Can healthy babies originate from oocytes with smooth endoplasmic reticulum aggregates? A systematic mini-review

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STUDY QUESTION: Is it time to reconsider whether oocytes affected by smooth endoplasmic reticulum aggregates (SERa) should still be destroyed?

SUMMARY ANSWER: At the time of writing, the literature shows that 171 apparently healthy babies have been born from SERa+ cycles amongst which 22 were from SERa+ oocytes.

WHAT IS KNOWN ALREADY: The SER dysmorphism has been associated with negative embryological, clinical and neonatal outcomes, which led to a recommendation in 2011 to avoid inseminating affected oocytes. The data in the literature are nevertheless conflicting and some centres have continued using SERa+ oocytes.

STUDY DESIGN, SIZE, DURATION: A systematic mini-review of the literature to 7 November 2013 was performed with the keywords ‘Smooth Endoplasmic Reticulum’ and ‘oocyte’, limited to humans and written in English.

PARTICIPANTS/MATERIALS, SETTING, METHODS: Articles (Pubmed) and major abstracts where the effect of the SER dysmorphism was studied as an individual feature on embryological, clinical or neonatal outcomes were included in this review.

MAIN RESULTS AND THE ROLE OF CHANCE: From a total of 297 publications identified, 13 were selected as being relevant to this review. One hundred eighty-three babies have been reported to be born from SERa+ cycles, 171 were healthy, 8 live births presented malformations, 3 were neonatal deaths, 1 was a stillborn and additionally 4 terminations of pregnancy occurred.

LIMITATIONS, REASONS FOR CAUTION: Data concerning SERa+ oocytes in the literature are scarce, the studies are small, heterogeneous and results are conflicting. The malformations observed could be due to over-reporting of scattered alarming results. Alternatively, an under-reporting of complications cannot be excluded.

WIDER IMPLICATIONS OF THE FINDINGS: Centres that have or that are including transfers of SERa+ embryos in their IVF procedures should publish their clinical and neonatal outcomes as well as the follow-up of children. The birth of healthy babies from SERa+ embryos is encouraging and might lead in the future to a revision of the current consensus on the SER dysmorphism. Further research is needed to understand the origin of this dysmorphism and help avoid its occurrence. Therefore, until we have a better understanding of the situation, transfers of affected embryos should be carried out with caution.

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Key words: smooth endoplasmic reticulum aggregates / oocyte dysmorphism / clinical outcome / embryology / assisted reproduction
Introduction

In 2011, it was recommended not to inseminate oocytes affected by smooth endoplasmic reticulum aggregates (SERa), since they might be associated with an increased risk of abnormal outcome (Alpha Scientists in Reproductive Medicine and ESHRE Special Interest Group of Embryology, 2011).

Several reports focusing specifically on SERa+ cycles/oocytes have indeed shown negative outcomes in terms of fertilization, embryo development and pregnancy rates as well as compromised obstetric and neonatal outcomes (Otsuki et al., 2004; Ebner et al., 2008; Akarsu et al., 2009; Wallbutton and Kasraie, 2010; Sá et al., 2011). However, a recent publication demonstrated that healthy babies could be obtained using oocytes that contain SERa (SERa+ oocytes) (Mateizel et al., 2013). The data in the literature are controversial and some authors present results for SERa+ cycles, others for SERa+ oocytes. Indeed, oocytes that appear at first sight using light microscopy as negative for SERa but which arise from the same cohort of SERa+ gametes (defined as SERa+ cycles), may in fact also be affected when studied using transmission electron microscopy (Otsuki et al., 2004). In order to have a clear overview of what is known, we performed a systematic mini-review analyzing clinical outcomes for SERa+ cycles and oocytes.

Methods

A PubMed systematic literature search using the keywords 'Smooth Endoplasmic Reticulum' and 'oocyte' limited to humans was performed (last search on 21st October 2013), as well as in the Human Reproduction, Fertility and Sterility and Reproductive Biomedicine Online Journals (last search on 7th November 2013).

English articles identified by the search were selected for inclusion in the review if they reported data concerning the effect of the SER dysmorphism on at least one of the following outcomes: fertilization rate, pregnancy rate, miscarriage rate, implantation rate, embryo quality and neonatal outcome.

SERa can be clearly distinguished morphologically by light microscopy from fluid-filled vacuoles as shown in Fig. 1.

