Effect of Postprepared Sperm Parameters and Insemination Specimen Volume on the Outcome of Intrauterine Insemination

Meng-Yin Tsai, Yung-Chieh Tsai, Jong-Chou Chang, Fu-Tsai Kung, Jik-Fuu Wu, Shiuh-Yang Chang, Ling-Cheng Yang*

***Background*: The purpose of the present study was to identify the postprepared sperm parameters affecting the outcome of intrauterine insemination and to find out whether the volume of insemination specimen was a determinant factor in the rate of successful conception.**
***Materials and Methods*: A retrospective study including 306 couples was designed. The patients were inseminated with either 1.0 or 0.5 ml of prepared specimens. The pregnancy rates were compared using the chi-square test. Logistic regression was chosen for multivariant analysis of the parameters.**
***Results*: The only parameter significantly affecting the success rate was the postprepared sperm motility (p=0.033). The pregnancy rate was 27.91% in cases with ≥95% sperm motility. Only two patients with less than 75% sperm motility conceived. The pregnancy rates in cases with 0.5 ml and 1.0 ml inseminations were 12.12% and 16.13%, respectively. This difference was statistically insignificant (p= 0.427).**
***Conclusion*: The postprepared sperm motility was the only parameter predicting the successful rate of intrauterine insemination. Seventy-five percent sperm motility can be used as a cut-off value for selecting patients. The volume of insemination specimen did not influence the outcome. Insemination with 1 ml of fluid was just as effective as insemination with 0.5 ml.**
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Key words: parameters, volume, intrauterine insemination, motility.

Intrauterine insemination has long been used as a competent and economic method for the treatment of cervical factor, immunologic factor, mild male factor and unexplained infertility. However, the efficacy of this treatment varies in different reports. In the 17 different series reviewed by Allen et al., the pregnancy rate ranged from 3.4% to 62%.[1] The wide range of the success rate could partly reflect a great heterogeneity in patient selection and different techniques of insemination and sperm preparation. Although many "guidelines" have been published to assist physicians in choosing couples who are suitable for the procedure, most of these recommendations are controversial or even contradictory.[2-12] The characteristics of semen, especially semen after preparation, are widely used to predict the outcome of insemination.[2-8, 10-15] Some of the parameters, including sperm motility, sperm morphology, and total motile sperm count have been suggested in several studies as predictors of the pregnancy rate. Conversely, sperm concentration surprisingly has displayed no correlation to the successful rate in most of the reports despite a high correlation to the total motile sperm count.

Regardless of the method of sperm preparation, the prepared specimens are usually diluted before insemination. The final volume varies at different centers. Although it is usually between 0.2 and 0.5 ml,[2,6,7,15-18] up to 4 ml of insemination specimen has been reported.[19-23] The physiology of sperm transportation in the uterine cavity is

From the Department of Obstetrics and Gynecology, Department of Anesthesiology*, Chang Gung Memorial Hospital, Kaohsiung.
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Address for reprints: Dr. Meng-Yin Tsai, Department of Obstetrics and Gynecology, Chang Gung Memorial Hospital. 123 Ta-Pei Road. Niaosung Hsiang, Kaohsiung, Taiwan, R.O.C. Tel.: 886-7-7317123; Fax: 886-7-7322915.

poorly understood. Naturally, only 83 to 180 μL of uterine fluid sustains thousands of spermatozoa at midcycle.[24] Whatever method we use in artificial insemination, the situation is obviously different. Millions of spermatozoa are suspended in a relatively large volume of fluid in this situation. A study of the physiologic change of the uterine cavity under this condition could be an interesting and challenging topic. However, what we clinicians are concerned about is whether or not a change in the volume and the concentration of the insemination fluid alters the pregnancy rate. In addition, disregarding volume and concentration, does any parameter play a critical role in determining the outcome? In order to answer these questions, we designed the following study.

