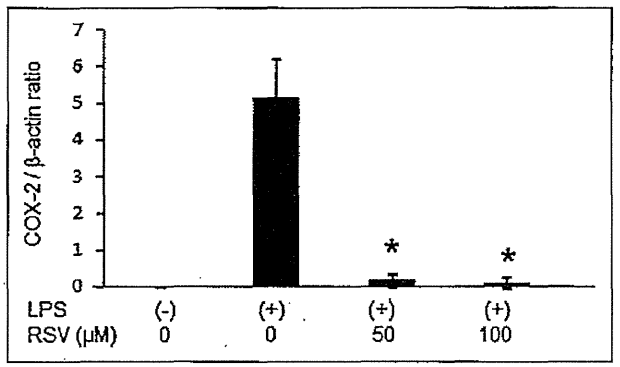

Figure 5. Eradication of COX-2 production in LPS-treated peritoneal macrophages by resveratrol exposure. Cultured peritoneal macrophages were incubated with resveratrol (final concentration: 50 or 100 μmol/L) for 12 hours. Resveratrol-treated or control macrophages were exposed to 100 nmol/L of LPS for 4 hours. COX-2 messenger RNA (mRNA) levels in macrophages were assessed using reverse transcription-quantitative polymerase chain reaction (RT-qPCR). Data are representative of 2 independent trials. *$P < .05$. COX-2 indicates cyclooxygenase 2; LPS, lipopolysaccharide.

eradication of LPS-induced COX-2 production in macrophages residing around the uterus.

A volume of 10 μg of LPS injection into the cervices caused preterm birth in 90% to 100% of pregnant mice. This LPS dose had been optimized for our preterm birth model in our previous study.[14] Resveratrol administration significantly reduced the rate of preterm birth rate by half. We investigated the rate of preterm birth using 2 doses of resveratrol: 20 and 40 mg/kg of body weight. More than 40 mg/kg of resveratrol was not able to be administered to mice because of the maternal toxicity of ethanol, which is requisite for solubilizing resveratrol. In this model, ethanol was administered equally to resveratrol-treated (as a solvent) and control (as a vehicle) mice. Approximately half of the fetuses did not have preterm birth only in resveratrol-treated mice. There was no difference in reduction rate of preterm birth between 20 and 40 mg/kg of resveratrol-treated mice. Considering ethanol toxicity, 20 mg/kg of resveratrol was thought to be the optimal dose for protection against preterm birth. Therefore, we chose this dose for the following examinations.

According to the report,[22] plasma concentration of resveratrol peaks 10 minutes after oral administration, and peak concentrations were 32 μmol/L in plasma when mice are administered 240 mg/kg of resveratrol. Although we didn't assess concentrations of resveratrol, the estimated concentration of resveratrol would be around 5 μmol/L. The concentration of resveratrol we used in vivo is lower than the concentration that resveratrol showed its anti-inflammatory effect on cultured peritoneal macrophages in vitro. We consider this discrepancy would be due to the complicated mechanism in vivo where cells like fibroblasts or other immunocytes work together with macrophages.

Proinflammatory cytokine levels were increased, with a peak of 4 hours after LPS injection. Preterm delivery occurred at 12 to 48 hours after LPS injection. The time lag between local inflammation and delivery can be explained by the fact that proinflammatory cytokine production accompanies COX-2 production, followed by uterine contraction and cervical ripening, and finally, preterm delivery. Therefore, transient supraphysiological inflammation around the uterus appeared to be critical for our LPS-induced preterm birth model. We found that resveratrol administration abolished LPS-induced local inflammation at 4 hours after LPS injection. This finding strongly suggested that anti-inflammatory effect of resveratrol contributed to a reduction in preterm birth in this model.

