

THE EFFECT OF INTERVALS FROM SPERM WASH TO INTRA UTERINE INSEMINATION (IUI) TIME ON PREGNANCY RATE

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SUMMARY

Background: The aim of our study was to assess the influence of intervals from sperm wash to intra uterine insemination on the IUI outcome.

Material and methods: This was prospective study of 1125 cycles in Obstetrics and Gynecology Department of Baskent University Medical Faculty between January 2011 and December 2011.

Results: In totally 1125 cycles IUI performed within < 30 minute in 202 cycle, 30-59 minute in 367 cycle, 60-89 minute in 381 cycle, 90-119 minute in 114 cycle, 120-180 minute in 61 cycle. Pregnancy rate was %4.5,%7.9,%11,%8.8, ve %11.5 respectively. We reanalyzed <30 minutes and other groups again we found that the difference reached statically significant level (%4.5 vs %9.5)(p: 0.01). In good prognostic patients with total motile sperm count was $\geq 10 \times 10^6$, pregnancy rates were lower in patients whose inseminated in <30 minute than the others (%5.2 vs %10.1)(p: 0.024).

Conclusion: The results of our study showed that if a washing sperm can be incubated minimum 30 minute at 37 C the pregnancy rate is optimum. The longer period up to 180 minute doesn't compromise the pregnancy rate.

Key words: IUI, pregnancy rates, sperm wash, timing

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SPERM HAZIRLIĞI İLE İNTRAUTERİN İNSEMINASYON ARASI SÜRENİN GEBELİK ORANLARINA ETKİSİ

ÖZET

Amaç: Yaygın olarak kullanılan intrauterin inseminasyon (IUI) sikluslarında semen hazırlama ve IUI arasındaki sürenin IUI sonuçlarına etkisini prospektif olarak araştırmayı amaçladık.

Gereç ve yöntem: Ocak 2011- Aralık 2011 tarihleri arasında Başkent Üniversitesi Tıp Fakültesi Kadın Hastalıkları ve Doğum Bölümü, YÜT merkezinde IUI programına alınan 1125 siklus prospektif olarak değerlendirmeye alınmıştır.

Bulgular: Çalışmaya alınan 1125 siklusun 202'sinde <30 dk, 367 'sinde 30-59 dk, 381'inde 60-89dk, 114'ünde 90-119dk, 61'inde 120-180 dk içerisinde IUI uygulandığı, bu gruplarda sırasıyla gebelik oranlarının %4.5,%7.9,%11,%8.8, ve %11.5 olduğu tespit edildi. Bu nedenle 30 dakikada yapılan IUI'lar ile diğer sikluslar toplam olarak tekrar analiz edildiğinde aradaki farkın istatistiksel olarak anlamlı düzeye eriştiği izlendi (%4.5 vs %9.5)(p: 0.01). IUI başarısında önemli prediktörlerden biri olan insemine edilen total motil sperm sayısının 10 milyon üzerinde olduğu sikluslarda da gebelik oranları ilk 30 dakikada IUI yapılanlarda istatistiksel olarak anlamlı ölçüde düşük tespit edildi (%5.2 vs %10.1)(p: 0.024).

Yorum: Sperm hazırlandıktan sonra IUI sikluslarında spermin 37 C de en az yarım saat bekletilmesi optimum gebelik oranlarını sağlamak ve 180 dakikaya kadar olan bekleme süreleri sonuçları olumsuz etkilememektedir.