MATERIALS AND METHODS

Patients

From July 1995 through September 1996, 306 couples who underwent intrauterine insemination in our hospital were enrolled in this study. All the couples completed an infertility work-up which included hysterosalpingography, basal body temperature, and hormone assay for the women and semenalysis for the men before undergoing intrauterine insemination. Laparoscopy was also performed in patients suspected of having tubal disease or pelvic endometriosis. All the patients accepted controlled ovarian hyperstimulation in the therapeutic cycle. Folliculometry was performed on day 11 of the cycle and was repeated if it was necessary to follow-up on the size of the follicles. Human chorionic gonadotropin (HCG) 10,000 IU was given when two of the dominant follicles grew to be 1.6 cm in diameter. Insemination was performed 30 to 38 hours after HCG injection.

The indications for intrauterine insemination varied among our patients. They included cervical factor, immunologic factor, ovulatory factor, mild male factor and mild peritoneal factor. Patients with a tubal factor, moderate or severe male factor, or moderate or severe endometriosis were excluded. These factors were believed to be detrimental to the outcome[25,26] and could have caused bias in our study. The "American Fertility Society Revised Classification of Endometriosis" was used to define moderate and severe pelvic endometriosis. Patients were defined as having a moderate and severe male factor if the total motile sperm count (TMSC) was less than 1 million or the normal morphologic sperm were less than 40% in the postprepared specimens. These criteria were set according to previous reports[4,6] and our own data. The number of the follicles was one of the factors to affect the outcome.[12,15,26] In order not to cause bias, we only chose patients with 2 to 6 dominant follicles (>1.5 cm in diameter) for the study.

Preparation of semen

The semen was prepared using a modified Percoll gradient method.[27] Briefly, Percoll (Pharmacia, Milwaukee, WI, USA) was diluted with human tubal fluid (HTF) medium to concentrations of 95% and 47.5%. Layered discontinued gradients of Percoll were prepared in a 16 x 125 mm tissue culture tube (Falcon Plastics, CA, USA) by carefully pipetting 1.5 ml of 95% and 47.5% Percoll consecutively. After layering the semen on top, the tube was centrifuged at 1350 rounds per minute (rpm) for 20 minutes. The supernatant of the top 2 layers was pipetted off. Only the bottom layer remained. After adding 11 ml of HTF medium, the tube was washed and centrifuged at 1250 rpm for 15 minutes twice. The supernatant was discarded. The pellet was finally suspended in either 0.5 ml or 1 ml HTF medium with 0.3% bovine serum albumin, ready for insemination. Semenalysis was conducted before and after the preparation.

Insemination

Couples were randomly inseminated with either 0.5 ml or 1 ml of prepared semen. The catheter used for insemination was a TDT catheter (Laboratoire, C.C.D., Paris, France).

Luteal phase support and pregnancy detection

The luteal phase was supported by micronized progesterone 400 mg per day. Urine β-HCG was tested 2 weeks after the insemination. The micronized progesterone was continuously given until 10 weeks of gestational age if the patient successfully conceived. The pregnancy was confirmed by sonography which demonstrated a gestational sac at 6 weeks of gestational age.

Data analysis

All statistical analysis was performed using the PC version of the Statistical Package for the Social Sciences (SPSS-PC). Patients were classified into two groups according to the volume of insemination fluid. The difference of the prepared sperm parameters in the two groups was compared using Student's t-test. Multiple variables, including the volume of insemination fluid, postprepared sperm concentration, motility, morphology,

percentage of forward progressive sperm, total motile sperm count, and the age of the female patients as independent variables and outcome as a noncontinuous dependent variable were analyzed using logistic regression. The chi-square test or Fisher's exact test, where appropriate, was used to compare various proportions, including the pregnancy rates of the 0.5 ml insemination group and the 1.0 ml insemination group.

RESULTS

The data of 306 patients were collected. Fifty-five couples that did not fulfill the criteria were excluded. Of the remaining 251 patients, 66 patients were inseminated with 0.5 ml of prepared specimen, and the other 185 were inseminated with 1.0 ml. Table 1 presents the causes of infertility in our couples. They were classified into 2 groups according to the volume of insemination fluid. The distribution in both groups showed no significant difference.

