ICSI and embryo development

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The process of fertilization in intracytoplasmic sperm injection (ICSI) treatment is largely different from that of physiological fertilization. It is also true that the rate of development of the embryo into the blastocyst after ICSI is lower than that after in vitro fertilization (IVF) [1,2]. It is unknown whether these facts are closely related. Although it is impossible to simply compare the results of IVF and ICSI due to the difference in their indications, it has been determined that pregnancy rates, the endpoint of fertilization failure treatment, are similar between ICSI and IVF treatments. This is because negative impacts of ICSI on fertilization do not become apparent due to a considerable effect of the in vitro culture environment. In this report, we discuss causes for the low development rate of the blastocyst after ICSI.

In the process of fertilization by ICSI, sperm egg fusion cannot be seen. In the physiological fertilization process, spermatozoa cause sperm egg fusion, followed by the incorporation of sperm nuclei into the ovum, and then by two major phenomena: oocyte activation and decondensation of sperm nuclei. These phenomena are delayed in ICSI treatment, and this is considered as a negative impact of ICSI on embryogenesis.

1. Oocyte activation

The oocyte activation mechanism in ICSI treatment seems to be different from that of normal fertilization. In general, the conducting system for oocyte activation works immediately after sperm egg fusion. In this mechanism, G proteins on the oolemma are involved. Via G proteins, PLC is activated and IP$_3$ is produced from PIP$_2$. IP$_3$ causes discharge of calcium ions through calcium ion channels on the endoplasmic reticulum membrane from the rough endoplasmic reticulum that stores calcium in a cell. After these processes, oocyte activation is caused. However, as sperm egg fusion is bypassed in ICSI treatment, oocyte activation cannot be caused by this mechanism. At present, oocyte activation is believed to be caused by IP$_3$ increases when sperm-type PLC$_z$ in spermatozoa is incorporated into oocyte [3]. An important factor in this process is the timing when PLC$_z$ is leaked into oocyte. It is considered that such leaking of PLC$_z$ can be seen only when the sperm cell membrane is sufficiently broken down. Although spermatozoa used for ICSI undergo immobilization process, it takes some time for the sperm cell membrane to be broken down after ICSI. Our investigation revealed that calcium oscillations would occur approx. 15 minutes after ICSI [4]. In the normal fertilization process, however, this event would occur 1 - 2 minutes after sperm egg fusion. In this way, calcium oscillations in ICSI treatment occur at a different timing from normal fertilization.
2. Decondensation of sperm nuclei

In the normal fertilization process, decondensation occurs immediately after sperm nuclei are incorporated into oocyte after sperm egg fusion. In ICSI treatment, however, decondensation occurs after immobilized spermatozoa are injected and the sperm cell membrane is sufficiently broken down. The level of such sperm immobilization has a large impact on the timing of decondensation commencement. When the immobilization level is low, decondensation commencement is delayed.

3. Effect of nucleoprotein of sperm nuclei on embryo development

The nucleoprotein of ejaculated spermatozoa is protamine that includes a large amount of cysteine. As cysteine includes many SH bonds, many SS bonds can be formed, contributing to the firm binding of DNA strands. On the other hand, nucleoprotein of spermatozoa in the testis is protamine, which is characterized by relatively few SS bonds. When ICSI is performed in a mouse model using spermatozoa in the testis, decondensation occurs earlier than that using spermatozoa in the epididymis (including many SS bonds as in ejaculated spermatozoa). In addition, formation of the pronucleus is promoted faster. Morphologic evaluation was performed on blastocysts developed 96 and 120 hours after ICSI. The blastocyst was treated with a double fluorescent staining technique that uses anti-cow splenocyte antibodies to examine complement fixation reaction. The evaluation was performed by counting cells in the inner cell mass (ICM) stained by Hoechst and trophoectoderm (TE) stained by PI. The total cell count tended to be high in the blastocysts 96 hours after sperm nuclei in the testis were injected (40.2 vs 33.4, p=0.0575). ICM cell ratio (ICM cells/total cell count) was significantly lower in the blastocysts 96 hours after sperm nuclei in the testis were injected (0.374 vs 0.470, p=0.0031). This suggests that proliferation of TE is promoted faster than in the blastocysts into which sperm nuclei in the cauda epididymidis are injected, and that the speed of blastocyst formation is significantly higher.

4. Clinical test results

The treatment was performed with 77 IVF and 63 ICSI treatment cases. For these cases, day-5 blastocysts developed using ejaculated spermatozoa were transferred to uterus. Eight or more oocytes were picked up from women aged 35 or younger. The results are shown in the Figure 1. The blastulation rate was significantly lower after ICSI (29.7% vs 41.5%, p=0.037).

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Figure 1. Comparison of blasturation rate among IVF and ICSI.

*ANOVA (mean± SD)

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Conclusion

The examination performed using a mouse model suggested that post-ICSI embryogenesis would be affected by the chromatin structure of injected sperm nuclei. A clinical results also showed that development rate of blastocysts in ICSI treatment is lower than in IVF treatment. In addition, it was suggested that decondensation of injected spermatozoa is delayed in ICSI treatment, and this unique fertilization process in ICSI treatment may have an impact on embryogenesis.
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References


