ORIGINAL

Effect of semen characteristics on pregnancy rate following intrauterine insemination

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Abstract : Objective : To assess the effects of semen characteristics on the success of intrauterine insemination (IUI). Design : A retrospective study. Settings : The Department of Obstetrics and Gynecology, Tokushima University Hospital, Japan. Patients : Between 2004 and 2008, 1,177 IUI cycles in 283 couples were studied. Intervention : IUI cycles were preceded with ovarian stimulation. Main Outcome Measure : Clinical pregnancy. Result : A total of 82 clinical pregnancies were obtained (7.0% pregnancy rate per cycle, 28.9% per case). Their subsequent outcomes of pregnancies were 18 miscarriages (21.9%), 2 ectopic pregnancies (2.4%) and 60 live births (73.2%). Of the 82 clinical pregnancies, 2 were twin pregnancies (2.4%). There was no triple or higher order multiple pregnancies. At the end of the sixth cycle, 73 clinical pregnancies had been achieved (89.0%). After diagnostic laparoscopy, the pregnancy rate per cycle for patients ≤35 years age was 18%, which is significantly higher than that of patients >35 years of age. Pregnancies occurred up to the fifth cycle after laparoscopy. The pregnancy rate (PR) per cycle was significantly higher in cases of sperm movement rates more than 30% (PR 9.3%) and total motile sperm counts more than 10×10^6/ml (PR 8.2%). A study comparing the washed and unwashed cases did not reveal any differences. Conclusion : In male sub-fertility cases of sperm parameters as motility rates ≥30% and motile sperm concentration ≥10×10^6/ml, IUI could be a useful option for infertility treatment J. Med. Invest. 58 : 127-133, February, 2011

Keywords **:** *Intrauterine insemination, sperm parameters, diagnostic laparoscopy*

INTRODUCTION

Intrauterine insemination (IUI) is a simple first line treatment for infertile couples (1). This inexpensive treatment, in comparison with other assisted reproductive techniques (ART), has been widely used to treat infertile couples with a variety of indications such as male subfertility, unexplained fertility, cervical mucus hostility and endometriosis-related infertility. (2-7) Pregnancy rates after IUI differ between studies according to patient selection criteria, the presence of various infertility factors, ovarian stimulation methods, number of cycles performed, different sperm parameters, and preparation

Abbreviations :
ART : Assisted reproductive technology, IUI : Intrauterine insemination, PR : Pregnancy rate, OH : Ovarian hyperstimulation, COH : Controlled ovarian hyperstimulation, CC : Clomiphene citrate, FSH : Follicle-stimulating hormone, LH : Luteinizing hormone, hMG : Human menopausal hormone, hCG : Human chorionic gonadotropin, HTF : Human tubal fluid, SSS : Serum Substitute Supplement, WHO : World Health Organization, SD : Standard deviation, TMSC : Total motile sperm count, IVF : In vitro fertilization

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technique.

IUI can be used with ovarian stimulation, which increases the number of available oocytes at the site of conception, and increase pregnancy rates than IUI alone. (8-11) Diagnostic laparoscopy is the standard procedure in diagnosing infertility. For infertile patients where pathologic abnormalities are considered, COH and IUI should be performed after the laparoscopic surgery.

In this retrospective study, we evaluate the relationships between IUI outcome and various parameters of sperm quality in relation to pregnancy rate.

MATERIALS AND METHODS

Patient selection

All patients were evaluated by physicians in the Department of Obstetrics and Gynecology, The University Hospital of Tokushima, Japan. The criteria for receiving ovulation stimulation with IUI treatment were as follows.

1. Infertility duration of at least 1 year.
2. Infertility evaluations including tube patency were confirmed by hysterosalpingography and/or laparoscopy. Laparoscopy was performed only when the hysterosalpingography or transvaginal ultrasonography revealed the possibility of pelvic adhesion or endometriosis. Other hormonal and immunological tests (serum FSH, LH, prolactin on cycle day 5, monitoring of ovulation by ultrasound and progesterone after ovulation) were also conducted.
3. Each male partner had at least one or two semen analyses. Normal semen analysis was defined by threshold values of the World Health Organization : concentration 20×10^6/ml, motility 50% and typical morphology 30%.

The medical records, including infertility duration (years), type of infertility (primary or secondary), number of spermatozoa, and number of IUI cycles per couple were reviewed. Finally, pregnancy rate and its associated factors were compared.

