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In patients with isthmocele undergoing IVF, the risk of ectopic pregnancy on the cesarean scar is reduced only if the ultrasound-guided transfer is performed on day 5 – A retrospective case-control study

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Abstract. – OBJECTIVE: The study aimed to demonstrate that the risk of Cesarean Scar Pregnancy (CSP) for patients with isthmocele decreases when the embryo transfer is performed on day 5 at the blastocyst stage.

PATIENTS AND METHODS: From January 2014 to December 2021, 167 patients who previously had an IVF treatment and delivered by cesarean section, were selected. The isthmocele was found in 98 of them. Firstly, we evaluated whether the isthmocele increases the risk of CSP. Subsequently, we investigated the possible correlation between the risk of the CSP with the day of the embryo transfer. Hence, the selected patients were divided into two groups: Group A where the embryo transfer was performed at the cleavage stage on day 3 and Group B where the embryo was transferred at the blastocyst stage on day 5.

RESULTS: The outcomes show that the isthmocele does not seem to increase the risk of CSP, while the embryo transfer on day 3 increases its rate.

CONCLUSIONS: When the isthmocele is diagnosed, according to our results, an embryo transfer on day 5 at the blastocyst stage seems to minimize the risk of the CSP.

Key Words:
Cesarean scar pregnancy, *In vitro* fertilization-embryo transfer, Transvaginal ultrasound, Embryo transfer.

Introduction

Reducing the rate of ectopic pregnancies is one of the main objectives of reproductive medicine, especially if the site of gestational chamber formation is near a previous scar formed by a cesarean section. Ectopic implantation on fibrotic tissue can generate profuse hemorrhage and even endanger the pregnant woman's life. Moreover, the possibility of a subsequent hysterectomy is high and would undermine any future possibility of pregnancy. The incidence of Cesarean Scar Pregnancy (CSP) is extremely low and has been estimated from 1:2216 to 1:1800[1-4]. Despite its low incidence, CSP can cause serious complications, such as uterine rupture associated with fetal death, even at early gestation[5,6]. The first cases of CSP were only reported in the late 1970s by Larsen and Solomon[7], but since then attention to this rare condition has been increasing.

Although several models have been proposed to explain the pathogenetic mechanism of CSP, its etiology remains unclear. According to some studies[8,9] the embryo, during its movement along the female reproductive tract, may enter the margins of a septum, placing itself on scar tissue. Predisposing risk factors for CSP are uterine trauma, cesarean section, myomectomies, manual removal of central placenta, adenomyosis and *in vitro* fertilization embryo transfer (IVF-ET)[10,11].

In recent years, the rate of cesarean sections has increased in many regions of the world[12], leading to an increase of CSP cases[13,14]. On the other hand, the growth in the number of IVF cycles has also increased the incidence of ectopic pregnancies by 1%, compared to natural conception, which varies between 1:50,000 and

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1:10,000[15,16]. In some cases, however, the cesarean scar causes a loss of wall substance known as an isthmocele. The isthmocele is a scarring condition caused by fibrotic tissue formed after a cesarean section. The sac-like formation develops in the area between the canal of the cervix and the isthmus, located near a previous scar[17]. There is still no standardized approach to assessing the anatomical features of the isthmocele. Usually, it is assessed by transvaginal ultrasound, and the prevalence among Cesarean Section (CS) patients is roughly estimated to be between 24 and 70%[18]. The increase in CS rates, and consequently the growth of isthmocele cases, is a major concern for associated complications. Scar tissue dehiscence and placenta accreta and percreta are among the main obstetric complications associated with the condition. In addition, isthmocele appears to worsen the course of scar pregnancies, as continued tissue erosion may facilitate placental invasion into nearby organs.

The increasing number of IVF treatments, ending in embryo transfer associated with increased rates of cesarean section pregnancies seem to raise the risk of ectopic scar pregnancies[19]. Recently, studies[20] have also shown that the risk of ectopic pregnancy in IVF may be correlated with the day of transfer. From a therapeutic point of view, in those cases, the most common practice has been the termination of pregnancy[21,22]. However, the risk of serious complications and morbidity of CSP are still at the center of scientific debate[5]. According to several recent reviews[23-25], a significant proportion of CSPs may progress to term or near term when patients are treated while waiting.

This may provide the opportunity to have a baby born, albeit with a high risk of placenta accreta and percreta and hemorrhage (although this hypothesis needs further verification in the future). Concerning the medically assisted procreation cycles, to date, no studies are comparing the risk of pregnancy on isthmocele about the day of transfer. Hence, the following study aimed to investigate the likely relationship between the CSP and the day of the transfer among patients with diagnosed isthmocele.