Inhibitory effects of resveratrol on NF-κB activity have been well reported in various types of disease models.[16-18] The NF-κB activity is observed not only in classical immune cells but also in the uterine myometrium, decidual stroma, and amniotic cells.[23] The NF-κB promotes expression of the proinflammatory cytokines TNF-α, IL-1β, IL-6, and IL-8, which play a central role in induction of term or preterm labor.[24-26] In turn, NF-κB is activated by various stimuli, including LPS and proinflammatory cytokines, such as TNF-α and IL-1β. These proinflammatory cytokines and the NF-κB pathway appear to create a positive feedback loop in the gestational tissues that are exposed to LPS. Resveratrol may inhibit the NF-κB/cytokine loop in this model.

The TNF-α, IL-1β, IL-6, IL-8, the IL-10 family, the IL-12 family, IL-15, and TGF-β are LPS-inducible cytokines that are produced by human monocytes and macrophages through innate immune pathway.[27] Among them, we focused on murine proinflammatory cytokines and examined LPS-induced productions of these cytokines. In peritoneal washes and cervical tissue, TNF-α, IL-1β, and IL-6 were significantly increased by LPS exposure to the cervices. In our in vivo model, resveratrol abolished LPS-induced elevation of TNF-α and IL-1β levels, whereas it slightly suppressed elevation of IL-6 levels. As mentioned previously, TNF-α and IL-1β are well-known proinflammatory cytokines that are secreted mainly by immune cells, such as macrophages, IL-6 is produced in various types of stromal cells, such as fibroblasts, decidual stromal cells, adipocytes, and the myometrium.[28-31] Furthermore, IL-6 is also induced by proinflammatory cytokines, such as TNF-α and IL-1β, as well as being directly induced by LPS stimulation.[32] Considering that LPS stimulation of toll-like receptors of macrophages is the first step of this preterm delivery model, and direct inhibition of this signal was represented by the suppression of TNF-α and IL-1β expression in peritoneal washes and the uterine cervix. Circulating IL-6 protein and mRNA levels are the result of various types of responses from various types of cells. Therefore, anti-inflammatory effects of resveratrol could not be shown clearly as suppression of IL-6 production.

Cyclooxygenase 2 is an inducible enzyme that is particularly responsive to inflammatory stimuli.[33] Cyclooxygenase 2 is upregulated in the amnion, choriodecidua, and myometrium.[34] Cyclooxygenase 2 produces PGE2 and PGF2α as potent inducers of spontaneous uterine contractility and cervical ripening.[35] Therefore, upregulation of COX-2 is associated with the pathological mechanism of preterm birth not only in

the mouse model but also in humans. A previous study reported that resveratrol suppresses LPS-induced COX-2 elevation in the gestational tissue[20] but the study did not focus on the macrophages. Macrophages are pivotal cells for production of COX-2 and consequent production of PGE2 and PGF2α. We have previously demonstrated that suppression of LPS-induced PGE2 and PGF2α elevation in gestational tissues is associated with suppression of macrophage function by omega-3 fatty acids.[14] Taken together, our data suggested that resveratrol acts mainly on macrophages residing around the uterus.

Recently, there are some reports that indicates the biophysical activity of resveratrol metabolites such as resveratrol-3-O-sulfate.[36] In this study, we didn't mention whether the anti-inflammatory effect is caused by resveratrol or resveratrol metabolites, therefore, further investigation is needed to clarify the precise mechanism of its anti-inflammatory effect.

In summary, resveratrol administration protects against LPS-induced preterm birth in a preterm mice model by suppression of local proinflammatory cytokines production. Our study suggests that the anti-inflammatory effect of resveratrol results in eradication of proinflammatory cytokine-mediated COX-2 elevation in macrophages residing around the uterus. The safety of resveratrol for pregnant women and the fetus remains to be determined, and further studies are needed for its administration to pregnant women. Resveratrol may be a potential therapeutic agent for preterm birth.

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Declaration of Conflicting Interests

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Supplemental Material

The online data supplements are available at http://rs.sagepub.com/supplemental.

