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Higher abnormal fertilization, higher cleavage rate, and higher arrested embryos rate were found in conventional IVF than in intracytoplasmic sperm injection

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Summary

Objective: The aim of this study was to investigate whether performing different fertilization technologies (intracytoplasmic sperm injection [ICSI] and in vitro fertilization [IVF]) may affect the result of fertilization in the normal fertilization cycles. *Study design:* The authors performed a retrospective analysis of 164 cycles using sibling oocytes in combined IVF/ICSI with achieved a normal fertilization (≥ 25%) both conventional IVF and ICSI in this infertility centre. *Results:* It was found that there were no differences in 2PN rate (70.25% *vs* 70.60%), but higher cleavage rate in ICSI than IVF insemination (98.99% *vs* 96.81%), higher arrested embryos rate in IVF than ICSI in 2PN group (20.00% *vs* 13.95%), and higher abnormal fertilization 1PN (3.87% *vs* 1.92%) and 3PN (3.63 *vs* 0.854%) in IVF than ICSI. *Conclusion:* there were some differences fertilization outcomes between ICSI and IVF, which may be related to different procedures between two techniques.

Key words: IVF; ICSI; Embryos cleavage; Arrested embryos; 1PN.

Introduction

In assisted human reproduction, there are two major technologies: conventional in vitro fertilization (IVF) and intracytoplasmic sperm injection (ICSI) [1]. Conventional IVF procedure, designed mainly to deal with female infertility, involves mixing individual eggs in a petri dish with spermatozoa in overnight incubator, in which sperm-egg fertilization will occur. ICSI is the procedure for injecting a selected sperm cell into the middle of an egg by micro-injection system. ICSI is being used mostly for two indications: severe male-factor infertility and failed or low fertilization in conventional IVF previously.

There are several differences between the two kind's fertilization technologies. ICSI is more invasive than IVF, and oocytes degeneration is a common phenomenon after performing ICSI [2]. During IVF, the more immature oocytes underwent maturation, and have an opportunity to fertilization with spermatozoa in overnight incubator [3]. During IVF, sperm-eggs fertilization is more natural and random, compared with ICSI. Therefore, can the aforementioned different procedures between ICSI and IVF affect the fertilization outcome? To address the issue, excluding the impact of fertilization failure, the present authors performed a retrospective analysis of 164 cycles using sibling oocytes in combined IVF/ICSI with achieved a normal fertilization (≥ 25%) both conventional IVF and ICSI (January 2011 and June 2012) in the present infertility centre.

Materials and Methods

Patients

Between January 2011 and June 2012, 164 cycles using sibling oocytes in combined IVF/ICSI with a normal fertilization (≥ 25%), both conventional IVF and ICSI, were considered for the study in the infertility centre. Patients' age ranged from 22 to 43 years, with a mean (±SD) of 32.30 ± 3.9 and main causes of infertility were tubal (n = 66), unexplained infertility (n = 11), endometriosis (n = 5), ovulatory dysfunction (n = 22), uterus (n = 4), and male factor (n = 56). All patients signed written informed consent. Only the first cycle's therapies were included in the current study.

Ovarian stimulation

Patients were down-regulated with gonadotropin releasing hormone (GnRH) agonist using long or short protocols and stimulated with follicle stimulating hormone (FSH) and human menopausal gonadotropin (hMG). Ovarian activity was monitored by regular ultrasound scans and serum sex hormone assays. A dose of 10,000 U of human chorionic gonadotropin (hCG) was administered when the leading cohort follicles reached a diameter of 18 to 20 mm. Oocyte retrieval was performed through vaginal puncture under ultrasound guidance 36-38 hours after hCG.