Ovarian stimulation and monitoring

All women in the study underwent ovarian stimulation using clomiphene citrate (Clomid, Shionogi, Japan) and/or gonadotropin (hMG, Mochida, Japan) and human chorionic gonadotropin (HCG, Mochida, Japan). For clomiphene citrate-stimulated cycles, 50 mg clomiphene citrate was given on days 5-9. For cycles managed by gonadotropins only, stimulation was started on day 5 with 75-150 IU HMG either alternate days or daily.

Cycles were monitored by transvaginal ultrasound for the mean follicular diameter and thickness of the endometrium on days 10, 12, and 14 of the cycle. To induce ovulation, a 5000 IU hCG injection was given when at least one or two follicles measured 17 mm or more. Standard IUI was scheduled 36 hours after hCG administration (10, 12).

Sperm examination and preparation

Semen samples were analyzed after complete liquefaction according to WHO guidelines. On the day of insemination, a semen specimen was collected in a sterile container following 3-5 days of sexual abstinence. After liquefaction at room temperature, initial volume, viscosity, mobility, progression, and sperm count were assessed.

Spermatozoa were prepared by conventional sperm wash technique. After liquefaction, fresh semen samples were diluted with 2 ml Human tubal fluid (HTF, NK System, Osaka, Japan) and centrifuged for 5 min. (1 ml sample to 2 ml HTF). The spermatozoa were centrifuged once more after resuspension in supplemented HTF. Supernatants were again discarded. Sperm concentration and mobility were recounted. The pellet was suspended with HTF and Serum Substitute Supplement (SSS ; Irvine Scientific Co., Santa Ana, CA, USA) into a final volume of 0.5 ml for IUI.

Insemination procedure

Intrauterine insemination was performed using an intrauterine catheter (Nipro Co., Osaka, Japan) with a 1- or 2-ml syringe, 36 ± 4 hours after hCG injection (10). The catheter was gently passed through the cervical canal and the sperm suspension slowly pushed into the uterine cavity. Insemination volume ranged from 0.5-2.0 ml. The catheter was subsequently withdrawn and the patient remained in the supine position for 15 min after insemination.

Statistical Analysis

The statistical analyses of data were done using the Excel program and statistical analyses were performed using GraphPad Prism version 5.00 for Windows (GraphPad Software, San Diego, CA, USA). Statistical significance was defined as $P < 0.05$.

RESULTS

We studied a total of 1,177 IUI cycles in 283 couples. On average, each couple underwent 4.1 (3.9 ± 0.1) IUI cycles. The patient characteristics are summarized in Table 1. Infertility was due to the female partner in 16.6% of cases, the male in 64% of cases, and unexplained infertility in 19.3% of cases. The female causes could be as follows in order of prevalence : ovulation disorder 43.1%, tubal infertility 13.7%, endometriosis 11.7%, other 31.4%. At the time of the first IUI cycle, the mean age of patients was 35.28 ± 0.12 years. Mean infertility duration was 34.03 ± 0.8 months.

As shown in Table 2, the 1,177 IUI cycles resulted in to 82 pregnancies for a 7.0% pregnancy rate per cycle (28.9% per cases). The pregnancies were divided into categories according to their subsequent outcome : 18 miscarriages (21.9%), 2 ectopic pregnancies (2.1%) and 60 live births (73.2%), and 2 unknown pregnancies (3.6%). Of the 82 clinical pregnancies, 2 were twin pregnancies (2.4%), but there were no triple or other multiple pregnancies. At the end of the sixth cycle, 73 clinical pregnancies had been achieved (89.0%) (Figure1).

If controlled ovarian stimulation (COH) with IUI

Table 1. Patient characteristics according to pregnancy rate.