Results

Design and limitations of the studies

The search identified a total of 297 records and 13 of these were selected for this review (Fig. 2). These included 5 articles, 7 abstracts and 1 case report. The design of the majority of the studies was retrospective (n = 9) (Table I), although for one of them, the authors claimed the study to be randomized.

Two studies were prospective, one specified that transfer of SERa+ embryo transfers was avoided (Ebner et al., 2008) and for the other one, no information on embryo selection was provided (Yang et al., 2008). Only two retrospective studies specified that embryos were selected for transfer irrespective of the presence of SERa (Bielanska and Leveille, 2011; Mateizel et al., 2013) and one specified that SERa+ embryo transfers were avoided (Otsuki et al., 2004).

In most studies, it was not mentioned whether information was provided to the patients concerning the presence of SERa nor if patients’ consent was obtained before transfer of affected embryos. In one study however, patients were informed about the presence of SERa+ oocytes and the risk of a reduced pregnancy rate even when SERa− embryos are transferred (Otsuki et al., 2004). In a second study, the authors specified that the rare transfers of SERa+ embryos only occurred after consultation with the patients (Ebner et al., 2008).

Preferential transfers of SERa− over SERa+ embryos introduce a bias for cycle comparisons and even more so for oocyte comparisons. When studying the effect of oocyte morphology on clinical outcome, the endpoint should at least be childbirth. Data concerning neonatal outcomes for SERa+ embryos and even SERa− embryos from SERa+ cycles are scarce in the literature. In 5 publications, mainly abstracts, no neonatal

Figure 1  Human metaphase II oocytes as viewed by light microscopy. (A) A metaphase II oocyte containing an SERa (arrow) (400× magnification). (B) A metaphase II oocyte with a fluid-filled vacuole (arrow) (400× magnification).
Methodology of studies on SERa

For 2 studies, the frequency of SERa was provided per embryo (375/5516); (Braga et al., 2013) or per patient (53/510); (Munaswamy et al., 2008) instead of per cycle (Table I). For 10 studies, authors analyzed either differences between SERa+ and SERa− cycles (n = 3) or between SERa+ and SERa− oocytes in the same cycle (n = 2) or data from both (n = 5). Two studies were only included for their data provided on neonatal outcome (Akarsu et al., 2009; Miwa et al., 2013) and finally a case–control study reported on blastocyst quality and pregnancy outcomes per studied embryo (Braga et al., 2013).

Table 1 Methodology employed and outcomes studied when evaluating smooth endoplasmic reticulum aggregate positive (SERa+) cycles in women.

<table>
<thead>
<tr>
<th>Authors, Year published</th>
<th>Study design</th>
<th>SERa+ cycles (n)</th>
<th>SERa+ cycles (%)</th>
<th>SERa+ oocytes (%)</th>
<th>FR</th>
<th>PR</th>
<th>IR</th>
<th>MR</th>
<th>EQ</th>
<th>PO</th>
</tr>
</thead>
<tbody>
<tr>
<td>Otsuki et al. (2004)</td>
<td>RS</td>
<td>18</td>
<td>9.4</td>
<td>34.4</td>
<td>y</td>
<td>y</td>
<td>y</td>
<td>y</td>
<td>y</td>
<td>y</td>
</tr>
<tr>
<td>De Los Santos et al. (2005)*</td>
<td>RS</td>
<td>107</td>
<td>9.2</td>
<td></td>
<td>y</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Maldonado et al. (2006)*</td>
<td>RRS¹</td>
<td>16</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
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</tr>
<tr>
<td>Yang et al. (2008)*</td>
<td>PS</td>
<td>71</td>
<td>2.8</td>
<td>19.0</td>
<td>y</td>
<td>y</td>
<td>y</td>
<td>y</td>
<td></td>
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<tr>
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<td>POS</td>
<td>30</td>
<td>6.2</td>
<td>25.0</td>
<td>y</td>
<td>y</td>
<td>y</td>
<td>y</td>
<td>y</td>
<td>y</td>
</tr>
<tr>
<td>Munaswamy et al. (2008)*</td>
<td>RS</td>
<td></td>
<td></td>
<td></td>
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<td></td>
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<tr>
<td>Akarsu et al. (2009)</td>
<td>CR</td>
<td>3</td>
<td></td>
<td></td>
<td>y</td>
<td></td>
<td></td>
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<tr>
<td>Sá et al. (2011)</td>
<td>RS</td>
<td>60</td>
<td>10.3</td>
<td>18.8</td>
<td>y</td>
<td>y</td>
<td>y</td>
<td>y</td>
<td>y</td>
<td>y</td>
</tr>
<tr>
<td>Bielanska and Leveille (2011)*</td>
<td>RS</td>
<td>121</td>
<td></td>
<td>19.3</td>
<td>y</td>
<td>y</td>
<td>y</td>
<td>y</td>
<td></td>
<td></td>
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<tr>
<td>Mateizel et al. (2013)</td>
<td>RS</td>
<td>394</td>
<td>5.4</td>
<td>17.6</td>
<td>y</td>
<td>y</td>
<td>y</td>
<td>y</td>
<td>y</td>
<td>y</td>
</tr>
<tr>
<td>Braga et al. (2013)</td>
<td>CCS</td>
<td></td>
<td></td>
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<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Miwa et al. (2013)*</td>
<td>RS</td>
<td>86</td>
<td>7.3</td>
<td></td>
<td>y</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hattori et al. (2013)*</td>
<td>RS</td>
<td>102</td>
<td>7.8</td>
<td>20.4</td>
<td>y</td>
<td>y</td>
<td></td>
<td></td>
<td></td>
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</tr>
</tbody>
</table>