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ASSISTED REPRODUCTION TECHNOLOGIES

Elective single-embryo transfer improves cumulative pregnancy outcome in young patients but not in women of advanced reproductive age

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Abstract
Purpose The purpose of the present study is to assess the significance of elective single-embryo transfer (eSET) in older women.
Methods The outcomes of assisted reproductive technology between 2001 and 2013 at single institution were retrospectively evaluated. Cumulative live birth rates (CLBRs) in one oocyte retrieval cycle were compared between those who underwent eSET and multiple embryo transfer (MET) in fresh cycles.
Results The outcomes of 429 eSET cycles and 965 MET cycles were compared. CLBRs in eSET were higher than those of MET in women under 37 and were comparable in women aged 37 and over. The analysis of the outcomes separately in three age subgroups showed a significantly higher CLBR in young eSET (aged under 37) than that in young MET and similar CLBR between older (aged 37–40 and over 40) eSET and MET. Multiple birth rates were lower in eSET in all age groups. Multivariate logistic regression analyses showed that, in women aged under 37, number of frozen embryos, presence of good-quality embryos, and eSET were significantly related to cumulative live birth. In women aged between 37 and 40, age and number of frozen embryos were significantly related, while eSET was not.
Conclusions eSET in women under 37 resulted in increased CLBR compared with MET. In women aged between 37 and 40, CLBR in eSET group was similar with that in MET group. In both age groups, eSET reduced multiple birth rates. The significance of eSET in older women is limited presently, and further research on the strategy to improve cumulative outcomes is necessary.

Keywords eSET · Age · Cumulative live birth rate · Multivariate logistic regression analyses

Capsule Elective SET in women aged 37 and over reduced multiple pregnancy rate but did not improve cumulative live birth rate.

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Introduction

Multiple pregnancy as a result of more than one embryo transfer is one of the most serious complications of assisted reproductive technology (ART), leading to increased maternal morbidity and mortality as well as perinatal complications [1]. In the last 15 years, in proportion as advancements in ART and increased implantation rate, reduce the number of transferred embryos has been of main concern for many of the clinicians involved in ART in the world [2, 3].

Elective single-embryo transfer (eSET) has been widely accepted as a strategy to reduce the risk of multiple birth especially in selected patients. Most of the previous reports regarding the efficacy of eSET have targeted younger patients with enough ovarian reserve, describing a significantly lower rate of multiple pregnancy than multiple embryo transfer cycles, as well as comparable cumulative pregnancy rates including subsequent frozen embryo transfer cycles [4, 5].

On the other hand, the significance of eSET in unselected or older patients is controversial. In a non-randomized study in women aged 36–39 years, clinical pregnancy rate and live birth rate in fresh transfer cycles were similar between eSET and double-embryo transfer (DET) groups [6]. Another

retrospective cohort study showed similar results in women aged 40–44 years [7]. In these two reports, women in eSET groups achieved significantly higher cumulative pregnancy and live birth rates including subsequent frozen-thawed embryo transfer cycles than those in DET groups. However, in a review from a Canadian study group, in women aged 38 years and over, eSET resulted in a significant reduction in live birth rate compared with DET [8]. There have been very few large-scale studies regarding eSET in older patients compared with those in younger patients.

In 2008, the Japan Society of Obstetrics and Gynecology (JSOG) issued guidelines regarding mandatory SET for women less than 35 years of age and in their first and second ETs to reduce multiple pregnancies. Since then, we have recommended patients of all ages to undergo SET according to the instructions of JSOG. We have expanded application of eSET to women of higher age because we considered that multiple pregnancies in older women might be associated with increased maternal morbidity [9] and that frozen embryo transfer after failed fresh transfer cycles could compensate low success rate of eSET in them.

In this retrospective study, the overall effectiveness of eSET was evaluated with cumulative live birth rate (CLBR) after fresh and frozen transfer cycles in one oocyte retrieval. We compared the outcome after eSET with that after multiple embryo transfer (MET) to examine the significance of eSET policy in unselected patients and to readdress the indication of eSET.