Characteristic	Number of cases	Treatment cycles	Pregnancy rate	Pregnancy rate per cycle	Pregnancy rate per cases	P value
Age (years)						
< 25	5	15	2	13.3%	40%	—
25-29	26	76	6	7.9%	23%	NS
30-34	100	458	35	7.6%	35%	NS
35-39	100	405	28	6.9%	28%	NS
> 40	52	223	11	4.9%	21.1%	NS
Type of infertility						
Primary	163	688	39	5.6%	23.9%	—
Secondary	120	489	43	8.8%	35.8%	NS
Infertility duration (years)						
< 1	39	162	14	8.6%	35.9%	—
1-3	144	585	50	8.5%	38.2%	NS
3-5	56	252	10	4.0%	17.8%	NS
≥ 5	44	178	8	4.5%	18.8%	NS
Indication for IUI						
Unexplained	56	218	20	9.2%	35.7%	—
Male factor	184	757	48	6.3%	26.1%	NS
Female factor :	43	202	14	6.9%	32.5%	NS
Ovulation disorder	18	75	8	10.7%	44.4%	NS
Tubal	6	16	1	6.25%	16.7%	NS
Endometriosis	5	23	0	0%	0%	NS
Other	14	88	5	5.7%	35.7%	NS

NS=not significant

Table 2. Pregnancy rate by intrauterine insemination protocol.

Pregnancy outcome	Number of patients
Pregnancies/cases	82/283 (28.8%)
Live births	60 (73.2%)
Miscarriages	18 (21.9%)
Ectopic pregnancies	2 (2.4%)
Multiple pregnancies	2 (2.4%)

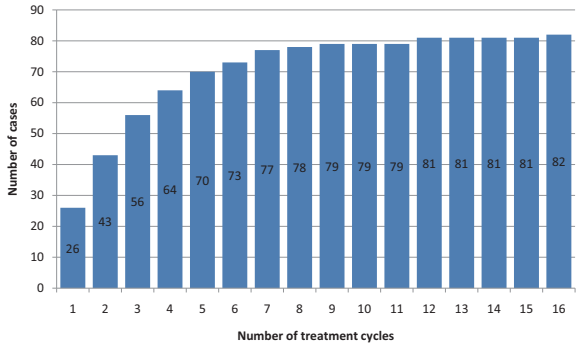

Figure 1. Accumulation of pregnancies by cycles.
At the end of the sixth cycle, 73 clinical pregnancies had been achieved (89.0%).

is unsuccessful, before shifting to the next treatment choice (i.e., ART), diagnostic laparoscopy was performed. For patients ≤35 years of age, after diagnostic laparoscopy, pregnancy rate per cycle was 18%, which is significantly higher than that of the >35 age group as this study did not include any pregnancies in the over than 35 age group. A limited number of pregnancies occurred up to the fifth cycle after laparoscopy, with the highest number of pregnancies detected at the first cycle (Table 3).

During the semen analysis, some of the cases did not undergo sperm washing as of technical difficulties (509 cycles, 37 pregnancies) as weekend practices. A study comparing the washed and unwashed cases did not reveal any differences. The success rate was not influenced by the different indications for IUI (female causes, idiopathic subfertility, male subfertility and combined problem).

Those who underwent 278 cycles of treatment did not have sperm examination records. These cycles were excluded from the sperm analysis. The mean number of spermatozoa determined in the semen analysis was 75.83±77.11 million/ml (mean±SD), with motile sperm proportion of 33.81±16.28% (mean±SD) and percentage of sperm with normal forms 12.57±12.30% (mean±SD). The TMSC was 26.25±29.30 million (mean±SD).

The relationship between sperm value and pregnancy is presented in Table 4. The pregnancy rate per cycle improved with sperm mobility rate ≥ 30% (PR 9.3%) and total motile sperm counts ≥ 10×10^6/ml (PR 8.2%).

Table 3. Comparison of pregnancy rate before and after laparoscopic surgery.

No. of treatment cycle	Before operation			After operation			
	No. of cases	No. of pregnancies	Pregnancy rate per cases %	No. of cases	No. of pregnancies	Pregnancy rate per cases %	P value
1	283	26	9.1	19	7	33.4	0.0002
2	226	15	6.6	11	1	9.1	NS
3	181	11	6.0	10	0	0	NS
4	130	7	5.5	7	0	0	NS
5	91	6	6.8	6	1	16.6	NS
6	64	2	3.2	4	0	0	—
7	38	4	7.9	1	0	0	—
8	24	0	0	1	0	0	—
9	18	0	0	0	0	0	—
≥10	63	2	3.0	0	0	0	—
Total	1118	73	6.5	59	9	15.3	

NS=not significant

Table 4. Relationship of initial sperm parameters for pregnancy rates per cycles.

