The relationship between pregnancy outcome and smooth endoplasmic reticulum clusters in MII human oocytes

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BACKGROUND: During ICSI, we occasionally observe pronucleus sized translucent vacuoles. We investigated why these vacuoles occur and determined the effect on pregnancy outcome. METHODS: Translucent vacuole-positive oocytes and the corresponding cohort were examined by transmission electron microscopy (TEM) and histochemical staining with DiI and ER-Tracker. Stimulation methods, hormonal levels, patients’ condition and grade of transferred embryos were compared between vacuole-positive and vacuole-negative cycles. RESULTS: By TEM, we confirmed that the vacuoles were tubular-type smooth endoplasmic reticulum clusters (sERCs). Numerous small sERCs were also observed in the oocytes from the same cohort. Veeck’s grades of transferred embryos were higher in sERC-positive cycles and fertilization rate was similar to those of sERC-negative cycles. However, in sERC-positive cycles, significantly lower pregnancy and higher biochemical pregnancy rates were shown. Serum estradiol levels on the day of hCG administration were significantly higher in sERC-positive cycles. CONCLUSIONS: The presence of sERCs is associated with lower chances of successful pregnancy, even in sERC-negative oocytes from the same cohort that are transferred along with the sERC-positive oocytes. High estradiol levels could be one of the causes of sERC formation.

Key words: biochemical pregnancy/high serum estradiol levels/idiopathic infertility/oocyte maturation/smooth endoplasmic reticulum cluster

Introduction

Early pregnancy loss is still regarded as a major problem of infertility. Since clear correlations are yet to be found, embryo selection remains one of the most arduous tasks in assisted reproduction.

After the denuding procedure of ICSI, we sometimes come across many kinds of dysmorphic oocytes. As dysmorphic phenotypes, seven cytoplasmic phenotypes [dark/granular, clustered organelles, smooth endoplasmic reticulum (sER) accumulation, vesiculated, necrotic regions, polarized cortical depletion of organelles, vacuolated] have been identified in human oocytes (Van Blerkom and Henry, 1992). It has been suggested that the dysmorphic phenotypes observed during stimulated cycles might reflect a high frequency of aneuploidy related to ovarian stimulation (Van Blerkom and Henry, 1992, 1998). A more recent report contends that oocyte morphology might not affect fertilization rates, embryo quality or implantation rates after ICSI (De Sutter et al., 1996; Balaban et al., 1998). There are some reports showing that fertilization and cleavage rates were not affected by oocyte morphological phenotypes; however, both the pregnancy and implantation rates were reduced when embryos derived from oocytes contained granular oocytes, and oocytes with inclusions (ERs, refractile bodies and vacuoles) were transferred (Serhal et al., 1997; Meriano et al., 2001). It has been suggested that there is a tendency towards a higher rate of spontaneous pregnancy loss in cases of oocyte dysmorphism (Alikani et al., 1995). Based on these findings, it is likely that a correlation exists between morphology, developmental competence and chromosomal abnormalities. However, the cause of each dysmorphic phenotype may result from different pathways, so that we should study the cause of each occurrence independently.

In this study, we occasionally (~10% of cycles) observe the cytoplasmic localization of the pronucleus sized translucent vacuoles at the metaphase II (MII) stage of human oocytes after the ICSI denuding procedure. It was reported that this vacuolar formation was the result of accumulation of sER (Van Blerkom, 1990; Sathananthan, 1997). This accumulation was also observed in cleaved embryos (Makabe et al., 2001) and post-vitrified 1 pronuclei (PN) and 3PN zygotes (Isachenko et al., 2003). In this study, we refer to this accumulation as an
sER cluster (sERC). sERCs can be clearly distinguished morphologically from fluid-filled vacuoles under the inverted microscope. Cytoplasmic vacuolation is considered as cellular degeneration (Zamboni et al., 1972) and oocyte atresia (Nayudu et al., 1989). The mechanism responsible for the occurrence of sERCs is not known.

Low pregnancy outcomes have been reported for oocytes with intracytoplasmic organelle clustering (Meriano et al., 2001), which is also described as granular cytoplasm (Kahraman et al., 2000). Patients who received embryos derived from granular oocytes, following embryonic transfer, exhibited low pregnancy outcome. Also, no pregnancy was reported in the patients who had oocytes with inclusions (sERs, refractile bodies and vacuoles), and it was suggested that these oocytes should not be used for ICSI (Serhal et al., 1997).

