Oocytes with smooth endoplasmic reticulum clusters originate blastocysts with impaired implantation potential

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Objective: To study whether embryos derived from oocytes presenting a smooth endoplasmic reticulum cluster (SERC) are less likely to develop into blastocysts and implant.
Design: Transversal study.
Setting: Private university-affiliated in vitro fertilization (IVF) center.
Patient(s): Total of 7,609 oocytes obtained from 743 intracytoplasmic sperm injection (ICSI) cycles.
Intervention(s): Oocytes split between the SERC-positive cycles (with at least one SERC-positive oocyte) and the SERC-negative cycles (only oocytes free of SERC).
Main Outcome Measure(s): Embryo implantation.
Result(s): A statistically significantly higher mean number of follicles (24.0 ± 10.5 vs. 19.6 ± 10.5), retrieved oocytes (17.8 ± 8.3 vs. 14.3 ± 8.0), and mature oocytes (13.5 ± 6.2 vs. 10.6 ± 5.9) were observed in the SERC-positive cycles as compared with SERC-negative cycles. The implantation rate was statistically significantly lower in SERC-positive cycles as compared with SERC-negative cycles (14.8% vs. 25.6%; odds ratio 0.61; 95% confidence interval, 0.44–0.86). When only cycles with in which none (0) or all the blastocysts transferred had implanted (100%) were analyzed, the mean implantation rate per transferred blastocyst in the SERC-negative group was 20.5%; no blastocysts derived from SERC-positive oocytes implanted.
Conclusion(s): The occurrence of SERC impairs embryo implantation. Careful oocyte observation that takes into account the presence of SERC should be part of embryo selection strategy before transfer. (Fertil Steril® 2016;106:1718–24. ©2016 by American Society for Reproductive Medicine.)
Key Words: Blastocyst, embryo implantation, ICSI, oocyte morphology, smooth endoplasmic reticulum

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Embryo selection for transfer is a key element in in vitro fertilization (IVF). The selection of high-quality embryos increases pregnancy rates and decreases multiple pregnancies as it enables restrictions on the number of transferred embryos. Usually embryos are selected for transfer based on their morphologic features on days 1, 2, 3, and 5 of development (1). Additionally, oocyte quality assessed on day 0 can also be taken into account for embryo selection, considering the vital role played by the oocyte in the embryo developmental process (2).

In fact, several studies have shown that embryo quality and implantation potential may be influenced by oocyte quality (3–8). The typical evaluation of the oocyte quality is based on extra and intracytoplasmic morphologic features, such as first polar body morphology, perivitelline space size and granularity, zona pellucida defects, shape anomalies, and the presence of refractile bodies, dense cellular granulation, vacuoles, and smooth endoplasmic reticulum (9).

Smooth endoplasmic reticulum is a type of organelle that forms an interconnected network of flattened, membrane-enclosed sacs or tubes. These can be

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distinguished from fluid-filled vacuoles because they are not filled with fluid and not separated from the cytoplasm by a membrane (10, 11). One of the key roles of smooth endoplasmic reticulum in the oocyte is storage and redistribution of calcium, which is responsible for cell activation in the course of fertilization (11, 12). A smooth endoplasmic reticulum cluster (SERC) is an intracytoplasmic dimorphism that has been suggested to interfere with calcium stores and oscillations during fertilization, and may have a negative effect on embryo development and implantation (13).

Previous studies have shown that compromised fertilization, embryo development, pregnancy rates, and obstetric and neonatal outcomes (8,14–17) result when oocytes presenting with SERC are injected and the embryo thus derived is transferred. In 2011 the Alpha Scientists in Reproductive Medicine and European Society of Human Reproduction and Embryology (ESHRE) Special Interest Group of Embryology recommended not inseminating oocytes that presented with SERC because they might be associated with an increased risk of abnormal outcomes (18). However, more recently a study showed that healthy babies could be obtained using oocytes presenting with SERC (13).

Thus, information regarding the clinical significance of oocytes presenting with SERC is sparse and controversial. We investigated whether embryos derived from oocytes presenting SERC are less likely to develop into blastocysts and implant. Additionally, we examined the predictive factors for the occurrence of SERC.

