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Effect of time interval between sperm collection, preparation and insemination on in vitro fertilization.

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Abstract

Objective To determine the effects of the time interval between sperm collection and preparation, collection and insemination, and preparation and insemination on IVF results.

Design Retrospective descriptive study.

Setting IVF Unit, Research Center, and Department of Obstetrics and Gynecology, Ramathibodi Hospital.

Subjects Sixty couples who underwent IVF-ET were recruited. The subjects were divided into normal and abnormal sperm parameters as followed.
Group 1 : sperm concentration $\geq 20 \times 10^6$/ml with % motility ≥ 50, (N=47)
Group 2 : sperm concentration $< 20 \times 10^6$/ ml and/or % motility < 50, (N=13)

Main outcome measures Time interval between sperm collection and insemination (T_1), after complete sperm preparation and insemination (T_2), sperm collection and preparation (T_3); fertilization rate; cleavage rate.

Results The female age, number of oocytes, T_1, T_2, and T_3 in both groups were not different. The fertilization rate in group 1 was significantly higher than that in group 2 (85.1±16, 65±29.3 ; respectively), where as cleavage rate was comparable. In group 1, there were no correlation between T_1, T_2, T_3, and fertilization or cleavage rate. In group 2, T_1 and T_2 also showed no correlation with fertilization and cleavage rate but there were significant inverse correlation between T_3 and both fertilization and cleavage rate (r = -0.75 and -0.575, respectively).

Conclusion Prolonged time interval between semen collection and preparation may effect IVF outcome, especially in cases with abnormal sperm parameters. Appropriate schedule for semen collection should be considered in IVF program to ensure the optimal outcome.

Key words : sperm preparation time, in vitro fertilization, fertilization rate, cleavage rate

In spite of quality of oocytes, sperm parameters are the important factors affecting outcome of IVF. Sperm processing which includes semen collection, examination and preparation therefore plays significant role in the procedure. Timing of semen collection varied from centers to centers. Some requested husband to collect semen prior to oocyte retrieval to ensure that sperm would be available. In case of masturbation failure the cycle may be cancelled. Regardless of masturbation problem, some centers required semen after oocyte retrieval approximately few hours before insemination.[1] Theoretically, after ejaculation semen was left for 30-90 minutes allowing complete liquefaction to take place.[2] An examination and sperm preparation would later be performed and kept incubating for insemination. The usual time of insemination was 2-5 hours for metaphase II oocytes after collection. Longer insemination time up to 24-36 hours was required for the immature oocytes.[3] It was noticed that there were wide ranges of time among the periods of time interval between semen collection, sperm preparation, and insemination.

It is consequently our interest to see whether those time intervals affect the IVF outcome. The purpose of this is to determine the effect of time interval between semen collection and preparation or collection and insemination or preparation and insemination on fertilization rate and cleavage rate.

Materials and Methods

This retrospective study was carried out among 60 couples who underwent IVF-ET at Ramathibodi IVF unit during December 1996 to October 1997.

Control ovarian hyperstimulation protocol

Ovarian stimulation was achieved by short protocol using hMG (150 to 300 IU/day) intramuscularly (IM) from day 2-3 of cycle concomitant with GnRH-a 800 μg/day intranasally from 1-2 day of cycle. The dose of hMG was adjusted based on follicular size. When three or more follicles had reached a size of ≥ 16 mm, 10,000 IU of hCG were administered IM and all other medication was discontinued. Oocyte retrieval was performed approximately 34-36 hours after hCG injection.[4]

Semen samples

Semen samples were obtained by masturbation and collected into sterile plastic container on the morning of oocyte retrieval between 8-10 AM. Following complete liquefaction, the samples were assessed for concentration and motility according to WHO standard 1992[5] by computerized assisted semen analysis (IVOS, Integrated Visual Optical Systems, Version 10, Hamilton Thorne research, MA, USA). Percoll gradient (Sigma, St.Louis, MO, USA) was prepared in 2 layers by overlaying 1.5 ml of 90% isotonic Percoll with 1.5 ml of 40% isotonic Percoll. The semen samples were added onto the top layer and centrifuged at 750 g for 15 minutes. In case of severe oligospermia (total sperm $< 5 \times 10^6$/ml, mini-Percoll, which was prepared by layers of 95, 70 and 50% (0.3 ml for each layer), was used.[6,7] After two times washing, sperm pellets were diluted to approximately 10×10^6/ ml (if available) by Earle's medium supplemented by 7.5% inactivated maternal serum. The final semen samples were then incubated in 5% CO_2 at 37 °C atmosphere until insemination.

