUBFERTILITY n = 55				
Parameters	Pre-Wash	Post-Wash 1	Final IUI Sample	Pre-Wash	Post-Wash 1	Final IUI Sample	p-values* (wash)	p-values (fertility?)
Volume (mL), mean ± SD	2.3 ± 0.5	–	1.1 ± 0.9	2.6 ± 2.4	–	1.0	0.777	
pH, median (IQR)	7.2	–	–	7.2	–	–	–	–
Concentration , n (%), mean ± SD	85.8 ± 50.6	39 ± 22.5	80.8 ± 53.2	89 ± 44.5	40.3 ± 18.7	81.2 ± 46.5	<.0001	
Rapid linear motility (%), median (IQR)	40 (25)	90 (10)	90 (10)	45 (25)	90 (5)	90 (5)	<.0001	
Normal morphology (%), mean ± SD	55.1 ± 5.4	83.3 ± 6.4	83.3 ± 6.4	56.5 ± 6.4	83.9 ± 3.3	83.9 ± 3.3	.0004	

*Significance deemed if ??<.05

?Final IUI Sample data was used to compare significance of fertility status on parameter outcomes

Discussion:

The purpose of sperm separation is to separate the sperms from seminal plasma, which is known to contain substances which inhibit capacitation and therefore prevent fertilization[10]. Few methods are available for separation of sperm namely, swim up, direct layering and gradient separation. Of these swim up with direct layering and gradient separation technique are the most popular. There is debate going on regarding which one is the best that lead to many controversies and encourage scientists to do more work in this field. The selection of sperm incase of swim up is based on sperm motility but density gradient centrifugation selects cells according to their density, which is also closely related to viability for sperm. It signifies that only highly motile sperms are able to reach the supernatant medium during the swim up technique until equilibrium for cell dispersion is reached while less motile spermatozoa can be separated by the gradient separation technique.

The value of intrauterine insemination in treating couples with male factor still remains questionable, even though a number of reports have been published in which pregnancies were achieved with a total sperm count of 1- 5 X 10[6.11]. There have been reported that a threshold number of approximately $1x10^6$ motile sperm is required to achieved a pregnancy [12].

In amulticentre trial comprising of the use of invitro fertilization (IVF) and intrauterine insemination (IUI) showed both techniques were suitable for couples suffering from primary subfertility due to oligzospermia, asthenozospermia or teratozospermia[13]. IVF yields higher pregnancy rates than intrauterine insemination for most indications. However, no significant differences were noted for male factor infertility without asthenozospermia. It has been reported that intrauterine insemination gives better result in male infertility cases due to oligozospermia and/or asthenozoospermia[14].

Patricia et al - concluded from their study that ovarian stimulation followed by intrauterine insemination in couples with male subfertility may increase the probability of conception as compared to ovarian stimulation followed by timed intercourse. The effective cumulative pregnancy rate of different methods of treatment of male infertility were seen and it was concluded that intrauterine insemination played an important role in the management of male subfertility. However, intrauterine insemination in male infertility is only recommended for moderate sperm disorder cases.[15]

The present knowledge, in male immunological infertility cases,is that treatment with ovarian stimulation and intrauterine insemination is a valuable first choice method to use before starting the more invasive and expensive as assisted reproduction technique (ART)[16].

It is already established that the recovered motile sperm is the deciding factor for semen quality and subsequently pregnancy outcome in male infertility.By using sperm wash swim up technique, number of recovered motile sperm is identified and can be used to select the appropriate modalities of treatment without wasting much time and money.

In a low resource setting like our country where the financial burden plays an important role in deciding the management protocol, early intervention by using recovered motile sperm number could be good diagnostic tool. However, we haven't followed up the cases for pregnancy outcome and also we did not compare the different methods of ART. There are limitations in this study. A large multicenter prospective study with different modalities of treatment would be of great help.

Conclusion:

The result of our study provides some interesting observations in the treatment modalities for male infertility. Relatively low cost, less time consuming Swim up sperm wash technique can improve the quality of semen to a great extent. They are particularly relevant when attempting to make a decision as to what would be the most ideal treatment approach especially in low resource setting.

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