ORIGINAL RESEARCH

The Relationship Between Smooth Endoplasmic Reticulum Clusters in Metaphase II Oocytes and Embryological and Birth Outcomes in Infertile Couples

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Background: To assess the relationship between oocytes with smooth endoplasmic reticulum cluster (SERc) and embryological and birth outcomes in infertile couples.

Methods: This was a descriptive study that included 231 infertile patients undergoing in vitro fertilization (IVF) with a total of 2447 mature oocytes (MII), of which 279 oocytes with SERc(+) from 100 patients, the remaining 2168 oocytes with SERc(-). Oocytes were evaluated for the presence or absence of the SERc simultaneously with intracytoplasmic sperm injection at 200x magnification using inverted microscopy – Observe D1.

Results: The mean age of patients was 32.05 ± 5.56 years. One hundred patients had at least one SERc(+) oocyte (with 279 SERc(+) and 956 SERc(-) oocytes). One hundred and thirty-one patients had 1212 SERc(-) oocytes. Fertilization outcomes and the rates of good-quality embryos on day 2 and day 5 did not differ between the SERc(+) and the SERc(-) groups. In the first frozen embryo transfer cycles, the clinical pregnancy rate in the group of patients with SERc(+) was not different with the SERc(-) group (61.1% vs 48.78%, $p = 0.074$, respectively). The live birth rate in the SERc(+) group was statistically significantly higher than the SERc(-) group (57.7% vs 43.9%, $p = 0.045$, respectively).

Conclusion: The fertilization rate, the quality of embryos on days 2 and 5 from oocytes with SERc(+) are similar to those with SERc(-). The live birth rate in the patients with SERc(+) group is statistically significantly higher than the SERc(-) group. There is no difference in clinical pregnancy rate between patients with and without SERc. Therefore, the exclusion of oocytes with SERc should not be recommended.

Keywords: Smooth reticulum endoplasmic cluster, SERc, Intracytoplasmic sperm injection, In vitro fertilization, IVF

Introduction

In vitro fertilization (IVF) is the only method by which oocytes and embryos can be directly observed under a bright-field microscope.[1] The technique of intracytoplasmic sperm injection (ICSI) allows for the most comprehensive evaluation of oocyte quality, as it eliminates all cytoplasmic particles and rays. Assessing the

basic quality of oocytes provides embryologists with insights into nuclear maturity, particularly through the appearance of the first polar body during the ICSI process.[1]

Embryo selection is a key element for transfer in IVF. Indeed, the selection of high-quality embryos might increase pregnancy rates. Typically, based on their morphological characteristics on days 1, 2, 3, and 5 of development, embryos are selected for transfer.[2] Additionally, regarding the important role of oocytes in embryonic development, oocyte quality assessed at day 0 can also be considered for embryo selection.[3]

Previous studies have shown that oocyte quality could affect embryo quality and implantation.[4–8] The typical evaluation of the oocyte quality is based on external and internal cytoplasmic morphological characteristics, such as first polar body morphology, size and granularity of the perivitelline space, zona pellucida defects, shape abnormalities, and the presence of refractile bodies, vacuoles, dense cellular granulation, and smooth endoplasmic reticulum.[7]

Information regarding oocyte morphology holds significant prognostic value, though there is a lack of consensus on the outcomes of various studies worldwide. Abnormal oocyte morphologies, including abnormalities in the nucleus and cytoplasm, have been associated with reduced fertilization rates, good embryo quality, and pregnancy rates, as reported in the literature.[9–12] Among the morphological abnormalities in oocyte cytoplasm, the presence of a smooth endoplasmic reticulum cluster (SERc) has garnered attention.

