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Compare the effectiveness of sperm preparation methods for Intrauterine insemination (IUI)

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Abstract: Intrauterine insemination is the deliberate introduction of semen into a female's cervix for the purpose of achieving a pregnancy through fertilization by means other than copulation. Contrary to IVF/ICSI methods, IUI is easy to perform, inexpensive and offers particular advantages such as the minimal equipment required, an easy technique to learn, being less invasive with a reduced psychological burden on the couple when compared to IVF/ICSI. Subsequently IUI has a good couple compliancy (low drop-out rate), a low risk for OHSS (ovarian hyper stimulation syndrome) and a low multiple pregnancy rate in natural cycles and clomiphene citrate or low-dose HMG (human menopausal gonadotrophins) ovarian stimulation protocols (Ombelet et al., 2008).

The present study aimed to compare pregnancy rates for couples undergoing intrauterine insemination (IUI) under different preparation conditions. , found that patients with successful IUI had significantly better sperm concentration and motility when compared with failed IUI. As regards the relation between pregnancy outcome and the sperm preparation parameters, we noted that couples with successful pregnancy had significantly higher frequency of abstinence prior to insemination, higher frequency of preparation time less than 60 minutes and higher frequency of gradient method of semen preparation.

Introduction

Artificial insemination has been used to treat infertile couples for almost 200 years. Intrauterine insemination (IUI) is now performed for several reasons. The cut-off level of semen parameters in predicting the likelihood of successful IUI is still unequivocal (Speroff and Fritz Marc, 2005). It is not determined which parameter of semen is essential for diagnosis in couples who will benefit from IUI (Van Weert et al., 2005).

Some pregnancy will occur after IUI even with sever male factor. Clinicians need tests that identify which sub-fertile couples are likely to benefit from IUI (Van Weert et al., 2004).

The effectiveness of IUI depends mainly on semen quality, which is assessed by the total motile sperm count (TMSC) and sperm morphology. TMSC in the ejaculate is the product of multiplying the semen volume by the sperm concentration by the percentage of progressively motile sperms. The best results are achieved when the number of TMSC exceeds a threshold of approximately 10 million (Speroff and Fritz Marc, 2005).

Sperm morphology is another factor that may influence the IUI result. Most studies have found a strong correlation between sperm morphology and the IUI result. In assessing sperms morphology by strict criteria, success rates with IUI are highest when 14% or more of the sperm have normal morphology, like the results observed in vitro fertilization (IVF) cycles (Lee et al., 2002).

The basic premise of any sperm preparation technique is to (a) eliminate any factors detrimental to fertilization, (b) to block factors such as prostaglandins that would otherwise cause uterine contractions, (c) to increase sperm concentration, motility and (d) to form sperm capacitation. This is to be achieved by separating the seminal plasma from spermatozoa in a rapid and efficient fashion (Mortimer, 1994).

Patients and methods.

The present study is a retrospective study. It was conducted at Mansoura Integrated Fertility Center.

The study comprised 200 couples undergoing intrauterine insemination. They were selected to participate in the study on the basis of the following criteria:

Inclusion criteria

Normal sperm count and motility according to the World Health Organization criteria (World Health Organization, 1992) and normal morphology according to the Kruger's criteria (Kruger et al., 1988).

- Mild male infertility (an average of more than 10 million motile sperm before preparation and average of more than 18 million motile sperm after semen processing for insemination).
- Hormonal profiles (serum FSH, serum LH, serum E2, serum Prolactin, serum TSH, serum T3, serum T4) on the third day of the cycle were within normal.

Exclusion criteria

Partner with a severe male factor (sperm concentration < 10 million /mL, motility < 25%, or normal forms < 15%).

Sperm preparation

Semen was obtained from male partners of couples who were undergoing IUI for treatment of infertility. The specimen was collected by masturbation into a sterile jar after 2–4 days of sexual abstinence. After liquefaction at room temperature, the semen samples were examined under a microscope to define their characteristics regarding concentration and motility.

The sperm preparation technique was randomized in all patients; either "swim-up" or the "gradient" technique was used. The sperm preparation technique for each patient was choosen by the laboratory staff in order to the randomization table and the physicians conducting the insemination were blinded to which preparation technique was to be performed. Once a couple was randomized to one of the two sperm preparation techniques they remained in the same group during the entire study. The randomization results (the sperm preparation technique appointed) were revealed and evaluated after the study was completed.

The sperm sample was centrifuged at 500 g for 15 min. The supernatant was discarded and the pellet diluted in 2.5 ml of medium and re-centrifuged. After removing the supernatant the final pellet was gently covered with medium and incubated for one hour at 37°C in an incubator (Shahrzad et al., 2009).