Table 2 demonstrates the means of postprepared sperm parameters and the pregnancy rates in both groups. They displayed significant difference in concentration which was directly influenced by the volume of dilution, but

Table 1 Indications for Insemination of the Different Groups

Indication for	No. of patients inseminated with 0.5 ml fluid (%)	No. of patients inseminated with 1.0 ml fluid(%)	*p* value
Male factor	13 (19.70)	35 (18.92)	0.890†
Mild or minimal endometriosis	20 (30.30)	52 (28.11)	0.735†
Anovulation	13 (19.70)	47 (25.41)	0.351†
Cervical or immunologic factor	4 (6.06)	14 (7.57)	0.464*
Combined male & female factors	3 (4.55)	8 (4.32)	0.588*
Unexplained infertility	13 (19.70)	29 (15.67)	0.452†
Total	66(100)	185(100)	

*= Fisher's exact test, †= Chi-square test.

Table 2 Postprepared Semen Profiles of the Different Insemination Groups

Semen profiles	0.5 ml insemination group	1.0 ml insemination group	*p* value
Concentration (Ø)	46.24±35.81	31.17±22.50	<0.001*
Morphology (%)	51.22± 6.64	55.81±11.49	0.120*
Motility (%)	86.39±10.37	86.60±10.69	0.874*
Progressive motility (%)	52.24±18.75	55.81±11.49	0.228*
TMSC (Ø)	21.28±17.83	26.97±22.03	0.052*
Pregnancy rate (%)	8/66 (12.12)	30/185 (16.21)	0.427†

Abbrev: TMSC= total motile sperm count
Ø = million / ml; *=t- test, †= X^2 test, $X^2 = 0.635$, p= 0.427, df = 1

Table 3 Logistic Analysis of the Postprepared Sperm Parameters and the Clinical Outcome

	OR	95% C.I.	*p* value
Concentration	0.774	(0.810, 0.740)	0.268
Motility	1.883	(1.996, 1.776)	0.033*
Total motile sperm count	1.000	(1.001, 0.999)	0.208
Progressive motility	0.896	(1.136, 0.710)	0.363
Morphology	1.015	(1.052, 0.978)	0.437
Volume	1.224	(4.446, 0.336)	0.759
Age of female patients	0.933	(1.042, 0.834)	0.219

Abbrev: OR= odds ratio; C.I.= confidence interval; p= probability
*=significant difference

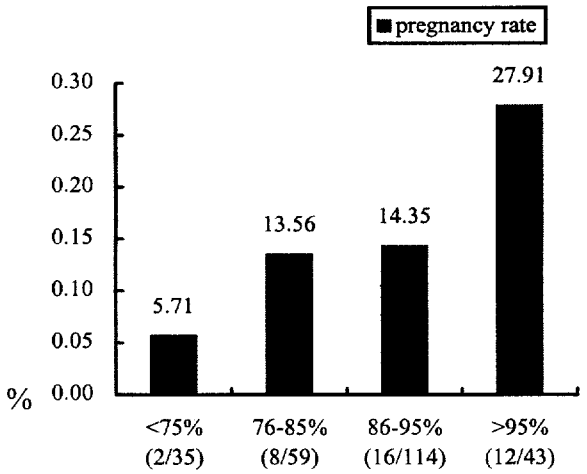

Fig. 1 Pregnancy rate and distribution of motility
* Number in parentheses indicate number of pregnancies/total number of couples; X^2 test = 8.1, p=0.044, df=3

correspondence in the others. The pregnancy rates were 12.12% in the 0.5 mL group and 16.13% in the 1.0 ml group. The difference was not statistically significant (p= 0.427).

The result of logistic regression is illustrated in Table 3. All but one parameter showed no significant correlation with the clinical outcome. The only one that displayed positive correlation was postprepared motility (p= 0.033). The distribution of motility is further stratified and presented in Figure 1. In comparison with the other 3 groups, the patients with less than 75% sperm motility had the worst outcome. Only two couples in the group conceived. The pregnancy rate was 5.71%. On the other hand, patients with over 95% sperm motility demonstrated the best result. This group achieved a 27.91% pregnancy rate.