Anahtar kelimeler: gebelik oranları, IUI, sperm yıkama, zamanlama

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INTRODUCTION

Intrauterine insemination (IUI), an assisted reproductive technique widely used all over the world, is a distinguished method which is cheaper and less invasive compared to other assisted reproductive techniques⁽¹⁾. Intrauterine insemination is widely used in mild-medium male factor infertility, unexplained infertility, cervical factor and ovulatory problems⁽²⁾. Several factors, such as maternal age, cause of infertility, uterine factor, sperm parameters, presence of endometriosis, number of cycles and ovulation induction agents, have been investigated as prognostic factors for IUI success⁽³⁻⁷⁾. However, there is limited number of studies on the effect of other parameters that may affect pregnancy rates with IUI, such as the effect of the place of semen collection (house, clinic), intervals from semen collection to sperm wash and intervals from sperm wash to IUI on the IUI outcomes⁽⁸⁻¹¹⁾. In a retrospective study, it was reported that semen collection at home rather than in the clinic, increasing the interval between semen collection and sperm wash from 30 minutes to 60 minutes, and increasing the interval between sperm wash and IUI from 90 minutes to 120 minutes resulted in a significant decrease in pregnancy rate in hMG-IUI cycles⁽¹¹⁾. On the other hand, it was found in another study that semen collection at home and delayed transport period did not have any effect on pregnancy rate⁽¹⁰⁾. In our clinic, we ask semen collection to be performed in the clinic in all IUI cycles. Yet, duration between sperm wash and IUI may vary depending on many factors (how busy the clinic and andrology laboratory are, patient's arrival time, etc.)

In this prospective study, we aimed to assess the effect of intervals between sperm wash and IUI on the IUI outcomes.

MATERIALS AND METHODS

The cycles included in the IUI program in the Obstetrics and Gynecology Department of Başkent University Faculty of Medicine between January 2011 and December 2011 were assessed prospectively. Approval was received for this study from the Ethics Committee of Başkent University (Project no: KA10/166). The study included the first two cycles of patients for whom gonadotrophin-controlled ovarian hyperstimulation

and IUI was planned for unexplained infertility or mild male factor. Patients with basal FSH values higher than 12 IU and with endometrial thickness lower than 6 mm on the day of HCG administration were excluded from the study. On the day of intrauterine insemination, the intervals between semen collection and sperm wash, and between sperm wash and IUI were recorded carefully. While assessing the study results, the duration between sperm wash and IUI was evaluated in five groups with 30 minute intervals.

Basic tests for infertility research included sperm analysis after an abstinence of 3-7 days, hormonal analysis and pelvic ultrasonography on day 3 of menstrual cycle, histerosalpingography (HSG) between days 7-11 of menstrual cycle, and mid-luteal progesterone measurement for ovulation assessment between days 7-11. Based on the findings, patients were categorized as either mild-medium male factor or unexplained infertility groups. Ovulatory cases with normal HSG and without male factor, and cases who were diagnosed with oligo-anovulation and who failed to achieve pregnancy despite at least 3 ovulatory cycles with ovulation induction were classified as "unexplained infertility". Cases with sperm count less than 20×10^6 (oligospermia), fast forward sperm percentage less than 25% and total percentage of slow forward moving sperm (A+B) less than 50% (asthenospermia) in motility assessment were evaluated as male factor. As per March 2010 SUT law, patients with sperm count over 5 million were included in the IUI program. Ovulation induction (OI) recombinant FSH preparations (Gonal-F, Serono & Puregon, Organon, Turkey) were used. According to the protocol applied in our clinic, treatment with rFSH in OI was initiated with doses ranging from 50 to 150 IU on day 3 or 4 of menstrual cycle, based on patients'weight, age and antral follicle count in USG. Monitorization was started on day 6 of the treatment. Monitorization intervals were determined according to follicle size. The presence of at least one follicle over 18 mm was taken as criterion to trigger ovulation. Cycle was cancelled in cases in which more than 2 follicles over 16 mm were detected in the follow-up. For this purpose, 10 000 IU hCG (Ovitrelle flk, Merck Serono, Pregnyl flk, MSD, Turkey) was used. On day 21 of menstrual cycle, ovulation and progesterone were measured and checked in all patients. Luteal phase support was not performed in any of the cycles. A single insemination was performed 36-40 hours after hCG was administered in all cycles.