Patients and Methods

This study was conducted in a private setting (MOMO' FertiLIFE IVF Center, Bisceglie, Italy); it was designed as a retrospective case-control study. The study was regularly approved by the Local Ethical Committee of MOMO' FERTILIFE Institution. The declaration was made by our Internal Committee with protocol number 031/2014. This study had been performed by the ethical standards described in an appropriate version of the 1975 Declaration of Helsinki, as revised in 2000. All patients received and signed informed consent.

From January 2014 to December 2021, 4678 IVF cycles were performed, resulting in 1778 pregnancies. Of these 1,778 pregnancies, 167 were achieved in patients who had already undergone a cesarean section. All patients underwent a two- and three-dimensional ultrasound scan before IVF treatment. Isthmocele was detected in 102 patients but 4 of them were excluded from the study since they had not performed the transfer under ultrasound guidance (Figure 1).

Inclusion Criteria

- Patients with a previous cesarean section;
- Isthmocele;
- Echo-guided transfer.

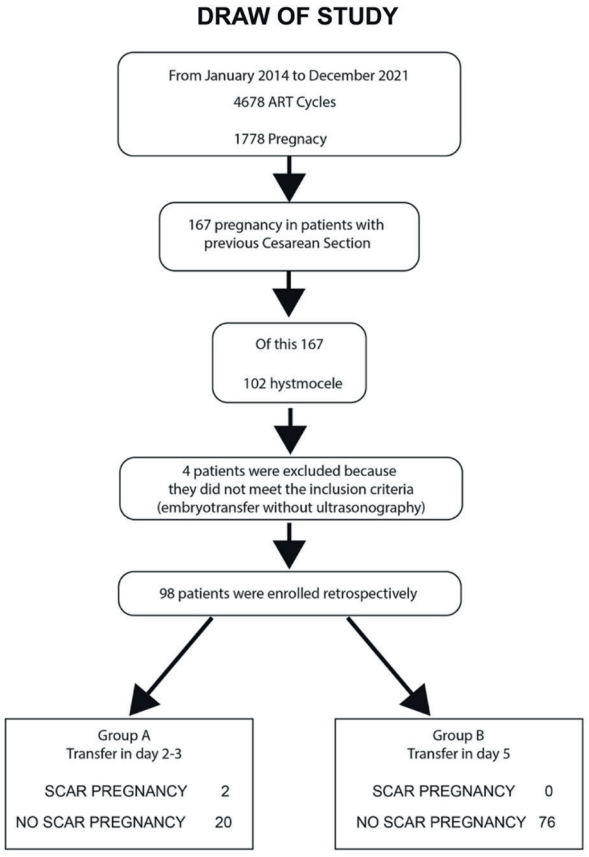

Figure 1. Study design.

Exclusion Criteria

- Presence of previous ectopic pregnancies;
- Congenital uterine malformations;
- Sepsis;
- Insertion of the intrauterine device.

Two groups were selected retrospectively: the first group (Group A) of 22 patients where the ultrasound-guided transfer was performed on day 3, and the second group (Group B) of 76 patients where the transfer was performed on day 5.

Statistical Analysis

The enrolled groups were subjected to statistical evaluation using the Student's *t*-test. Both groups did not show statistically significant differences confirming so the homogeneity of the latter. Subsequently, we wanted to evaluate whether the presence of isthmocele increases the risk of CSP in the population of IVF patients who have already undergone a cesarean section. Besides, we wanted to assess whether the presence of isthmocele increases the risk of CSP on the day of the transfer. Both statistical evaluations were carried out with the Fisher exact test applied on a 2x2 contingency table. Subsequently, to verify if the result was statistically significant, Exact Chi^2 was applied. This test was preferred over Pearson's Chi^2 test because the sample sizes were small (below 400 patients). The software used to perform the statistical evaluation was SSPS (IBM, Armonk, NY, USA).

