EFFECTS OF SPERM MORPHOLOGY AND TOTAL MOTILE SPERMATOZOA NUMBER ON THE RATE OF PREGNANCY THROUGH ARTIFICIAL INSEMINATION

XUAN-CHENG MAI, LEI DING, YONG-FANG XU, PENG CENG, DAN TAO*
Department of Reproductive Medicine, the first people's Hospital of Yunnan Province, China

ABSTRACT

***Objective**: To investigate correlation of pregnancy rate through intrarauterine insemination (IUI) with the ratio of normal sperm and total motile spermatozoa number (A and B).*

***Methods**: A retrospective study was carried out with the IUI data of 330 cycles between January 1st and August 31st in 2017 to compare the effects of the ratio of normal sperm and total motile spermatozoa number (A and B) on the pregnancy rates of IUI.*

***Results**: a) For patients with normal sperm ratio less than 6%, their clinical pregnancy rate of IUI was significantly different from those with normal sperm ratio between 6% and 10%, or not less than 10% (p<0.05); b) during the treatment cycle, the clinical pregnancy rates of patients whose total motile spermatozoa numbers were not less than 10×106 and those whose total motile spermatozoa numbers were less than 10×106 were 21.6% and 9.2% before and after ovulation, and the difference had statistical significance (p<0.05); for those whose total motile spermatozoa numbers before ovulation were not less than 10×106, and after ovulation were less than 10×106, their pregnancy rate was 14.8%, which was also significantly different from those rates above (p<0.05).*

***Conclusion**: a) A high clinical pregnancy rate can be attained in IUI for those whose ratio of normal sperm is not less than 6%; b) IUI requires that seminal fluid should contain at least 10×106 sperms in total motile spermatozoa number, and the inseminations before and after ovulation are similar in importance.*

***Keywords**: Sperm morphology, total motile spermatozoa number, IUI, clinical pregnancy rate.*

DOI: 10.19193/0393-6384_2018_3_135

***Received** November 30, 2017; **Accepted** January 20, 2018*

Introduction

Intrarauterine insemination (IUI) is a simple but effective assisted reproductive technology for infertility caused by factors of males, including sexual dysfunction, retrograde ejaculation and idiopathic oligoasthenospermia, and female factors, including immune factors, ovulation failure and endometriosis[1, 2]. Compared with other complicated assisted reproductive technology (in-vitro fertilization and intracytoplasmic sperm injection), IUI is excellent for its non-invasive, operability, low cost and proximity to the natural pregnancy, and has been accepted by the patients. However, IUI is inferior in the pregnancy rate.

Therefore, in this study, a retrospective study was performed for the outcomes of IUI of a total of 330 cycles between January 1st and August 31st in 2007 to figure out the correlation of clinical pregnancy rate of IUI with the ratio of normal sperms and the total motile spermatozoa number (A and B) after treatment, and the detailed information is reported as follow:

Data and methods

Clinical data

IUI data of a total of 330 cycles between January 1st and August 31st in 2017 were obtained in accordance with the inclusion criteria: Before treatment, male and female subjects were required to fulfill the examinations according to the requirement of Ministry of Health. Before treatment, contraindications of artificial fertilization had already been excluded. For female subjects, natural ovulation or ovulation induction was adopted; before ovulatory cycle, laparoscope, hysteroscope or uterotubography was carried out to examine the tubal patency, and those who had unobstructed oviduct on at least one side; endocrine examination and chromosome test revealed no anomalies, and no genital malformation, myoma of uterus or endometriosis was identified. For male subjects, they were required to withdraw all medications; after treatment, they regular analysis of seminal fluid showed normal concentration and activity of sperms, and the forward movement sperm number exceeded 10×106. All parameters were analyzed by computer, and averaged as the results.

Analysis of sperm

Sperm analysis and treatment

After 3 to 7 days of abstinence, seminal fluid was collected into a septic container through masturbation, and following adequate liquidation at room temperature, regular analysis of the seminal fluid was analyzed routinely. Sperms were separated through the density gradient centrifugation, and seminal fluid was mixed into the upper layers of 80% and 40% density, followed by centrifugation at 1500 g for 15 min. Then the supernatant was abandoned, and 1 mL SAGE-1005 was dripped in the sediment and well mixed. Thereafter, centrifugation at 1500 g was performed for 5min with the supernatant being discarded. In the sediment, 0.4 to 0.5 mL HTF was added, and the sperm was preserved in a 5% CO_2 incubator for artificial fertilization.

