Evaluation of Rescue ICSI on Oocytes without Extrusion of the Second Polar Body in Cases of Moderately Disturbed Fertilization

Yuka Hirabayashi[1], Fumiko Tawara[1], Misato Ishii[1], Hirotaka Muramatsu[1] and Naohiro Kanayama[2]*

[1]*Takeuchi Hospital Toyota Infertility Center, 1-2-1 Kosaka-cho, Toyota-shi, Aichi 471-0034, Japan*
[2]*Department of Obstetrics and Gynecology, Hamamatsu University School of Medicine, 1-20-1 Handayama, Hamamatsu, Shizuoka 431-3192, Japan*

Abstract: *We predicted the success or failure of fertilization by the presence or absence of a second polar body 6 hours after insemination, and we performed intracytoplasmic sperm injection (ICSI) for oocytes that did not produce a second polar body to investigate the efficacy of such rescue ICSI. Cases in which ICSI was performed as scheduled after oocyte collection were classified together as the ICSI group. Those in which the second polar body was confirmed in at least half of the oocytes 6 hours after insemination by in vitro fertilization (IVF) were classified as the IVF group, and those in which the second polar body was not confirmed in at least half of the oocytes 6 hours after insemination and on which ICSI was thus performed were classified as the rescue ICSI group. The fertilization rates in the ICSI group, IVF group and rescue ICSI group were 73.8%, 75.4% and 69.0% without significant differences. Pregnancy rates were 41.0%, 41.0% and 29.8%, in the respective groups without significant differences. Our results strongly suggest that cancellation of embryo transfer is avoided by rescue ICSI when IVF results in all unfertilized oocytes.*

Key words: *Intracytoplasmic sperm injection, Rescue ICSI, Second polar body, In vitro fertilization*

Introduction

During the initial attempt at *in vitro* fertilization (IVF), it is extremely difficult to determine whether there is a fertilization disorder. Cancellation of embryo transfer due to non-fertilization puts both psychiatric and financial burdens on the patient. Male fertility can be assessed by semen analysis before IVF. To predict a fertilization disorder, the Kruger test, which investigates the morphology of sperm (Kruger *et al.* [1]), and a computer-assisted semen analyzer to mechanically assess the motility of sperm on the basis of straightforward movement and the amplitude of the sperm head are reported to be useful (Itagaki *et al.* [2]). Egashira, *et al.* [3] evaluated indications for intracytoplasmic sperm injection (ICSI) and reported that ICSI is indicated when the number of sperms that show straightforward movement is less than $1–5 \times 10^6$/ml. To predict sperm disorder, i.e., sperm which cannot pass through the zona pellucida, and oocyte membrane fusion disorder, for which both oocyte and sperm disorders are reportedly responsible, Franken [4] and Sifer *et al.* [5] conducted a zona pellucida penetration test using hamster ovum (hamster test) and hemizona assay (binding of sperm to the human pellucid zone) and the zona pellucida-binding test, and the results were reported to be useful.

Furthermore, as a rescue treatment for intractable fertilization disorder after IVF, 1-day-old ICSI, has been reported. That is ICSI into an oocyte that does not produce a second polar body or oocyte pronuclei are not confirmed the day after insemination. In 1996, Lundin *et al.* reported that the fertilization rate and pregnancy rate by this method were 46.5% and 6.9% [6], respectively; the respective rates reported for 1997–2004 were 44.0–82.7% and 12.5–14.8% [7–10]. ICSI is considered clinically impractical because the pregnancy

Received: August 31, 2006
Accepted: September 20, 2006
*To whom correspondence should be addressed.
e-mal: ivf-take@hm2.aitai.ne.jp

rate is still low even though the fertilization rate achieved by 1-day-old ICSI is gradually improving. Kuczynski *et al.* [11] performed rescue ICSI for cases in which oocytes remained unfertilized after IVF, but the pregnancy rate was 0%. In 2003, Chen *et al.* [12] performed ICSI for cases in which the second polar body was not observed in any oocyte 6 hours after insemination and reported that the pregnancy rate improved to 48%.