RS, retrospective study; RRS, randomized retrospective study; PS, prospective study; POS, prospective observational study; CCS, case control study; CR, case report; FR, fertilization rate; PR, pregnancy rate; IR, implantation rate; MR, miscarriage rate and/or BCR biochemical rate; EQ, embryo quality; PO, perinatal outcome; % SERa+ cycles = n SERa+ cycles/n total cycles; % SERa+ oocytes = n SERa+ oocytes/n total oocytes in SERa+ cycle; y = yes, outcome studied.

*Abstracts. The authors claim that their study was an RR study, but we have no way to clarify this point.
For SER+ cycles, the first case described decreased embryo cleavage and blastocyst formation (Sá et al., 2011). The second case reported a reduced cycle efficiency (selection of embryos for transfer or cryopreservation) when embryos were cultured to Day 5 (Mateizel et al., 2013). Concerning SER+ oocytes, a reduced cleavage rate (Sá et al., 2011), blastocyst formation (Ebner et al., 2008; Sá et al., 2011), inner cell mass quality (Braga et al., 2013) and blastocyst expansion (Braga et al., 2013) were observed.

Perinatal outcomes, for embryos, originating from SER+ cycles
A total of 171 healthy babies were born from SER+ cycles and perinatal complications occurred in 16 pregnancies (Table III). These complications included 1 stillborn, 2 neonatal deaths and 13 malformations, which led to 4 pregnancy terminations and 1 neonatal death (Table III). Altogether, 22 healthy babies were born specifically from SER+ embryos, including fresh and cryopreserved transfers and also twin births originating from mixed transfers. Malformations were observed in 3 pregnancies with SER+ embryos.

Three studies (Ebner et al., 2008; Sá et al., 2011; Mateizel et al., 2013) allowed us to calculate the malformation rate per live birth for SER+ cycles: a malformation rate of 5.6% (7/126) was obtained, compared with 2.2% (38/1762) for SER− cycles.

### Discussion

#### Origin of SERA and the role of calcium

The appearance of SERA may be due to ovary hyper-stimulation (Van Blerkom and Henry, 1992). Indeed, several studies have demonstrated that the duration of ovarian stimulation was found to be longer and the total amount of gonadotrophins administered higher for cycles with affected gametes (Otsuki et al., 2004; Ebner et al., 2008; Sá et al., 2011). Analysis of the occurrence of SERA in spontaneous cycles as well as determining the effect of a change of stimulation protocol on aggregate recurrence would help our understanding of the origin of this pathology (Ebner et al., 2013). In normal oocytes, SER vesicles, small SERA and associated mitochondria act as Ca$^{2+}$ stores (Sá et al., 2011; Sathananthan, 2013). The fundamental role of Ca$^{2+}$ in fertilization and early embryo development has been demonstrated by numerous studies (Homa et al., 1993; Carroll et al., 1996). The presence of SERA disturbs Ca$^{2+}$ stores and oscillations (Van Blerkom, 2011). When normal metaphase II oocytes and SERA+ oocytes were loaded with a fluorescent Ca$^{2+}$ reporter and activated with the Ca$^{2+}$ ionophore A23187, instead of a normal transient and uniform signal, free Ca$^{2+}$ increases occurred in distinct regions for longer durations in affected oocytes (Van Blerkom, 2011). Otsuki et al. (2004) showed that SERA disappear 16–20 h after ICSI. Indeed, when fertilization of an affected egg occurs, the calcium release would be accompanied by the disappearance of the aggregates. On the other hand, several authors describe the presence of SERA in unfertilized eggs after conventional IVF (Van Blerkom and Henry, 1992; Meriano et al., 2001). In this case, the absence of a Ca$^{2+}$ release might explain the unfertilized status of the egg and the continued presence of the SERA. Although the rescue of unfertilized eggs containing SERA with Ca$^{2+}$ ionophores might be considered, extreme caution should be taken, since calcium patterns may be far from normal.