According to our experience and other published reports, assisted reproductive technology (ART) for those patients whose oocytes display sERCs tends to be unsuccessful (Serhal et al., 1997; Meriano et al., 2001), even though transferred embryos are derived from sERC-negative oocytes in the same cycle cohorts. In this study, we investigated the origin of sERCs and determined their effect on embryonic development and clinical pregnancy rate. We report, for the first time, that the pregnancy rate remains low with sERC-negative cohort oocytes in sERC-positive cycles.

Materials and methods

Stimulation protocols

During the study period, three stimulation protocols were used. A long protocol was utilized for the majority of the cycles (174 cycles), while a short protocol was utilized for 17 cycles. For the long protocol, pituitary desensitization was induced from the mid-luteal phase of pre-treatment. For the short protocol, pituitary desensitization was induced on the first day of gonadotrophin administration. Exogenous gonadotrophins were administered from the first day of menstrual bleeding in the treatment cycle. For one cycle, clomiphene citrate was used for follicular growth induction. When the average dominant follicle size reached 18–20 mm, hCG was administrated. The exact stimulation protocol was chosen by the gynaecologist according to gynaecologist and/or patient preference.

Hormonal assays

The concentrations of estradiol and progesterone in the serum on the day of hCG administration were determined by enzyme-linked fluorescent assay (ELFA; BioMérieux, Japan).

Oocyte retrieval and denuding

Oocytes were retrieved by transvaginal ultrasound-guided aspiration 36 h after the hCG injection. Follicular fluid was aspirated to Flashing Medium (Vitrolife). Oocytes were collected from follicular fluid, washed in G1.2 medium (Vitrolife), equilibrated and incubated for 2–5 h with 6% CO₂/5% O₂/89% N₂ at 37°C. Denuded oocytes were classified morphologically into sERC positive and negative oocytes.

Culture of an oocyte with medium sized sERCs

An oocyte with medium sized sERCs was cultured in IVC-TWO medium (InVitro Care). The size of the sERC was measured 6, 18 and 30 h after the denuding procedure.

Conventional IVF and ICSI

We performed ICSI on at least 20% of the oocytes in the majority of patients according to our clinic’s criteria for sperm quantity and quality. For the ICSI procedure, G1.2 and G2.2 media (Vitrolife) were used, and for the IVF procedure, IVF medium, G1.2 and G2.2 media were used.

Morphological observation of sERC-positive oocytes after ICSI

ICSI was performed on 11 sERC-positive oocytes, taking care that sperm were not injected into the sERCs. These oocytes that had been cultured for 16–20 h were examined for fertilization success and morphological changes of the sERCs. To reveal the timing of sERC disappearance, three out of 11 sERC-positive oocytes were checked every 2 h after ICSI until the 2PN appeared.

Embryo transfer

Embryonic development was evaluated 2 or 3 days after oocyte retrieval using Veeck’s classification. (Veeck, 1991). One to three embryos, depending on the number of cleaved embryos obtained, were transferred to the uterus. In 18 sERC-positive cycles, sERC-negative oocytes were selected and embryos transferred.

On the basis of the embryo selection, transfer of embryos derived from morphologically abnormal oocytes including sERC-positive oocytes was avoided. In the case of ICSI, we did not transfer embryos that had been derived from sERC-positive oocytes. In the case of IVF, we explained a possible risk of pregnancy failure to the patients when cohort oocytes were affected. We transferred such embryos only when the patients had agreed to the transfer.

Continuous culture of oocytes that failed to be fertilized after the IVF or ICSI procedure

Twenty sERC-negative oocytes (obtained from 12 patients) that were not fertilized in our IVF or ICSI procedure were cultured for a further 2–5 days in IVF-TWO medium (InVitro Care) to check for the presence of sERCs.

Transmission electron microscopy (TEM)

We obtained informed consent from all patients when we used oocytes and embryos for experimental and analytical purposes.

To examine the morphology by TEM, six large sized sERC-positive oocytes and one sERC-negative oocyte (MII stage) were obtained from the sERC-positive cycle. They were fixed in 2.5% glutaraldehyde in phosphate-buffered saline (PBS; pH 7.4) for 2 h, rinsed with PBS three times, and post-fixed with 1% OsO₄ in PBS for 2 h. They were rinsed thoroughly in distilled water, then dehydrated in ethanol, and finally embedded in epoxy resin. Ultra thin sections were stained with uranyl acetate and lead citrate and examined under a transmission electron microscope (JEM 1200EX Nihon Denshi).