Two- and Three-Dimensional Vaginal Ultrasound (TVUS) Before Treatment

All patients enrolled on this study underwent a two dimensional and three-dimensional transvaginal ultrasound before IVF treatment; the aim of the assessment was to identify the presence of isthmocele. The examination was always performed by the same doctor with a GE S8 ultrasound system (GE Co Ltd., Boston, MA, USA) equipped with a 4-9 MHz transvaginal probe. The diagnosis of isthmocele was confirmed when the examination revealed a hypoechoic area (filling defect) within the myometrium of the lower uterine segment at the site of a previous cesarean section. Concerning the assessment of isthmocele types, we used the classification published by Bij de Vaate et al[24,25]. The type of isthmocele has been classified as: triangle (Figure 2A), semicircle (Figure 2B), rectangle (Figure 2C), circle (Figure 2D), droplet (Figure 2E) and inclusion cyst (Figure 2F).

Residual myometrial thickness (RMT), isthmocele depth (DI), isthmocele width (WI), cervical thickness (CT), distance from fundus to isthmocele (DFUI) and distance from the isthmocele to the cervix (DCI), were measured in the sagittal plane of the isthmocele[26,27].

The RMT was defined as the shortest distance between the serous surface of the uterus and the endometrium at the level of the scar. During the execution of the TVUS, the measurements of position, length, width, endometrial thickness, and the presence of intrauterine fluid in the uterus

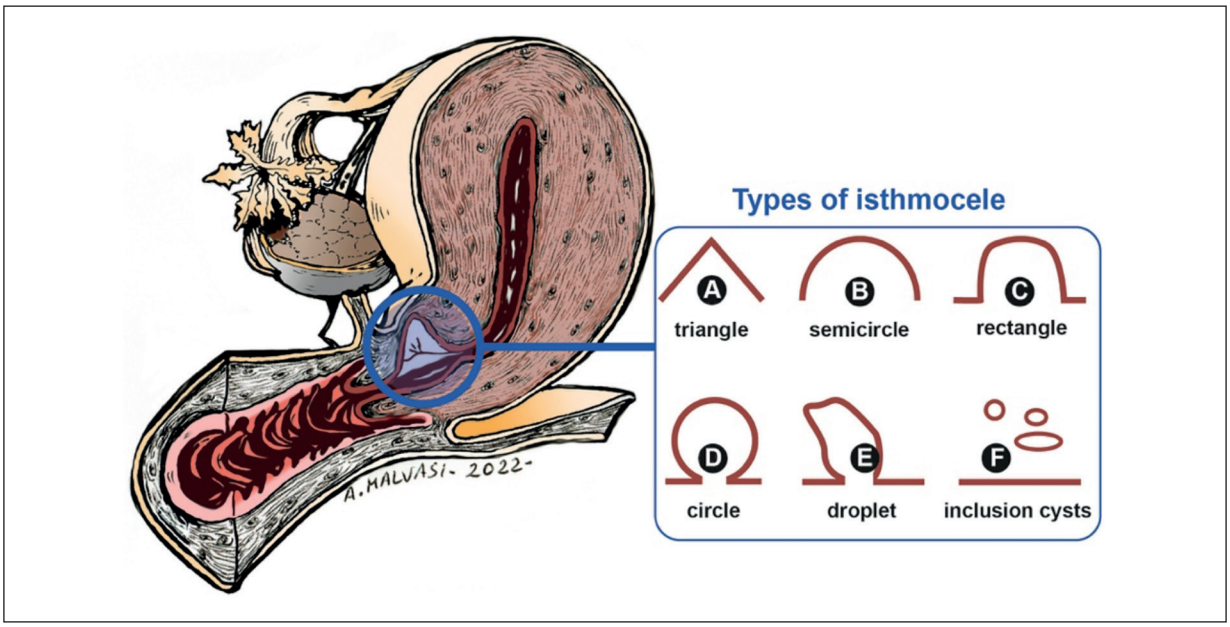

Figure 2. Schematic representation of isthmocele types.

were taken. A three-dimensional scan[28] was subsequently performed to make a reconstruction of the defect related to the isthmocele.

Stimulation Procedure

All patients were stimulated with recombinant follicle stimulating hormone (FSH) (GONAL-f, Merck Serono, Merck KGaA, Darmstadt, Germany) in a protocol with gonadotropin-releasing hormone (GnRH) antagonists (Cetrotide, Merck Serono, Merck KGaA, Darmstadt, Germany).

The administration of the antagonist from day 7 of stimulation and, eventually, final maturation was induced with a choriogonadotropin (Gonasi HP-IBSA, Lugano, Switzerland) when at least three follicles were larger than 18 mm. Patients included in the study with BMI > 30 were required to lose weight before starting stimulation[29].