Material and methods

This retrospective study included all women who underwent ART treatment in the IVF Unit of University of Tokyo Hospital between January 2001 and December 2013. The Institutional Review Board approval was obtained at University of Tokyo Hospital. Controlled ovarian stimulation (COS) was mainly performed using long protocol. Nafarelin nasal spray (Nasanyl®; Pfizer Pharmaceuticals, Japan) was started in the mid-luteal phase. On the third day of the following menstruation cycle, ultrasound examination was performed to confirm pituitary suppression, followed by the administration of human menopausal gonadotropin (hMG) (hMG Teizo®; ASKA Pharmaceutical Co. Ltd., Japan) or pure follicle-stimulating hormone (FSH) (Gonapure®; ASKA Pharmaceutical Co. Ltd., Japan). In patients with diminished ovarian reserve, clomiphene citrate and/or hMG combined with GnRH antagonist was used. Clomiphene citrate (Clomid® ; Shionogi & Co. Ltd., Japan) or hMG was started on the third day of a menstrual cycle, followed by the combination with ganirelix acetate (Ganirest®; MSD Co. Ltd., Japan) 0.25 mg s.c. When the size of the lead follicle reached 18 mm in mean diameter as measured by transvaginal ultrasound, 10,000 IU of human chorionic gonadotropin (hCG) was administered. Oocytes were retrieved transvaginally with ultrasound guidance 34 h after hCG injection. They were inseminated or injected with husband's spermatozoa, depending on semen quality. Number of cells and morphology of each embryo were evaluated according to the criteria reported by Veeck [10] on day 3 after oocyte retrieval. Seven or eight cell embryos with grade 1 or 2 were defined as good-quality embryos. Other embryos with grades 1–3 were defined as fair-quality and those with grades 4–5 as poor-quality embryos. Embryo transfer was performed under ultrasound guidance on day 3 after oocyte retrieval, and the supernumerary embryos were cryopreserved in day 3 or day 5 using vitrification method. Thawed embryo transfer was performed in natural or hormone replacement cycles. The luteal phase was supported with progesterone suppository (200 mg/day) and transdermal estradiol (Estraderm M®; Kissei Pharmaceuticals, Japan). Luteal support was continued up to 7 weeks of gestation, when fetal heartbeat was detected with transvaginal ultrasound.

Between 2001 and 2007, number of embryos transferred was 2 or 3, depending on patients' age, embryo quality, and previous results of treatment. eSET was performed only for parous women or for those for whom multiple pregnancy was contraindicative for medical or other reasons. In and after 2008, women of all ages who had good-quality embryos were strongly recommended to undergo eSET to minimize the risk of multiple pregnancies. They were informed of possible risks associated with multiple pregnancies and of chances of success after subsequent frozen embryo transfer cycles. Double-embryo transfer (DET) was performed for patients under 39 years of age with more than four previous implantation failures and for patients aged 39 years and over with more than three previous failures. When there were no good-quality embryos suitable for transfer, embryo transfer was performed or cancelled after taking the couples' opinion into consideration. In such cases, SET was performed in the patients under 39 years of age with less than three previous failures, and DET was performed in other patients. The supernumerary embryos were incubated until day 5, when they were evaluated again, and blastocysts suitable for transfer were cryopreserved.

Data on fresh embryo transfer and subsequent frozen-thawed embryo transfer were collected as cumulative results in one oocyte retrieval cycle. Cumulative live birth was defined as ever occurrence of a live birth as a result of single oocyte retrieval and treated as a bivariate outcome. Cumulative results were analyzed as of December 2014. Ongoing pregnant cases in more than 12 weeks of pregnancy using frozen embryos were counted for live birth.