DiI labelling and confocal imaging

Five sERC-positive MII stage oocytes and five unfertilized sERC-negative oocytes were stained by injection of DiI. A saturated solution of DiIC₁₆(3) (DiI) (1,1′-dioctadecyl-3,3′,3′-tetramethylindocarbocyanine perchlorate; Molecular Probes) was prepared by dissolving the dye in soybean oil (see Terasaki and Jaffe, 1991). The DiI solution was injected into oocytes until the oil drop reached ~20 μm in diameter (~10–15% of the total volume). After injection, the oocytes were incubated for 1 h to allow DiI to diffuse throughout the cytoplasm. DiI-injected oocytes were observed under the fluorescence microscope using a He/Ne laser (543 nm) for excitation and a long pass filter (560 nm) for emission fluorescence.
Acquisition and deconvaltion of ER-Tracker images
Three sERC-positive oocytes were loaded with ER-Tracker Blue-White DPX (Molecular Probes). A stock solution of ER-Tracker was prepared at 1 mM in dimethylsulphoxide (DMSO). Three sERC-positive oocytes were loaded with 100 nmol/l ER-Tracker in pre-warmed culture medium for 30 min at 37°C. The specimens were excited at 386 nm and the images of the ER-Tracker fluorescence were captured by a digital high-speed microscope.

Comparative studies
Consecutive ICSI attempts and combined IVF and ICSI attempts from January 2000 to April 2001 in our clinic were covered retrospectively by this study. All patients anonymously agreed to have their data analysed for scientific reasons. Subjects were labelled as sERC-negative (when there were no sERCs in the denuded oocytes) or positive (when there was at least one sERC-positive oocyte in the denuded oocytes) cycles. The two groups were compared by follicular stimulation protocols, serum hormonal levels, age, number of retrieved oocytes, existence of endometriosis in ovaries, and thickness of the endometrium in order to identify factors correlating to sERC formation in oocytes. The fertilization rate, cell division rate, grade of transferred embryos (using Veeck's classification), clinical pregnancy rate, implantation rate and biochemical pregnancy rate were compared between the two groups to determine if the sERC had an effect on these parameters.

Statistical analysis
The statistical package used for data analysis was StatView Ver. 5.0 (SAS Institute Inc.). Clinical characteristics were analysed using the unpaired Student’s t-test or χ² analysis. A P-value of <0.05 was considered statistically significant.

Results
When oocytes were subjected to the ICSI denuding procedure, we occasionally (9.4% of oocytes) observed morphologically abnormal oocytes in which pronucleus sized translucent vacuoles were present in the centre of the cytoplasm at the MII stage (Figure 1A). These sERCs could be clearly distinguished from fluid-filled vacuoles (Figure 1B). By examining the sERC-positive oocytes using TEM, we confirmed that they were, in fact, tubular-type sERCs (Figure 2A, b). In addition to pronuclear sized larger (18 µm) sERCs, medium sized sERCs ~8 µm in diameter were found in the same oocyte. These larger sERCs were not always found in the oocyte in the same patients or even in the same cycles. An oocyte that had been collected from the same cycle of the patient showed no visible sERC (Figure 2B, a). When this oocyte was investigated by TEM, numerous small sERCs of 2–5 µm in diameter were seen in the cortical area (Figure 2B, b). Figure 2C and D shows a bright staining of the sERCs with DiI and ER-Tracker, respectively, strongly suggesting that they were derived from the ER.

When sERC-negative oocytes that were not fertilized in our IVF or ICSI procedure were cultured continuously for a further 2–5 days, large sERCs appeared in the centre of the cytoplasm (six out of 20 oocytes), or in the cortical area (11 out of 20 oocytes, Figure 3A). Only one oocyte had three medium sized sERCs (Figure 3B), and the remaining oocytes (two out of 20) had no sERC. We next examined if sERCs increased in size during culture at the MII stage. A medium (10 µm) sized sERC was marked and its size measured during culture. This medium sized sERC grew to an approximate diameter of 25 µm after 18 h, and no further increase in size was seen up to 30 h (Figure 3C).