MATERIALS AND METHODS

Experimental Design, Patients, and Inclusion and Exclusion Criteria

Our transversal study included data from patients undergoing intracytoplasmic sperm injection (ICSI) from July 2011 to June 2014 at a private university-affiliated IVF center located in Brazil. The inclusion criteria were as follows: patients undergoing ICSI with fresh embryo transfer performed on day 5 of development. Patients who were undergoing ICSI with vitrified/thawed or donated oocytes, surgical sperm retrieval, sperm without progressive motility, vitrified/thawed embryo transfer, donated embryo transfer, or preimplantation genetic diagnosis or screening were excluded from the analysis.

Two analyses were performed. In the first analysis, the obtained oocytes were split between the SERC-positive group, in which the oocytes presented with SERC, and the SERC-negative group, in which the oocytes were free of SERC. The ICSI cycles' characteristics, such as female age, total dose of follicle-stimulating hormone (FSH) administered, number of follicles and oocytes obtained, fertilization rate, high-quality embryos available on day 2 and 3, blastocyst formation rate, number of high-quality blastocysts, and number of embryos transferred, were compared between the SERC-positive and SERC-negative oocytes.

In the second analysis, the ICSI cycles were split between the SERC-positive cycles, in which the cycles had at least one SERC-positive oocyte, and the SERC-negative cycles, in which the cycles had only SERC-free oocytes. The ICSI cycles' characteristics such as female age, total dose of FSH administered, number of follicles and oocytes obtained, fertilization rate, high-quality embryos on day 2 and 3, blastocyst formation rate, high-quality blastocysts, number of embryos transferred, and implantation, pregnancy, and miscarriage rates were compared between the SERC-positive and SERC-negative cycles. In a further analysis, to investigate the implantation potential of the embryos derived from SERC-positive oocytes, only cycles in which none (0) or all embryos transferred had implanted (100%) were analyzed. All patients signed a written informed consent form, and the study was approved by the local institutional review board.

Controlled Ovarian Stimulation

Ovarian stimulation was achieved by the administration of recombinant FSH (Gonal-F; Serono) on a daily basis until the visualization of at least one follicle $\geq$14 mm, at which time we began the administration of gonadotropin-releasing hormone (GnRH) antagonist, cetrorelix acetate (Cetrotide; Serono).

The ovulation trigger was provided by injection of recombinant human chorionic gonadotrophin (hCG, Ovidrel; Serono) when at least three follicles $\geq$18 mm were observed. Oocyte retrieval was performed 35 hours after the administration of hCG, through transvaginal ultrasonography.

Oocyte Preparation

Retrieved oocytes were maintained in culture medium (Global for fertilization; LifeGlobal) supplemented with 10% protein supplement (LGPS; LifeGlobal) and covered with paraffin oil (Paraffin oil P.G.; LifeGlobal) for 2 to 3 hours before cumulus cell removal. The surrounding cumulus cells were removed after exposure to a HEPES-buffered medium containing hyaluronidase (80 IU/mL; LifeGlobal). The remaining cumulus cells were then mechanically removed by gently pipetting with a hand-drawn Pasteur pipette (Humagen Fertility Diagnostics).

Oocyte morphology was assessed using an inverted Nikon Diaphot microscope (Eclipse TE 300; Nikon) with a Hoffmann modulation contrast system under $\times$400 magnification, just before sperm injection (5 hours after retrieval). The presence of SERC in the ooplasm was recorded (Fig. 1). Oocytes that had released the first polar body were considered mature and were used for ICSI.