SER is an interconnected network of flattened membrane-enclosed vesicles or tubes. They can be distinguished from fluid-filled vacuoles by the fact that they are not filled with fluid and not separated by a membrane from the cytoplasm.[12] One of the most important roles of SER is to store and redistribute calcium in the oocyte, which is responsible for cell activation during fertilization.[3] A SERc is an intracytoplasmic dimorphism that has been suggested to interfere with calcium stores and oscillations during fertilization. It may have a negative impact on embryo implantation and development.[13]

Clinically, oocytes containing SERc have been linked to abnormal live births, including Beckwith-Wiedemann syndrome and congenital abnormalities in the brain, ears, and kidneys. However, the direct mechanisms underlying these conditions remain unknown.[9,14,15] During the process of embryo division, some types of abnormal divisions can be observed through time-lapse techniques, such as direct division and reverse division. These embryos tend to have lower pregnancy success rates. IVF cycles with SERc-containing oocytes have a reverse division rate more than twice that of cycles without SERc oocytes. In addition, errors in the meiotic division and failure to extrude the 2nd polar body are also 5 times higher in oocytes with SERc than in those without SERc.[16]

According to the recommendations of the European Society of Human Reproduction and Embryology (ESHRE), there is a perceived risk associated with oocytes containing SERc, and they advise against fertilizing these oocytes.[17] This partly explains the limited research on SERc-containing oocytes in Europe. However, previous studies reported that the impact of SERc on reproductive outcomes is not entirely clear.[13,18,19] Indeed, the blastocyst formation rates between two patient groups with and without SERc during ovarian stimulation were similar. Moreover, the rate of single, full-term pregnancies with live, healthy babies is also relatively high in pregnancies by IVF using SERc-containing oocytes.[19]

In Vietnam, the infertility rate is increasing,[20] leading to a growing demand for IVF. However, the evaluation of reproductive outcomes of oocytes containing SERc remains limited. Therefore, we conducted a study to assess whether oocytes with SERc affect embryo development and IVF outcomes.

Subjects and Methods
Study Population

The study included 231 infertile female patients from 18 to 45 years old undergoing IVF with a total of 2447 mature oocytes (MII) from January 2020 to December 2023. Among these, 279 MII oocytes had SERc. Patients were divided into two groups: 131 patients with oocytes without SERc and 100 patients with oocytes containing SERc.

Exclusion criteria included the use of frozen sperm, sperm retrieved through surgery, oligoasthenoteratozoospermia according to the World Health Organization 2021 criteria,[21] thin endometrial thickness, and uterine abnormalities. Therefore, the cause of infertility identified in this study was due to female factors, including polycystic ovary syndrome (PCOS), menstrual disorders, blocked fallopian tubes, primary ovary insufficiency, and even idiopathic causes.

Study Design

The study employed an analysis prospective research method, and oocytes and embryos were described according to the Alpha consensus – Istanbul, Turkey.[22] The research procedures were approved by the hospital's Ethics Committee (No. 865/2016/QĐ-BVNH).

$$\text{Sample size calculation}: n = Z^2_{1-\frac{\alpha}{2}} \frac{p(1-p)}{\Delta^2}$$

With $Z_{1-\alpha/2} = 1.95$ for 95% confidence level, p = 0.851 was the fertilization rate according to Rienzi's study,[23] the range Δ is from 0.024 to 0.039, therefore, the minimum number of oocytes needed was 1892.3. The number of mature oocytes in our study was 2447.

Methods

Ovarian Stimulation Protocol

Patients underwent controlled ovarian stimulation with a GnRH antagonist protocol, starting with a combination of FSH at a dose ranging from 150 IU to 300 IU from day 2 of the menstrual cycle.[17] The Gonadotropin starting dose was determined based on age, ovarian function, and previous ovarian stimulation responses. GnRH antagonist subcutaneous injections began when the largest follicle reached 14 mm in diameter. Triggering of final oocyte maturation occurred when at least two largest follicles reached 17 mm in diameter, using 250 µg of hCG (Ovitrelle; Merck Serono) or 0.2 mg of Diphereline. Oocyte retrieval was performed 35–36 hours after trigger.

Sperm Preparation for ICSI

Sperm preparation included 2 steps. First, the density gradient method in the Sil-Select plus medium (FertiPro, Belgium) was used, then the swim-up method in G-IVF medium (Vitrolife, Sweden) was used to select motile sperm for ICSI.

Assessment of Oocyte Morphology, ICSI Implementation, and Embryo Culture

Oocytes were retrieved using GMOPs medium (Vitrolife, Sweden) and incubated for 2 hours in G-IVF medium (37°C, 6% CO_2, and 5% O_2) (Vitrolife, Sweden). Subsequently, the cumulus-oocyte complexes were denuded using the HYAS medium (Vitrolife, Sweden). The ICSI procedure was performed after a 2-hour denudation using an ICSI medium (Vitrolife, Denmark) covered with OVOIL (Vitrolife, Sweden). The medium system utilized during ICSI was the G-Series (Vitrolife, Sweden). Oocytes were evaluated for the presence or absence of the SERc simultaneously with ICSI at 200x magnification using inverted microscopy – Observe D1 (Carl Zeiss).