Patient selection and statistics

Only women who underwent fresh embryo transfer were included in the present study. The inclusion criterion for eSET group was defined as single-embryo transfer with one or more frozen embryos. The criterion for multiple embryo transfer (MET) group is defined as two or three embryo transfers in fresh cycles. In both groups, women who underwent three or more previous oocyte retrieval (OR) cycles were excluded from the study. Clinical pregnancy and implantation rates of the patients in the study groups in each year were analyzed with Speaman's rank correlation coefficient. In the two study groups, live birth rate following fresh transfer cycles and CLBR were compared in different age groups. According to the result of the first analysis, patients were divided into six groups—young eSET, intermediate eSET, old eSET, young MET, intermediate MET, and old MET. Next, the characteristics and the outcomes of ART following eSET or MET were compared in each age group. Chi-square tests were used to compare insemination method (IVF or intracytoplasmic sperm injection (ICSI)), controlled ovarian stimulation (COS) protocols (long protocol, antagonist protocol or others), presence of good-quality embryos, clinical pregnancy rate, live birth rate, and multiple live birth rate in fresh, frozen transfer cycles and cumulative outcomes. Number of retrieved oocytes was compared using t test. Thirdly, significance of eSET in three age groups to achieve cumulative live birth was examined using logistic regression analysis. We adjusted for confounders including year of treatment, maternal age, insemination method, COS protocol, number of retrieved oocytes, number of frozen embryos, and presence of good-quality embryos. In each age group, multilevel univariate analyses were conducted with cumulative live birth as the dependent variable. Variables with $p<0.20$ in the univariate analysis were selected for variables in subsequent multivariate analysis. Excel Statistics Ver.6.0® and Excel Multivariate Analysis Ver. 6.0® (Esumi Co. Ltd., Tokyo, Japan) were used as a statistical software program. Statistical significance was defined as $p<0.05$.

Results

During the study period, there were 2624 OR cycles conducted in the IVF Unit of University of Tokyo Hospital. The age of the patients was between 24 and 46 years old. The patient selection chart for the two study groups is shown in Fig. 1. In the study period, there were 1987 fresh embryo transfer cycles, 788 of which were conducted as SET and others as MET. In SET cycles, 437 were accompanied by frozen embryos. Eight of 437 eSET cycles were excluded from the study because of three or more previous OR cycles. Of 1199 MET cycles, 234 cycles with three or more previous OR cycles were excluded from the further analysis. Accordingly, 429 eSET cycles and 965 MET cycles met the criteria for the present study.

The study period in the present research was as long as 13 years, so we analyzed clinical pregnancy and implantation rates per transfer in fresh cycles of women aged under 37 included in the study in each year (Fig. 2). Clinical pregnancy rate did not change ($r=0.374$, $p=0.21$), while implantation rate significantly increased during the study period ($r=0.769$, $p=0.002$). Live birth rate in fresh transfer cycles and CLBR of the two groups stratified by age are shown in Fig. 3. In both groups, live birth rates declined according to maternal age. In eSET group, live birth rates in fresh transfer cycles were slightly higher than those of MET group in women aged 36 and under. In ICSI, the differences of live birth rate between eSET and MET in young women (aged under 31) were bigger than those in IVF (Fig. 3a, b). However, in women aged 37 and over, live birth rates in eSET fresh cycles rapidly dropped and fell below those of MET cycles in both IVF and ICSI. Rapid declines of success rate in older women with eSET were also observed in CLBR (Fig. 3a–c). CLBRs in eSET group were higher than those of MET group in women under 37 and were comparable in women aged 37 and over (Fig. 3c).

According to the result of initial analysis, significance of eSET policy was examined separately in three age groups—younger (under 37), intermediate (between 37 and 40), and older (over 40) patients. The characteristics and outcomes of treatment cycles in six groups—young eSET (group A), young MET (group B), intermediate eSET (group C), intermediate MET (group D), old eSET (group E), and old MET (group F)—are shown in Table 1. Insemination methods (IVF or ICSI) were similar between groups A and B, while significantly more women in group D underwent ICSI than those in group C. In all age groups, use of GnRH antagonist was significantly more frequent in eSET cycles than in MET cycles. Number of retrieved oocytes was similar in all age groups. In eSET, significantly more ETs were performed with good-quality embryos in all age groups.