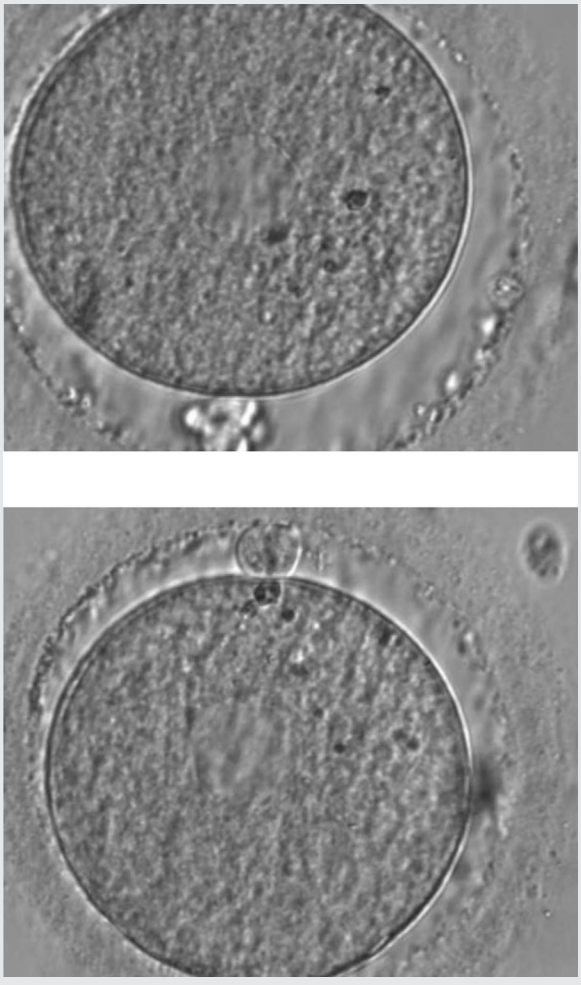
ICSI

We performed ICSI according to the methods described by Palermo et al. (19). Sperm selection was analyzed at $\times$400 magnification using an inverted Nikon Eclipse TE 300 microscope. The injection was performed in a microinjection dish prepared with 4-μL droplets of buffered medium (Global with HEPES; LifeGlobal) and covered with paraffin oil on a heated stage at 37.0°C $\pm$ 0.5°C in an inverted microscope. Fertilization was confirmed by the presence of two pronuclei and the extrusion of the second polar body approximately 16 hours after ICSI.

Embryo Quality and Transfer

To evaluate the cleavage-stage morphology, the following parameters were recorded: the number of blastomeres, the

FIGURE 1



Smooth endoplasmic reticulum clusters (SERC) in mature human oocyte.

Setti. Oocyte with SERC and embryo implantation. Fertil Steril 2016.

percentage of fragmentation, the variation in blastomere symmetry, the presence of multinucleated blastomeres, and defects in the zona pellucida and cytoplasm. The high-quality cleavage-stage embryos were defined as those with all of the following characteristics: 4 cells on day 2 or 8–10 cells on day 3, <15% fragmentation, symmetric blastomeres, the absence of multinucleated blastomeres, colorless cytoplasm with moderate granulation and no inclusions, the absence of perivitelline space granularity, and the absence of zona pellucida dimorphisms. Embryos lacking any of these characteristics were considered to be of low quality.

To evaluate the blastocyst morphology, embryos were given a numeric score from 1 to 6 based on their degree of expansion and hatching status, as follows: [1] an early blastocyst with a blastocoel that is less than half the volume of the embryo; [2] a blastocyst with a blastocoel that is greater than half the volume of the embryo; [3] a full blastocyst with a blastocoel that completely fills the embryo; [4] an expanded blastocyst; [5] a hatching blastocyst; and [6] a hatched blastocyst. The inner cell mass of full, expanded, hatching, and hatched blastocysts was classified as either high quality (tightly packed with many cells) or low quality (loosely grouped with several or few cells). Similarly, the trophectoderm was also classified as either high quality (many cells forming a cohesive epithelium) or low quality (few cells forming a loose epithelium or very few cells) (18).

Embryos were placed in a 50-μL drop of culture medium (Global; LifeGlobal) supplemented with 10% protein supplement, and they were covered with paraffin oil in a humidified atmosphere under 7.5% CO_2 at 37°C for 5 days. Embryo transfer was performed on day 5 of development using a soft catheter with transabdominal ultrasound guidance. One to four embryos were transferred per patient, depending on embryo quality and maternal age.

Clinical Follow-up

A pregnancy test was performed 10 days after embryo transfer and repeated 48 hours later. All women with a positive test received a transvaginal ultrasound scan after 2 weeks. A clinical pregnancy was diagnosed when the fetal heartbeat was detected. Implantation rates were calculated per patient. Pregnancy rates were calculated per transfer. Miscarriage was defined as a pregnancy loss before 20 weeks.

Data Analysis and Statistics

Data are expressed as the mean ± standard deviation (SD) for continuous variables, and percentages are used for categorical variables. Mean values were compared by Student's parametric *t*-test or Mann-Whitney nonparametric test, according to the result obtained in the Kolmogorov-Smirnov normality test. Percentages were compared by the chi-square or Fisher's exact test when the expected frequency was five or lower.