For sperm wash, 2 ml was taken from 80% gradient medium (Suprasperm, Tek Medical Service, Denmark) by using a 1 ml pipette (Falcon, 7521, Aksuvar and Assist Medical, USA) and slowly dropped into a conic tube (Falcon, 2095, Aksuvar and Assist Medical, USA). Then, 2 ml was taken from 55% gradient medium (Suprasperm, Tek Medical Service, Denmark) and slowly dropped into the tube. The liquefied semen sample was slowly stirred and counted by the help of a 2 ml pipette (Falcon, 7507, Aksuvar and Assist Medical, USA) and then slowly dropped on gradient medium and centrifuged at 300 g (1200 rpm) for 230 minutes. At the end of this period, supernatant part was taken and added on the 3 ml pellet from the Medi-cult IVF (Tek Medical Service, Denmark) washing medium placed in a 15 ml round-bottom tube (Falcon 2001, Aksuvar and Assist Medical, USA) on the pellet at the bottom and then resuspended. Supernatant part was removed after centrifuge. Medi-cult IVF washing medium (Tek Medical Service, Denmark) was added to leave 0.6 ml final volume on the pellet and then resuspended. It was placed in a 5 ml tube (Falcon 2003, Aksuvar and Assist Medical, USA) by using a 1 ml pipette (Falcon 7521, Aksuvar and Assist Medical, USA) and allowed to stand in laminar flow after the final count was performed. All patients were asked to come to the clinic with a full bladder. After the vulva and vagina were washed with physiologic saline, the prepared sperm was injected slowly into intrauterine cavity, accompanied by transabdominal ultrasonography (USG), using artificial insemination catheter (Wallace(r), Smiths Medical International Ltd, UK), to reach a total volume of 0,5 ml. The patient was allowed to rest for 10-15 minutes and then was told to return to normal daily activities. Patients was subjected to β -hCG test if they had not experienced a menstrual cycle in 14 days following the day of hCG. Patients with positive test results were called back to the clinic two weeks later for transvaginal USG. Patients whose pregnancy was confirmed with USG were accepted as pregnant in this study.

Statistical method

Results were given as mean \pm standard deviation. In order to analyze pregnancy rated in IUI cycles, chi-square test or Fisher's exact test, if necessary, were used. Pearson correlation test was used to assess the relationship between the variables and pregnancy. $P < 0.05$ was accepted to be significant.

Results

1125 cycles in total were analyzed. In the correlation analysis, there was no statistically significant difference between the groups in terms of age, infertility period, basal FSH, follicle count, and inseminated mean TMSS (total motil sperm count) among the parameters that affect pregnancy rates (Table I). The interval from sperm wash to IUI was examined in five groups, with 30 minute intervals, and it was found that the lowest pregnancy rates were in the cycles performed in the first 30 minutes (Table II). Thus, the IUIs performed in 30 minutes and other cycles were re-analyzed, and this difference was observed to have reached to a statistically significant level (Table III) ($p < 0.01$). When we re-analyzed the data in inseminated cycles with more than 10 million sperm count in total, we found that pregnancy rates decreased in cycles in which IUI was administered in the first 30 minutes and this decrease was statistically significant (Table IV).

Table I: Demographic data.

	Interval between sperm wash and IUI (minute)				
	120-180	90-119	60-89	30-59	<30
Woman's age	29.1 \pm 4.4	28.8 \pm 5.3	29.3 \pm 4.5	29.2 \pm 4.6	29.3 \pm 5.1
Infertility period	4.6 \pm 3.6	4.2 \pm 2.8	4.6 \pm 3.3	4.5 \pm 3.1	4.5 \pm 3.5
Basal FSH	5.5 \pm 1.7	5.6 \pm 2.1	5.5 \pm 1.1	5.5 \pm 1.9	5.4 \pm 1.7
Inseminated					
TMSS	37.4 \pm 23.7	35.5 \pm 23.9	32.8 \pm 24.3	31.8 \pm 24.3	25.8 \pm 19.8
Follicle count	1.6 \pm 0.6	1.7 \pm 0.8	1.6 \pm 0.7	1.5 \pm 0.7	1.7 \pm 0.9

TMSS (total motil sperm count)

Table II: Interval between sperm wash and IUI and pregnancy rates.

	Interval between sperm wash and IUI (minute)				
	120-180	90-119	60-89	30-59	<30
Number of cycles					
(1125)	61	114	381	367	202
Pregnancy rates	7	10	42	29	9
	(%11.5)	(%8.8)	(%11)	(%7.9)	(%4.5)

Table III: Negative effect of > 30 minute interval between sperm wash and IUI on pregnancy rates.

	Interval between sperm wash and IUI (minute)		p
	≥ 30	<30	
N(1125)	923	202	
Pregnancy rates	88 (%9.5)	9(%4.5)	0.01

Table IV: Effect of the interval between sperm wash and IUI on pregnancy rates in cycles with TMSS>10 million after sperm wash.