A study in rabbits, where the first calcium rise was increased experimentally did not appear to affect preimplantation embryo development. However, during the fetal stages, major developmental and differentiation defects in organogenesis were observed (Ozil and Huneau, 2001).

### Role of SERA in adverse clinical outcomes

Even though there are conflicting results in the literature, the data show that SERA do affect clinical and embryological outcomes to a certain extent. Variation in population, fertility etiology, stimulation protocols and study design and size can probably explain part of these discrepancies. In terms of perinatal complications, a total of 16 incidents out of 187 pregnancies were found in the literature when studying SERA. One must keep in mind though, that the majority of transfers included in these studies concern SERA− embryos originating from SERA+ cycles. The largest study by Mateizel et al. (2013) described a major malformation rate per live birth for SERA− cycles of 2.1%. If we include pregnancy terminations and stillborns, this rate is increased to 4.2%
Table III  Perinatal outcomes for babies born from fresh or cryopreserved SERa+ cycles.

<table>
<thead>
<tr>
<th>Authors, year published</th>
<th>Type of embryo transfer</th>
<th>Perinatal outcomes</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>HB</td>
</tr>
<tr>
<td>Akarsu et al. (2009)</td>
<td>SERa+</td>
<td>2</td>
</tr>
<tr>
<td>Sä et al. (2011)</td>
<td>SERa+</td>
<td>1</td>
</tr>
<tr>
<td>Bielanska and Leveille (2011)</td>
<td>SERa+</td>
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</tr>
<tr>
<td>Mateizel et al. (2013)</td>
<td>SERa+</td>
<td>10</td>
</tr>
<tr>
<td>Hattori et al. (2013)</td>
<td>SERa+</td>
<td>4</td>
</tr>
<tr>
<td>Otsuki et al. (2004)</td>
<td>SERa−</td>
<td>1</td>
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<td>Ebner et al. (2008)</td>
<td>SERa−</td>
<td>3</td>
</tr>
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<td>Bielanska and Leveille (2011)</td>
<td>SERa−</td>
<td>16</td>
</tr>
<tr>
<td>Sä et al. (2011)</td>
<td>SERa−</td>
<td>23</td>
</tr>
<tr>
<td>Miwa et al. (2013)</td>
<td>SERa−</td>
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<tr>
<td>Mateizel et al. (2013)</td>
<td>SERa−</td>
<td>59</td>
</tr>
<tr>
<td>Ebner et al. (2008)</td>
<td>Mix</td>
<td>2</td>
</tr>
<tr>
<td>Sä et al. (2011)</td>
<td>Mix</td>
<td>5†</td>
</tr>
<tr>
<td>Bielanska and Leveille (2011)</td>
<td>Mix</td>
<td>13³</td>
</tr>
<tr>
<td>Mateizel et al. (2013)</td>
<td>Mix</td>
<td>23²</td>
</tr>
<tr>
<td>Total</td>
<td></td>
<td>171</td>
</tr>
</tbody>
</table>

HB, healthy babies; PC, perinatal complications; Mix, mixed transfer of SERa+ and SERa− embryos; PT, pregnancy termination.

*†²² Twin pregnancies for mixed transfers are specified. *2 twin pregnancies; *3 twin pregnancies.
*²²² Included the following: 1 47 XY+21, 1 duplicated kidney; 2 brachialis paresis, 1 hypospadias.