Binary regression analyses were used to investigate the influence of maternal age, total dose of FSH administered, estradiol levels on the day of hCG administration, aspirated follicles, and retrieved oocytes on the occurrence of SERC. A multivariate logistic regression was used to investigate the influence of SERC on the odds of implantation. This analysis was adjusted for maternal age, total FSH dose administered, estradiol levels on the day of hCG trigger administration, number of retrieved oocytes, and embryo quality because these variables were considered as potential confounders. The results are expressed as odds ratio (OR) with 95% confidence intervals (CI) and *P* values. $P<.05$ was considered statistically significant. Data analyses were performed using the Minitab version 17 statistical program (Minitab, Inc.).

RESULTS

A total of 2,155 ICSI cycles were performed during the period proposed for this study. Subsequent to the application of inclusion and exclusion criteria, 743 ICSI cycles performed in 583 patients were included in the analysis. The descriptive analysis of the included cycles is shown in Table 1.

TABLE 1

Descriptive analysis of patient demographics (n = 583) and ICSI cycle outcomes (n = 743).

Variable	N	Mean	SD	Range
Patients' demographics				
Maternal age (y)	583	34.3	4.1	21–45
Paternal age (y)	583	37.2	5.6	25–63
Total FSH administered (IU)	–	2,279	605	600–4,950
Estradiol level (pg/mL)	–	2,124	1,538	125–10,000
COS outcomes				
Aspirated follicles	14,905	20.1	10.6	2–64
Retrieved oocytes	10,868	14.6	8.1	1–50
Mature oocyte rate (%)	8,072/10,868	74.3	–	–
Injected oocytes	7,609	10.2	5.0	1–36
Laboratory outcomes				
Fertilization rate (%)	5,848/7,609	76.9	–	–
Blastocyst formation rate (%)	3,072/5,848	52.5	–	–
Transferred embryos	1,659	2.2	0.6	1–4
Clinical outcomes (n, %)				
Clinical pregnancy rate	308/743	41.5		
Miscarriage rate	38/308	12.3		
Implantation rate	407/1,659	24.5		

Note: COS = controlled ovarian stimulation; FSH = follicle-stimulated hormone; ICSI = intracytoplasmic sperm injection; SD = standard deviation.

Setti. Oocyte with SERC and embryo implantation. Fertil Steril 2016.

Incidence and Predictive Factors of SERC

From a total of 743 ICSI cycles, 7,609 oocytes were morphologically evaluated, and 167 oocytes were found to present with SERC (2.2%). Seventy-eight cycles showed at least one oocyte presenting with SERC (10.6%). No patients had only SERC-positive oocytes. The number of SERC-positive oocytes per cycle ranged from 0 to 12, and the prevalence ranged from 0.0 to 66.7%.

From 474 patients undergoing only one ICSI cycle (n = 474 cycles), 417 had no SERC-positive oocytes, and 57 patients had at least one SERC-positive oocyte. In patients with multiple cycles, the occurrence of SERC was repetitive. From 88 patients undergoing two ICSI cycles (n = 176 cycles), 82 had no SERC-positive oocytes in both cycles (164 cycles), and 6 patients had SERC in the first and second cycles (12 cycles). From 31 patients undergoing three ICSI cycles (93 cycles), 28 had no SERC-positive oocytes in all cycles (84 cycles), and 3 patients had SERC-positive oocytes in the first, second, and third cycles (9 cycles).

Maternal age (OR 1.02; 95% CI, 0.99–1.07), the total dose of FSH administered (OR 1.01; 95% CI, 0.99–1.01), and the estradiol levels on the day of hCG administration (OR 1.01; 95% CI, 1.00–1.01) were not associated with the occurrence of SERC. However, the number of follicles (OR 1.03; 95% CI, 1.02–1.04; $P<.001$) and retrieved oocytes (OR 1.03; 95% CI, 1.01–1.04; $P=.002$) were determinants to the increased odds of SERC occurrence.