Following ICSI, each oocyte was placed in a droplet of 20 µL to 30 µL of LP 50 medium, covered with LightOil (Life Global, USA), and cultured in a Benchtop mini-incubator – BT37 from Origio, using a gas mixture of 6% CO2, 5% O2, and 37°C. Fertilization assessment was performed 16 to 18 hours after ICSI, and embryo evaluation on day two, blastocyst formation, and blastocyst quality on day five were based on the Istanbul 2011 consensus criteria.[22] Cryopreservation of usable blastocysts was performed using the vitrification technique (Kit Kitazato).

Embryo Transfer and Pregnancy Outcome Measurements

The thawing protocol was performed using Kitazato VT602 thawing media (Kitazato, Japan). After thawing, 1 to 2 blastocysts were transferred into the patient's uterus under abdominal ultrasound guidance. Clinical pregnancy was determined by the detection of a gestational sac via transvaginal ultrasound. Live birth was the complete expulsion or extraction from a woman of a product of fertilization after 22 completed weeks of gestational age, which, after such separation, breathed or showed any other evidence of life.

Statistical Analysis

Statistical analysis was performed using Microsoft Excel and SPSS 21.0. Numerical data were described as mean and standard deviation, while categorical data were described by frequency and percentage. Chi-square test was used for comparing proportions, and one-tailed *t*-test was used for comparing mean values. A p-value <0.05 in comparisons was considered statistically significant.

Results

A total of 231 patients undergoing controlled ovarian stimulation using GnRH antagonists were included, the mean age of patients was 32.05 ± 5.56 years. The average LH concentration in the patients with SERc(+) group was statistically significantly higher than the patients with SERc(-) group (p = 0.032). There were no statistically significant differences between the 2 groups in terms of age, FSH, AMH, estradiol, progesterone concentrations and the number of AFC (p > 0.05) (Table 1).

A total of 100 patients had at least one SERc(+) oocyte (with 279 SERc(+) and 956 SERc(-) oocytes). One hundred and thirty-one patients had 1212 SERc(-) oocytes. The overall fertilization rate of the 2447 MII oocytes was 76.7% (Figure 1).

MII SERc(+) oocytes had a fertilization rate of 73.83%, which did not differ significantly from MII SERc(-) oocytes in patients with SERc(+) (78.97%) and patients with SERc(-) (75.58%) (p > 0.05). In addition, the quality of day 2 embryos, blastocyst formation rate on day 5, and good blastocyst quality among the three groups were not significantly different (p > 0.05) (Table 2).

During the study period, there were 90 first frozen embryo transfer cycles from the patient group with SERc(+) and 123 cycles from the group with SERc(-). In the first frozen embryo transfer cycles, the clinical pregnancy rate in the group of patients with SERc(+) was not different with the SERc(-) group (61.1% vs 48.78%, p = 0.074, respectively). However, the live birth rate in the SERc(+) group of patients was statistically significantly higher than the SERc(-) group (57.7% vs 43.9%, p = 0.045, respectively) (Table 3).

Table 1 Characteristics of Patients with or Without SERc

Characteristics	All patients (n = 231)	Patients with SERc(+) (n = 100)	Patients with SERc(-) (n = 131)	*P-value*
Age (years) (Min - Max)	32.05 ± 5.56 (19–44)	31.5 ± 5.35 (19–44)	32.47 ± 5.70 (19–44)	0.188
FSH (IU/L) (Min - Max)	6.57 ± 1.99 (1.28–16)	6.53 ± 1.93 (2.58–16)	6.59 ± 2.04 (1.28–14.40)	0.805
LH (IU/L) (Min - Max)	5.56 ± 2.37 (0.62–16.58)	5.93 ± 2.77 (1.5–16.58)	5.26 ± 1.97 (0.62–13.60)	0.032
AMH (ng/mL) (Min - Max)	3.52 ± 2.42 (0.22–13.21)	3.78 ± 2.74 (0.22–13.21)	3.32 ± 2.13 (0.31–10.92)	0.161
Estradiol (pg/mL) (Min - Max)	40.59 ± 22.03 (14.46 −160)	40.96 ± 23.34 (14.46 −160)	40.31 ± 21.07 (14.16–153.70)	0.824
Progesterone (ng/mL) (Min - Max)	1.05 ± 0.53 (0.10–4.15)	1.09 ± 0.46 (0.27–2.18)	1.01 ± 0.57 (0.10–4.15)	0.231
AFC (follicle) (Min - Max)	11.89 ± 6.80 (2–40)	11.17 ± 6.51 (3–40)	11.16 ± 7.00 (2–40)	0.067

