
GYNECOLOGY

The Predictive Value of Post Processing Total Motile Sperm Count of Semen on the Success of Intrauterine Insemination

Ukris Towised, M.D.*,
Chatchai Treetampinich, M.D.*,
Matchuporn Sukprasert, M.D.*,
Wicharn Choktanasi, M.D.*,
Chonthicha Satirapod, M.D.*.

* Department of Obstetrics and Gynaecology, Faculty of Medicine Ramathibodi Hospital, Mahidol University, Bangkok 10400, Thailand

ABSTRACT

Objectives: To study the predictive value of post-processing total motile sperm count (TMSC) on pregnancy rate in intrauterine insemination (IUI).

Materials and Methods: Medical records were reviewed from August 2013 to January 2015. All unexplained infertile women or anovulation less than or equal to 35 years old, and initially treated with IUI were included. Small myoma, adenomyosis, adenomyoma, endometrial thickness less than 6 mm at 2-3 days before IUI and patients with previous pelvic surgery were excluded. The predictive values of post-processing TMSC parameters were analyzed regarding pregnancy outcomes with a receiver operating characteristic (ROC) curve.

Results: The overall pregnancy rate was 9.5% for all 218 couples with 314 IUI cycles. The cause of female infertility was unexplained for 75.16% of the sample population with anovulation at 24.84%. All parameters including duration of infertility, number of dominant follicles, largest dominant follicle diameter and endometrial thickness were unrelated to pregnancy outcomes. The ROC showed sensitivity, specificity, positive predictive value and negative predictive value of post-processing TMSC at 20x106/mm, with pregnancy outcomes of 50, 45, 48, and 47%, respectively.

Conclusion: Post-processing TMSC was an unreliable predictive factor for IUI outcome.

Keywords: total motile sperm count, pregnancy outcome, intrauterine insemination.

Correspondence to: Wicharn Choktanasi, M.D., Department of Obstetrics and Gynaecology, Faculty of Medicine Ramathibodi Hospital, Mahidol University, Bangkok 10400, Thailand , E-mail: jajaja2545@gmail.com

การทำนายความสำเร็จของการฉีดอสุจิเข้าไฟฟ์ฟองมดลูกด้วยจำนวนอสุจิที่เคลื่อนที่หลังการเตรียมน้ำอสุจิ

อุกฤษฎ์ ติวิเศษ, ฉัตรชัย ตรีธรรมพนิจ, มัชชุพร สุขประเสริฐ, วิชาญ โชคธนัศริ, ชลธิชา สถาระพจน์

บทคัดย่อ

วัตถุประสงค์: เพื่อศึกษาความสัมพันธ์ระหว่างจำนวนอสุจิที่เคลื่อนที่หลังการเตรียมน้ำอสุจิและการตั้งครรภ์ของการฉีดอสุจิเข้าไฟฟ์ฟองมดลูก

วิธีดำเนินการวิจัย: เก็บบันทึกข้อมูลจากเวชระเบียนผู้ป่วยระหว่าง ลิงหาคม 2556 จนถึง มกราคม 2558 ผู้ป่วยที่มีอายุน้อยกว่า 35 ปี มีบุตรยากโดยไม่สามารถหาสาเหตุได้หรือภาวะไม่มีการตกไข่ จำนวนวิเคราะห์ผล ส่วนคนที่มีความผิดปกติของตัวมดลูกและรังไข่ เช่น เนื้องอกกล้ามเนื้อมดลูก เยื่อบุไฟฟ์ฟองมดลูกเจริญผิดที่ หรือ ผนังมดลูกบางกว่า 6 มิลลิเมตร ก่อนทำหัตถการ ฉีดอสุจิเข้าไฟฟ์ฟองมดลูก 2-3 วัน หรือ เคยมีประวัติการผ่าตัดด้วยวิธีซึ่งกรานมาก่อน จะถูกนำออกจากงานวิจัย การทำนายความสำเร็จของการฉีดอสุจิเข้าไฟฟ์ฟองมดลูกด้วยจำนวนอสุจิที่เคลื่อนที่หลังการเตรียมน้ำอสุจิโดยใช้ receiver operating characteristic (ROC) curve