SERC and ICSI Outcomes

First analysis. Mean female age and total dose of FSH administered were similar between the SERC-positive and the SERC-negative oocytes. A statistically significantly higher mean number of follicles (23.6 ± 11.4 vs. 27.7 ± 11.2, $P<.001$), obtained oocytes (17.7 ± 8.8 vs. 19.9 ± 8.2, $P=.001$), and mature oocytes (13.4 ± 6.7 vs. 15.0 ± 5.8, $P<.001$) were observed in the SERC-positive oocytes as compared with the SERC-negative oocytes. On day 1 of development, 5,848 zygotes were obtained from 7,609 injected oocytes (76.9%). There were no statistically significant differences between the SERC-positive and SERC-negative oocytes regarding the fertilization rate and high-quality embryos rate on days 2 and 3. On day 5 of embryo development, 3,072 blastocysts were obtained from (52.5%). The blastocyst formation rate, high-quality blastocyst rate and number of transferred embryos were similar between the groups (Table 2).

Second analysis. Mean female age and total dose of FSH administered were similar between the SERC-positive and the SERC-negative cycles. A statistically significantly higher mean number of follicles (24.0 ± 10.5 vs. 19.6 ± 10.5, $P=.001$), retrieved oocytes (17.8 ± 8.3 vs. 14.3 ± 8.0, $P=.001$), and mature oocytes (13.5 ± 6.2 vs. 10.6 ± 5.9, $P<.001$) were observed in the SERC-positive cycles as compared with the SERC-negative cycles. There were no statistically significant differences between the SERC-positive and SERC-negative cycles regarding the fertilization rate and high-quality embryos rate on days 2 and 3. The blastocyst formation rate, high-quality blastocyst rate and number of transferred embryos were similar between the groups.

The implantation rate was statistically significantly lower in SERC-positive cycles as compared with SERC-negative cycles (14.8% vs. 25.6, $P<.001$). Multivariate logistic regression results demonstrated that implantation was statistically significantly less likely to occur in the SERC-positive cycles as compared with SERC-negative cycles (OR 0.61; 95% CI, 0.44–0.86; $P<.001$). The pregnancy and miscarriage rates were similar between the groups (Table 3).

SERC and Implantation

For the association of SERC and chances of implantation, only cycles in which none (0) or all the blastocysts transferred

TABLE 2

Comparison of ICSI outcomes between SERC-negative and SERC-positive oocytes.

Variable	SERC-negative oocytes (n = 7,442)	SERC-positive oocytes (n = 167)	*P* value
Maternal age (y)	34.1 ± 4.01	34.5 ± 3.9	.214
Total FSH administered (IU)	2,252 ± 614	2,264 ± 563	.776
Aspirated follicles	23.6 ± 11.4	27.7 ± 11.2	<.001
Retrieved oocytes	17.7 ± 8.8	19.9 ± 8.2	.001
Mature oocyte	13.4 ± 6.7	15.0 ± 5.8	<.001
Fertilization rate	76.2	76.4	.447
High-quality embryos rate (D2)	50.0	53.9	.570
High-quality embryos rate (D3)	43.1	46.1	.433
Blastocyst formation rate	43.2	43.8	.722
High-quality blastocysts	82.5	73.8	.076
Transferred embryos	2.2 ± 0.6	2.2 ± 0.5	.833

Note: FSH = follicle-stimulating hormone; SERC = smooth endoplasmic reticulum clusters.

Setti. Oocyte with SERC and embryo implantation. Fertil Steril 2016.

had implanted (100%) were included in the analysis. Out of 1,659 embryos transferred in all included cycles, 767 blastocysts were transferred to patients who had a 0 (351 ICSI cycles) or 100% (83 ICSI cycles) implantation rate. A total of 618 blastocysts were transferred in the 0 implantation rate group, and 149 blastocysts were transferred in the 100% implantation group. Of the 767 blastocysts transferred, 745 derived from SERC-negative oocytes and 22 from SERC-positive oocytes. The mean implantation rate per transferred blastocyst in the SERC-negative group was 20.5% whereas no blastocysts derived from SERC-positive oocytes implanted.

DISCUSSION

Despite the conflicting results published regarding the effect of SERC on ICSI outcomes, the data indicate that embryo development is compromised to a certain extent when SERC-positive oocytes are injected. We investigated whether embryos derived from oocytes presenting with SERC are less likely to develop into blastocysts and implant. Our results demonstrated that although oocytes displaying SERC normally reached the blastocyst stage, the implantation rate was statistically significantly lower in SERC-positive cycles compared with SERC-negative cycles. This result was confirmed by a multivariate regression analysis that demonstrated that implantation is 40% less likely to occur in the SERC-positive cycles as compared with SERC-negative cycles.