In the present study, we predicted fertilization in IVF cases by the presence or absence of a second polar body 6 hours after insemination, and we investigated the effect of ICSI in cases in which the second polar body was not confirmed in at least half of the oocytes after IVF.

Materials and Methods

The study subjects were 539 patients who provided their informed consent between February 2004 and August 2005. A total of 752 ICSI cycles were investigated. Ovulation was induced by stimulation with concomitant use of GnRH antagonist or clomifenecitrate and hMG. When the ovarian follicle diameter reached 18 mm or more, hCG or GnRH agonist was used, and oocytes were collected after 35–36 hours. Oocyte maturity was confirmed, and mature oocytes were pre-incubated in K-SIFM fertilization medium (Cook Co., Queensland, Australia) for about 4 hours. Sperms were obtained on the day of oocyte collection. After precipitation of semen on 90%, 68% and 45% PureCeption sperm washing medium (Sage BioPharma., Bedminster, NJ, USA), the sample was centrifuged at 450 g for 15 minutes. The precipitates were then added to 3 ml K-SIFM and washed by centrifugation at 300 g for 5 minutes. The collected sperm were maintained at 37°C on a warming plate until the time of insemination. In cases of IVF, the sperms were inseminated after pre-incubation of about 2 hours or more subsequent to washing. The sperm concentration for insemination was adjusted to 10–15 $\times$ 10^4/ml. Six hours after insemination, the oocytes were aspirated and extruded several times with the use of a pipette for oocyte transfer, and the cumulus cells were detached to an extent that allowed detection of the second polar body. Presence or absence of the second polar body was confirmed under an inversion microscope.

Six hours after insemination, the culture solution was exchanged in cases in which extrusion of the second polar body was confirmed in at least half of the oocytes, and incubation was continued until the next day. Cases in which extrusion of the second polar body was not confirmed in at least half of the oocytes 6 hours after insemination were considered cases of insemination failure, and ICSI was performed only for the oocytes that did not show a second polar body at that time. In principle, such rescue ICSI was performed when extrusion of the second polar body was not confirmed in at least half of the oocytes that were inseminated.

For the ICSI procedure, the cumulus was detached by processing them with 60 IU/ml hyaluronidase (Sigma, Chemical Co., St. Louis, MO, USA). ICSI was then performed, and this was followed by incubation until the next day. Oocytes in which 2 pronuclei were confirmed about 20 hours after insemination or after ICSI were considered normally fertilized. G-1™ v3PLUS and G-2™ v3PLUS (Vitrolife Co., Kungsbacka, Sweden) medium or the Blast Assist System (MediCult, Jyllinge, Denmark) was used for incubation for 5–6 days until formation of a blastocyst. The embryos were incubated in a CO_2 water jacket incubator at a temperature of 37°C in 6% CO_2 in air (Fig. 1).

Cases in which ICSI was performed as scheduled were classified together as the ICSI group. Those in which the second polar body was confirmed in at least half of the oocytes 6 hours after insemination were classified as the IVF group, and those in which the second polar body was not confirmed in at least half of the oocytes 6 hours after insemination and on which ICSI was thus performed for these oocytes were classified as the rescue ICSI group.

Because oocytes fertilized by IVF were included in the rescue ICSI group in addition to oocytes that underwent rescue ICSI, this group was further divided into 2 subgroups, the IVF + rescue ICSI subgroup (cases in which some oocytes were fertilized by IVF) and the rescue ICSI-only subgroup (cases in which rescue ICSI was performed for all oocytes), and differences in the results between these 2 subgroups were investigated.

Differences in the number of oocyte collection and patient age were analyzed by Student's t-test. Differences in the rates of fertilization, blastocyst formation and pregnancy were analyzed by the F-test.