Sperm parameters	Number of the cycles	Number of pregnancies	Pregnancy rate per cycle (%)	P value
Concentration (10^6/ml)				
<20.0	145	6	4.1	
≥20.0	754	55	7.3	0.1
Mobility (%)				
<30.0	392	14	3.6	
≥30.0	507	47	9.3	0.001
Total motile sperm count				
$<10\times10^6$	291	11	3.8	
$\geq10\times10^6$	608	50	8.2	0.01

DISCUSSION

Intrauterine insemination using the male partner's sperm is commonly performed to overcome factors, as well as to increase the probability of conception in diverse etiologies (5, 9, 13). Therefore, several semen parameters have been evaluated as predictors of successful outcome with intrauterine insemination. This study sought to evaluate the effects of sperm characteristics on IUI-related pregnancy and also attempted to determine good parameters to predict pregnancy rate . A wide range success in achieving pregnancy was reported IUI for various indications (9). Guzick *et al*. reported superovulation and IUI had a higher pregnancy rate (33%) than IUI alone (18%).

Herein, the pregnancy rates achieved by 1,177 cycles of ovarian stimulated IUI and associated factors are described. Favorable patient characteristics for treatment success in our sample were younger age, as well as minimal duration of infertility and male infertility factors. For semen characteristics, successful pregnancy rate per cycle was related to a sperm movement rate over 30% (PR 9.3%) and total motile sperm count higher than 10×10^6/ml (PR 8.2%). The minimum recommended number of motile sperm after preparation in a number of reports varies from 0.8-10×10^6/ml (2, 14, 15). In the present study, the number of motile sperm was 10×10^6/ml (PR 8.2%). Most authors recommend IVF when the number of motile sperm is $\leq 1\times10^6$/ml. (14-17) Some authors also report that the couples should be referred directly to IVF treatment in the presence of $< 5\times10^6$/ml motile sperm. Van Voorhis *et al*. (2) and Miller *et al*. (18) demonstrated that the processed total motile sperm count independently predicts IUI success because cycles with $< 10\times10^6$/ml are significantly less likely to result in pregnancy.

Several studies have reported threshold fertility values for IUI after sperm were processed. Other researchers found progressive mobility (19, 20) and total motile sperm count (17, 20) to be the principal predictors of pregnancy after sperm processing. Huang *et al*. (21) determined a threshold level after sperm wash of $\geq 5\times10^6$/ml total motile sperm. Francavilla *et al*. (22) reported no pregnancies when the total motile sperm count $< 5\times10^6$/ml after swim-up. Some studies did report on the pre- and post-wash value of the sperm parameters, (23-25) but did not link these to pregnancy rates. Others found no correlations (5). In the present study, during the sperm analysis, some of the sperm was not washed during the sperm analysis (509 cycle 37 pregnancies).] Therefore there were no correlations between the washed (393 cycles 24 pregnancies) and unwashed cases. However, usually prefer the washing procedure is necessary to remove prostaglandins, infectious agents and antigenic proteins, without influencing spermatozoa quality and quantity.

When patients with unexplained infertility following standard infertility screening test underwent diagnostic laparoscopy, 21-28% of these patients were found to have pathologic abnormalities, which included endometriosis and tubal disease (26, 27). Nakagawa *et al*. (28) performed diagnostic laparoscopy for unexplained infertility patients and compared the pregnancy rate following laparoscopic surgery with that following ART treatment performed in the same center for patients of differing age groups, because they obtained a significantly higher rate following laparoscopy (75%) than ART treatment (33.3%) in women 26-30 years of age. In our study for patients ≤ 35 years of age, after diagnostic laparoscopy, the pregnancy rate per cycle was 18% which is significantly higher than that of > 35 age group, since there were no pregnancies in woman > 35 years older investigated in this study.

Laparoscopy proves to be a useful tool considering the significantly higher pregnancy rate after such a procedure, in patients < 35 years of age with unexplained infertility or mild male infertility (30-50% motility rate). IUI with washed spermatozoa involves bypassing the cervical mucus barrier resulting in an increased gamete density at the fertilization site. This washing procedure is necessary to remove prostaglandins, infectious agents and antigenic proteins. This might contribute to enhanced sperm quality by reducing the formation of free oxygen radicals after sperm washing (29).

In conclusion, for male sub-fertility cases of sperm parameters as motility rates $\geq$ 30% and motile sperm concentration$\geq 10\times10^6$/ml, IUI could be a useful option for infertility treatment.

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