Considering that embryos derived from both SERC-positive and SERC-negative oocytes were transferred in the SERC-positive cycles, we could suggest that the presence of SERC is associated with a lower chance of successful implantation, even in SERC-negative oocytes from the same cohort that are transferred along with the SERC-positive oocytes. These findings corroborate with the study by Otuski et al. (14) that demonstrated statistically significant lower pregnancy rates and higher biochemical pregnancy rates in cycles presenting at least one SERC-positive oocyte compared with cycles that had only oocytes free of SERC. Additionally, we observed that no blastocysts derived from SERC-positive oocytes implanted.

The endoplasmic reticulum is a multifunctional organelle responsible for storage and release of calcium in the oocyte, which contributes to fertilization. Additionally, complexes of

TABLE 3

Comparison of ICSI outcomes between SERC-negative and SERC-positive cycles.

Variable	SERC-negative cycles (n = 665)	SERC-positive cycles (n = 78)	*P* value
Maternal age (y)	34.2 ± 4.1	34.6 ± 3.9	.422
Total FSH administered (IU)	2,271 ± 612	2,338 ± 525	.306
Aspirated follicles	19.6 ± 10.5	24.0 ± 10.5	**.001**
Retrieved oocytes	14.3 ± 8.0	17.8 ± 8.3	**.001**
Mature oocyte	10.6 ± 5.9	13.5 ± 6.2	**<.001**
Fertilization rate	82.3	79.5	.172
High-quality embryos rate (D2)	32.5	33.7	.500
High-quality embryos rate (D3)	55.9	58.3	.217
Blastocyst formation rate	37.4	34.8	.177
High-quality blastocysts	83.4	81.6	.475
Transferred embryos	2.2 ± 0.6	2.2 ± 0.5	.879
Implantation rate	382/1,490 (25.6)	25/169 (14.8)	**<.001**
Pregnancy rate	282/665 (42.4)	26/78 (33.3)	.145
Miscarriage rate	36/281 (12.8)	2/26 (7.7)	.754

Note: FSH = follicle-stimulating hormone; ICSI = intracytoplasmic sperm injection; SERC = smooth endoplasmic reticulum clusters.

Setti. Oocyte with SERC and embryo implantation. Fertil Steril 2016.

endoplasmic reticulum and associated mitochondria are essential for energy accumulation, protein synthesis and assembly, lipid synthesis, and production of cytosolic and nuclear membranes through early embryo development (11, 17, 20–22). However, it has been suggested that the presence of SERC interferes with calcium storage and distribution, resulting in a negative effect on fertilization, embryo development, and implantation (14, 15).

It has been demonstrated that when an oocyte with SERC is fertilized, the SERC fades after the release of calcium (14); conversely, the permanence of SERC in unfertilized eggs after conventional IVF has been described (23, 24). In fact, oocytes displaying this particular dimorphism have been shown to originate embryos with poor developmental potential (7, 24). Otsuki et al. (14) found comparable fertilization rates in SERC-negative and SER-positive oocytes, but in SERC-positive cycles statistically significantly lower pregnancy and higher biochemical pregnancy rates were shown. Sjöblom et al. (25) observed that the blastocyst rate of oocytes displaying SERC was nil.

In a study population with a SERC prevalence of 13%, Munaswamy et al. (26) found no statistically significant rates of fertilization, cleavage, or high-quality embryos in SERC oocytes compared with normal oocytes. However, the clinical pregnancy rate in the SERC group was more than 50% lower than that of the normal oocyte group (26). On the other hand, Ebner et al. (15) observed lower fertilization and blastulation rates in SERC oocytes compared with unaffected oocytes. Conversely, the study by Yang et al. (27) found no association between the presence of SERC and ICSI outcomes, from fertilization to ongoing pregnancy rates.

Braga et al. (3) found an association between SERC and blastocyst development; the presence of SERC resulted in a 35% decrease in the quality of the inner cell mass. Moreover, a 67% decrease in the chance of pregnancy and a 20% increase in the odds of miscarriage were observed for SERC-positive oocytes (3). More recently, it was demonstrated that despite similar fertilization and embryo development rates, oocytes with SERC had lower blastocyst formation and high-quality blastocyst rates than SERC-negative oocytes (28).