These larger sERCs of pronuclear size were found in 18 (15 patients) out of 192 cycles (134 patients), representing 9.4% (11.2%) of the total cycles performed. In the sERC-positive cycles, the percentage of sERC-positive oocytes per denuded oocytes for ICSI was 34.4% (42 out of 122). All (42 cases) sERCs appeared in oocytes only in the MII stage oocytes and were never seen at the MI or germinal vesicle (GV) stage. With the ICSI procedure, all 11 sERC-positive oocytes were fertilized successfully when sperm injection was carried out carefully into the cytoplasm avoiding the sERC area. In all cases, sERCs disappeared within 16–20 h after the ICSI procedure. Two of 11 sERC-positive oocytes matured to expanded blastocysts and others were arrested at the 4-cell to morula stages. Three oocytes were examined further for the timing of sERC disappearance during development. sERCs in these oocytes disappeared prior to the appearance of the 2PN.

A statistical analysis showed that the formation of sERCs was not related to patient age, mean number of retrieved oocytes, existence of endometriosis in ovaries or thickness of the endometrium (Table I). The level of serum estradiol on the day of hCG administration was significantly higher in sERC-positive cycles than in negative cycles (Figure 4A). In contrast, no difference in the levels of serum progesterone was seen (Figure 4B).

Next, we examined whether different stimulation protocols may affect the occurrence of sERC-positive cycles. Due to a limitation of samples tested, no significant difference was detected between groups; however, the number of sERC-positive oocytes obtained by the short protocol was about three times larger than that by the long protocol (Table II).

According to the Veeck’s classification, we found that the rate of transferred grade I embryos in sERC-positive cycles (82.0%) was significantly higher than in sERC-negative cycles (62.1%). In contrast, the rate of grade II embryos in sERC-positive cycles (12.0%) was lower than in negative cycles (27.7%) (Figure 5).

Although the fertilization and cell division rates were almost identical in the two groups, the biochemical pregnancy rate in
Figure 2. The translucent vacuoles were smooth endoplasmic reticulum clusters (sERCs). Light microscopic observation of MII stage oocyte with a large translucent vacuole (arrow, A-a) and its cohort oocyte (B-a) obtained from the same patient. Transmission electron microscope images of translucent vacuoles (A-b). A tubular type of large sERCs (***) and a medium-sized sERC (**) were observed within the cytoplasm. The inset is an enlarged image of a part of a large sERC structure (box). M: mitochondria. A transmission electron microscope image of a cohort oocyte (B-b). Numerous small sERCs (*), 2–5 μm in diameter, were seen in a cortical area. The large translucent vacuoles were brightly stained by DiI (C) and ER tracker (D).

Figure 3. Appearance and growth of the sERCs in cultured oocytes. The denuded oocytes in which no sERCs had been seen were cultured for 2–5 days. A large sERC appeared after 4 days in the cortical area (A), and three medium sized sERCs appeared after 3 days (B). The light microscopic images (left) and the confocal images stained with DiI (right) are shown. An oocyte in which a medium sized sERC had been observed during the denuding procedure was cultured (C). The time course images of this oocyte are shown. The size of a growing sERC indicated by the arrows was measured during incubation (0–30 h).
sERC-positive cycles (22.2%, four out of 18 cycles) was significantly higher than in sERC-negative cycles (3.5%, six out of 170 cycles, \( P < 0.05 \)). The clinical pregnancy rates in sERC-negative cycles and sERC-positive cycles were 28.2% (48 out of 170 cycles) and 5.6% (one out of 18 cycles), respectively (Table III). In the one sERC-positive case that proceeded to clinical pregnancy, the baby was diagnosed with Beckwith–Wiedemann syndrome.

**Discussion**

We have shown by TEM that the translucent vacuoles present in denuded oocytes were sERCs. This was confirmed by DiI and ER-Tracker, dyes that stain the ER predominantly (Henson et al., 1989; Jaffe and Terasaki, 1994; Kline et al., 1999). The sERCs were clearly distinguished from fluid-filled vacuoles under the inverted microscope (Figure 1). The sERCs were not always found in all embryos obtained from sERC-positive cycles. There are at least three forms of sERC, which can be classified, for convenience, by size, using light or electron microscopy. The large (18 \( \mu \)m) and the medium (10–17 \( \mu \)m) sized sERCs are visible by light microscopy. However, the small (2–9 \( \mu \)m) sERCs were not visible under the conditions used. The oocytes obtained from sERC-positive cycles were used for ICSI and transferred if no large and/or medium sized sERCs were found. These oocytes were fertilized successfully and showed normal cleavage rates in early developmental stages (Table III).