Swim up method

The swim up is the most common technique used in IVF laboratories and is preferred if the semen sample has a normal number of good sperms (normozoospermia). By this technique, the sperms are selected on their motility and the capability to swim out of the seminal plasma. If the "direct swim up" is performed, after the fluidification of the sample, the entire volume (well mixed) is divided in fractions of 1 ml into centrifuge tubes (round bottom is preferred). 1, 3 ml of culture medium is placed over the semen with extreme attention in each tube. The tubes must be put in the incubator, inclined at an angle around 45°and incubated at 37°C for 30-60 min. By inclining the tubes at 45°, surface between the medium and the semen is increased and improved the capability of the sperms to swim out of the semen and to reach the medium. After that, the tube must be returned in the vertical position and 1 ml of the supernatant of each tube can be gently removed, aspirating the sperms from the upper meniscus downwards with a sterile pipette (Henkel et al., 2003).

Density gradient centrifugation

This is the preferred technique to select the greater number of motile spermatozoa in cases of severe oligozoospermia, teratozoospermia or asthenozoospermia. In this method, good quality sperms can be separated from dead sperms, leukocytes and the other components of the seminal plasma by a density discontinuous gradient. Cells with different density and motility can be selected during the centrifugation by the colloidal silica coated with silane of the gradient; the sperms with high motility and good morphology are at the bottom of the tube, finally free from dead spermatozoa, leukocytes, bacteria and debris. After the centrifugation, most of the supernatant must be gently removed and the pellet is placed into a new, clean tube; here, the pellet is well resuspended in 5 ml of medium to remove the density gradient medium. It is centrifuged at 200g for 10 minutes. At the end of the centrifugation, the supernatant is removed and 5 ml of new medium are added (Zini et al., 2000).

- **Mode of insemination**

The sperm suspension can be deposited in the cervix, the uterus, the peritoneum or the Fallopian tube. IUI is by far the most common method. It is performed by introducing a 0.2–0.5 ml sperm suspension into the uterus with a small catheter, usually without imaging guidance. With Fallopian tube sperm perfusion (FSP), the inseminate is 4 ml, so that with this large volume of fluid the inseminate may fill not only the uterine cavity and Fallopian tubes, but also some of the volume may even end up inside the peritoneal cavity (Cantineau et al., 2013).

Timing of insemination

Insemination can be done at various time points around ovulation and can be done once or several times. In the majority of the published studies, the insemination is done 32–36 h following HCG administration. It is assumed that the timing of insemination relative to ovulation is critical for an optimal success rate, so it is rather surprising that few studies were designed to find the optimal time for insemination (Ragni et al., 2004).

A systematic review found no difference in the pregnancy rate per couple with two inseminations compared with one (Cantineau et al., 2003).

Insemination procedure and detection of pregnancy

Intrauterine insemination was performed using an intrauterine catheter: a Soft-pass insemination catheter (Cook,J-SPI-068015, Indiana, USA) or a Frydman classical catheter with a 1- or 2-ml syringe. The catheter was gently passed through the cervical canal and the sperm suspension

expelled into the uterine cavity. Insemination volumes ranged from 0.5 to 2 ml. All techniques were carried out using sterile procedure. The IUI was performed with the patient in the dorsal lithotomy position. The women remained supine for 10–15 min after IUI. After insemination, each patient received progesterone daily (400 mg vaginal or rectal suppository or 100 mg intramuscular), followed as the same dosage after pregnancy for 6 – 12 weeks. IUI were performed at a private center for assisted reproduction, Mansoura Integrated Fertility Center, (MIFC), (Mansoura, Egypt)

Statistical analysis

Data obtained from the present study were computed using SPSS versions 17 under the platform of Microsoft Windows XP, Professional Edition. Continuous data were expressed in the form of mean ± SD while categorical data were expressed in the form of count and percent. Comparisons of continuous data were performed utilizing student t test, while categorical data were done using Chi-square test. P value less than 0.05 was considered statistically significant.

Results

Table 1: Sperm parameters in the studied groups

	Range	Mean ± SD
Before preparation		
Concentration(× 106/ml)	10.0 - 120.0	26.4 ± 18.7
Motility (%)	25.0 - 70.0	37.6 ± 8.4
Normal (%)	20.0 - 75.0	39.6 ± 8.3
After preparation		
Concentration(× 106/ml)	9.0 - 100.0	22.3 ± 15.2
Motility (%)	30.0 - 75.0	44.6 ± 8.2
Normal (%)	28.0 - 80.0	47.8 ± 8.3

This table shows the sperm parameters before and after semen preparation.