Analysis of the sperm morphology

Before treatment of seminal fluid, the liquidated seminal fluid collected from the same subject was spread on two clean glass slides, followed by drying at room temperature and improved Pap staining. Analysis was carried out in strict accordance with the standards of *WHO laboratory manual for the examination and processing of human semen* (5^{th} edition).

Ovulatory induction and IUI

Normal ovulation

Normal ovulation was adopted for patients with normal period.

Ovulatory induction protocol and detection of follicle

Ovulatory induction was carried out for patients with ovulation failure, irregular menstruation, abnormal follicular development or those who failed in pregnancy after IUI in natural period. In ovulatory induction protocol, clomiphene (CC), letrozole (LE) and human menopausal gonadotrophin (HMG) were used, and when the diameter of follicle reached 18 mm, 10000 U of hCG was administrated through intramuscular injection, followed by IUI after 24 h or 36 h.

IUI

With patients in lithotomy position, uterine neck was exposed. Then, 1 mL injector in connection with non-invasive catheter was used to draw 0.4 to 0.5 mL sperm suspension, and then the catheter was slightly injected into the cervix. Sperm suspension was then injected within 2 to 3 min, and the catheter was slowly removed. Afterwards, subjects were required to stay in supine position with hip being lifted for 30 min.

Luteal support

All subjects received the luteal support, so as to promote the transition of endometrium from the proliferative stage into the secretory stage, and from the ovulatory day, progesterone at a dose of 40 mg was injected intramuscularly per day for a total of 15 d.

1.3.5 Pregnancy evaluation

At 14 d after IUI, examination of the β-hCG in urine or blood was performed to determine the pregnancy, and ultrasound B examination was carried out at the 4th week after IUI. Patients with gestational sac, vitelline sac or cardiac impulse were deemed as clinical pregnancy.

Statistical analysis

Original data in this study were analyzed by SPSS 13.0. Chi-square test was performed for comparison of the rates among groups, and $p<0.05$ suggested that the difference had statistical significance.

Results

Effect of sperm morphology on the pregnancy rate of IUI

For patients with normal sperm ratio less than 6%, their clinical pregnancy rate of IUI was significantly different from those with normal sperm ratio between 6% and 10%, or not less than 10% ($p<0.05$; Table 1).

Group (Ratio of sperms in normal morphology	Treatment cycles	Pregnancy cycles	Clinical pregnancy rate in single cycle (%)
1 (<6%)	82	7	8.5
2 (6%～10%)	130	20	15.4
3 (≥10%)	65	12	18.5

Table 1: Effect of sperm morphology on the pregnancy rate of IUI.

Effect of total motile spermatozoa number on the pregnancy rate of IUI

During the treatment cycle, the clinical pregnancy rates of patients whose total motile spermatozoa numbers were not less than 10×10^6 and those whose total motile spermatozoa numbers were less than 10×10^6 were 21.6% and 9.0% before and after ovulation, and the difference had statistical significance ($p<0.05$); for those whose total motile spermatozoa numbers before ovulation were not less than 10×10^6, and after ovulation were less than 10×10^6, their pregnancy rate was 14.8%, which was also significantly different from those rates above ($p<0.05$; Table 2, 3).

Group (Total number of sperms in Grade A and B)	Treatment cycles	Pregnancy cycles	Clinical pregnancy rate in single cycle (%)
1 (≥20×10⁶)	37	9	21.6
2 (10×106～20×10⁶)	32	6	18.8
3 (5×106～10×10⁶)	25	3	12
4 (<5×10⁶)	41	3	7.3

Table 2: Effect of total number of sperms (A and B) on the clinical pregnancy rate of IUI.