However, the pregnancy rates were lower in sERC-positive cycles than in negative cycles. No pregnancy and low implantation rates were reported when the oocytes that had possessed sERCs (described as SER cumulus) proceeded to ICSI/embryo transfer (Serhal et al., 1997; Meriano et al., 2001). The present study showed that the pregnancy rates were associated with cycles regardless of the presence or absence of visible sERCs. When oocytes were cultured, medium sized sERCs grew to large sERCs in 18 h (Figure 3C), suggesting that the three forms of sERCs were derived from the same origin. The sERCs appeared in 90% of oocytes in which no sERCs had been observed at the denuding procedure when cultured for 2–5 days. These findings imply the possibility of cytoplasmic deterioration and changes known to occur in ageing human oocytes during extended culture. Because an sERC is most likely to be associated with an aberrant event, we propose that the transfer of embryos derived from sERC-positive oocytes should be avoided.

Reports on several kinds of animal oocytes, including human, have shown the following functional and structural alterations of the ER during oocyte maturation: (i) an increase in the sensitivity of the IP3 receptor for Ca\(^{2+}\) (Fujiwara et al., 1993; Fissore et al., 1999; Goud et al., 1999); (ii) an increased storage of Ca\(^{2+}\) which is released during oscillation (Carroll et al., 1994, 1996); and (iii) changes in the structure of a sheet-like form to a spherical form in starfish oocytes (Jaffe and Terasaki, 1993) and distribution of the ER in mouse oocytes (Mehlmann et al., 1995). In the human oocytes, the localization of mobilizable Ca\(^{2+}\) was detected in the small vesicles beneath

**Table I.** Patient characteristics in sERC negative and positive cycles

<table>
<thead>
<tr>
<th>sERC (−)</th>
<th>sERC (+)</th>
<th>( P )-value(^a )</th>
</tr>
</thead>
<tbody>
<tr>
<td>( n = 174 )</td>
<td>( n = 18 )</td>
<td></td>
</tr>
<tr>
<td>Age</td>
<td>33.8 ± 4.7</td>
<td>33.1 ± 4.3</td>
</tr>
<tr>
<td>Mean no. of retrieved oocytes</td>
<td>8.2 ± 5.4</td>
<td>9.4 ± 5.0</td>
</tr>
<tr>
<td>Endometriosis in ovary</td>
<td>9/174 (5.2%)</td>
<td>1/18 (5.6%)</td>
</tr>
<tr>
<td>Thickness of endometrium (mm)</td>
<td>11.8 ± 2.9</td>
<td>10.5 ± 2.5</td>
</tr>
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\(^a\)Statistical analysis: Student’s \( t \)-test or \( \chi^2 \) test; NS = not significant (\( P > 0.05 \)).

**Table II.** A comparison of stimulation protocols

<table>
<thead>
<tr>
<th>sERC (−)</th>
<th>sERC (+)</th>
</tr>
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<tbody>
<tr>
<td>Long protocol</td>
<td>160/174 (92.0%)</td>
</tr>
<tr>
<td>Short protocol</td>
<td>13/17 (76.5)</td>
</tr>
<tr>
<td>Clomiphene citrate</td>
<td>1/1</td>
</tr>
<tr>
<td>Natural</td>
<td>0/1</td>
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![Figure 4](https://link.to/image4.png)

**Figure 4.** Serum estradiol (A) and progesterone (B) concentrations in sERC negative (−) and positive (+) cycles. \( *P < 0.05 \) (significance determined by the \( \chi^2 \) test). NS = not significant.

![Figure 5](https://link.to/image5.png)

**Figure 5.** Grade of transferred embryos in sERC negative and positive cycles by Veeck’s classification. \( *P < 0.05 \) (significance determined by the \( \chi^2 \) test).
the plasma membrane of sERC. In pronuclear zygotes and blastomeres of cleaving embryos, Ca2+-rich vesicles were no longer present close to the plasma membrane, and the entire cell periphery was poor in Ca2+-containing organelles which, however, were abundant in the perinuclear region (Sousa et al., 1996, 1997). Because Ca2+ release from sER plays pivotal roles in oocyte maturation, fertilization and early embryonic development (Homa et al., 1993; Tesarik, 1997), studies of Ca2+ signalling in sERC-positive oocytes may contribute to understanding the cause and the effect of sERC formation. Ca2+ oscillations in human GV oocytes are facilitated by 17β-estradiol (Tesarik and Mendoza, 1995) and inhibited by androstendione (Tesarik and Mendoza, 1997). From our hormonal data, high estradiol concentrations on the day of hCG administration in the sERC-positive cycles may be one of the clues to sERC formation. Although no research has been attempted regarding how sERCs are involved in the low pregnancy rates, they would be, more importantly, a clinical marker for diagnosis of oocyte quality and implantation failure due to the delay of oocyte retrieval.