ผลการวิจัย: คุณสมรรถที่มีบุตรยากทั้งหมด 218 คู่ ทำการฉีดอสุจิเข้าไฟฟ์ฟองมดลูกทั้งหมด 314 รอบ อัตราการตั้งครรภ์เท่ากับร้อยละ 9.5 (30/314) สาเหตุมีบุตรยากของฝ่ายหญิงแบ่งเป็น ไม่สามารถหาสาเหตุได้ร้อยละ 75.16 ภาวะไข่ไม่ตกร้อยละ 24.84 ปัจจัยต่างๆ อาทิ เช่น ระยะเวลาการมีบุตรยาก จำนวนของฟอลลิเคิล ขนาดของฟอลลิเคิลที่ใหญ่ที่สุด หรือ ความหนาของเยื่อบุไฟฟ์ฟองมดลูกนั้น ไม่มีความสัมพันธ์กับการตั้งครรภ์โดยมีค่าความไว, ความจำเพาะ, ค่าพยากรณ์ผลบวก, ค่าพยากรณ์ผลลบ ของจำนวนอสุจิที่เคลื่อนที่หลังการเตรียมน้ำอสุจิต่อการตั้งครรภ์ที่ $20 \times 106/\text{mm}$ เท่ากับร้อยละ 50, 45, 48 และ 47 ตามลำดับ **สรุป:** จำนวนอสุจิที่เคลื่อนที่หลังการเตรียมน้ำอสุจิใช้ทำนายการตั้งครรภ์ใน การฉีดอสุจิเข้าไฟฟ์ฟองมดลูกได้ไม่ดี **คำสำคัญ:** จำนวนอสุจิที่เคลื่อนที่หลังการเตรียมน้ำอสุจิ, ผลลัพธ์ของการตั้งครรภ์, การฉีดอสุจิเข้าไฟฟ์ฟองมดลูก

Introduction

Intrauterine insemination (IUI) is one of the most common and cost-effective methods for infertility treatment⁽¹⁻³⁾. Reasons for IUI procedure include mild male infertility, mild endometriosis and unexplained infertility⁽⁴⁻⁶⁾. IUI treatment enhances the chance of conception by increasing the density of healthy sperm at the site of fertilization⁽⁷⁾. Numerous studies have listed the predictive independent variables for conception after standard infertility treatment including age, endometrial thickness, number of dominant follicles, type, duration and cause of infertility, and semen quality^(8, 9).

There is, however, no consensus on parameters that can reliably predict IUI outcome. A recent systematic review validated total motile sperm count (TMSC) as an important predictor of pregnancy by IUI⁽¹⁰⁾. However, the cut-off level of TMSC in predicting the likelihood of the pregnancy is still unequivocal^(10, 11). The minimum TMSC after preparation recommended for IUI varied from 0.8-10 million⁽¹²⁻¹⁴⁾. Independent predictions for pregnancy outcome after IUI suggested that cycles with under 10 million TMSC were significantly less likely to result in pregnancy⁽¹⁵⁻¹⁷⁾.

This study investigated the correlation between the post-process sperm level and pregnancy outcome. The lowest level of processing sperm which resulted in pregnancy was also evaluated.

Materials and Methods

All couples undergoing IUI at Ramathibodi hospital fertility center during August 2013-January 2015 were enrolled into the database on the day of insemination for evaluation of semen quality. All had experienced primary or secondary infertility for at least 1 year. This study was approved by the Ethics Committee of the Faculty of Medicine Ramathibodi Hospital, Mahidol University.

Medical histories of all the couples were physically evaluated with a pelvic examination, transvaginal ultrasonography and semen analysis.

A total of 218 couples underwent 314 IUI cycles. Baseline semen analysis was normal following the 2010 World Health Organization (WHO) parameters⁽¹⁸⁾. The couples underwent IUI with ovulation induction or superovulation using clomiphene citrate, an aromatase inhibitor or gonadotropin injection. Inclusion criteria were primary unexplained infertility or anovulation with normal semen analysis. Couples were excluded from analysis if the woman was over 35 years old, had a previous history of uterine surgery, a uterine myoma, adenomyosis, stage 3 or 4 endometriosis, endometrium thickness less than or equal to 6 mm 2-3 days before IUI or the donor sperm from IUI was frozen.