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Table 1. — *Fertilization, cleavage, and embryos results after the164 cycles using sibling oocytes in combined IVF/ICSI with fertilization rate ≥ 25% obtained both IVF and ICSI.*

Parameter	2PN		0PN		1PN		(≥)3PN	
	IVF	ICSI	IVF	ICSI	IVF	ICSI	IVF	ICSI
Oocytes (%)	70.25 (1162/1654)	70.60 (992/1405)	16.99 (281/1654)	15.94 (224/1405)	3.87[c] (64/1654)	1.92 (27/1405)	3.63[d] (60/1654)	0.854 (12/1405)
Cleavage oocytes (%)	96.81[a] (1125/1162)	98.99 (982/992)	29.54 (83/281)	26.34 (59/224)	73.44 (47/64)	81.48 (22/27)	98.33 (59/60)	91.66 (11/12)
Arrested embryos (%)	20.00[b] (225/1125)	13.95 (137/982)	61.45 (51/83)	67.80 (40/59)	65.96 (31/47)	63.64 (14/22)		
Available embryos (%)	98.44 (886/900)	97.40 (823/845)	84.38 (27/32)	78.95 (15/19)	81.25 (13/16)	75.00 (6/8)		
Good-quality embryos (%)	73.70 (653/886)	75.21 (619/823)						

Values are % (n/total). [a] $p = 0.001$, between IVF and ICSI in 2PN cleavage oocytes. [b] $p = 0.000$, between IVF and ICSI in 2PN arrested embryos. [c] $p = 0.001$, between IVF and ICSI in 1PN oocytes. [d] $p = 0.000$, between IVF and ICSI in 3PN oocytes.

Conventional IVF and ICSI

The oocytes retrieved from each patient were collected into the center of a four-well dish which contained medium, and then divided evenly into two (if up to ten oocytes) or four wells (if more than ten oocytes), and more than 20 oocytes were divided two four-well dish, and so on. Embryos were divided into two groups by allotting the oocytes from the first well to conventional insemination or ICSI in alternating order. In IVF, oocytes were inseminated by conventional IVF three to four hours after oocyte retrieval. Spermatozoa were collected by the swim-up technique, using 100,000 motile spermatozoa per insemination dish. In ICSI, the removal of cumulus cells from oocytes was performed two hours after retrieval, and, ICSI was performed as previously described [1].

Fertilization and embryo assessment

Between 16–18 hours after insemination, both the IVF and the ICSI oocytes were examined for evidence of normal fertilization (the presence of two pronuclei) or abnormal fertilization (0 and 1, ≥ three pronuclei, respectively) or whether the oocyte had been degenerated after ICSI.

The cleavage of the 2PN, 0PN, 1PN, ≥3PN oocytes, and the quality of the embryos were evaluated on day 3 (day 0 was the day of oocyte retrieval). Day 3 embryos were assessed and graded as available embryos (≥ 5 cells and ≤ 30% fragmentation, or four cells and ≤ 20% fragmentation), or arrested embryos (< three cells and regardless of volume of fragmentation). Those embryos with equal-sized six to eight cells and ≤ 10% fragmentation were defined as good-quality embryos. The embryos cleavage rate was calculated as the number of 2PN, 0PN, 1PN, or ≥3PN cleavage embryos divided by the number of 2PN, 0PN, 1PN, or ≥3PN oocytes, respectively. The arrested embryos rate was calculated as the number of arrested embryo divided by the number of cleavage embryos (in order to eliminate the effects of cleavage stage, cleavage embryos are the denominators). The available embryos rate was calculated as the number of available embryos divided by the number of non-arrested embryos (in order to eliminate the effects of arrested stage, non-arrested embryos are the denominators). The good-quality embryos rate was calculated as the number of good-quality embryos divided by the number of available embryos.

Statistical Analysis

Data were analysed using the chi-squared test. All analyses were performed with Statistical Package for Social Sciences version 17.0 (SPSS). Statistical significance was defined as $p < 0.05$.

Results

There were 164 cycles using sibling oocytes in combined IVF/ICSI cycles with the normal fertilization in IVF in the current study; 1,710 and 1,715 oocytes complexes were randomly subjected to IVF and ICSI, respectively.

There were no difference in 2PN rate and 0PN rate between IVF and ICSI (70.25% *vs* 70.60% and 16.99% *vs* 15.94 %). The 1PN rate and 3PN rate was significantly higher in IVF than ICSI (3.87% *vs* 1.92% and 3.63 *vs* 0.854%). The 2PN cleavage rate was significantly higher in ICSI than IVF (98.99% *vs* 96.81%). The arrested embryos rate was significantly higher in IVF than ICSI (20.00% *vs* 13.95%) (Table 1).

In group ICSI, there were 89 (54.29%) cycles with oocytes degeneration occurring after ICSI, and the degeneration rate was 10.46% (147 / 1,405).

