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The Effect of Preincubation Period of Oocytes on Nuclear Maturity, Fertilization Rate, Embryo Quality, and Pregnancy Outcome in IVF and ICSI[1]

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***Purpose*:** To clarify the effect of preincubation of oocytes on the results of IVF and ICSI.
***Methods*:** A total of 176 IVF and 64 ICSI cycles received long protocol ovarian stimulation. The oocytes were incubated for 1–8 h before insemination or sperm injection. Metaphase II (MII) percentage was evaluated in the ICSI arm; fertilization rates, embryo quality, and pregnancy outcomes were analyzed in both IVF and ICSI arms according to the preincubation period duration of oocytes.
***Results*:** The MII percentage of the ICSI arm was significantly lower ($P < 0.05$) in the group with preincubation period of <2.5 h. The fertilization rates in groups with preincubation for 2.5–5.5 h were significantly higher ($P < 0.001$) for IVF. Embryo quality and pregnancy outcomes were not significantly different between the IVF or ICSI arm.
***Conclusions*:** The preincubation of oocytes for at least 2.5 h is beneficial to both IVF and ICSI outcomes by increasing the nuclear maturity of oocytes.

KEY WORDS: Fertilization rates; ICSI; IVF; metaphase II; preincubation period.

INTRODUCTION

Delayed insemination in human in vitro fertilization (IVF) was originally introduced by Trounson *et al.* (1) in 1982 in a study demonstrating better fertilization rates of oocytes and higher embryo quality when the retrieved oocytes received preinsemination incubation. This method was later adopted by most IVF centers producing the best IVF results when oocytes were inseminated 4–6 h after retrieval (2–4). The preincubation of oocytes was considered to complete the final nuclear and cytoplasmic maturation of the oocyte. However, controversy about the necessity of preincubation in IVF arose because some studies showed no effect of preincubation of oocytes on fertilization rate or pregnancy outcome unless the preincubation period was longer than 9 h (5,6).

When intracytoplasmic sperm injection (ICSI) was first introduced in 1992, many procedures were based on previous experience with IVF, including the concept of preincubation of oocytes (7). In the beginning, preincubation for 3–7 h was adopted in most centers (8,9), but later studies contradicted this opinion. Although improved fertilization rate after ICSI on preincubated human oocytes was previously described (10), other researchers supported opposing conclusions (11–13).

Complete nuclear and cytoplasmic maturation of oocytes is essential for the activation of oocytes at fertilization and the development of embryos (14). In a natural cycle, the lutenizing hormone (LH) surge initiates the resumption of meiosis in the oocyte and hence completes the final maturation of the oocyte. In

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the stimulating cycle, human chorionic gonadotropin (hCG) has been used to induce oocyte maturation in a controlled environment. An oocyte is considered to reach nuclear maturity when its meiosis is arrested again at metaphase II (MII) with the presence of an extruded first polar body. Because cytoplasmic maturity may not be synchronous with nuclear maturity in the stimulating cycle (15,16), the fertilizing ability of an oocyte with a mature nucleus is not necessarily at maximum potential.

In IVF cycles, the level of nuclear maturity is usually unknown because the oocytes are surrounded by cumulus and corona cells. On the other hand, denuding procedures must be performed before ICSI to remove surrounding cumulus and corona cells and to ascertain nuclear maturity. As a result, factors other than nuclear maturity seem to affect the fertilizing ability of oocytes in ICSI since only MII oocytes receive sperm injection.

During the 3-year course of this study, oocyte retrievals were performed uniformly 34 h after hCG administration. The insemination or injection procedures were performed randomly 1–8 h after oocyte retrieval. Because all patients enrolled in this study received the same ovarian stimulating protocol, we assumed that the same preincubation period in the IVF arm would result in a similar degree of nuclear maturity to that which would occur in the ICSI arm if the dosage of gonadotropin and ovarian response were similar. To elucidate how preincubation would influence nuclear maturity, we retrospectively analyzed the MII oocyte percentage within the ICSI arm according to varying oocyte preincubation periods. To further clarify the effect of the duration of the preincubation period on the prognosis, we conducted a retrospective analysis on the fertilization rate, embryo quality, and pregnancy outcome in IVF and ICSI cycles with varying oocyte preincubation periods.