Abbreviations: SERc, smooth endoplasmic reticulum cluster; FSH, Follicle-stimulating hormone; LH, Luteinizing hormone; AMH, Anti-Mullerian hormone; AFC, antral follicle count.
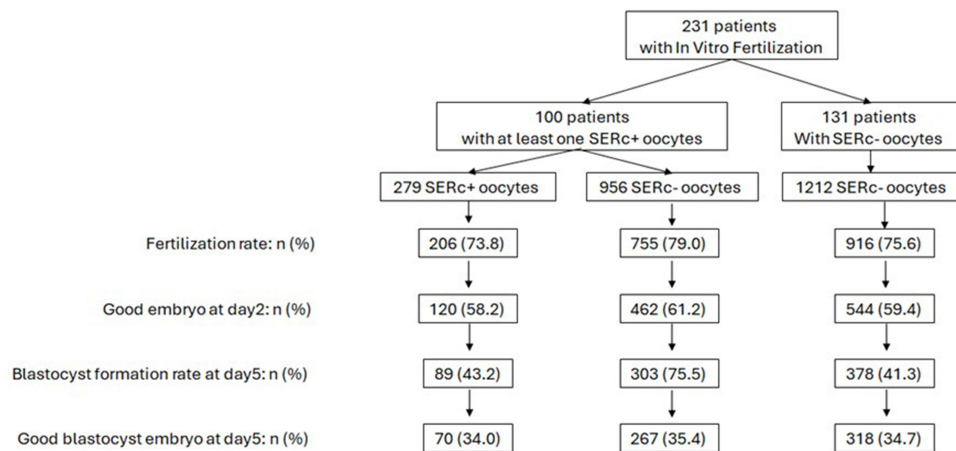
Figure 1 Flow-chart of study.

Discussion

Our results show that the incidence rate of SERc(+) in MII oocytes is 11.4%. Embryo development outcomes, including fertilization rate, rate of good-quality embryos at day 2, blastocyst formation rate at day 5, and rate of good-quality blastocysts, did not differ between oocytes with SERc and those without SERc among patients with SERc and oocytes from patients without SERc. In the first embryo transfer cycles, the clinical pregnancy rate did not differ between the SERc(+) group and the SERc(-) group. However, there was a significantly higher live birth rate in the SERc(+) group.

The endoplasmic reticulum is one of the most common organelles in the cytoplasm. It constitutes a system of tubules, serving an essential function in storing and distributing calcium ions, facilitating cellular activities during fertilization, and early embryo development stages.[24,25] The endoplasmic reticulum plays a role in material transport, and the appearance of clusters indicates the concentration of transport at a specific location within the oocyte. Therefore, smooth endoplasmic reticulum (SER) does not affect the genetic structure of the oocyte.[26] When sperm cells penetrate the oocyte, the activation of tyrosine kinase triggers phospholipase C, leading to the release of calcium ions.[27] Phospholipase C causes the hydrolysis of phosphatidylinositol 4.5-bisphosphate (PIP2) into inositol 1,4,5-triphosphate (IP3) and diacylglycerol (DAG). Another hypothesis suggests that phospholipase C isoform zeta (PLCζ) from the sperm cell

Table 2 Comparison of Embryological Outcomes Between SERC (+) and SERC (-) Oocytes Groups