Oocyte Collection Procedures

Patients underwent sedation for oocyte retrieval. Oocytes were collected by follicular puncture using a 17-gauge aspiration needle (COOK Medical, Bloomington, IN, USA) connected to a guide on the transvaginal ultrasound device (GE Voluson S8, GE Co Ltd., Boston, MA, USA). Cumulus-oocyte complexes were exposed to a hyaluronidase solution (25 IU/ml) to remove the corona radiata. The cleaned oocytes were inspected and evaluated under the stereomicroscope (Nikon SMZ 1500, Tokyo, Japan) to select those in metaphase II (MII). Semen was collected after 3 to 4 days of sexual abstinence and prepared according to the method used in our centre[30,31]. The eggs were incubated in LGGF medium (Global Fertilisation, Cooper Surgical, Trunbull, CT, USA) and injected 2 hours later after the egg collection.

Insemination Procedure

In both groups, the ICSI procedure was performed at 37°C under an inverted microscope (Nikon eclipse, TE 200, Tokyo, Japan) using a microinjection system with 400x magnification.

After insemination, fertilized oocytes were cultured in LGG medium (Global, Cooper Surgical, Trumbull, CT, USA) for up to 6 days. Patients having a high progesterone value on the triggering date were excluded from the study and therefore the embryos were frozen[32].

Transfer Procedure

On the third and fifth days of culture, fresh embryos were transferred as single embryo transfer (SET). The transfer was performed under ultrasound guidance (GE Voluson S8, GE Co Ltd., Boston, MA, USA) with the transabdominal probe. After 12 days, a beta hCG blood assay was performed to assess the presence or absence of pregnancy. When the beta hCG value reached 1500/ml two-dimensional ultrasound was performed to verify the position of the pregnancy (Figure 3).

Results

Firstly, we did not find a correlation (Chi^2 p = 0.5175; $p > 0.05$ not significant) between the presence of the isthmocele and an increased risk of CSP, compared with the CSP rates established on the classic cesarean scar (Table I). Subsequently, the sample adequacy of the groups used in the study was analyzed (Table II), and we did not find a significant Student's t-test between them. In fact, both groups were homogeneous. It is important to underline that in both groups the number of

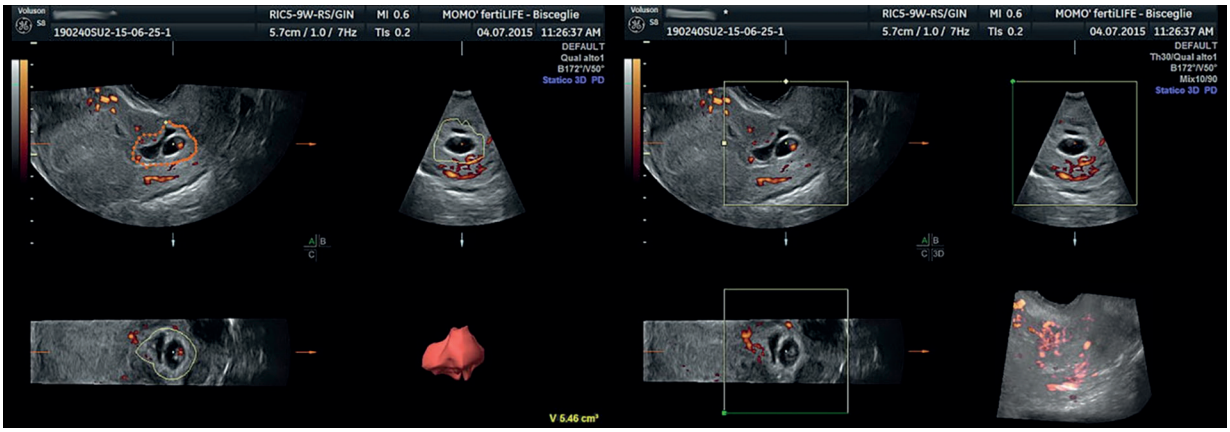

Figure 3. Two-dimensional and three-dimensional scans of the implantation area of the pregnancy.

Table I. This table shows that isthmocele does not increase the risk of CSP ($p > 0.05$).

	CSP	No CSP	Total
Transfer in patients with CT Without isthmocele	0	65	65
Transfer in patients with CT With isthmocele	2	96	98
Total	2	161	163

Fisher's test was applied and Chi^2 $p = 0.5175$ with $p > 0.05$ so not significant.

cesarean sections previously performed for every patient was only one and the type of suture was always the same (i.e., single layer).