MATERIALS AND METHODS

Subjects

We retrospectively analyzed data collected from patients who received IVF or ICSI between January 1999 and December 2001 at the Department of Obstetrics and Gynecology, Taichung Veterans General Hospital, Taiwan. Patients registered for IVF or ICSI were enrolled in this study, except for those with the following conditions: (i) embryo transfer using previous cryopreserved embryo; (ii) failure to retrieve oocytes; (iii) fertilization using rescue ICSI technique in a cycle with IVF failure; and (iv) blastocyst transfer; (v) inadequate documentation of preincubation period; (vi) different stimulation protocol. The patient selection criteria for ICSI were (i) zero or very low normal fertilization rate (less than 20%) in two previous standard IVFs; (ii) poor sperm parameters (lower than 5×10^5 progressively motile spermatozoa with normal morphology) after sperm preparation; and (iii) testicular sperm. A total of 176 IVF and 64 ICSI cycles from 180 patients were included in this analysis. The patients were categorized into five groups in each of the IVF and ICSI arms according to the period between oocyte retrieval and insemination or injection: Group I, <2.5 h; Group II, 2.5 to <3.5 h; Group III, 3.5 to <4.5 h; Group IV, 4.5 to <5.5 h; Group V, ≥5.5 h.

Sperm Preparation

Semen samples were collected at the time of oocyte retrieval by masturbation after 2–5 days of abstinence. The semen parameters were evaluated according to published protocols (17), including (i) manual evaluation of sperm concentration and percentage motility by counting with a Makler chamber according to World Health Organization (1992) criteria; (ii) evaluation of sperm vitality using the conventional eosin–nigrosin stained smear; and (iii) evaluation of sperm morphology using Kruger's strict criteria (Diff–Quik stain). The sample was layered onto a discontinuous Pure-Sperm (Nidacon International, Gothenburg, Sweden) two-layer gradient (40 and 80%) and centrifuged at 300*g* for 30 min. The pellet was washed twice with IVF™-20 (IVF Science Scandinavia, Gothenburg, Sweden) for insemination.

IVF and ICSI Protocols

In all cycles, ovarian stimulation was carried out by a downregulation protocol using gonadotropin-releasing hormone analogue (GnRHa) leuprolide acetate (Lupron®, Takeda Chemical Industries, Osaka, Japan) followed by gonadotropins including human menopausal gonadotropin (hMG; Pergonal®, FSH 75 IU and LH 75 IU in each ampoule, Serono, Aubonne, Switzerland) and follicle-stimulating hormone (FSH; Metrodin®, FSH 75 IU, Serono, Aubonne, Switzerland). The patient received 0.5 mg of GnRHa by daily subcutaneous injection starting at the mid-luteal phase of the previous cycle. Reduction of GnRHa dosage to 0.25 mg daily and 4–6 ampoules of gonadotropin daily was started on Day 3

of the menstrual cycle. Gonadotropin dosage was adjusted accordingly by monitoring the follicles using transvaginal ultrasound starting on Day 9 of the menstrual cycle. Ovulation was triggered by using 10,000 IU hCG (Profaci® , Serono, Aubonne, Switzerland) when two leading follicles reached a mean diameter of 18 mm. Oocyte retrieval was performed 34 h after hCG administration.

The cumulus–corona–oocyte complexes were pooled and incubated in IVF medium. In the case of ICSI, the oocytes received denudation procedures using enzymatic digestion with 80 IU/mL hyaluronidase and capillary pipette dissection just before the injection of sperm. Insemination of oocytes in IVF was performed with spermatozoa adjusted to a concentration of 1×10^5 motile spermatozoa/mL in 100 μL of IVF medium. ICSI was performed essentially as described by Van Steirteghem *et al.* (18). A micromanipulator (Research Instrument Ltd., Penryn, Cornwall, England) and an inverted microscope (Nikon, Tokyo, Japan) with Hoffman modulation were used for ICSI. Only motile sperm cells with normal morphology were selected, and specifically MII oocytes were injected.