Since the sperm parameters generally influence IVF outcome, the patients were divided into normal and abnormal group according to WHO criteria.[5] The abnormalities included both oligospermia and asthenospermia. There were 47 cases with normal semen analysis where as 13 cases were abnormal. Therefore, they were grouped as followed : Group 1 consisted of sperm concentration $\geq 20 \times 10^6$ /ml and % motility ≥ 50 (N=47) and Group 2 consisted of sperm concentration $< 20 \times 10^6$/ml and/or % motility <50 (N=13).

In Vitro Fertilization, Insemination, and Embryo transfer

The retrieved oocytes were evaluated for maturity according to the criteria previously proposed.[8] More than 95% of them were metaphase I and II. Not more than 4 oocytes per well were cultured in IVF Medium (Medicult, Cat, No.10315060, Copenhagen,

Denmark) in the inner well of organ culture dish (Falcon cat. No.3037, New Jersey, USA). Three to four hours after collection, the oocytes were inseminated with approximately 250,000 sperm per well.

Time interval between semen collection, preparation and insemination were recorded and defined as T_1 for time interval between semen collection and insemination, T_2 for complete sperm preparation and insemination, and T_3 for semen collection and sperm preparation.

Fertilization and embryo cleavage were examined at 18 and 48 hours post insemination, respectively. Up to 4 embryos were selected for transfer using a Frydman catheter (CCD, Paris, France). Micronized progesterone (Utrogestran®, Piette laboratorie, Brussels, Belgium) 400 mg/day was given orally as luteal support. Fourteen days later, serum β-hCG determinations were performed for initial diagnosis of pregnancy.

Statistical Analysis

Statistical analysis was performed using the SPSS for windows. Data were analysed by unpaired t-test to compare means of T_1, T_2, T_3, fertilization rate, and cleavage rate between groups. Relationship between T_1 or T_2 or T_3 and fertilization rate or cleavage rate were carried out by correlation test (Pearson and Kendall). Statistical significance required p-value < 0.05.

Results

Female ages and number of retrieved oocytes in both groups were shown in Table 1. There were no differences between the groups. Clinical indications were presented in Table 2. Majority of the cases in both groups had the problems of tubal occlusion and unexplained infertility. Percent of endometriosis cases that may cause a detrimental effect on fertilization were comparable in both groups.

Table 3 demonstrated T_1, T_2, T_3, fertilization and cleavage rate of the two groups. Fertilization rate in group with normal sperm parameters was higher than that in group with abnormal sperm parameters ($p<0.05$). T_1, T_2, T_3 and cleavage rate were not diferent in both groups.

Correlation coefficients (r) between various time intervals and IVF outcome of group 1 and 2 were shown in Table 4. Significant inverse correlation between T_3 and fertilization rate and between T_3 and cleavage rate in group with abnormal sperm parameters were observed at $r = -0.75$ and -0.575, respectively.

Table 5 displayed 3 of 13 cases in group 2 whose sperm concentration was $< 20 \times 10^6$/ml and $< 50\%$ motility (severe oligoasthenozoospermia). In case 25, fertilization rate and cleavage rate were zero and T_2 was longer than 3 hours. In case 12, who gave very good fertilization rate and cleavage rate (100% both) T_2 was less than 3 hours and T_3 was within 1 hour. In case 36, who had longer T_3, although T_2 was less than 3 hours, fertilization was much lower than case 12 and cleavage rate was zero.

Table 1. Female ages and number of oocytes in cases with normal (group 1) and abnormal semen parameters (group 2)

	group	N	mean ± SD	range	95% CI for mean	p-value
Female age (years)	1	47	35.4±4.0	26-42	34.2-36.6	0.78
	2	13	35.1±3.1	30-40	33.2-37.0	
No. of oocytes	1	47	7.6±4.5	1-18	6.2-9.0	0.36
	2	13	6.6±3.2	3-15	4.6-8.6	

Table 2. Clinical indications in cases with normal (group 1) and abnormal semen parameters (group 2)

Clinical indications	Group 1 (N %)	Group 2 (N %)
Tubal occlusion	21(44.6)	8(61.5)
Male factor	1(2.1)	1(7.7)
Unexplained	13(27.7)	3(23.1)
endometriosis	3(6.4)	1(7.7)
ovarian factor	6(12.8)	-
uterine factor	3(6.4)	-
Total	47(100)	13(100)

Table 3. T_1, T_2, T_3, fertilization rate and cleavage rate in cases with normal (group 1) and abnormal semen parameters (group 2)