Uterus and isthmocele characteristics were also evaluated (Table III), and even in this case, no statistically significant differences were found, hence, we can consider the samples homogeneous.

Furthermore, the number of embryos transferred, and the volume of media injected was identical in the two groups. Subsequently, we wanted to correlate the risk of CSP on isthmocele between transfer performed on day 3 and day 5, and the statistical result was significant (Chi^2 $p = 0.0486$; $p > 0.05$ not significant).

As a matter of fact, from the results obtained, it seems that day 3 (d3) embryo transfer increases the risk of ectopic pregnancy on the isthmocele (Table IV).

Discussion

Since the 1970s, with the first *in vitro* fertilization cycle, the transfer of an embryo into the uterine cavity has been associated with increased rates of extrauterine pregnancies, compared with those obtained naturally[33]. The mechanisms underlying these ectopic pregnancies remain uncertain, and strategies to reduce their incidence are still limited. The main actions proposed to reduce the risk of it are based on standardizing the procedure of embryo transfer.

For example, adjusting the depth of transfer prevents the catheter from touching the bottom of the uterine wall. Injecting a reduced volume of transfer medium prevents the embryo from dislodging in inappropriate sites for rooting.

Table II. This table shows that Group A and Group B are homogeneous.

Day of transfer patients	Group A D3 22	Group B D5 76	*p*-value
Age	36.6 ± 4.76	35.9 ± 4.82	0.749
BMI	27.00 ± 5.39	27.5 ± 5.22	0.901
Infertility duration (in months)	26.1 ± 5.2	25.9 ± 5.8	0.879
Infertility causes			
Tubal factor	1 (4.5%)	4 (5.2%)	-
Uterine factor	0	1 (1.3%)	
Endometriosis	5 (22.7%)	20 (26.3%)	
Male factor	7 (31.8 %)	25 (32.9%)	
Ovarian dysfunction	3 (13.6%)	9 (11.8%)	
DOR	4 (18.1%)	12 (15.7%)	
Other	0	1 (1.3%)	
Unexplained	2 (9.3%)	4 (5.2%)	
Time to interval from last CS (in months)	23.7 ± 3.08	22.9 ± 3.22	0.625
Previous TC	1	1	-
Type of suture			
Single layer	22	76	-
Double layer	0	0	
N° Embryos transferred	1	1	-
Endometrial thickness	8.15 ± 2.19	8.32 ± 2.36	0.791
Total gonadotrophin	1942.80 ± 411	1996.3 ± 438	0.921
E2 day trigger	1562 ± 503	1862 ± 487	0.849
Pregnancy	8 (36.3%)	30 (38.4%)	0.428
Insemination method	icsi	icsi	-

$p < 0.05$ was considered statically significant.

Table III. The size of the uterus and the characteristics of the isthmocele were evaluated in the two groups, in both groups there was no significant difference

Day of transfer patients	Group A D3 22	Group B D5 76	*p*-value
Uterine length (mm.)	78 ± 3.26	78 ± 3.08	0.949
Uterine width (mm.)	44.3 ± 2.78	45.1 ± 2.64	0.872
Isthmocele size (mm.)			
RMT	4.6 ± 2.70	4.7 ± 2.77	0.196
OF	3.9 ± 2.1	4.0 ± 1.94	0.883
WI	6.21 ± 1.65	6.02 ± 1.87	0.829
CT	9.44 ± 3.16	9.42 ± 3.21	0.785
DFUI	51.53 ± 7.57	51.67± 7.11	0.912
DCI	25.4 ± 6.65	25.3 ± 6.48	0.845
DFUI/DCI ratio	2.04 ± 0.56	2.07 ± 0.48	0.278
Easy of transfer	22	76	-
Catheter type	Cook	Cook	-
Volume of injected culture liquid	0.1 ml	0.1 ml	-

RMT=residual myometrial thickness; DI=Depth of isthmocele; WI=Width of isthmocele; CT=Cervical thickness; DFUI=Distance from the uterine fundus to isthmocele; DCI=Distance from the cervix to isthmocele. $p < 0.05$ was considered statically significant.

Abstaining from using cervical forceps during embryo transfer would allow a reduced genesis of uterine contractures[34-37]. Although these strategies are logical, (as they should avoid displacement of the embryo into the lower or middle endometrial cavity and minimize peristaltic waves in the uterus), it is also essential to consider the basic physiology of human reproduction and implantation.