Results

Patient backgrounds are shown by group (ICSI group, IVF group and rescue ICSI group) in Table 1. The mean age of patients per group was 35.3 $\pm$ 5.1 years, 33.9 $\pm$ 4.7 years and 33.3 $\pm$ 4.5 years, respectively, with no significant differences between groups. Infertility in

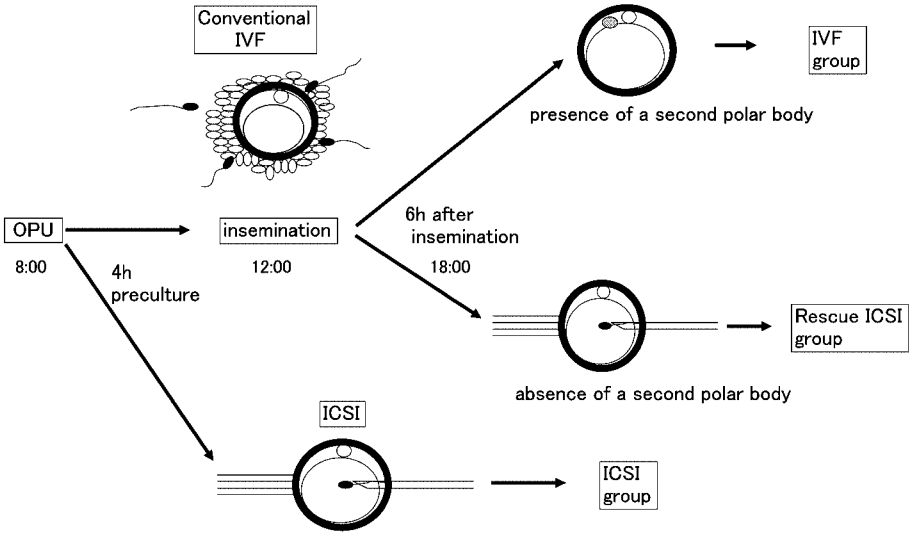

Fig. 1. OPU: (8:30) oocytes were collected, and the oocytes were pre-incubated for about 4 hours. Insemination: (12:00) the sperm concentration for insemination was adjusted to 10–15 × 10^4/ml. Observation of the second polar body: (18:00) six hours after insemination, the second polar body was observed microscopically. Rescue ICSI: ICSI was performed on oocytes without a second polar body if more than half of the oocytes did not have the second polar body. Classification: Cases in which ICSI was performed as scheduled after oocyte retrieval were classified together as the ICSI group. Those in which the second polar body was confirmed in at least half of the oocytes 6 hours after insemination by IVF were classified as the IVF group, and those in which the second polar body was not confirmed in at least half of the oocytes 6 hours after insemination and on which ICSI was thus performed were classified as the rescue ICSI group.

Table 1. Patient's backgrounds per group

	ICSI group	IVF group	Rescue ICSI group
Mean age (years)	35.3 (± 5.1)	33.9 (± 4.7)	33.3 (± 4.5)
Number of cases	162	249	128
Cause no. and percentage of patients of infertility			
Tubal disease	19 (11.7)	71 (28.5)	28 (21.8)
Endometriosis	10 (6.2)	17 (6.8)	23 (18.0)
Male factor	110 (67.9)	20 (8.0)	16 (12.5)
Unknown	16 (9.9)	133 (53.4)	59 (46.1)
Other	7 (4.3)	8 (3.2)	2 (1.6)
Frequency of oocyte collection	1.6 (1–6)	1.4 (1–5)	1.1 (1–2)
Number of oocytes collected	3.5 (1–29)	4.1 (1–33)	4.8 (1–12)

the ICSI group was mainly male infertility. There was no significant difference in the type of infertility between the IVF group and the rescue ICSI group. The frequency of oocyte collection per group was 1.6, 1.4, and 1.1, respectively, and the mean number of oocytes collected per group was 3.5, 4.1 and 4.8, respectively.