Characteristics	SERc(+) oocytes[a]	SERc(-) oocytes from SERc(+) patients[b]	Oocytes from SER(-) patients[c]	*p-value*
Number of MII oocytes	279	956	1212	
Fertilization rate (%)	206/279 (73.83%)	755/956 (78.97%)	916/1212 (75.58%)	p_{a-b} = 0.069 p_{b-c} = 0.062 p_{a-c} = 0.543
Good quality day 2 embryo rate (%)	120/206 (58.25%)	462/755 (61.19%)	544/916 (59.38%)	p_{a-b} = 0.444 p_{b-c} = 0.454 p_{a-c} = 0.764
Blastocyst formation rate (%)	89/206 (43.20%)	303/755 (40.13%)	378/916 (41.26%)	p_{a-b} = 0.427 p_{b-c} = 0.639 p_{a-c} = 0.610
Good quality blastocyst rate (%)	70/206 (33.98%)	267/755 (35.36%)	318/916 (34.71%)	p_{a-b} = 0.712 p_{b-c} = 0.782 p_{a-c} = 0.841

Abbreviations: SERc, smooth endoplasmic reticulum cluster; MII oocyte, metaphase II oocyte.

Table 3 Comparison of Embryological and Neonatal Outcomes in the SERC(+) and SER(-) Cycle Groups

Characteristics	Patients with SERc(+) (n = 100)	Patients with SERc(-) (n = 131)	p-value
Number of MII oocytes	1235	1212	
Fertilization rate (%)	961/1235 (77.81%)	916/1212 (75.58%)	0.20
Good quality day 2 embryo rate (%)	588/961 (61.18%)	544/916 (59.38%)	0.426
Blastocyst formation rate (%)	386/961 (40.16%)	378/916 (41.26%)	0.628
Good quality blastocyst rate (%)	340/961 (35.37%)	318/916 (34.71%)	0.763
Clinical pregnancy rate (%)	90 first frozen embryo transfer cycles	123 first frozen embryo transfer cycles	
	55/90 (61.10%)	60/123 (48.78%)	0.074
Live birth rate (%)	52/90 (57.70%)	54/123 (43.90%)	0.045

Abbreviations: SERc, smooth endoplasmic reticulum cluster; MII oocyte, metaphase II oocyte.

enters the oocyte and cleaves PIP2. In either case, IP3 binds to receptors on the surface of the endoplasmic reticulum, resulting in calcium release.[28] The phenomenon of smooth endoplasmic reticulum clustering in MII oocytes varies significantly, ranging from 4% to 23%.[29] Using time-lapse imaging in the IVF and ICSI cycles, the disappearance of smooth endoplasmic reticulum clusters occurs a few hours after the appearance of the second polar body in the perinuclear area and disappears entirely before the formation of pronuclei.[30] In our study, the incidence rate of SER clusters in M2 oocytes falls within the range reported in previous studies, at 11.4%.

The appearance of SERc is a sign of prolonged maturation of the cumulus-oocyte complex before the LH surge in controlled ovarian stimulation cycles. Generally, the ideal size of the follicle before ovulation is between 18 and 20 mm. However, the negative impact on oocyte quality when follicle size exceeds 21 mm is still not clear. Large follicles, along with increased progesterone and estradiol levels, are associated with the presence of SERc in ovarian stimulation cycles.[31,32] Previous studies[9,10] have shown that high estradiol levels in individual follicles predict the appearance of smooth endoplasmic reticulum clustering upon triggering ovulation.

Regarding fertilization and embryo development outcomes showed that MII SERc(+) oocytes had a fertilization rate of 73.83%, which did not differ significantly from MII SERc(-) oocytes in patients with SERc(+) and patients with SERc(-). The quality of day 2 embryos, blastocyst formation rate on day 5, and good blastocyst quality among the three groups were not significantly different (p > 0.05). Gurunath et al conducted research on 951 GnRH antagonist-controlled ovarian stimulation cycles, comparing cycles with and without SERc. The results showed similar rates of fertilization, embryo cleavage, blastocyst formation, clinical pregnancy rate, and live birth rate.[33] Other studies have also reported similar findings.[34,35] However, there are also some publications with results contrary to ours. Sá et al found significantly decreased fertilization rate, embryo cleavage rate, and blastocyst formation rate in cycles with SERc compared to cycles without SERc.[36] Mateizel et al also observed a significantly lower blastocyst formation rate in SERc(+) cycles compared to SERc(-) cycles.[13]