The embryo in a natural conception enters the uterine cavity when it reaches the blastocyst stage[38]. Therefore, embryo transfer on day 5 has been proposed as a method to reduce the rates of extrauterine pregnancies[39]. Although current results have not demonstrated a statistically significant difference in extrauterine pregnancy risks when transferring embryos on day 5 compared to day 3, we cannot exclude the possibility of a clinically significant association between the risk of an extrauterine pregnancy and the day of embryo transfer[40]. The blastocyst has a larger diameter and remains inside the tubes for a limited time in natural cycles. These factors limit the chances of displacement and also ectopic implantation along with the tubal[41]. Several high-resolution three-dimensional ultrasound experiments have shown the presence of alterations in uterine contractility during the menstrual cycle[38,42]. The directional flow of uterine contractile waves after ovulation is directed from the cervix towards the uterine fundus. These peristalses progressively decrease in frequency and amplitude to a state of approximate quiescence by day 6 or 7 after triggering with HCG[35,43,44]. Therefore, according to these theories, embryo transfer on day 5 may reduce the probability of ectopic implantation compared to embryo transfer on day 3.

Ishihara et al[43] found a statistically significant reduction in ectopic pregnancies following blastocyst transfer. Other studies in the literature did not show statistically significant differences between embryo transfer on day 3 and day 5. However, the retrospective nature of almost all the papers on this subject provides inferior level of evidence compared to prospective studies.

As a matter of fact, other factors may influence the outcome, such as the use of ultrasound, the type of embryo transfer catheter and the volume of medium injected. In our study, all these data,

Table IV. Group A with transfers on the third day and Group B with transfers on the fifth day were evaluated. CSP risk appears statistically significant in group A ($p = 0.0486$).

	CSP	No CSP	Total
Group A - Echo-guided transfer in D3	2	20	22
Group B - Echo-guided transfer in D5	0	76	76
Total	2	96	98

The Fisher's exact test was applied and Chi^2 $p = 0.0486$ with $p < 0.05$ therefore significant.

even if retrospectively, were present. From a retrospective study published in 2020[45,46] performed on 22476 patients undergoing IVF, it emerged that the transfer on day 5 reduces the risk of ectopic pregnancies. It is important to underline that all the studies previously cited, refer to tubal pregnancy which is the most frequent and studied among ectopic pregnancies. In our investigation instead, we wanted to evaluate the risk of ectopic pregnancies on the isthmocele, which to date does not seem to be analyzed in other studies. The CSP rates are generally very low, therefore, the limitations of our study are linked to the retrospective analysis and the small number of cases, although it appeared that this was statistically significant. Another bias could be the dissymmetry between group A and group B in terms of numbers. In any case, it must be clear that ectopic pregnancy on a cesarean section scar is a very rare phenomenon. Our study shows that where there is an isthmocele and *in vitro* fertilization treatment, it is advisable to perform the transfer always under ultrasound guidance, on day 5 at the blastocyst stage. Indeed, by ultrasound guidance, pregnancy rates increase and despite the absence of statistically significant differences, there is a reduction in the number of ectopic pregnancies. Besides, the type of ultrasound (two and three dimensional) does not seem to affect the outcome[47,48]. Given the fact that the CSP is already recognized as a dangerous pregnancy, we decide to perform only SET on those patients enrolled in the study.

A multiple CSP would increase remarkably the health risk of the woman. Instead of following the classic choice to perform a therapeutic abortion[49-52], in one of the two cases of CSP, we decided to carry the pregnancy to term, and we were rewarded with the birth of a healthy and viable fetus. Immediately after birth, the patient has been undertaken to hysterectomy. Considering the rarity of the CSP, further studies with a bigger number of cases are needed to have stronger data from a statistical point of view. Therefore, in this case, a multicentric study is advisable. However, the presence of isthmocele must be considered a risk of CSP, hence, an embryo transfer on day 5 at the blastocyst stage seems to be mandatory.

Conclusions

Our work suggests that single embryo transfer on day 5 should be performed in patients with isthmocele. This decision may decrease the CSP risk. In any case, studies with more numerous samples will be necessary in order to have statistically more significant results.

Conflict of Interest
The Authors declare that they have no conflict of interests.

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Informed Consent
Each patient was given specific informed consent and signed.

Authors' Contribution
Giorgio Maria Baldini: research idea and study design, editing the text; Antonio Malvasi: editing of the text and iconographic illustration; Safak Hatirnaz: drafting the text; Ioannis Kosnas: data collection and statistical evaluation; Andrea Tinelli: Critical review; Domenico Baldini: Critical review and reference evaluation.

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