The fertilization rate and blastocyst formation rate are shown per group in Table 2. The normal fertilization rates were 73.8% (679/920), 75.4% (1085/1439) and 69.0% (470/681) in the respective groups, with no significant differences between groups. The incidence of tripronucleate (3PN) formation was 3.2% (29/920) in the ICSI group, 5.5% (79/1,439) in the IVF group and 5.3% (36/681) in the rescue ICSI group. The rate was significantly lower in the ICSI group than in the other 2 groups ($p<0.05$), but there was no significant difference between the IVF group and the rescue ICSI group. The number of cultured embryos per group was 382, 606

Table 2. Fertilization and blastocyst formation rates per group

	ICSI group	IVF group	Rescue ICSI group
Number of cycles	264	349	139
Number of oocytes	920	1439	681
Normal fertilization rate	73.8% (679/920)	75.4% (1085/1439)	69.0% (470/681)
Abnormal fertilization rate 1PN	3.3% (30/920)	4.0%* (58/1439)	2.1% (14/681)
3PN	3.2%** (29/920)	5.5% (79/1439)	5.3% (36/681)
Blastocyst formation rate	52.1%*** (199/382)	66.3% (402/606)	60.6% (146/241)

* vs. ICSI and Rescue ICSI, P<0.05. ** vs. IVF and Rescue ICSI, P<0.05. *** vs. IVF and Rescue ICSI, P<0.05.

Table 3. Clinical results per group

	ICSI group	IVF group	Rescue ICSI group
Transfer of day2–3 embryo	33.3% (33/99)	34.0% (49/144)	23.0% (14/61)
Transfer of blastocyst	51.4% (39/76)	52.9% (45/85)	47.8% (11/23)
Total pregnancy rate	41.1% (72/175)	41.0% (94/229)	29.8% (25/84)

Table 4. Results per rescue ICSI subgroup

	IVF + Rescue ICSI	Rescue ICSI-only
Number of cycles	98	41
Number of oocytes	608	73
Normal fertilization rate	67.3%* (409/608)	83.6% (61/73)
Abnormal fertilization rate 1PN	2.0% (12/608)	2.7% (2/73)
3PN	5.4% (33/608)	4.1% (3/73)
Blastocyst formation rate	59.4% (133/224)	76.5% (13/17)
Transfer of day2–3 embryo	20.5% (9/44)	29.4% (5/17)
Transfer of blastocyst	40.0% (6/15)	62.5% (5/8)
Total pregnancy rate	25.4% (15/49)	40.0% (10/25)

* vs. Rescue ICSI-only, P<0.05.

and 241. In comparison to a blastocyte formation rate of 52.1% (199/382) in the ICSI group, rates were significantly higher at 66.3% (402/606) in the IVF group and 60.6% (146/241) in the rescue ICSI group (p<0.05).

Clinical results are shown per group in Table 3. The pregnancy rate per cycle after transfer of the day2–3 embryo was 33.3% (33/99), 34.0% (49/144) and 23.0% (14/61) in the ICSI group, IVF group and rescue ICSI group, respectively, with no significant differences between groups. The pregnancy rate per cycle after blastocyst transfer was 51.4% (39/76), 52.9% (45/85) and 47.8% (11/23), respectively, with no significant differences between groups. The 72 pregnancies in the ICSI group resulted in 5 sets of twins, 55 singletons and 12 spontaneous abortions. The 94 pregnancies in the IVF group resulted in 8 sets of twins, 69 singletons and 17 spontaneous abortions. The 25 pregnancies in the rescue ICSI group resulted in 3 sets of twins, 16 singletons, 5 spontaneous abortions and 1 unknown outcome.

Results obtained in the 2 rescue ICSI subgroups (IVF + rescue ICSI and rescue ICSI-only) are shown in Table 4. The normal fertilization rate increased significantly to 83.6% (61/73) in the rescue ICSI-only subgroup and 67.3% (409/608) in the IVF + rescue ICSI subgroup (p<0.05). The incidence of 3PN formation was 5.4% (33/608) in the IVF + rescue ICSI subgroup and 4.1% (3/73) in the rescue ICSI-only subgroup, with no significant difference between the 2 subgroups. The blastocyst formation rate was 59.4% (133/224) in the IVF + rescue ICSI subgroup and 76.5% (13/17) in the rescue ICSI-only subgroup. The pregnancy rate per day2–3 embryo transfer cycle was 20.5% (9/44) and 29.4% (5/17), respectively. The pregnancy rate per blastocyst transfer cycle was 40.0% (6/15) and 62.5% (5/8), respectively. When both subgroups were

compared respectively a result better than 40% were obtained in them. Healthy babies were born from patients who were treated until the time of birth.

