ORIGINAL ARTICLE
https://doi.org/10.5653/cerm.2021.04469
pISSN 2233-8233 · eISSN 2233-8241
Clin Exp Reprod Med 2021;48(3):255-261

CERM

Predictive value of sperm motility before and after preparation for the pregnancy outcomes of intrauterine insemination

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Objective: This study aimed to investigate sperm motility and its changes after preparation as predictors of pregnancy in intrauterine insemination (IUI) cycles.
Methods: In total, 297 IUI cycles from January 2012 to December 2017 at a single tertiary hospital were retrospectively analyzed. Patient and cycle characteristics, and sperm motility characteristics before and after processing were compared according to clinical pregnancy or live birth as outcomes.
Results: The overall clinical pregnancy rate per cycle was 14.5% (43/297) and the live birth rate was 10.4% (30/289). Patient and cycle characteristics were similar between pregnant and non-pregnant groups. Sperm motility after preparation and the total motile sperm count before and after processing were comparable in terms of pregnancy outcomes. Pre-preparation sperm motility was significantly higher in groups with clinical pregnancy and live birth than in cycles not resulting in pregnancy (71.4%±10.9% vs. 67.2%±11.7%, p=0.020 and 71.6% ±12.6% vs. 67.3%±11.7%, p=0.030, respectively). The change in sperm motility after processing was significantly fewer in the non-pregnant cycles, both when the comparison was conducted by subtraction (post–pre) and division (post/pre). These relationships remained significant after adjusting for the female partner's age, anti-Müllerian hormone level, and number of pre-ovulatory follicles. According to a receiver operating characteristic curve analysis, an initial sperm motility of ≥72.5% was the optimal threshold value for predicting live birth after IUI.
Conclusion: Initial sperm motility, rather than the motility of processed sperm or the degree of change after preparation, predicted live birth after IUI procedures.

Keywords: Intrauterine insemination; Pregnancy; Semen analysis; Sperm motility

Introduction

Intrauterine insemination (IUI) is considered as a treatment option in patients with unexplained infertility or mild to moderate male factor infertility. Compared with *in vitro* fertilization (IVF) and intracytoplasmic sperm injection, IUI is known to be a more simple, less invasive, and inexpensive procedure [1]. Despite its widespread use in infertility practice, there is still controversy regarding the effectiveness of IUI and its use as a first-line treatment due to its relatively low success rate [2].

Prognostic factors for IUI success have been examined in a number of studies. Factors such as the female partner's age, duration or etiology of infertility, ovarian stimulation method, number of preovulatory follicles, and sperm parameters have been suggested to determine the outcome of IUI [3-5]. Many studies have investigated semen characteristics that can predict the prognosis of IUI procedures, but they have shown conflicting results. Among semen parameters, the effects of sperm morphology by strict criteria, sperm motility, and initial total motile sperm count (TMSC) on IUI cycles have mostly

Received: February 23, 2021 · Accepted: April 6, 2021
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been studied, and cut-off values were suggested in some studies [6-11]. In addition, post-wash TMSC was also reported as a prognostic factor for pregnancy after IUI [9,11-13]. However, some studies reported that most semen parameters could not properly predict the IUI outcome [14,15].

Semen preparation techniques have been developed to separate morphologically normal motile spermatozoa from seminal plasma. It is known that preparation improves sperm quality, and the pregnancy rate can be improved accordingly [16-18]. Indeed, while motility of sperm initially collected on the insemination day varies from patient to patient, the percentage of motile sperm left after preparation generally increases and becomes relatively homogeneous. Although many studies have investigated the effect of initial and post-preparation semen parameters separately, few studies have examined the impact of changes after semen preparation on the outcomes of IUI. The purpose of this study was to examine whether changes in sperm motility characteristics during the preparation process could predict pregnancy outcomes in IUI.

Methods

1. Study population

This study included infertile couples who underwent IUI cycles at Seoul National University Hospital from January 2012 to December 2017. Cases were included only if the IUI procedure was completed and the pregnancy outcome was confirmed during this period. This study was approved by the Institutional Review Board of Seoul National University Hospital (IRB No. 2101-189-1194). This was an observation-only study using medical chart review and the need of informed consent was waived. The medical records of the subjects were reviewed retrospectively by a clinician.