Assessment of Fertilization, Embryo Quality, and Pregnancy Outcome

On the morning after insemination or injection, the oocytes were checked for fertilization. The number of polar bodies and pronuclei were documented. Normal fertilization was defined as the presence of two pronuclei and two individualized or fragmented polar bodies. The fertilization rate was defined as the ratio of normal fertilized oocytes to the total number of inseminated or injected oocytes. The embryo quality was evaluated on the morning of Day 2 after oocyte retrieval using the embryo grading criteria established by Veeck (19). The cumulative embryo score (CES) established by Steer *et al.* (20) was derived from the following formula:

$$\mathrm{CES} = \sum_n ((\mathrm{ES} \times \mathrm{CN})_\mathrm{a} + (\mathrm{ES} \times \mathrm{CN})_\mathrm{b} + (\mathrm{ES} \times \mathrm{CN})_\mathrm{c} + \cdots)$$

The ES is the embryo score, and CN is the blastomeric number of each of the embryos. The embryo score was defined as follows: Grade 1 embryo – 4 scores, Grade 2 embryo – 3 scores, Grade 3 embryo – 2 scores, Grade 4 embryo – 1 score, and Grade 5 embryo – 0 score. Average CES was obtained by dividing the CES by the number of normally fertilized oocytes in each cycle.

Pregnancy test using serum hCG assay was performed on Day 12 after embryo transfer. A serial rise in serum hCG concentration would suggest a positive pregnancy outcome. Ongoing pregnancy refers to successful progress of pregnancy beyond the twelfth week of gestation.

Statistics

Statistical analysis was performed with SPSS statistical software (SPSS v 5.0 for Windows). Statistical significance was assessed using the Student's t test, χ^2 test, and one-way analysis of variance (ANOVA). At $P < 0.05$, the difference was considered to be statistically significant.

RESULTS

The results are summarized in Tables I–III. Data collected from 176 IVF cycles and 64 ICSI cycles were included. Among both IVF and ICSI arms and within the five groups of each arm, there were no statistically significant differences observed with regard to the mean age of the patients, the dose of FSH or hMG, or the number of oocytes retrieved in each cycle.

In the IVF arm, 1883 oocytes were retrieved from 176 cycles (10.7 oocytes/cycle). Of the total retrieved, 1477 oocytes (78.4%) were normally fertilized. Because of total fertilization failure, seven cycles (3.97%) in the IVF arm did not receive embryo transfer. The fertilization rates were 67.9, 80.5, 82.0, 84.5, and 73.0% in Groups I, II, III, IV, and V, respectively, with statistically significant differences ($P < 0.001$, using χ^2 test). The fertilization rates of Groups I and V were significantly lower than that of Group II ($P < 0.05$, using χ^2 test and Fisher's exact test, one-tailed). There was no statistically significant difference among Groups II, III, and IV regarding fertilization rates.

In the ICSI arm, 802 oocytes retrieved from 64 cycles (12.5 oocytes/retrieval) received denuding after preincubation; 706 oocytes (88%) were identified as MII stage. The percentages of MII oocytes in each group were significantly different ($P < 0.05$, using χ^2 test). The lowest percentage (80.0%) was observed in Group I ($P < 0.05$, when compared with Group II using χ^2 test and Fisher's exact test). A total of 381 oocytes (54.0%) were fertilized with normal diploid morphology on the second day after injection. The fertilization rate in each group showed no statistically significant difference. Because of total

Table I. Comparison of Age, Gonadotropin Dosage and Response of Ovarian Stimulation, and Outcome Between IVF and ICSI

	IVF	ICSI	*P* value
No. of patients	137	43	—
No. of retrieved cycles	176	64	—
Average age (SD)	33.07 (3.95)	32.95 (5.31)	NS[a]
Ampoules of FSH (SD)	16.49 (7.88)	18.50 (9.16)	NS[a]
Ampoules of hMG (SD)	18.51 (7.15)	17.61 (9.42)	NS[a]
Ampoules of FSH+hMG (SD)	35.00 (12.21)	36.11 (13.43)	NS[a]
Total sperm counts $\times 10^6$	63.31	11.39[b]	—
Total motile sperm counts $\times 10^6$	38.79	3.29[b]	—
No. of total oocytes retrieved	1883	802	—
No. of retrieved oocytes/cycle (SD)	10.7 (5.9)	12.5 (7.1)	NS[a]
Positive hCG (% per retrieval)	68 (38.64)	16 (25)	—
Ongoing pregnancy rate (%)	46 (26.14)	11 (17.2)	—