The rates of the transferred embryos that had been judged as grade I according to Veeck’s classification were higher in sERC-positive cycles than in negative cycles, suggesting that sERCs are somehow associated with the status of oocyte maturity. Although the implantation rates can be improved by various methods of embryo selection, based on evaluations of embryo morphology, prior to transfer, developmental failure is frequent even for healthy looking embryos. This discrepancy may explain an important part of idiopathic infertility. Furthermore, sERCs have been overlooked and considered insignificant on the basis of the high grade of embryos produced from sERC-negative cohorts. By observing denuded oocytes during ICSI procedures, we can more easily and reliably check for the presence of sERCs in oocytes than in the IVF procedure.

One baby born in sERC-positive cycles was diagnosed with Beckwith–Wiedemann syndrome, a model imprinting disorder resulting from mutations or epigenetic events affecting the imprinted genes on chromosome 11p15.5. Questions arise as to whether inheritance of this disease is causally related to the use of ART (Gosden et al., 2003; Maher et al., 2003). At present, there are no data concerning the relationship between sERC and the genomic imprinting defect. Although possible causes of such a disorder should be carefully considered, it would be interesting to study the correlation between sERC and imprinting disorders. It would be important to examine the unusual distribution pattern of the sERC formation that may be involved in the abnormal regulation of Ca2+ signalling. It is clear that sERCs in human oocytes require future studies due to important functions of the sER in oocyte maturation and Ca2+ signalling for embryonic development.

### Table III. Fertilization rate, cell division rate and pregnancy rate in sERC negative and positive cycles

<table>
<thead>
<tr>
<th></th>
<th>sERC (−)</th>
<th>sERC (+)</th>
<th>P-value*</th>
</tr>
</thead>
<tbody>
<tr>
<td>No. of cycles</td>
<td>174</td>
<td>18</td>
<td></td>
</tr>
<tr>
<td>No. of patients</td>
<td>119</td>
<td>15</td>
<td></td>
</tr>
<tr>
<td>Mean no. of transferred embryos</td>
<td>2.3 ± 0.7</td>
<td>2.7 ± 0.6</td>
<td>NS</td>
</tr>
<tr>
<td>Mean no. of ART attempts</td>
<td>2.2 ± 1.5</td>
<td>3.4 ± 1.6</td>
<td></td>
</tr>
<tr>
<td>Fertilization rate by IVF</td>
<td>693/813 (85.2%)</td>
<td>93/114 (81.6%)</td>
<td>NS</td>
</tr>
<tr>
<td>Fertilization rate by IVF</td>
<td>441/569 (77.8%)</td>
<td>36/45 (80.0%)</td>
<td>NS</td>
</tr>
<tr>
<td>Cell division rate by ICSI at 72 h</td>
<td>662/693 (95.5%)</td>
<td>90/93 (96.8%)</td>
<td>NS</td>
</tr>
<tr>
<td>Cell division rate by IVF at 72 h</td>
<td>42/441 (96.1%)</td>
<td>35/36 (97.2%)</td>
<td>NS</td>
</tr>
<tr>
<td>Clinical pregnancy/OPU</td>
<td>48/174 (27.6%)</td>
<td>1/18 (5.6%)</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>Clinical pregnancy/embryo transfer</td>
<td>48/170 (28.2%)</td>
<td>1/18 (5.6%)</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>Implantation rate (implanted)</td>
<td>57/445 (13.0%)</td>
<td>1/48 (2.1%)</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>Biochemical pregnancy/embryo transfer</td>
<td>6/170 (3.5%)</td>
<td>4/18 (22.2%)</td>
<td>&lt;0.05</td>
</tr>
</tbody>
</table>

*aStatistical analysis: Student’s t-test or χ2 test; NS = not significant (P > 0.05).

*b Beckwith–Wiedemann syndrome.

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### References


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