The indications for IUI were mostly unexplained infertility, infertility with combined factors, and mild male factor or ovulatory factor infertility. Couples were considered eligible if the female partner had at least one patent fallopian tube and had a normal endometrial cavity. Cases with severe endometriosis (stage III or IV) were excluded from the analysis. Patients with severe male factor infertility whose TMSC was lower than 5×10^6 or those who used cryopreserved or donor sperm were also excluded.

For all female patients, a detailed medical history was taken and a physical examination was performed. In order to identify infertility factors, transvaginal ultrasonography, serum hormonal assays including anti-Müllerian hormone (AMH), and hysterosalpingography were performed. Male partners underwent a medical interview and semen analysis. We recorded general patient information such as the patient's age, gravidity, and body mass index. We also examined cycle-specific information such as the number of pre-ovulatory follicles (≥ 17 mm in diameter) on the day of ovulation triggering and the sperm characteristics of both the initial semen sample and inseminated semen on the day of IUI.

2. Semen analysis and preparation

After at least 2 days of ejaculatory abstinence, semen samples were obtained by masturbation and collected in sterile containers. After liquefaction for 30 minutes at room temperature, semen samples were processed using the density gradient technique with SpermGrad (Vitrolife, Gothenburg, Sweden), and the total volume was adjusted to 0.3 mL using a wash medium. Before and after semen preparation, each sample was analyzed for volume, concentration, and motility according to the World Health Organization criteria [17]. The semen evaluation was performed manually by one of three IVF laboratory researchers, each of whom had more than 10 years of experience.

3. Ovarian stimulation and IUI

Ovarian stimulation was conducted using a single agent or combination among clomiphene citrate, letrozole, and human menopausal gonadotropin (IVF-M; LG Life Sciences, Seoul, Korea). In most cases, clomiphene citrate (100–150 mg/day) was administered for 5 days beginning on menstrual cycle day 3, and gonadotropin (75–150 IU/day) injections were started thereafter. The gonadotropin dosage was determined based on ovarian reserve markers and the female partner's age. All patients were monitored using serial transvaginal ultrasonography. When at least one follicle diameter reached 18 mm or more, ovulation was triggered using human chorionic gonadotropin (hCG), via either 250 μg of recombinant hCG (Ovidrel; Merck Serono, Darmstadt, Germany) or 10,000 IU of hCG (IVF-C; LG Life Sciences).

IUI was performed 36 hours after hCG administration with a soft IUI catheter. A single IUI procedure was performed for all cycles. The luteal phase was supported vaginally using either Crinone (Merck Serono) 8% vaginal gel or Lutinus (Ferring, Saint-Prex, Switzerland) 100 mg vaginal tablets beginning on the insemination day. After confirmation of pregnancy, luteal phase support was continued until the 10th week of gestational age.

Pregnancy test was done by measuring serum hCG 14 days after IUI and transvaginal ultrasound was performed one week later if the test was positive. Confirmation of an intrauterine gestational sac on transvaginal ultrasonography was considered as indicating clinical pregnancy. Abortion was defined as fetal demise or loss of fetal heart tones before the 20th week of pregnancy. Live birth was defined when delivery of a live fetus at a gestational age of 24th week or more was confirmed.

4. Outcome measures and statistical analysis

The main outcome measures were clinical pregnancy and live

https://doi.org/10.5653/cerm.2021.04469

birth. Each sperm parameter, especially motility, and its change during processing were examined. The effect of sperm motility characteristics before and after preparation on pregnancy outcomes was analyzed. All statistical analyses were performed with IBM SPSS ver. 21.0 (IBM Corp., Armonk, NY, USA). Continuous variables were analyzed using either the Student *t*-test or the Mann-Whitney *U*-test. Categorical variables were analyzed using the chi-square test. Receiver operating characteristic (ROC) curve analysis was conducted to calculate clinically acceptable cut-off values for sperm motility characteristics to predict IUI outcomes. Multivariable logistic regression analysis was performed to control for possible confounding factors that could affect the pregnancy outcomes of IUI. Numerical data are presented as mean ± standard deviation, and categorical variables are expressed as numbers or percentages. A p-value <0.05 was considered to indicate statistical significance.