[a] NS, Not significant, Student's t test, df = 238.
[b] Data from 29 patients were excluded because of testicular sperm or previous total fertilization failure in IVF.

fertilization failure, three cycles (4.68%) in the ICSI arm did not receive embryo transfer.

The embryo quality evaluated by the average CES had no statistically significant difference within groups in the IVF arm, or in the ICSI arm. In the IVF arm, positive hCG tests and ongoing pregnancies were found in 68 cases (38.6% per retrieval) and 46 cases (26.2% per retrieval), respectively. In the case of ICSI, positive hCG tests and ongoing pregnancies were found in 16 cases (25% per retrieval) and 11 cases (17.2% per retrieval), respectively. In each arm, no statistically significant differences were observed among the five groups with regard to positive pregnancy tests or ongoing pregnancies.

DISCUSSION

Clear evidence suggests that the maturity of the oocytes affects the outcome of IVF in both the fertilization rate and embryo quality (14). The oocytes resume meiosis and undergo a process of nuclear maturation from germinal vesicle to metaphase I (MI), and finally arrest again at MII after hCG administration. In general, by 20 h after hCG injection, the oocyte has reached MI and at 35 h after hCG administration, the oocyte is at MII (21). To prevent ovulation before retrieval of oocytes, retrieval was usually arranged 32–36 h after hCG injection. This was based on a study showing that ovulation may occur 32–36 h

Table II. Results of IVF Rate, Embryo Quality, and Pregnancy Outcome

	Preincubation time					
	Group I	Group II	Group III	Group IV	Group V	*P* value
No. of retrieved cycles	7	23	31	47	68	—
No. of embryo transferred cycles	7	22	29	46	65	—
Average age (SD)	32.43 (3.36)	32.96 (4.35)	31.39 (3.62)	33.43 (3.94)	33.71 (3.89)	NS[a]
Ampoules of FSH (SD)	17.71 (6.78)	18.13 (7.96)	15.58 (7.19)	17.49 (8.20)	15.54 (8.05)	NS[a]
Ampoules of hMG (SD)	16.57 (6.19)	18.22 (6.67)	18.03 (6.31)	19.83 (7.65)	18.10 (7.45)	NS[a]
No. of total oocytes retrieved	56	195	372	497	763	—
No. of retrieved oocytes/cycle (SD)	8.00 (5.77)	8.48 (3.50)	12.00 (5.23)	10.57 (5.85)	11.24 (6.60)	NS[a]
No. of total fertilized oocytes	38	157	305	420	557	—
Fertilization rate (%)	67.9[b]	80.5[c]	82.0[c]	84.5[c]	73.0[d]	<0.001[e]
Average cumulative embryo score (SD)	8.86 (5.58)	9.53 (3.23)	10.19 (4.84)	10.46 (5.08)	10.73 (6.01)	NS[a]
No. of transferred embryos (SD)	4.1 (1.86)	4.7 (1.74)	4.9 (1.86)	5.2 (2.02)	4.7 (2.25)	NS[a]
Positive hCG (% per retrieval)	1 (14.3)	7 (30.4)	16 (51.6)	16 (34.0)	28 (41.2)	NS[e]
Ongoing pregnancy (% per retrieval)	1 (14.3)	7 (30.4)	12 (38.7)	9 (19.1)	17 (25.0)	NS[e]

[a] One-way ANOVA, df = 4.
[b] $P < 0.05$, compared with Group II (χ^2 test and Fisher's exact test, one-tailed).
[c] NS (χ^2 test, df = 2).
[d] $P < 0.001$, compared with Group II (χ^2 test and Fisher's exact test).
[e] χ^2 test, df = 4.