Table 2: Comparison between females with failed and successful pregnancy regarding the sperm parameters

	Successful pregnancy (n=30)	Failed pregnancy (n=170)	Student t test	
			t	P
Before preparation				
Concentration	50.6 ± 37.5	29.0 ± 14.9	2.1	0.0013*
Motility	50.3 ± 13.9	36.5 ± 7.0	3.7	0.0018*
Normal	36.6 ± 13.8	40.7 ± 7.9	-1.1	0.28
After preparation				
Concentration	42.2 ± 30.7	24.3 ± 11.9	2.2	0.0042*
Motility	56.3 ± 13.9	43.4 ± 6.9	3.5	0.003*
Normal	43.6 ± 13.5	49.2 ± 8.26	-1.55	0.14

This table shows that women with successful pregnancy had significantly better sperm concentration and motility before and after preparation when compared with failed pregnancy.

Table 3 Comparison between women with failed and successful pregnancy regarding the sperm preparation parameters

		Successful pregnancy (n=30)	Failed pregnancy (n=170)	Chi-square test	
				X^2	P
Previous abstinence	+	22	60	15.2	0.0001*
	-	8	110		
Preparation time	≤ 60 min.	28	127	5.1	0.024*
	> 60 min.	2	43		
Preparation method	Gradient	23	77	10.0	0.002*
	Swim up	7	93		

This table shows that couples with successful pregnancy had significantly higher frequency of abstinence prior to insemination, higher frequency of preparation time less than 60 minutes and higher frequency of gradient method of semen preparation.

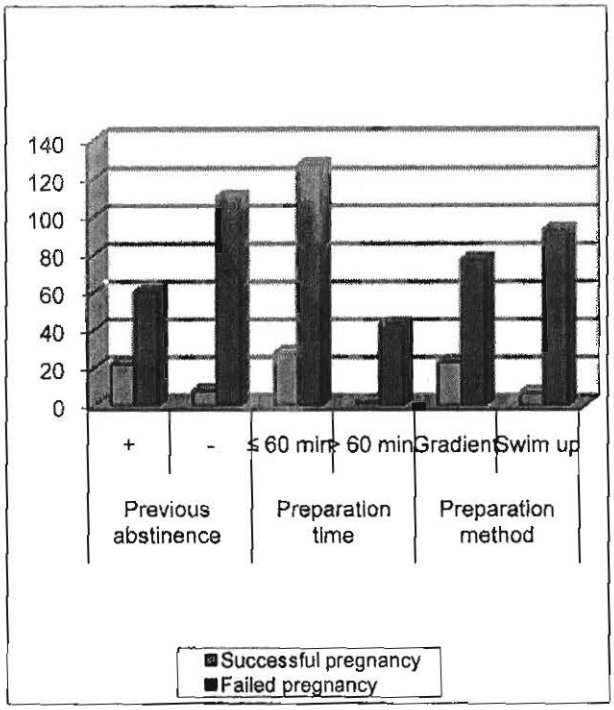

Fig. (1) Comparison between semen preparation parameters in women with successful and failed pregnancy.

Discussion

In our study, comparison between patients with failed and successful pregnancy regarding the sperm parameters, found that patients with successful IUI had significantly better sperm concentration and motility when compared with failed IUI. This is line with the study of Guven et al., (2008) who aimed to identify the predictors of pregnancy rate (PR) among women undergoing intrauterine insemination (IUI) cycles. Two hundred thirty-two women undergoing 255 IUI cycles were retrospectively evaluated according to clinical and semen characteristics in terms of PRs. The PR increased in accordance with the total motile sperm count before sperm preparation.

As regards the relation between pregnancy outcome and the sperm preparation parameters, we noted that couples with successful pregnancy had significantly higher frequency of abstinence prior to insemination, higher frequency of preparation time less than 60 minutes and higher frequency of gradient method of semen preparation.

This is in agreement with the study of Marshburn et al., (2010) who found that An ejaculatory abstinence period of≤ 2 days before IUI produced the highest pregnancy rates per cycle compared with longer intervals of ejaculatory abstinence. This higher conception rate occurred despite a lower total number of motile spermatozoa inseminated.

Also, the study of Fauque et al., (2014) aimed to determine the impact of the time interval from the end of sperm preparation (TSP) to intrauterine insemination (IUI) on the outcome. Eight hundred sixty-two IUI cycles (709 patients) managed by gonadotropins were studied. Cycles were stimulated by either FSH or hMG, and hCG was administrated when the leading follicle diameter measured >15 mm. IUIs were performed ~36 hours after ovulation triggering. Generalized linear mixed models for binary outcomes were used to model clinical pregnancy (CP) to assess the effect of TSP adjusted for other predictors (such as maternal age, semen quality, and indication of IUI treatment). The TSP effect was significant, featuring an inverse U-shaped curve admitting an optimum interval of ~40–80 minutes improving CP compared with other values. Other significant predictors were total motile spermatozoa inseminated, maternal age, and unexplained infertility.