Results

In total, 184 infertile couples who underwent 297 IUI cycles were included in the study. The overall clinical pregnancy rate was 14.5% (43/297) per completed cycle. Of these, eight cases were lost to follow-up until delivery or termination of pregnancy, so the live birth outcome could not be confirmed. In the other 289 cycles, the abortion rate was 4.2% (12/289) and the live birth rate per cycle was 10.4% (30/289). Basal clinical and cycle-specific characteristics were compared between the two groups according to clinical pregnancy or live birth outcome (Table 1). Basal AMH levels and the number of follicles 17 mm or more in size on the hCG trigger day were significantly higher in cycles with clinical pregnancy than in those without clinical pregnancy, and the same tendency was observed for live birth. Other characteristics were not significantly different between pregnant and non-pregnant groups.

Sperm motility characteristics including initial and inseminated sperm motility or TMSC, along with the change in sperm motility after processing, were compared according to clinical pregnancy outcome (Table 2). Initial TMSC, inseminated TMSC, and post-preparation sperm motility were comparable according to clinical pregnancy outcome. Sperm motility before preparation was significantly higher in cycles that resulted in clinical pregnancy than in those that did not (71.4% ± 10.9% vs. 67.2% ± 11.7%, $p=0.020$). Cycles that resulted in clinical pregnancy showed significantly fewer changes in sperm motility throughout preparation, as assessed using both subtraction (post-pre) and division (post/pre) (19.9% ± 11.5% vs. 23.4% ± 11.9%, $p=0.043$ and 1.3 ± 0.2 vs. 1.4 ± 0.3, $p=0.039$, respectively). These re-

Table 1. Clinical and cycle characteristics according to the pregnancy outcome of cycles

Variable	Clinical pregnancy			Live birth		
	Yes (n = 43)	No (n = 254)	*p*-value	Yes (n = 30)	No (n = 259)	*p*-value
Female partner's age (yr)	33.4 ± 3.4	34.5 ± 3.9	0.131	33.2 ± 3.6	34.5 ± 3.9	0.127
Male partner's age (yr)	36.2 ± 4.9	36.9 ± 4.5	0.204	36.1 ± 5.2	36.9 ± 4.5	0.150
BMI (kg/m^2)	22.8 ± 3.4	22.6 ± 4.0	0.452	22.9 ± 3.4	22.6 ± 4.0	0.485
AMH (ng/mL)	7.0 ± 4.4	5.3 ± 4.3	0.004	7.7 ± 4.4	5.3 ± 4.3	0.001
No. of IUI cycles	1.6 ± 0.8	1.6 ± 0.8	0.917	1.6 ± 0.8	1.6 ± 0.8	0.666
Primary infertility	33 (76.7)	182 (71.7)	0.582	23 (76.7)	185 (71.4)	0.670
Infertility diagnosis			0.543			0.204
Unexplained	14 (32.6)	103 (40.6)		7 (23.3)	105 (40.5)	
Male factor	5 (11.6)	24 (9.4)		4 (13.3)	25 (9.7)	
Ovulatory factor	7 (16.3)	26 (10.2)		7 (23.3)	26 (10.0)	
Tubal factor	3 (7.0)	21 (8.3)		1 (3.3)	22 (8.5)	
Combined and others	14 (32.6)	80 (31.5)		11 (36.7)	81 (31.3)	
Ovulation induction			0.705			0.982
Natural cycle	2 (4.7)	7 (2.8)		2 (6.7)	7 (2.7)	
CC only	2 (4.7)	26 (10.2)		0	27 (10.4)	
Letrozole only	1 (2.3)	10 (3.9)		1 (3.3)	10 (3.9)	
Gn only	5 (11.6)	13 (5.1)		5 (16.7)	13 (5.0)	
CC+Gn	30 (69.8)	190 (74.8)		20 (66.7)	193 (74.5)	
Letrozole+Gn	3 (7.0)	8 (3.1)		2 (6.7)	9 (3.5)	
No. of follicles ≥ 17 mm	1.6 ± 0.9	1.3 ± 0.6	0.048	1.6 ± 0.9	1.3 ± 0.7	0.039

Values are presented as mean±standard deviation or number (%).
BMI, body mass index; AMH, anti-Müllerian hormone; IUI, intrauterine insemination; CC, clomiphene citrate; Gn, gonadotropin.