Discussion

Porter *et al.* [13] reported that the incidence of 3PN formation was 2.5% in ICSI cases but 8.1% in IVF cases. The possibility of polyspermy is considered to be higher with IVF than with ICSI, in which sperm is injected into one oocyte.

The blastocyst formation rate in the present study was slightly lower in the ICSI group than in the other 2 groups, and there was no significant difference between the IVF group and the rescue ICSI group. The pregnancy rate was somewhat lower in the rescue ICSI group than in the other 2 groups. However, in view of the rate achieved by blastocyst transfer and considering that the pregnancy rate reported for 1-day-old ICSI is as low as 12.5–20.7% [6–8, 10], the rate achieved by means of rescue ICSI is sufficient to show that this method is clinically applicable. With the conventional method, oocytes may deteriorate during the 24-hour incubation period, but deterioration of the oocytes may be negligible in cases of rescue ICSI because a judgment is made 6 hours after insemination.

It is possible to some extent to predict the fertilization by a semen analysis before IVF. However, a prediction of the fertilization with an individual oocyte is difficult. The results of this study suggest that non-fertilization and 1-day-old ICSI can be avoided by rescue ICSI.

Healthy babies were born from patients who were treated until the time of birth. In our patients, approximately 3 oocytes were collected; this is a relatively small number. There is some possibility that we could not achieve embryo transfer in such cases. Recently, the number of oocytes collected has decreased in many hospitals. Therefore, we believe that rescue ICSI is clinically meaningful for obtaining an embryo.

The fertilization rate and blast cyst formation rate tended to be higher in the rescue ICSI-only group than in the IVF + rescue ICSI group. However, there was no difference in the rate of 3PN formation between these 2 groups. Therefore, the quality of oocytes might have differed between IVF + rescue ICSI and rescue ICSI-only.

The pregnancy rate, after both day2–3 embryo transfer and blastocyst transfer tended to be lower in the IVF + rescue ICSI subgroup than in the rescue ICSI-only subgroup. This is probably because of a fertilization disorder (sperm not entering the oocyte) in the rescue ICSI- only subgroup in which rescue ICSI was performed for all oocytes. However, some oocytes were fertilized by IVF in the IVF + rescue ICSI subgroup, indicating that a chromosomal aberration or aberration in the oocyte rather than a lack of sperm motility was affecting the oocytes that did not produce a second polar body after rescue ICSI. In many cases, oocytes with a fertilization disorder include those with a chromosomal aberration. In some cases, the sperm has been noted to enter such an oocyte before pronucleus formation [14].

There would be no significant difference in the abnormal fertilization rate between the IVF + rescue ICSI and rescue ICSI-only subgroups if rescue ICSI were performed in all cases except those uirlving complete fertilization failure. Thus, it is assumed that rescue ICSI is effective.

3PN development is due to retention of the second polar body or polyspermy. Although the 3PN fertilization rate in the rescue ICSI cases was not high in the present study, further investigation into methods for judging the presence or absence of a second polar body and careful observation are necessary to reduce the abnormal fertilization rate caused by polyspermy. Although 1-day-old ICSI has been performed as a measure against non-fertilization by IVF, a low pregnancy rate has been a problem in such cases. There was no significant difference in the 3PN development rate between the IVF group and the rescue ICSI group in the present study. However, the existence of a delayed fertilized oocyte could not be ruled out. When 2 polar bodies are seen, rescue ICSI is not appropriate. It is difficult to distinguish discharge of establish a method to save the second polar body from fragmentation. It will be necessary to objectively assess of the second polar body in anticipation of rescue ICSI. The achieved rate by rescue ICSI in the present study was higher than that by 1-day-old ICSI, indicating that rescue ICSI is an effective treatment method.

We got similar results to Chen *et al.* in cases of lack of a second polar body 6 hours after insemination. Furthermore we succeeded in obtaining fertilized oocytes in IVF cases in which the second polar body was not observed in more than 50% oocytes.