In another study, Koyun et al., (2014) aimed to determine whether semen parameters (concentration, motility) were affected by the interval between the onset of postwash sperm incubation and intrauterine insemination (IUI) time. Semen specimens of 100 normozoospermic men collected at the clinic were allowed 20 minutes for liquefaction at room temperature. Semen samples were subjected to both macroscopic and microscopic examinations. After centrifugation in a density gradient column and sperm-washing medium, the samples were kept in an incubator. After 30 minutes, 60 minutes, and 120 minutes, the concentration and motility were recorded. According the results of the Bonferroni post hoc test, there were significant differences in values of mean sperm count, percent progressive sperm motility, and total motile sperm count between 30 minutes and 120 minutes ($p=0.000$, $p=0.000$, and $p=0.000$) and between 60 minutes and 120 minutes ($p=0.000$, $p=0.000$, and $p=0.001$), but there was no significant difference between 30 minutes and 60 minutes ($p=1$, $p=0.173$, and $p=1$). This study demonstrated that sperm parameters are negatively affected from prolonged incubation time. A maximum 60-minute limit of the interval between the onset of postwash sperm incubation and IUI time may increase pregnancy rates.

Moreover, the study of Karamahmutoglu et al., (2014) compare the efficacy of gradient and swim-up semen preparation techniques on pregnancy rates in couples undergoing intrauterine insemination (IUI) cycles with low dose gonadotropin stimulation with the diagnosis of unexplained or mild male subfertility. Two hundred and twenty three couples were randomized into swim up or gradient technique groups for sperm preparation. The clinical and ongoing pregnancy rates per cycle and per patient were evaluated. Both clinical and ongoing pregnancy rates per cycle were significantly higher in the "gradient" group (19 %and 16.9

%) in comparison with the "swim up" group (9.7 % and 6.9 %) ($p<0.05$). Clinical pregnancy and on-going pregnancy rates per patient were higher in the "gradient" group (26.1 % and 23.4 %) when compared to the "swim up" group (15.2 % and 10.7 %), ($p<0.05$). In the subgroup of 191 unexplained subfertile couples with 290 cycles; the "gradient" group also revealed significantly higher clinical and ongoing pregnancy rates per cycle (21.6%and 17.9 %) when compared with the "swim up" group (10.3 % and 7.1 %) ($p<0.05$).

Conculsion

Our study showed that couples with successful pregnancy had significantly higher frequency of abstinence prior to insemination, higher frequency of preparation time less than 60 minutes and higher frequency of gradient method of semen preparation. We propose that a "golden standard" for optimal results is using gradient method with less than 60 minutes for sperm preparation.

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مقارنة فعالية أساليب إعداد الحيوانات المنوية للتلقيح داخل الرحم

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التلقيح داخل الرحم هو إدخال متعمد لسائل المنوي الزوج إلى عنق الرحم الأنثى لغرض تحقيق الحمل عن طريق وسائل أخرى غير الجماع. وخلافا لأساليب الإخصاب داخل سيتوبلازم البويضة والتخصيب في المختبر، التلقيح داخل الرحم من السهل القيام بها، وغير مكلف، ويوفر الحد الأدنى من الأجهزة المطلوبة، وهي تقنية سهلة للتعلم، وكذلك أقل في التجهيز مع تخفيض العبء النفسي على الزوجين عند مقارنتها مع الإخصاب داخل سيتوبلازم البويضة والتخصيب في المختبر.كما أن التلقيح داخل الرحم له مخاطر منخفضة لمتلازمة فرط تحفيز المبيض وانخفاض معدل الحمل المتعدد في الدورات الطبيعية وبروتوكولات تنشيط المبيض تتم فيه بجرعة منخفضة من عقار سترات الكلوميفين أو عقار موجه للغدد التناسلية (HMG). هدفت الدراسة الحالية لمقارنة معدلات الحمل للأزواج بواسطة التلقيح داخل الرحم (IUI) في ظل ظروف إعداد مختلفة للسائل المنوي. ، وجدنا أن المرضى الذين لديهم زيارة في تركيز الحيوانات المنوية والحركة يكون لديهم نجاح في التلقيح داخل الرحم من أصحاب التركيزات والحركة الاقل . وفيما يتعلق بالعلاقة بين نتائج الحمل ومعلمات إعداد الحيوانات المنوية، لاحظنا أن الأزواج مع حمل ناجح امتنعوا عن الجماع لفترة أطول ، وكذلك زيادة نسب النجاح مع وقت تحضير للسائل المنوي اقل من ٦٠ دقيقه ، وكذلك مع استخدام طريقة التدرج لإعداد المني.