Table III. Results of ICSI Metaphase II Oocyte Percentage, Fertilization Rate, Embryo Quality, and Pregnancy Outcome

	Preincubation time					
	Group I	Group II	Group III	Group IV	Group V	*P* value
No. of retrieved cycles	9	23	10	12	10	—
No. of embryo transferred cycles	9	22	10	11	9	—
Average age (SD)	33.00 (5.34)	31.96 (5.90)	33.40 (6.55)	34.25 (3.91)	33.20 (4.52)	NS[a]
Ampoules of FSH (SD)	12.33 (7.14)	21.52 (9.00)	19.20 (10.02)	16.33 (8.55)	19.00 (9.24)	NS[a]
Ampoules of hMG (SD)	16.44 (9.94)	17.00 (10.32)	17.50 (7.56)	20.00 (10.27)	17.30 (8.74)	NS[a]
No. of total oocytes retrieved	120	281	131	149	121	—
No. of retrieved oocytes/cycle (SD)	13.33 (10.98)	12.22 (6.02)	13.10 (8.02)	12.42 (5.92)	12.10 (7.45)	NS[a]
No. of MII oocytes (% of retrieved oocytes)	96 (80.0)[b]	256 (91.1)[c]	117 (89.3)[c]	132 (88.6)[c]	105 (86.8)[c]	<0.05[d]
No. of total fertilized oocytes	54	140	62	66	59	—
Fertilization rate (per injected oocytes; %)	56.3	54.7	53.0	50.0	56.2	NS[d]
Average cumulative embryo score (SD)	8.27 (4.56)	8.87 (4.80)	8.75 (4.28)	10.53 (6.05)	7.89 (5.60)	NS[a]
No. of transferred embryos (SD)	4.4 (2.35)	4.4 (2.06)	4.7 (2.06)	4.1 (2.43)	3.9 (2.60)	NS[a]
Positive hCG (% per retrieval)	2 (22.2)	6 (26.1)	5 (50)	1 (8.3)	2 (20)	NS[d]
Ongoing pregnancy (% per retrieval)	2 (22.2)	3 (13)	3 (30)	1 (8.3)	2 (20)	NS[d]

[a] One-way ANOVA, df = 4.
[b] $P < 0.05$, compared with Group II, III, IV (χ^2 test and Fisher's exact test).
[c] NS (χ^2 test, df = 2).
[d] χ^2 test, df = 4.

after hCG injection in clomiphene citrate or hMG superovulation cycles (22). To maximize the maturity of oocytes, efforts to extend the time between hCG injection and insemination of sperm in IVF cycles have been made (1–3,23,24). Prolongation of the time from hCG administration to retreival of oocytes is regarded as in vivo maturation, and extension of the time from oocyte retrieval to insemination is regarded as in vitro maturation (25).

Since ICSI was introduced as an assisted fertilization technique, the nuclear maturity of the oocyte was routinely evaluated because only oocytes at MII received the microinjection procedure. Most centers performed preincubation of oocytes in ICSI as they did in IVF for two reasons. First, the nuclear and cytoplasmic maturation seem to be asynchronous in stimulated cycles, even though they are assumed to be coordinated in natural cycles (14). In animal models, it was also reported that MII mouse oocytes progressively develop sensitivity to parthenogenetic stimuli (26). Thus, the presence of a MII oocyte does not ensure maximum susceptibility to activation by sperm. Second, incubation of the denuded MI oocytes may allow the extrusion of the first polar body. When the retrieved oocytes received preinjection incubation, it was expected that the percentage of MII oocytes would increase before they were denuded. Two studies conducted by Van de Velde *et al.* (11) and Yanagida *et al.* (12) demonstrated that the fertilization rate of MII oocytes was not influenced by the preincubation period in ICSI. This may imply two possible mechanisms: first, ovarian stimulation with GnRHa/FSH/hMG protocol can result in synchronized nuclear and cytoplasmic maturation, or second, the ICSI procedure may be able to bypass some pathway, which is associated with cytoplasmic maturation. Meanwhile, they concluded that there was no need for preincubation of oocytes because the percentage of MII oocytes was not different among differing preincubation period groups when the oocyte was retrieved 35 or 36 h after administration of hCG. However, an evaluation of the relationship between nuclear maturity and preincubation period by Veeck has demonstrated that the percentage of MII oocytes increased from 63.4% to 85% if they were incubated after retrieval for 4 more hours (27). In another study comparing the percentage of MII oocytes in ICSI patients with a 2-h preincubation period and differing times between hCG administration and oocyte retrieval, Mansour *et al.* found that in vivo maturation for 36 h produced a statistically significantly higher percentage of MII oocytes than in vivo maturation for 35 h (28). Our data shows that the percentage of MII oocytes in the group with the preincubation period of <2.5 h is significantly lower than that in the groups with the preincubation period of ≥ 2.5 h in ICSI patients. This result supports the conclusion that preincubation of oocytes for >2.5 h is beneficial for nuclear maturation. The major difference between this study and that of Van de Velde is that over the course of this study, oocytes were retrieved 34 h after hCG administration, which may have allowed for observation of lower MII percentages in those oocytes receiving less time for in vivo maturation. As a result, we conclude