Table 2. Sperm motility characteristics according to the clinical pregnancy outcome

Variable	Clinical pregnancy			aOR[a] (95% CI)	*p*-value
	Yes (n=43)	No (n=254)	*p*-value		
TMSC before preparation (×10^6)	282.0±205.5	285.6±193.2	0.646	1.000 (0.998–1.001)	0.603
TMSC after preparation (×10^6)	51.4±22.9	51.2±19.4	0.849	1.001 (0.984–1.019)	0.883
Sperm motility before preparation (%)	71.4±10.9	67.2±11.7	0.020	1.037 (1.006–1.069)	0.019
Sperm motility after preparation (%)	91.3±5.9	90.6±7.5	0.929	1.014 (0.962–1.069)	0.608
Δ Sperm motility (post–pre) (%)	19.9±11.5	23.4±11.9	0.043	0.972 (0.945–0.999)	0.044
Δ Sperm motility (post/pre)	1.3±0.2	1.4±0.3	0.039	0.227 (0.055–0.943)	0.041

Values are presented as mean±standard deviation.
aOR, adjusted odds ratio; CI, confidence interval; TMSC, total motile sperm count.
[a]Multiple logistic regression after adjusting for confounding factors (female partner's age, anti-Müllerian hormone, and number of follicles ≥17 mm).

Table 3. Sperm motility characteristics according to the live birth outcome

Variable	Live birth			aOR[a] (95% CI)	*p*-value
	Yes (n=30)	No (n=259)	*p*-value		
TMSC before preparation (×10^6)	256.7±210.0	288.7±194.6	0.143	0.999 (0.997–1.001)	0.391
TMSC after preparation (×10^6)	45.2±21.0	51.4±19.3	0.065	0.986 (0.967–1.005)	0.150
Sperm motility before preparation (%)	71.6±12.6	67.3±11.7	0.030	1.038 (1.002–1.074)	0.037
Sperm motility after preparation (%)	90.5±6.6	90.6±7.5	0.512	0.997 (0.944–1.053)	0.907
Δ Sperm motility (post–pre) (%)	18.9±13.1	23.3±11.8	0.025	0.966 (0.936–0.998)	0.036
Δ Sperm motility (post/pre)	1.3±0.3	1.4±0.3	0.025	0.222 (0.044–1.123)	0.069

Values are presented as mean±standard deviation.
aOR, adjusted odds ratio; CI, confidence interval; TMSC, total motile sperm count.
[a]Multiple logistic regression after adjusting for confounding factors (female partner's age, anti-Müllerian hormone, and number of follicles ≥17 mm).

Table 4. Predictive power of sperm motility characteristics regarding live birth using ROC curve analysis

Variable	Cut-off	AUC	95% CI	*p*-value	Sensitivity	Specificity
Sperm motility before preparation (%)	72.5	0.619	0.506–0.733	0.032	0.533	0.703
Sperm motility after preparation (%)	93.8	0.467	0.362–0.572	0.553	0.467	0.448
Δ Sperm motility (post–pre) (%)	21.5	0.376	0.266–0.485	0.026	0.333	0.452
Δ Sperm motility (post/pre)	1.3	0.375	0.265–0.485	0.025	0.333	0.425

ROC, receiver operating characteristic; AUC, area under the curve; CI, confidence interval.

lationships remained significant after adjusting for the female partner's age, basal AMH level, and number of pre-ovulatory follicles larger than 17 mm.

Similar results were obtained when examining sperm motility parameters and the live birth outcome (Table 3). Pre-preparation sperm motility was higher in cycles with live birth than in cycles without live birth (71.6%±12.6% vs. 67.3% ±11.7%, $p=0.030$), and this remained significant after controlling for the female partner's age, AMH level, and number of follicles ≥17 mm. Cycles that resulted in live birth showed significantly fewer changes in sperm motility throughout preparation, as assessed using both subtraction (post-pre) and division (post/pre) (18.9%±13.1% vs. 23.3%±11.8%, $p=0.025$ and 1.3±0.3 vs. 1.4±0.3, $p=0.025$, respectively), but marginal significance was found for the latter measure of change after adjusting for confounding factors.