Group (Total number of sperms in Grade A and B)	Treatment cycles	Pregnancy cycles	Clinical pregnancy rate in single cycle (%)
1 (≥10×10⁶)	106	23	21.7
2 (≥10×10⁶ fertilization before ovulation and <10×10⁶ fertilization after ovulation)	54	8	14.8
3 (<10×10⁶ fertilization before ovulation and ≥10×106 fertilization after ovulation)	57	8	14
4 (<10×10⁶)	109	10	9.2

Table 3: Effects of the total number of sperms in Grade A and B before and after ovulation on the pregnancy rate of IUI in a single cycle.

Discussion

IUI is a kind of assisted reproductive technique to inject the sperms with strong motility gained through in-vitro treatment through non-sexual method into the uterus of females in ovulatory stage where sperm binds to the follicle, so as to realize the pregnancy, which conforms to the normal physiological process of reproduction but has the advantages with low cost, mini-invasion and operability.

There are various indications for IUI, including:

a) infertility caused by male conditions, such as oligozoospermia, asthenospermia, abnormal liquefaction of semen or genital deformity;

b) infertility caused by female conditions, like anomaly in secretion of cervical mucus, deformity of genital tract or psychological factors;

c) immune factors; d) unknown factors.

IUI is conducive to the capacitation of sperm without any adverse effect of cervical factors on the movement of sperm, thus shortening the distance of sperm, and increasing the success rate of pregnancy. For patients with infertility (oligozoospermia or asthenospermia), acquirement of the sperms with strong motility from the seminal fluid is the key link in pregnancy through IUI. Treatment of seminal fluid can remove the factors adverse to the fertilization, reduce the prostaglandin, immunocompetent cells, anti-sperm antibodies, bacteria or fragments, and minimize the viscosity of seminal fluid, thus to promote the capacitation of sperm and improve the fertilization ability. Therefore, sperms with high activity are enriched to attain a density that conforms to the requirement of fertilization, and sperms in a higher density can bind to the follicle in oviduct, thus increasing the pregnancy rate. Hence, quality of seminal fluid that has received the in-vitro treatment is closely correlated with the pregnancy rate[3, 4].

Routine analysis of seminal fluid (including sperm density, active ratio of sperm and motility of sperm) serves as the major examination for evaluating the fertility of males. Seminal fluid is affected severely by various factors, and no available method has yet been developed to assess the fertility of males exactly. In clinical practice, fertility of males is usually evaluated just by the comprehensive analysis of the seminal fluid. Sperm morphological analysis has gradually recommended as one of the indicators in evaluating the fertility of males, and initially, classification of sperm morphology was firstly put forward by Macleod et al[5] in 1951.

Thereafter, many researchers have stipulated various classification methods for sperm morphology, which have also make the role of sperm morphology in male infertility more controversy. Some scholars believed that sperm morphology is significant for prediction of pregnancy rate of IUI[6-8]. The results of this study indicated that for patients with normal sperm ratio less than 6%, their clinical pregnancy rate of IUI was significantly different from those with normal sperm ratio between 6% and 10%, or not less than 10% (p<0.05)

Affected by various factors, IUI is maximally limited by the parameters of seminal fluid. Generally, to acquire the expected pregnancy rate of IUI, post total motile sperm (PTMS) should attain to a certain number, which, however, is still under debate. According to the literatures, they believed that PTMS>10×106/mL can achieve a high clinical pregnancy rate of IUI[9]. Nevertheless, *Guidelines for Human Assisted Reproductive Technique* stipulates that sperms for injection in artificial fertilization should exceed 10 million.

The aim of this study is to explore the correlations of the pregnancy rate of IUI with the PTMS (A and B) and ratio of normal sperms, thus to provide more beneficial consultation and rational options for patients. A retrospective study was carried out with the IUI data of 330 cycles between January 1st and August 31st in 2017 to compare the effects of the ratio of normal sperm and total motile spermatozoa number (A and B) on the pregnancy rates of IUI. 330 cycles of treatment were divided into four groups: PTMS<5×10^6/mL, 5×10^6/mL ≤ PTMS < 10×10^6/mL, 10×10^6/mL ≤ PTMS < 20×10^6/mL and PTMS≥20×106/mL, and the pregnancy rates of these groups were 7.3%, 12.0%, 18.8% and 21.6%, respectively. Comparisons between the first two groups and the remaining two groups showed that difference had statistical significance (p<0.05), suggesting that PTMS ≥10×106/mL can achieve an ideal pregnancy rate.