that 34-h in vivo maturation alone is inferior to 34-h in vivo maturation plus at least 2.5-h preincubation in vitro for nuclear maturation of oocytes. The fertilization rates of MII oocytes and the average CES of embryos were not significantly different among each of the groups in the ICSI arm of this study. This revealed that the preincubation of the oocytes provided a higher percentage of available MII oocytes for injection, which had the same fertilizing ability and obtained the same embryo quality.

As for the IVF arm in this study, fertilization rates were significantly lower in the group with a preincubation period of <2.5 h than in the other groups, suggesting that a delay of insemination for 2.5–5.5 h is beneficial to fertilization rates. This finding is compatible with the suggestion that Trounson *et al.* have advocated, that a short culture period in vitro may allow the completion of oocyte maturation and improve the results of IVF. Although this study did not examine the nuclear maturation by denudation in the IVF arm, it may be assumed that the nuclear maturity profile of the oocytes is similar in IVF as in ICSI, since all the patients received the same ovarian stimulation protocol and achieved similar ovarian response. Fewer MII oocytes in the group receiving <2.5 h than those receiving > 2.5 h of preincubation is considered to be one of the factors affecting the fertilization rate in the IVF arm. In the current study, a much lower fertilization rate in Group I (67.9%) than in Groups II–IV (80.5–84.5%) was observed in the IVF arm. It seems that this effect was not solely due to the level of nuclear maturity. Cytoplasmic immaturity may be associated with polyspermy when luteinizing stimulus is inadequate, thus reducing the normal fertilization ratio. In normal fertilization of IVF, polyspermy is prevented by the mechanism of cortical reaction (29). Migration of cortical granules is dependent upon the time of initiation of the luteinizing stimulus (25).

There was a lower fertilization rate ($P < 0.001$) in Group V with the preincubation period of >5.5 h than that in Group II in the IVF arm. Prolonged incubation of oocytes may lead to hardening of zona pellucida in mouse oocytes (30). In human oocytes, Manna *et al.* showed prolonged metaphase oocyte incubation results in spontaneous zona pellucida hardening and prolonged zona pellucida dissolution time, which was significantly correlated with a low IVF rate (31). The fertilization rate of the oocytes in Group V with a preincubation period of >5.5 h in the ICSI arm was not significantly different from the fertilization rates of those in the other groups in the current study. This suggests that the ICSI procedure might overcome the obstacles of a hardened zona pellucida.

An important finding in this study that the embryo quality in both the IVF and ICSI arms was not significantly different among varying preincubation periods. Consequently, the pregnancy rates and ongoing pregnancy rates were not significantly different among each group within the IVF and ICSI arms. In this study, the cumulus cells were not removed until the injection of sperm in the ICSI arm, and they were not removed in the IVF arm. Although the role of the cumulus cells in the maturation of the oocytes has been questioned by some researchers (11), the cumulus cells have been commonly considered to be beneficial to oocyte maturation (32–34). In the current study, the beneficial effect of preincubation was considered to be partly provided by the surrounding cumulus cells.