In ROC curve analysis, sperm motility after preparation and the change in sperm motility showed poor predictive power for the pregnancy outcome. The optimal threshold value of initial sperm motility to predict live birth was ≥72.5% with a sensitivity of 53.3% and a specificity of 70.3% (area under the curve, 0.619; $p=0.032$) (Table 4). A dichotomous variable was then created by dividing the initial sperm motility into two groups (≥72.5% or <72.5%) and multivariable analysis was done including this variable and other confounding factors. As a result, the predicted odds of live birth were significantly higher in cases where the pre-preparation sperm motility was ≥72.5% than in cycles where the initial sperm motility was <72.5% (adjusted odds ratio, 2.741; 95% confidence interval, 1.241–6.057).

https://doi.org/10.5653/cerm.2021.04469

Discussion

This study revealed that initial sperm motility was the best sperm motility parameter for predicting pregnancy as an outcome of IUI compared with other parameters such as TMSC or the change in sperm motility after preparation. Pre-preparation sperm motility was higher in the pregnant group, and perhaps in this context, the change in sperm motility throughout preparation was fewer in the pregnant group. The optimal cut-off value of initial sperm motility to predict live birth was confirmed to be ≥ 72.5%.

Concerns have been raised that the risk of multiple pregnancy may increase in IUI procedures, especially in cycles with superovulation. We analyzed whether sperm characteristics were associated with multifetal pregnancy, and found that sperm motility parameters were not significantly different according to the outcome of multiple pregnancy (data not shown).

The sperm preparation process can yield as many motile spermatozoa as possible. Sperm motility characteristics before and after preparation have been evaluated as predictors of pregnancy after IUI. A retrospective study that investigated 1,007 IUI cycles reported that initial sperm motility and forward progression of processed sperm were independently associated with clinical pregnancy [19]. However, another retrospective study of 383 IUI cycles stated that initial sperm concentration, motility, and the percentage of rapid sperm were significantly different according to whether pregnancy was achieved, but no significant relationship was found for the sperm parameters after preparation [20].

As described above, several studies have investigated various sperm motility characteristics before and after processing, but we could only discover one study that analyzed the impact of changes in parameters after sperm preparation. Freour et al. [15] reported that computer-assisted sperm analysis (CASA) parameters and their changes after processing were comparable according to the pregnancy outcome, whereas improvement in the amplitude of lateral head displacement (ALH) of spermatozoa during preparation predicted clinical pregnancy well in IUI cycles with frozen-thawed donor semen.

In the present study, we found that couples with higher pre-preparation sperm motility showed better pregnancy outcomes in IUI cycles. This finding is consistent with the results of previous studies [19-22]. The change in motility during sperm preparation, which we initially tried to study, was found to be fewer in cycles that resulted in pregnancy than in those that did not. A reasonable interpretation of these results is that higher initial motility, rather than insufficient improvement in sperm motility through processing, can better predict pregnancy in IUI cycles.

Regarding the threshold of initial sperm motility for predicting conception after IUI, previous studies mostly suggested a threshold of 30%. In our study, the optimal cut-off value for pre-preparation sperm motility was 72.5%, which is considerably higher than that of existing studies. This may be related to differences in patient characteristics. This study was conducted at one of the largest university-based hospitals in Korea, and patients with a relatively old age or long duration of infertility tend to visit. Indeed, in two papers that previously proposed a 30% cut-off for sperm motility, the female participants were much younger than those in the current study, and thus they were probably patients with a better prognosis [21,22]. Zhao et al. [19] suggested 80% as a threshold of initial sperm motility, similar to our study, and the average age of female subjects in their study was 35.2 ± 4.5 years, also similar to our study.