DISCUSSION

There were two aims of this study. The first one was to identify the prognostic parameters of prepared semen with regard to the successful rate of intrauterine insemination. The second good was to determine whether or not the volume of insemination fluid influenced the outcome.

Logistic regression analysis revealed that postprepared motility was the only parameter affecting the clinical outcome (Table 3). By stratifying its distribution, the pregnancy rate was 27.91% in the group with ≥95% and 5.71% in the group with < 75% motility. Only two couples with less than 75% sperm motility were successful in conceiving. This result is compatible with several previous studies.[2-5,10] However, unlike some other reports,[8,14,15,28,29] we failed to prove any significant correlation between the pregnancy rate and the total motile sperm count. We believe there are two reasons for this. First, the total motile sperm count may not be an independent variant. It is equal to volume times concentration times motility. It could just reflect the combining effect of the three parameters. Since concentration and volume are not determinant factors in our study, the total motile sperm count reveals no correlation either. Second, two recent studies have mentioned that the total motile sperm count can only disturb the pregnancy rate if it is very low. The critical level was 0.8×10^6 in one study[4] and 1.0×10^6 in the other.[6] Since we eliminated cases with a TMSC of less than 1.0×10^6, it is no wonder that no statistical significance was displayed.

Traditionally, most physicians use 0.2 to 0.5 ml of specimen for insemination. With this volume, the physician can easily inject the prepared semen into the uterine cavity without causing reflux or patient discomfort.[1] However, Sahmay et al. have indicated that insemination with up to 1 ml of specimen can effectively augment the uterine activity which is helpful for sperm transportation without inducing patient discomfort.[30] Using a tight cervical cap and controlling the injection rate, Fanchin et al. successfully inseminated 4 ml into the uterine cavity without regurgitation.[31] Nevertheless, the efficacy of large volume insemination is controversial. Some studies suggested that it could improve the pregnancy rate,[19-21] but some others did not.[22,23] We have tried two different volumes for insemination in this study, 0.5 ml and 1 ml. The pregnancy rate was 12.12% with 0.5 ml and 16.13% with 1 ml. The difference was not statistically significant (p=0.427). This implies that insemination with 1.0 ml was just as effective as insemination with 0.5 ml.

In summary, the only important factor affecting the outcome of intrauterine insemination was postprepared sperm motility in this study. Insemination with up to 1 mL of specimen did not improve the pregnancy rate. However, further studies are needed to determine whether insemination with more than 1 ml would or would not have an influence on the outcome.

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精液洗滌後精蟲係數與體積對人工受精結果之影響

蔡孟穎 蔡永杰 張榮州 龔福財 吳介甫 黃富仁 張旭陽 楊寧正*

背景：本研究之目標，乃希望發現精蟲洗滌後各項係數，包含注射體積和人工受精結果的關係。
方法：本研究是一含306對不孕夫婦之回溯性研究。患者行人工受精時隨機地注射0.5或1.0毫升的洗滌後精蟲，然後以卡方檢驗比較這兩組的懷孕率的優劣。另外並以多變數複迴歸方式，取得其他各項精蟲係數和臨床結果的關係。
結果：惟一和臨床結果有相關的係數是洗滌後精蟲的活動力，其 p 值為 0.0331 。在活動力大於95%時懷孕率達27.91%，小於75%者中則只有2人懷孕。注射0.5毫升和1.0 毫升的懷孕率分別是12.12%及16.13%，無統計學上的差別，p 值是 0.427 。
結論：洗滌後精蟲的活動力是惟一可以預估成功率的係數。75% 的活動力可做為一篩選標準，以下者懷孕率極低，應考慮以其他方式治療。注射1.0毫升的洗滌後精蟲和注射0.5毫升者其臨床結果一樣好，並無顯著差異。
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長庚紀念醫院 高雄院區 婦產系，麻醉科*
受文日期：民國86年11月6日；接受刊載：民國87年3月19日。
索取抽印本處：蔡孟穎醫師，長庚紀念醫院 婦產系。高雄縣鳥松鄉大埤路123號。Tel.：(07) 7317123；Fax：(07) 7322915