In conclusion, the results of the current study suggest that preincubation between oocyte retrieval and sperm injection in ICSI may increase the percentage of MII oocytes available for injection. However, the fertilization rate of MII oocytes, as well as the subsequent embryo development, is not influenced by differing preincubation periods. The time interval between oocyte retrieval and insemination may influence the fertilization rates in IVF, and the best fertilization rates were obtained when the oocytes were inseminated between 2.5 and 5.5 h after retrieval. This finding is related to the percentage of MII oocytes in each group since preincubation for 2.5 h may be able to increase the nuclear maturity of the oocytes with hCG administration 34 h earlier. It awaits further study whether an extension of the time between hCG administration and oocyte retrieval from 34 to 36 h would be advantageous to the nuclear maturity of oocytes, and consequently increase the fertilization rates of IVF and MII oocyte ratios in ICSI.

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REFERENCES

1. Trounson AO, Mohr LR, Wood C, Leeton JF: Effect of delayed insemination on in-vitro fertilization, culture and transfer of human embryos. J Reprod Fertil 1982;64:285–294

2. Marrs RP, Saito H, Yee B, Sato F, Brown J: Effect of variation of in vitro culture techniques upon oocyte fertilization and embryo development in human in vitro fertilization procedures. Fertil Steril 1984;41:519–523
3. Veeck LL, Wortham JW, Jr, Witmyer J, Sandow BA, Acosta AA, Garcia JE, Jones GS, Jones HW, Jr: Maturation and fertilization of morphologically immature human oocytes in a program of in vitro fertilization. Fertil Steril 1983;39:594–602
4. Khan I, Staessen C, Van den Abbeel E, Camus M, Wisanto A, Smitz J, Devroey P, Van Steirteghem AC: Time of insemination and its effect on in-vitro fertilization, cleavage and pregnancy rates in GnRH agonist/HMG-stimulated cycles. Hum Reprod 1989;4:921–926
5. Harrison KL, Wilson LM, Breen TM, Pope AK, Cummins JM, Hennessey JF: Fertilization of human oocytes in relation to varying delay before insemination. Fertil Steril 1988;50:294–297
6. Fisch B, Kaplan-Kraicer R, Amit S, Ovadia J, Tadir Y: The effect of preinsemination interval upon fertilization of human oocytes in vitro. Hum Reprod 1989;4:954–956
7. Palermo G, Joris H, Devroey P, Van Steirteghem AC: Pregnancies after intracytoplasmic injection of single spermatozoon into an oocyte. Lancet 1992;340:17–18
8. Van Steirteghem AC, Liu J, Joris H: Higher success rate by intracytoplasmic sperm injection than by subzonal insemination. Report of a second series of 300 consecutive treatment cycles. Hum Reprod 1993;8:1061–1066
9. Payne D, Flaherty SP, Jeffrey R: Successful treatment of severe male factor infertility in 100 consecutive cycles using intracytoplasmic sperm injection. Hum Reprod 1994;9:2051–2057
10. Rienzi L, Ubaldi F, Anniballo R, Cerulo G, Greco E: Preincubation of human oocytes may improve fertilization and embryo quality after intracytoplasmic sperm injection. Hum Reprod 1998;13:1014–1019
11. Van de Velde H, De Vos A, Joris H, Nagy ZP, Van Steirteghem AC: Effect of timing of oocyte denudation and micro-injection on survival, fertilization and embryo quality after intracytoplasmic sperm injection. Hum Reprod 1998;13:3160–3164
12. Yanagida K, Yazawa H, Katayose H, Suzuki K, Hoshi K, Sato A: Influence of oocyte preincubation time on fertilization after intracytoplasmic sperm injection. Hum Reprod 1998;13:2223–2226
13. Jacobs M, Stolwijk AM, Wetzels AMM: The effect of insemination/injection on the results of IVF and ICSI. Hum Reprod 2001;16:1708–1713