References

1) Kruger, T.F., Menkveld, R., Stander, F.S., Lombard, C.J., Van der Merwe, J.P., van Zyl, J.A. and Smith, K. (1986): Sperm morphologic features as a prognostic factor in in

vitro fertilization. Fertil. Steril., 46, 1118–1123.
2) Itagaki, K., Takeuchi, H., Kikuchi, B., Saitou, Y., Sakurai, A., Kuwahara, H. and Tachibana, Y. (2000): Investigation of the usefulness of a new automatic sperm analysis device (C-MEM™) in semen test. Japan Society of Fertilization and Implantation Review, 17, 227–300.
3) Egashira, A., Motoishi, M., Sugioka, M., Ohtsu, K., Nagafuti, E., Nishigaki, A., Yoshioka, N. and Kuramoto, T. (2004): Evalution of sperm fertilizing ability by using motility sperm concentration in In Vitro fertilization programs. J. Mamm. Ova Res., 21, 214–219.
4) Franken, D.R. (1998): The clinical significance of sperm-zona pellucida binding. Front Biosci., 3, E247–253.
5) Sifer, C., Sasportes, T., Barraud, V., Poncelet, C., Rudant, J., Porcher, R., Cedrin-Durnerin, I., Martin-Pont, B., Hugues, J.N. and Wolf, J.P. (2005): World Health Organization grade 'a' motility and zona-binding test accurately predict IVF outcome for mild male factor and unexplained infertilities. Hum. Reprod., 20, 2769–2775.
6) Lundin, K., Sjogren, A. and Hamberger, L. (1996): Reinsemination of one-day-old oocytes by use of intracytoplasmic sperm injection. Fertil. Steril., 66, 118–121.
7) Morton, P.C., Yoder, C.S., Tucker, M.J., Wright, G., Brockman, W.D. and Kort, H.I. (1997): Reinsemination by intracytoplasmic sperm injection of 1-day-old oocytes after complete conventional fertilization failure. Fertil. Steril., 68, 488–491.
8) Yuzpe, A.A., Liu, Z. and Fluker, M.R. (2000): Rescue intracytoplasmic sperm injection (ICSI)-salvaging in vitro fertilization (IVF) cycles after total or near-total fertilization failure. Fertil. Steril., 73, 1115–1119.
9) Li, R., Zhuang, G. and Zhou, C. (2000): Reinsemination by intracytoplasmic sperm injection 24-hour after conventional in vitro fertilization failure. Zhonghua Fu Chan Ke Za Zhi, 35, 404–405.
10) Kasai, T., Fujie, M., Enkhmaa, D., Suzuki, M., Suzuki, K., Shima, T., Shimadu,Y., Wada, M., Miyake, M., Shoda, T., Nagai, S., Hirata, S. and Hoshi, K.(2005): One-day-old intracytoplassmic sperm injection of oocytes after fertilization failure. Japan Society of Fertilization and Implantation Review, 22, 158–162.
11) Kuczynski, W., Dhont, M., Grygoruk, C., Pietrewicz, P., Redzko, S. and Szamatowicz, M. (2002): Rescue ICSI of unfertilized oocytes after IVF. Hum. Reprod., 17, 2423–2427.
12) Chen, C. and Kattera, S. (2003): Rescue ICSI of oocytes that failed to extrude the second polar body 6 h post-insemination in conventional IVF. Hum. Reprod., 18, 2118–2121.
13) Kunathikom, S., Makemaharn, O., Suksompong, S. and Laokirkkiat, P. (2001): Chromosomal analysis of "failed-fertilized" human oocytes resulting from in vitro fertilization and intracytoplasmic sperm injection. J. Med. Assoc. Thai., 84, 532–538.
14) Porter, R., Han, T., Tucker, M.J., Graham, J., Liebermann, J. and Sills, E.S. (2003): Estimation of second polar body retention rate after conventional insemination and intracytoplasmic sperm injection: in vitro observations from more than 5000 human oocytes. J. Assist. Reprod. Genet., 20, 371–376.