Oral or injected medication was started at day 2 or 3 of the menstrual cycle for superovulation, or at day 5 of the cycle for ovulation induction. Mature follicles were evaluated by transvaginal ultrasonography at day 10-14 of the cycle. Human chorionic gonadotropin (hCG) of 5,000-10,000 units was injected 38-42 hours before IUI, when the transvaginal ultrasound revealed the largest follicle with a mean diameter of greater than or equal to 15 mm.

The males were recommended to follow abstinence for 2-4 days prior to collection of the sperm sample after masturbation in a room at the fertility clinic. The fresh sperm was allowed to liquefy before semen analysis, following WHO 2010 except for the morphology parameter. The semen was then processed using the density gradient technique. Finally, the processed samples were analyzed according to WHO 2010 before IUI, except for the morphology test.

Semen preparation was performed with the density gradient centrifugation technique at room temperature. 1.5 ml of 40% Sil-Select Stock solution (FertiPro N.V., Belgium) was dispensed into a sterile conical-bottomed tube and underlay 1.5 ml of 90% Sil-Select Stock solution. The semen was gently overlaid on a two-layer discontinuous gradient. The tube was centrifuged at 350 x g for 10 minutes. The

supernatant was removed and the remaining pellet was transferred to a fresh tube containing 1.5 ml of FertiCult Flushing medium (FertiPro N.V., Belgium) and then centrifuged at 300 x g for 5 minutes twice. The last pellet was resuspended by transferring to a fresh tube containing 1 ml of FertiCult Flushing medium.

Insemination was performed using a soft catheter with the patient remaining supine in the dorsal lithotomy position for at least 10 minutes after insemination. A urine pregnancy test was performed 13-16 days after IUI.

Anovulation was diagnosed by history of oligomenorrhea or no premenstrual symptom or transvaginal ultrasonography not seen dominant follicle in the mid cycle or transvaginal ultrasonography not seen corpus luteum in luteal phase.

We compared mean of baseline characteristics and semen parameter. T-test was used to analysis of continuous data between pregnant and non-pregnant groups. P value was statistical significance if < 0.05 .

Statistical Analysis

All statistical analyses were performed using SPSS version 17 (SPSS, Inc., Chicago, IL).

Results

A total of 218 couples underwent 314 IUI

Table 1. Baseline characteristics of pregnant and non-pregnant groups.

	Pregnant (30)	Non-pregnant (284)	p value ^a
Age (years)*	31.4 ± 2.2	31.7 ± 2.2	0.49
Duration of infertility (year)**	2.5 (1-8)	2.8 (1-10)	0.43
Number of dominant follicle **	2 (1-3)	2 (1-3)	0.65
Diameter of largest dominant follicle (mm)*	18.3 ± 4.6	19.5 ± 3.4	0.11
Endometrium thickness (mm)*	8.4 ± 1.9	8.9 ± 1.7	0.10

* Data are presented as mean \pm standard deviation,

** Data are presented as median (range),

^a = T-Test

cycles (1 cycle in 150 couples, 2 cycles in 50 couples, 3 cycles in 11 couples, 4 cycles in 5 couples, 5 cycles in 1 couple and 6 cycles in 1 couple). The cause of female infertility was 75.16% unexplained and 24.84% from anovulation. Overall pregnancy rates were 9.5% (30/314), with 21 singletons, 3 twins, 3 abortions and 3 antenatal care (ANC) at other hospitals.

Baseline characteristics including age, duration of infertility, number of dominant follicles (DF), diameter of largest DF, and endometrium thickness were not significantly different between the pregnant and non-pregnant groups (Table 1). The pregnancy rates were 9.16, 17.64, 8.33, and 10.00% following the different ovarian stimulation protocols for clomiphene citrate, gonadotropin, an aromatase inhibitor, and clomiphene citrate with a gonadotropin cycle, respectively (Table 2). All pre- and post-processing semen analysis parameters were compared for pregnancy outcome. No statistical significance was recorded for all semen parameters in terms of pre-processing volume, post-processing concentration, pre/post processing, and TMSC on the pregnancy outcome (Table 3).