	Interval between sperm wash and IUI (minute)		p
	≥30	<30	
N(988)	814	174	
Pregnancy rates	82 (%10.1)	9(%5.2)	0.024

DISCUSSION

The objective of our prospective study was to find the optimal time between sperm wash and insemination in intrauterine insemination cycles. Contrary to the general view, we have demonstrated that having an interval of at least 30 minutes, rather than initiating IUI immediately after sperm wash, increases success rate in IUI cycles.

In our literature review, in a single retrospective study conducted with 102 patients to determine the effect of the interval between sperm wash and IUI on pregnancy rates, it was reported that there was no difference between performing IUI in intervals of 30 minutes and 31-60 minutes, but pregnancy rates were higher in these two groups compared to the group in which IUI was administered after 60 minutes, and that no pregnancy was observed in the group in which hMG was used after 180 minutes⁽¹¹⁾. In this study, in the groups with 30 minutes, 31-60 minutes, >60 minutes interval, pregnancy rates were 6%, 6% and 15%, respectively, in the CC group; and 67%, 42% and 7%, respectively, in the hMG group. It is striking that in our study pregnancy rates were also lower in IUIs performed in the first 60 minutes in the CC group. In addition, high pregnancy rates in cycles with hMG were also noteworthy. In a study conducted by Rannall GW et al., it was reported that pregnancy rates were not affected in gonadotropine-administred cycles by the two-hour duration before the sample was brought to the clinic after sperm wash was performed following sperm collection at home⁽¹⁰⁾. In our study, as we aimed to determine the effect of the waiting period after sperm wash on pregnancy rates, all samples were collected in the clinic and sperm wash procedure was initiated immediately after the ejaculate was liquefied. Since ovulation induction with gonadotropines and IUI are required to prepare IVF report for patients in line with the new SUT law, ovulation induction with

gonadotropines was performed in all cycles. Owing to this design of the study, we had the opportunity to assess the effect of the interval after sperm wash by eliminating additional factors such as the effect of ovulation induction agents, transfer of sperm from home to the clinic.

In the correlation analysis we performed for parameters that could affect pregnancy rates in intrauterine insemination cycles, we found that age, infertility period, basal FSH, follicle count, TMSS (total motil sperm count) in inseminated medium, and waiting period of the inseminate were effective. There was no statistically significant difference between the groups in terms of age, infertility period, basal FSH, follicle count, inseminated mean TSS. However, when the intervals between sperm wash and IUI were examined, it was striking that the lowest pregnancy rates were observed in the cycles performed in the first 30 minutes.

Our aim in using sperm preparation techniques was to distinguish normal sperms in terms of motility and morphology, and to increase sperm capacitation and, thus, pregnancy rates by removing prostaglandin, dead sperms and infectious agents⁽¹³⁾. If we consider that sperms separated from the seminal plasma and liquid preserved their motility and viability for longer periods compared to unprocessed ones, and that sperms, which could not be capacitated when incubated in room temperature, achieved capacitation by incubation at 37°C (14); our higher pregnancy rates obtained with sperms which were allowed to stand at 37°C for an interval longer than 30 minutes can be explained by the interval long enough to enable the capacitation of sperms. After in vivo sperm capacitation, hyperactive motility and acrosome reaction develops in the sperm by stimuli such as human zona pellucida, progesterone or follicle liquid. Capacitation and acrosome reaction may develop in vitro when suitable conditions are provided. There are animal and human studies demonstrating that incubation temperature is a modulator in capacitation and acrosome reaction^(15,16). In a study which investigated the effect of sperm incubation at 37°C and room temperature, progressive sperm rates were similar in both groups, but those kept in room temperature did not show any hyperactive movement when examined in CASA system⁽¹⁶⁾.

Another reason for the higher pregnancy rate in the group with longer interval before IUI may be the increased possibility that insemination occurred after

ovulation. Because, in a recent study, pregnancy rates were reported to be higher in IUIs performed after ovulation was detected by USG on the day of insemination⁽¹²⁾. Consequently, we demonstrated in our study that the interval of at least 30 minutes at 37°C was optimal in IUI cycles, and intervals up to 180 minutes did not affect the outcomes negatively.

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