Discussion

In current study, there were no differences in 2PN rate, available embryos, and good-quality embryos between IVF insemination and ICSI (70.25% *vs* 70.60%, 98.44 % *vs* 97.40 %, and 73.70 % *vs* 75.21 %), but, in 2PN group, the cleavage rate was significantly higher in ICSI than IVF insemination (98.99% *vs* 96.81%) and arrested embryos rate was significantly higher in IVF than ICSI (20.00% *vs* 13.95%). The differences in cleavage rate and arrested embryos rate may be partly due to the different procedures between ICSI and IVF.

It is well known that ICSI is more invasive than IVF and oocyte degeneration is a common phenomenon after performing ICSI. As previously reported, oocytes with a fragile oolemma was considered a precipitant of degeneration [1, 4, 5], which was demonstrated by no resistance upon needle entry or sudden breakage. Those oocytes with sudden breakage oolemma, which would be degeneration after ICSI, are “rescued” by performing IVF, and what is

final fate for those "rescued" oocytes during IVF? The Palermo study [5] showed that oocytes with sudden breakage membrane was lower number of cleaved embryos compared to the other two membrane patterns (successful injection and difficult breakage) that resulted a similar cleavage ability. Therefore, it is may be that the oocytes tending to degenerate may have had a lower cleavage rate. In the current study, 2PN embryo cleavage rate was lower in IVF than ICSI, and it is probable due in part to the fact that the more oocytes, which would degenerate after ICSI, were "temporarily rescued" by performing IVF. Moreover, during IVF, the more immature oocytes underwent maturation, and had an opportunity to be fertilized with spermatozoa in overnight incubator; it is therefore difficult to study the developmental potential of in vitro matured oocytes during IVF. However, there were some studies addressing in vitro matured oocytes in ICSI cycles, which may give some explanation [6-8]. Balakier *et al.* found that the cleavage rate was lower in in-vitro matured oocytes compared with MII oocytes at denudation [6] . Shu *et al.* and Strassburger *et al.* found that in vitro matured oocytes had higher percentages of arrested embryos than in vivo matured oocytes [7, 8]. Based on the above results, it is possible that the more number of in vitro matured oocytes obtain normal fertilization during IVF than ICSI, so it would result in a lower cleavage rate and in more arrested embryos during IVF than ICSI. The results of the present study agree with the above assumption, as it was demonstrated that there was a lower cleavage rate and more arrested embryos in IVF compared with ICSI. All in all, IVF have a lower cleavage rate and the more arrested embryos compared with ICSI, probably partly due to the mode of fertilization.

There are no differences in 0PN cleavage rate between IVF and ICSI (29.54% *vs* 26.34 %), which suggest that some oocytes fail in fertilization in the normal cycles due to intrinsic oocyte factors and not due to whether the sperm enters the egg.

In the current study, the notable finding was that the 1PN rate was significantly higher in IVF insemination than ICSI (3.87% *vs* 1.92%), which is however difficult to explain. There were some suggested mechanisms for the appearance of a 1PN zygote, including parthenogenetic activation, asynchronous development of pronuclei, failure of either male or female pronucleus formation, and "pronuclear fusion" [9, 10]. Nagy *et al.* found that a higher proportion of oocytes develop two pronuclei asynchronously after IVF than after ICSI [11]. They explained that there was a much greater time-span in IVF oocytes between the formation of the male pronucleus and the formation of the female pronucleus, compared with ICSI [11]. It was perhaps because, with ICSI, there were not only sperm factors present in the oocyte to initiate activation, but also a mechanical stimulus in itself. While in IVF oocytes this process was initiated only by the sperm factors, which might require a longer time to trigger the same effect. Hence, the higher 1PN rate in IVF insemination than ICSI was related to a higher proportion of oocytes developing two pronuclei asynchronously after IVF than after ICSI. Another possibility is the procedures of formation of "pronuclear fusion". Male nuclear envelope and female nuclear envelope were formed just in the same area at the same time. So a common membranous envelope was formed to surround the male or female pronucleus [12]. Levron *et al.* suggested that it may occur when a spermatozoon enters or is deposited very close to the oocyte spindle [12]. However during ICSI, the sperm is injected away from the meiotic spindle and there is a less chance of "pronuclear fusion" phenomenon occurring, which partly resulted in the more 1PN occurring after IVF, as compared to after ICSI.