14. Zenzes MT, Belkien L, Bordt J, Kan I, Schneider HG, Nieschlag E: Cytological investigation of human in vitro fertilization failures. Fertil Steril 1985;43:883–891
15. Sundstrom P, Nilsson BO: Meiotic and cytoplasmic maturation of oocytes collected in stimulated cycles is asynchronous. Hum Reprod 1988;3:613–619
16. Eppig JJ, Schultz RM, O'Brein M, Chesnel F: Relationship between the developmental programs controlling nuclear and cytoplasmic maturation of mouse oocytes. Dev Biol 1994;164:1–9
17. Chen MJ, Bongso A: Comparative evaluation of two density gradient preparations for sperm separation for medically assisted conception. Hum Reprod 1999;14:759–764
18. Van Steirteghem AC, Joris H, Liu J: Protocol for intracytoplasmic sperm injection. Hum Reprod (Update) 1995;1(3), CD-ROM
19. Veeck LL: Atlas of the Human Oocyte and Early Conception, Vol. 2. Baltimore, Williams &Wilkins, 1991
20. Steer CV, Mills CL, Tan SL, Campbell S, Edwards RG: The cumulative embryo score: A predictive embryo scoring technique to select the optimal number of embryos to transfer in an in vitro fertilization and embryo transfer programme. Hum Reprod 1992;7:117–119
21. Bomsel-Helmreich O, Salat-Baroux J, Huyen LVN, Antoine J, Durand-Gasselin I: Timing of nuclear maturation and cumulus dissociation in human oocytes stimulated with clomiphene citrate, human menopausal gonadotropin, and human chorionic gonadotropin. Fertil Steril 1987;48:586–595
22. Edwards RG, Steptoe PC: Control of human ovulation, fertilization, and implantation. Proc R Soc Med 1974;67:932–936
23. Gudmundsson J, Fleming R, Jamieson ME, McQueen D, Coutts JR: Luteinization to oocyte retrieval delay in women in whom multiple follicular growth was induced as part of an in vitro fertilization/gamete intrafallopian transfer program. Fertil Steril 1990;53:735–737
24. De Vits A, Gerris J, Joostens M, Aytos A: Comparison between two hCG-to-oocyte aspiration intervals (36 versus 38) on the outcome of in-vitro fertilization. Hum Reprod 1994;9(suppl. 4):12
25. Jamieson ME, Fleming R, Kader S, Ross KS, Yates RW, Coutts JR: In vivo and in vitro maturation of human oocytes: Effects on embryo development and polyspermic fertilization. Fertil Steril 1991;56:93–97
26. Kubiak JZ: Mouse oocytes gradually develop the capacity for activation during the metaphase II arrest. Dev Biol 1989;136:537–545
27. Veeck LL: Oocyte assessment and biological performance. Ann NY Acad Sci 1988;541:259–274
28. Mansour RT, Aboulghar MA, Serour GI: Study of the optimum time for human chorionic gonadotropin-ovum pickup interval in in vitro fertilization. J Assist Reprod Genet 1994;11:478–481
29. Sathananthan AH, Trounson AO: Ultrastructure of cortical granule release and zona interaction in monospermic and polyspermic human ova fertilized in vitro. Gamete Res 1982;6:225–232
30. Fukuda A, Roudebush WE, Thatcher SS: Influences of in vitro oocyte aging on microfertilization in the mouse with reference to zona hardening. J Assist Reprod Genet 1992;9:378–383
31. Manna C, Rienzi L, Greco E, Sbracia M, Rahman A, Poverini R, Siracusa G, Defelici M: Zona pellucida solubility and cortical granule complements in human oocytes following assisted reproductive techniques. Zygote 2001;9:201–210
32. Yamazaki Y, Wakayama T, Yanagimachi R: Contribution of cumulus cells and serum to the maturation of oocyte cytoplasm as revealed by intracytoplasmic sperm injection (ICSI). Zygote 2001;9:277–282
33. Hwang JL, Lin YH, Tsai YL: In vitro maturation and fertilization of immature oocytes: A comparative study of fertilization techniques. J Assist Reprod Genet 2000;17:39–43
34. Hassan HA: Cumulus cell contribution to cytoplasmic maturation and oocyte developmental competence in vitro. J Assist Reprod Genet 2001;18:539–543