The receiver operating characteristic (ROC) (Fig. 1) gave 50% sensitivity, 45% specificity, 48% positive predictive value and 47% negative predictive value when the cut-off for post-processing TMSC was 20 million.

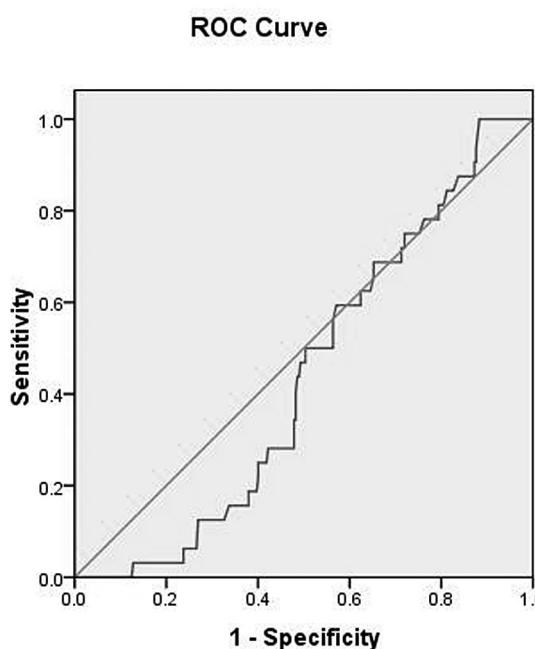
Table 2. Protocol of ovarian stimulation.

	N	Pregnancy	Pregnancy (%)
Clomiphene citrate (CC)	251	23	9.16
Gonadotropin injection (Gn)	17	3	17.64
Aromatase inhibitor (AI)	36	3	8.33
CC + Gn	10	1	10.00

Table 3. Comparison of pre- and post-processing semen parameters between pregnant and non-pregnant groups.

	Pregnant (30)	Non-pregnant (284)	p value ^a
Pre vol (ml)	2.4 ± 0.9	2.7 ± 1	0.06
Pre conc (mil/ml)	2.5 (1-8)	2.8 (1-10)	0.43
76.2 ± 35	78.6 ± 37.7	0.74	0.65
Pre motility (%)	61.4 ± 12.3	62.6 ± 12.4	0.61
Pre TMSC (mil)	103.8 ± 51	130 ± 76.9	0.06
Post conc (mil/ml)	52.3 ± 20.2	57.3 ± 28.8	0.36
Post motility (%)	95.6 ± 2.8	95.2 ± 4.3	0.55
Post TMSC (mil)	20.4 ± 7.9	23.5 ± 12.9	0.20

Data are presented as mean ± standard deviation,

^a = T-Test**Fig. 1.** The receiver operating characteristic (ROC) curve of post-processing total motile sperm count (TMSC) against pregnancy outcome.

Discussion

The pregnancy rate determined in this study was comparable with other reports^(9, 16, 19, 20). Inclusion and exclusion criteria were designed to minimize the confounding factors of age, type of infertility, number of dominant follicles and endometrial thickening⁽⁹⁾ previously reported to affect the pregnancy rate.

The best value of post-processing TMSC was 20 million, giving a high pregnancy rate. We used post-processing TMSC at 20 million because it was a mean of pregnant groups. This result was similar to previous studies^(21, 22). The lowest post-processing TMSC which resulted in pregnancy was over 8 million. Similarly, other studies reported similar post-processing TMSC values for IUI affecting the pregnancy outcome^(13, 14). A post-processing TMSC value below 5 million did not justify IUI treatment⁽²³⁾. However, only 33 cycles of post-processing TMSC below 8 million were recorded in this study; insufficient to predict a direct impact on pregnancy.

The ROC curve was not a good predictor of pregnancy outcome showing low sensitivity and low specificity. This result may be attributed to other factors including the strict morphology of sperm on the IUI day. Association of the fertilization rate with in vitro fertilization^(24, 25) and sperm morphology as a factor that may influence IUI outcome was also reported^(23, 26). These topics remain areas for future study.

Conclusion

Post-processing TMSC was not a good predictor of pregnancy outcome in IUI cycles with low sensitivity and low specificity.

Potential conflicts of interest

The authors declare no conflict of interest.

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