In the present study, 3PN rate was significantly higher in IVF than ICSI (3.63% *vs* 0.854%), which is easy to explain. Among the several mechanisms giving rise to triploid zygotes, dispermy is recognized to be the most common, which was overcome by performing ICSI. Jun *et al.* suggested performing ICSI in patients with a high incidence of triploidy in prior IVF cycles [13]

All in all, higher cleavage rate in ICSI than IVF insemination and higher arrested embryos rate in IVF than ICSI in 2PN group and higher abnormal fertilization (1PN or 3PN) in IVF than ICSI may be partly related to different procedures between ICSI and IVF.

References

[1] Palermo G., Joris H., Devroey P., Van Steirteghem A.C.: "Pregnancies after intracytoplasmic injection of single spermatozoon into an oocyte". *Lancet*, 1992, *340*, 17.

[2] Rosen M.P., Shen S., Dobson A.T., Fujimoto V.Y., McCulloch C.E., Cedars M.I.: "Oocyte degeneration after intracytoplasmic sperm injection: a multivariate analysis to assess its importance as a laboratory or clinical marker". Fertil. Steril., 2006, *85*, 1736.

[3] Taylor T.H., Wright G., Jones-Colon S., Mitchell-Leef D., Kort H.I., Nagy Z.P.: "Comparison of ICSI and conventional IVF in patients with increased oocyte immaturit". *Reprod. Biomed. Online*, 2008, *17*, 46.

[4] Ebner T., Yaman C., Moser M., Sommergruber M., Jesacher K., Tews G.: "A prospective study on oocyte survival rate after ICSI: influence of injection technique and morphological features". J. Assist. Reprod. Genet., 2001, *18*, 623.

[5] Palermo G.D., Alikani M., Bertoli M., Colombero L.T., Moy F., Cohen J., Rosenwaks Z.: "Oolemma characteristics in relation to survival and fertilization patterns of oocytes treated by intracytoplasmic sperm injection". *Hum. Reprod.*, 1996, *11*, 172.

[6] Balakier H., Sojecki A., Motamedi G., Librach C.: "Time-dependent capability of human oocytes for activation and pronuclear formation during metaphase II arrest". *Hum. Reprod.*, 2004, *19*, 982.

[7] Shu Y., Gebhardt J., Watt J., Lyon J., Dasig D., Behr B.: "Fertilization, embryo development, and clinical outcome of immature oocytes from stimulated intracytoplasmic sperm injection cycles". *Fertil. Steril.*, 2007, *87*, 1022.

[8] Strassburger D., Friedler S., Raziel A., Kasterstein E., Schachter M., Ron-El R.: "The outcome of ICSI of immature MI oocytes and rescued in vitro matured MII oocytes". *Hum. Reprod.*, 2004, *19*, 1587.

[9] Feenan K., Herbert M.: "Can 'abnormally' fertilized zygotes give rise to viable embryos?" *Hum. Fertil. (Camb)*, 2006, *9*, 157.

[10] Noyes N., Fino M.E., Krey L., McCaffrey C., Adler A., Grifo J.: "Embryo biopsy: the fate of abnormal pronuclear embryos". *Reprod. Biomed. Online*, 2008, *17*, 782.

[11] Nagy Z.P., Janssenswillen C., Janssens R., De Vos A., Staessen C., Van de Velde H., Van Steirteghem A.C.: "Timing of oocyte activation, pronucleus formation and cleavage in humans after intracytoplasmic sperm injection (ICSI) with testicular spermatozoa and after ICSI or in-vitro fertilization on sibling oocytes with ejaculated spermatozoa". *Hum. Reprod.*, 1998, *13*, 1606.

[12] Levron J., Munne S., Willadsen S., Rosenwaks Z., Cohen J.: "Male and female genomes associated in a single pronucleus in human zygotes". *Biol. Reprod.*, 1995, *52*, 653.

[13] Jun S.H, O'Leary T., Jackson K.V., Racowsky C.: "Benefit of intracytoplasmic sperm injection in patients with a high incidence of triploidy in a prior in vitro fertilization cycle". *Fertil. Steril.*, 2006, *86*, 825.

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