With a large number of potential embryos formed from oocytes with SERc, completely excluding these embryos would pose significant disadvantages for patients if following the recommendations of ESHRE. The success of embryo transfer beyond the quality of the endometrium relies on the normal embryo quality regarding chromosomal abnormalities. Chi Chiu et al analyzed 30 blastocysts originating from oocytes with SERc using the array comparative genomic

hybridization (aCGH) technique and found that 9 out of 30 (30%) euploid embryos were not significantly different from 31 out of 84 (36.9%) embryos originating from oocytes without SERc (p > 0.05).[19] Previous reports also did not observe differences in the rate of euploid embryos between these two groups.[31,37] The systematic mini-review by Shaw-Jackson et al reported that out of 183 newborns born from SERc(+) cycles, 171 were healthy, 8 were born with congenital anomalies, 3 neonates died, 1 was stillborn, and additionally, there were 4 cases of terminated pregnancies.[38]

In our study, regarding the outcomes of frozen embryo transfer cycles, the group of patients with SERc(+) did not exhibit a significantly different clinical pregnancy rate compared to the group with SERc(-). However, there was a significantly higher live birth rate, which contrasts with some publications reporting poorer pregnancy outcomes when SERc is present. Wang Xue et al conducted a study on 2097 ICSI cycles,[39] showing no difference in clinical pregnancy and live birth rates between the groups with and without SERc. However, the fertilization rate (73.9%), rate of good-quality blastocysts (26.7%), and blastocyst development rate on day 5 (44.4%) of oocytes with SERc (217 MII oocytes) were significantly lower (p < 0.05) than those without SERc (822 MII oocytes) with corresponding rates of (86.2%, 44.1%, and 63.4%), respectively. Both groups of oocytes originated from the same patients undergoing ovarian stimulation. Wang Xue et al used both GnRH agonist and antagonist protocols, with 59.3% of cycles using a mid-luteal GnRH agonist protocol.[39] Our ovarian stimulation protocol exclusively utilized GnRH antagonist, with recombinant FSH injections starting from cycle day 2. Hattori et al[34] also drove to the same conclusion as Wang Xue. Gurunath et al categorized cycles based on the proportion of M2 oocytes with SERc (<30%, 30–50%, >50%), and while fertilization and embryo cleavage rates were not different, the clinical pregnancy rate decreased inversely with the proportion of M2 oocytes with SERc, with live birth rates of 44.23%, 27.27%, and 0%, respectively, indicating no live births when over 50% of M2 oocytes had SERc.[32] In addition, the presence of oocytes with SERc reduced the pregnancy rate after embryo transfer.[3] The results in our study differ from previous publications and could be explained by the inability to determine whether transferred embryos originated from oocytes with or without SERc. Therefore, we can only compare the first frozen embryo transfer cycles between the two groups of patients with and without SERc.

One of the strengths of our study is its prospective design, focusing on evaluating live birth rates, which is the ultimate outcome of assisted reproductive treatment. However, the study has some limitations. Firstly, the sample size of the group with SERc-present is relatively small compared to the other group, which may affect the comparability of results. Secondly, our study lacked inclusion criteria for BMI and hormonal status for female partners in order to have a normal profile in ICSI program. Another limitation of this study is the lack of evaluation of preimplantation genetic testing (PGT) results, so it cannot ensure that all transferred embryos were euploid. Therefore, a study with a larger sample size and the ability to classify the origin of embryos, as well as evaluate additional PGT results, is necessary in the future to obtain more objective results.

Conclusion

The fertilization rate and the quality of embryos on days 2 and 5 from oocytes with SERc are similar to those without SERc. Furthermore, there is no difference in clinical pregnancy and live birth rates between patients with and without SERc. Therefore, the exclusion of oocytes with SERc should not be recommended.

Abbreviations

IVF, In vitro fertilization; ICSI, intracytoplasmic sperm injection; SERc, smooth endoplasmic reticulum cluster; ESHRE, European Society of Human Reproduction and Embryology; FSH, Follicle stimulating hormone; LH, Luteinizing hormone; GnRH, Gonadotropin releasing hormone; hCG, human chorionic gonadotropin; AFC, antral follicle count; AMH, Anti-Mullerian hormone; aCGH, array comparative genomic hybridization; MII oocyte, metaphase II oocyte.

Data Sharing Statement

The data used to support the findings of this study are available from the corresponding author upon request.

Ethical Statements

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