(63/1490) compared with 8.6% for SERa+ cycles reported in this review. Amongst the variety of pathologies detected, one case of a Beckwith–Wiedemann syndrome was found. An increased incidence of imprinting diseases may be correlated with the use of assisted reproduction techniques (Manipalviratn et al., 2009). Whether there is an association between SERa and imprinting disorders is unknown. Akarsu et al. (2009) published a case report where multiple fetal malformations were found for two pregnancies where the patient systematically had all oocytes affected in 3 consecutive cycles. In a second case report (Wallbutton and Kasraie, 2010), a patient had fertilization failure by conventional IVF and practically all of her 15 oocytes displayed large SERa. In two subsequent ICSI cycles, again most oocytes were affected and poor fertilization, embryonic arrest and no pregnancies were reported. In this study, polscope analysis did not detect a displacement of the meiotic spindle and the authors concluded that a genetic element would help to explain the relative homogeneity of SERa formation within successive oocyte cohorts. These observations raise the question of whether a threshold exists between the frequency and/or size of the aggregates for which the risk of a negative outcome might exist (Ebner et al., 2013). On the other hand, malformations have been reported for transfers of non-affected embryos originating from SERa+ cycles (Otsuki et al., 2004; Ebner et al., 2008; Mateizel et al., 2013).

Frequency of SERa+ cycles/oocytes and ethical considerations

This review shows that up to 10% cycles can present SERa, and a range of 19–34% of oocytes per affected cycle are shown to be positive for aggregates by observation under light microscopy. These percentages are underestimates since oocytes that are fertilized by conventional IVF would generally not show the dysmorphism at the time of fertilization assessment (Van Blerkom and Henry, 1992; Meriano et al., 2001; Otsuki et al., 2004). In addition, more subtle re-organization of the SER might go unnoticed by light microscopy (Otsuki et al., 2004). Cases other than those described above (Akarsu et al., 2009; Wallbutton and Kasraie, 2010) where a whole cohort of oocytes is affected by this dysmorphism have been reported. Indeed, Maldonado et al. (2006) published 16 cycles where patients had mixed ICSI/IVF cycles. All oocytes were affected in the ICSI cycles, but the status regarding SERa of oocytes treated by conventional IVF could not be assessed before Day 1. The chances that all oocytes were affected by SERa are high. Mateizel et al. (2013) reported a healthy birth from one patient in whom all 3 oocytes were affected. Finally, our own unpublished data revealed that over a period of 2 years, 24 cycles occurred with all oocytes affected. The majority of patients had few oocytes, though 7 women had over 3. Additionally, 11 transfers took place with affected embryos since no other embryo of equivalent quality was available (Shaw-Jackson et al., unpublished data). Eight healthy births and 2 ongoing pregnancies occurred from these affected embryo transfers. Taking into account the fact that the presence of SERa+ oocytes has been shown to be recurrent in just under 40% of subsequent cycles (Ebner et al., 2006; Sä et al., 2011), if the ESHRE and Alpha guidelines were to be followed, some couples would have to consider oocyte donation or adoption to achieve a family. Although, these patients represent a minority of those undergoing treatment, each couple should be treated as unique and offered a fair chance of conceiving with their own gametes. This is especially relevant in countries where the
number of fertilized oocytes is restricted. Moreover, supernumerary affected embryos of good quality in larger oocyte cohorts, capable of possibly developing into healthy babies could be cryopreserved and transferred in frozen cycles.

Oocyte dysmorphisms affect an important proportion of oocytes. The majority of studies in the past has analyzed pooled data for different dysmorphisms and has not allowed determining which ones are relevant in embryo selection (Rienzi et al., 2011). There is clearly a need to clarify the issue concerning clinical outcomes for SERa+ embryos in order, on the one hand, to reassure the couples involved and on the other hand, to avoid oocyte or embryo wastage.

**Conclusion**

Data in the literature concerning clinical outcomes for SERa as a unique feature are sparse and controversial. It remains unclear whether there is truly an association between SERa and fetal malformations. It is possible that only scattered alarming results are published and that they might not be related to SE-Ra, thus resulting in a biased conclusion. Alternatively, an under-reporting of complications cannot be excluded.

Laboratories should record information concerning SERa, and report the clinical outcome or destroy affected gametes. Data should be published with details on frequency and size of aggregates, and a follow-up of babies carried out, in order to determine whether SERa transfers are associated with adverse outcomes. As recently suggested in a letter to the editor (Ebner et al., 2013), the birth of apparently healthy babies from affected gametes might lead to a revision of the current consensus in the future. In the meanwhile, if transfers of affected embryos are performed, they should take place with caution and only when no alternative embryos of sufficient quality are available.

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**Authors’ roles**

C.S.-J. did the literature search, data analysis and wrote the manuscript. A.-L.T. and N.V.B. helped with the literature search, C.A. and S.R. were involved in the data analysis. All authors contributed to the scientific discussions.

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**Conflict of interest**

None declared.

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