Parameters	group	N	mean±SD	range	95% CI for mean	p-value
T_1 (min)	1	47	252±69	90-375	231-272	0.56
	2	13	239±70	120-360	197-281	
T_2 (min)	1	47	135±70	10-240	114-155	1
	2	13	135±78	15-240	87-182	
T_3 (min)	1	47	117±51	45-255	102-132	0.42
	2	13	104±45	60-210	77-131	
Fertilization rate (%)	1	47	85.1±16.0	33-100	80.4-90.0	0.037
	2	13	65.0±29.3	0-100	47.3-82.7	
Cleavage rate (%)	1	47	95.7±11	54-100	92.5-99.0	0.208
	2	13	81.3±37	0-100	58.9-103.7	

Table 4. Correlation coefficients (r) between T_1, T_2, T_3, and fertilization or cleavage rate in cases whose normal (group 1) and abnormal semen parameters (group 2)

	Group	N	Correlation Coefficient (r)	p-value
T_1 VS fertilization rate	1	47	0.094	0.530
	2	13	-0.167	0.452
T_1 VS cleavage rate	1	47	0.151	0.312
	2	13	-0.3	0.221
T_2 VS fertilization rate	1	47	-0.028	0.851
	2	13	0.247	0.262
T_2 VS cleavage rate	1	47	0.094	0.529
	2	13	0.091	0.703
T_3 VS fertilization rate	1	47	0.164	0.271
	2	13	-0.750	0.001
T_3 VS cleavage rate	1	47	0.075	0.618
	2	13	-0.575	0.018

Table 5. Number of retrieved oocytes, T_1, T_2, T_3, fertilization rate, and cleavage rate in cases that sperm concentration was <20 x 10^6/ml and <50% motility.

Case no.	No. of oocytes	T_1 (min)	T_2 (min)	T_3 (min)	Fertilization rate (%)	Cleavage rate (%)
12	3	210	150	60	100	100
25	5	360	220	140	0	0
36	9	270	120	150	44	0

Discussion

In this study, it was observed that time interval between semen collection and preparation had adverse effect on fertilization rate and cleavage rate especially in group with abnormal sperm parameters. Although semen was theoretically to be underwent sperm preparation within 60-90 minutes after ejaculation,[2] sometimes the schedule was practically prolonged. In ejaculated semen, there are decapacitation factors that can diminish the occurrence of acrosome reaction and hence, affect the fertilizing capacity of the sperm.[9,10] It has been shown that longer exposures to seminal environment in vitro can permanently diminish the fertilizing capacity of sperm[11] particularly, in the oligospermia.[12] The finding agreed to that was proposed by Mortimer[13] that sperm need to be separated from the seminal plasma quickly and efficiently. Although no correlation between time interval between semen collection and preparation and fertilization rate or cleavage rate were found in the group with normal sperm parameters in this study, it did not implied that this time interval had no effect on IVF outcome in the normospermia. A certain period of time of this interval perhaps beyond the range that we studied may affect fertilizing capacity of the sperm.

When considering time interval between sperm preparation and insemination, even though there was no correlation between this interval and fertilization or cleavage rate, some interesting findings were noticed. Data of 3 cases of oligoasthenozoospermia were presented in Table 5 in order to draw an attention to Takahasi and Kitao's work.[14] They proposed that in abnormal sperm there was a rapid increase of acrosome reaction with a maximal value at 3 hours after sperm washing then quickly decrease to less than 10% at approximately 6 hours while in normal sperm there was gradually increase of acrosome reaction with a maximum value at 6 hours or longer. Considering case 25 and 36, they had comparable T_3 but T_2 in cases 25 was more than 3 hours and fertilization rate was zero. According to their hypothesis, the left over % acrosome reaction may be too low for fertilization. In case 12, there was good fertilization rate though T_2 was a little bit more than that of case 36 and almost 3 hours. Based on our result, it could be explained that T_3 of case 12 was much lower than that of the other two cases. Therefore, semen collection before oocyte retrieval probably affected IVF outcome in cases with abnormal semen parameters since after preparation sperm would be incubated and waited for insemination. Such duration may take for 4-6 hours.

After the effects of prolonged time interval between semen collection and preparation and between preparation and insemination had revealed, we rearranged schedule for semen collection, preparation and insemination in our laboratory to the appropriate conditions.

In conclusion there were the evidences that prolonged time interval between semen collection and preparation especially in abnormal sperm may effect IVF outcomes. More attention should be paid on timing of semen collection, preparation and insemination. However, prospective study with control of the affecting factors and specific time interval are needed to confirm our findings.

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