Regarding the outcomes of pregnancy, a trend toward earlier delivery and statistically significantly reduced birth weight were observed by Ebner et al. (15). Additionally, abnormal outcomes such as cases of Beckwith-Wiedemann syndrome (14), diaphragmatic hernia (15), multiple malformations (16), and ventricular septal defect (17) have been associated with embryos derived from SERC-positive oocytes.

After the recommendation to avoid the injection of oocytes displaying smooth endoplasmic reticulum (18), Mateizel et al. (13) showed that, despite lower development rates, embryos derived from SERC-positive oocytes can develop normally and may lead to newborns free of major malformations. In a systematic review that included 13 relevant studies, it was reported that 183 babies were born from SERC-positive cycles, 171 babies were healthy, 8 presented with malformations, 3 were neonatal deaths, and 1 was stillborn (29). The investigators concluded that the birth of healthy babies derived from SERC-positive oocytes is encouraging and might lead to a future revision of the recommendations previously provided (18). Later, one study showed a nearly threefold higher incidence of transfer cancelation when SERC-positive oocytes are discarded, mainly due to the absence of suitable oocytes for insemination. The clinical outcomes were similar between the SERC-positive and SERC-negative oocytes; however, one stillbirth was observed in the SERC-positive group, and no major malformations were recorded among newborns (30).

Further investigation is needed to understand the origin of SERC and prevent its occurrence. In our study, a statistically significant higher mean number of follicles, retrieved oocytes, and mature oocytes were observed in the SERC-positive cycles as compared with the SERC-negative cycles. In fact, it has been suggested that the occurrence of SERC is a consequence of inadequate ovarian stimulation (31) related to the duration and dosage of stimulation (15).

Much has to be learned before any explanation can be offered regarding the association between SERC-positive oocytes and impaired embryo implantation. In human oocytes, small vesicles containing calcium are located beneath the plasma membrane of the smooth endoplasmic reticulum, whereas in zygotes and embryo blastomeres calcium-containing organelles are abundant in the perinuclear region (32, 33). It could be suggested that oocytes presenting SERC might reflect a high frequency of aneuploidy that prevents the implantation of the embryos. In fact, it has been previously demonstrated that women with SERC have a higher risk of miscarriage (16).

Despite controlled ovarian stimulation for ICSI leading to the production of heterogeneous oocytes, it has been previously shown that 5% of ICSI cycles show at least one SERC-positive oocyte (13). Even though we have observed that almost 11% of the cycles show at least one SERC-positive oocyte, the relatively rare occurrence of SERC in oocyte cohorts limits the experimental analysis of the dimorphism. Therefore, that could be considered the main drawback of our study. Moreover, it might be argued that a threshold exists between the number and size of SERC for which the risk of a negative outcome might exist (34).

Additionally, a bias might have been introduced because oocyte morphology in this study was punctually accessed by light microscopy; as previously described elsewhere, there are at least three forms of SERC that are classified by size: the large (18 μm) and medium (10–17 μm) sizes are visible by light microscopy, but the small (2–9 μm) sizes are not visible by light microscopy (14).

A previous study identified different types of smooth endoplasmic reticulum by transmission electron microscopy: isolated tubules, small vesicles, medium vesicles, large vesicles, and very large vesicles (35). That study showed that smooth endoplasmic reticulum tubular aggregates associated or not with mitochondria, and smooth endoplasmic reticulum vesicles associated with mitochondria were not observed in immature (germinal vesicle) oocytes (35). Accordingly, another study showed that mitochondria-smooth endoplasmic reticulum aggregates (M-SER) were only found in mature oocytes (12). Thus, the presence of smooth endoplasmic reticulum tubules and underdeveloped smooth endoplasmic reticulum aggregates in germinal vesicle oocytes

could be justified as oocyte immaturity (12). On the other hand, mature oocytes showed the presence of large M-SER (12). These aggregates could contribute to the generation of the typical sperm-induced calcium oscillations and could be interpreted by the mature oocyte as a trigger for the fertilization process (12).

The discrepancies observed in the published studies might be explained by differences in study population, infertility etiology, or stimulation protocols. Nevertheless, as stated by Shaw-Jackson et al. (29), "Until we have a better understanding of the situation, transfers of affected embryos should be carried out with caution."

CONCLUSION

The presence of SERC is associated with lowered chances of successful implantation. Careful oocyte observation that takes into account the presence of SERC should be part of the strategy for embryo selection for transfer.

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