In the current study, neither pre- nor post-preparation TMSC was correlated with the pregnancy outcomes of IUI. Initial or post-wash TMSC was previously reported to predict pregnancy after IUI in some studies. According to a systematic review, the most commonly suggested cut-off values for initial TMSC were 5–10 $\times 10^6$, and 0.8–5 $\times 10^6$ for processed TMSC in predicting IUI success [1]. The mean initial and post-processed TMSC of our subjects were approximately 285 $\times 10^6$ and 51 $\times 10^6$, respectively, both much higher than the previously suggested thresholds. Zhao et al. [19] stated that inseminated specimens with TMSC values ranging from 11–100 $\times 10^6$ resulted in the highest pregnancy rate after IUI, and the conception rate fell when TMSC was higher than 100 $\times 10^6$. Hansen et al. [23] also reported that the final TMSC available for IUI was significantly higher in the live birth group, with TMSC up to 20 $\times 10^6$, but that the live birth rate did not increase after the TMSC exceeded 20 $\times 10^6$. Among our study subjects, only 10% had male factor infertility, and even only mild male factors that exceeded threshold values were included, so which might explain why TMSC did not significantly differ according to the pregnancy outcome. Furthermore, the overall low live birth rate (and thus, the small number of cycles with live births) could have limited our ability to detect significant differences. Additional large-scale prospective studies with appropriate subjects are needed.

Two existing studies reported that post-wash sperm motility was related to pregnancy after IUI [24,25]. In our study, post-preparation sperm motility was similar in cycles regardless of whether pregnancy was achieved. These observed differences may have resulted from different patient populations and sperm preparation methods. Two previous studies used the sperm wash technique and our study used the density gradient method for sperm preparation. Moreover, rather than improving sperm motility, a small number of progressively motile spermatozoa are filtered out and sperm may become hyperactivated during preparation. Therefore, it may be hyperactivated sperm, not final sperm motility itself, that affects the pregnancy outcomes of IUI.

Hyperactivation of sperm is known to be critical to achieve fertilization because it assists sperm in reaching oocytes and penetrating

the zona pellucida. CASA can identify hyperactivated sperm using threshold values for curvilinear velocity and path linearity. As an indirect measure of flagellar bend amplitude, ALH can also be used to assess hyperactivation [26]. As mentioned above, Freour et al. [15] reported that ALH progression during sperm preparation was a good predictor of IUI success. This might indirectly confirm the importance of sperm hyperactivation through processing. It is necessary to evaluate various CASA parameters, especially regarding hyperactivation, and to further study the effect of their changes in the preparation process on pregnancy outcomes.

To the best of our knowledge, this is the first study to explore the effects of changes in motility during sperm preparation on IUI outcomes using autologous semen. This study was performed at a single center, so the overall IUI regimen (specifically the methods of ovulation induction or luteal phase support) was relatively consistent. In addition, while the majority of the aforementioned studies investigated clinical pregnancy as a pregnancy outcome, it is meaningful that we examined both the clinical pregnancy rate and the live birth rate as outcomes in the present study.

However, this study has the inherent limitations of a retrospective design. The relatively small sample size and inability to confirm the cumulative live birth rates are also limitations of this study. Furthermore, previous studies that highlighted the effects of sperm motility on IUI outcomes mostly emphasized the importance of rapid progressive and linear motility [20,27,28]. Unfortunately, since our center performed semen analysis manually on the IUI day, we could not properly evaluate a full variety of sperm motility parameters.

In conclusion, initial sperm motility better predicted live birth after IUI cycles than the change in motility after preparation or the final sperm motility. According to our study, even though sperm motility is substantially improved by the preparation process, pregnancy results may not be favorable if the initial sperm motility is relatively low. With this in mind, we can provide appropriate consultations to infertile couples undergoing IUI procedures. Further well-designed prospective studies should be conducted to confirm these findings.

Conflict of interest

Byung Chul Jee is an Editor-in-Chief and Seul Ki Kim is an Associate Editor of the journal, but they were not involved in the peer reviewer selection, evaluation, or decision process of this article. No other potential conflicts of interest relevant to this article were reported.

Acknowledgments

The authors would like to thank the staff of the Laboratory of Reproductive Medicine, Seoul National University Hospital, Seoul, Korea.

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