Fertilization should be performed through the combination between the follicle and sperm, and the time of IUI is a fundamental condition guaranteeing a higher pregnancy rate. Generally, ovulation occurs at about 36 h after HCG injection, and egg discharged from the follicle can only survive for 24 h. In addition to the asynchronized ovulation time, the first time of fertilization can provide the most active sperm with the first-discharged egg, while the second time can provide the following eggs with active sperms, thus increasing the pregnancy rate[10].

In this study, two times of fertilization showed that during the treatment cycle, the clinical pregnancy rates of patients whose total motile spermatozoa numbers were not less than 10×10^6 and those whose total motile spermatozoa numbers were less than 10×10^6 were 21.6% and 9.2% before and after ovulation, and the difference had statistical significance (p<0.05); for those whose total motile spermatozoa numbers before ovulation were not less than 10×10^6, and after ovulation were less than 10×10^6, their pregnancy rate was 14.8%, which was also significantly different from those rates above (p<0.05).

IUI is more economic and conforming to the physiological process of normal reproduction. IUI is usually the first choice for patients with primary infertility who are invalid for treatment, but when compared with IVF-ET and ICSI, the application range of IUI remains smaller, and the pregnancy rate is lower. Thus, for patients who have little probability in fertilization through IUI through screening in advance, they could seek for more efficient and reliable assisted reproductive technique, which can avoid the unnecessary physical damage, mental or economic burden of IUI to patients.

This study is limited by the number of cases. Furthermore, we did not investigate the effect of female factors on pregnancy rate, which might be correlated with the pregnancy rate of IUI according to some reports. These parameters are expected to be further investigated in the future studies.

References

1) Meriviel P, Heraud MHGrenier N, et al. Predictive factors for pregnancy after intrauterine insemination (IUI) : An analysis of 1038 cycles and a review of the literature. Fertil Steril, 2010, 93 (1): 79-88.
2) Nuojua-Hutttunen S, Tomas C, Bloigu R, et al. Intrauterine insemination treatment in subfertility: An analysis of factors affecting outcome. Hum Reprod, 1999, 14(3) : 698-703.
3) Guven S, Gunalp GS, Tekin Y. Factors influencing pregnancy rates in intrauterine insemination cycles. J Reprod Med, 2008, 53(4) : 257-265.
4) Van Voorhis BJ, Barnett M, Sparks AE, et al. Effect of the total motile sperm count on the efficacy and cost-effectiveness of intrauterine insemination and in vitro fertilization (J). Fertil Steril, 2001, 75(4): 661- 668.
5) Macleod J, Gojd RZ. The male factor in fertility and infertility. IV.Sperm morphology in fertile and infertile marriage (J). Fertil Steril,1951, 2(5): 394- 414.

6) Wiser A, Shalom-Paz E, Reinblatt SL, et al. Ovarian stimulation and intrauterine insemination in women aged 40 years or more. Reprod Biomed Online, 2012, 24(2): 170-173.
7) Bonnet R, Kruger TF, Franken DR. Atlas of human sperm morphology evaluation. London: Taylor & Francis Group Press, 2004. 27-34.
8) Wainer R, Albert M, Dorion A, et al. Influence of the number of motile spermatozoa inseminated and of their morphology on the success of intrauterine insemination(J). Hum Reprod, 2004, 19(9): 2060- 2065.
9) Yavuz A, Demirci O, S zen H, et al. Predictive factors influencing pregnancy rates after intrauterine insemination. Iran J Reo-rod Med, 2013, 11(3): 227-234.
10) Lee RK, Hou JW, Ho HY, et al. Sperm morphology analysis using strict criteria as a prognostic factor in intrauterine insemination(J). International Journal of Andrology, 2002, 25(5): 277-280.

Corresponding author
DAN TAO
Faculty of Environmental Science and Engineering,Kunming University of Science and Technology
Ophthalmology department of Kunming Children's Hospital
No. 288 Qian Xing Road, Xishan District, Kunming, Yunnan
Email: taodan1975@163.com
(China)