Clinical pregnancy rate in fresh transfer cycles was not significantly different in younger age groups (groups A and B), and live birth rate was significantly higher in group A than in group B ($p=0.033$). There was no multiple birth in group A while 23.6 % resulted in multiple deliveries in group B. In group A, number of frozen embryos was significantly higher than in group B. Over one third of frozen embryo cycles (35.3 %) resulted in live birth following subsequent transfer cycles, and CLBR reached 66.1 % in group A, significantly higher than those in group B.

In women aged 37–40, clinical pregnancy rate and live birth rate in fresh transfer cycles were significantly lower in eSET group (group C) than in MET group (group D). In group C, significantly more embryos were frozen, and 20.0 % of

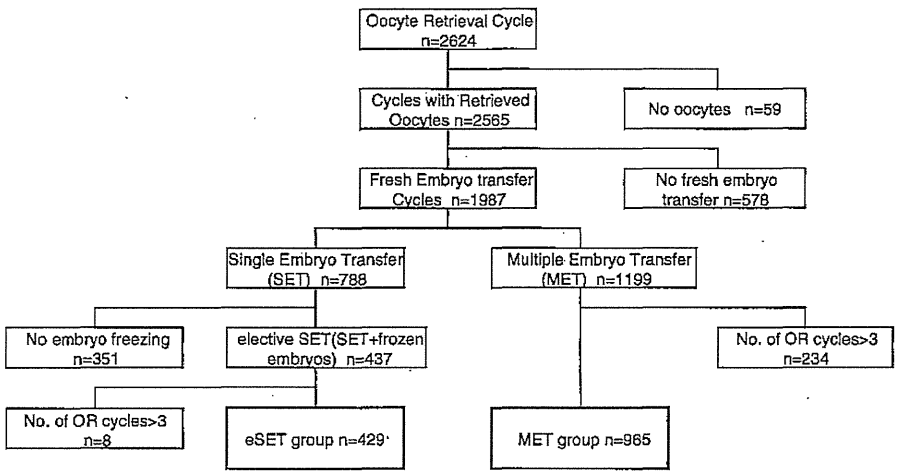

Fig. 1 The patient selection chart for the two study groups

frozen cycles resulted in live birth. CLBR in group C was slightly higher than that in group D, though not statistically significant (31.9 vs. 25.3 %, p=0.13). Multiple birth rates in fresh transfer cycles, frozen transfer cycles, and cumulative results were significantly lower in group C than those in group D. In women aged over 40, there were no statistically significant differences in live birth rate in fresh transfer cycles or CLBR between eSET and MET. Cumulative multiple birth rate in eSET group (group E) was lower than that in MET group (group F), though not statistically significant (0 and 9.5 %, respectively).

As shown in Table 1, there were several possible confounding factors in eSET and MET groups.

Therefore, to elucidate the significance of eSET in each age group, we subsequently investigated independent factors associated with cumulative live birth using logistic regression analysis. Results of univariate analysis in three age groups are shown in Table 2. In women aged under 37, year of treatment, age, number of retrieved oocytes, number of frozen oocytes, presence of good-quality embryos, and eSET were significantly related to cumulative live birth (p<0.05). In women aged between 37 and 40, age, ICSI, number of retrieved oocytes, number of frozen embryos, and presence of good-quality embryos were significantly related to cumulative live birth, while eSET was not (p=0.130). In women aged over 40, age and number of retrieved oocytes tended to be related to cumulative live birth, though not statistically significant (p=0.067 and 0.063, respectively).

Results of subsequent multivariate logistic regression analysis in each age group using variables with p<0.20 in the

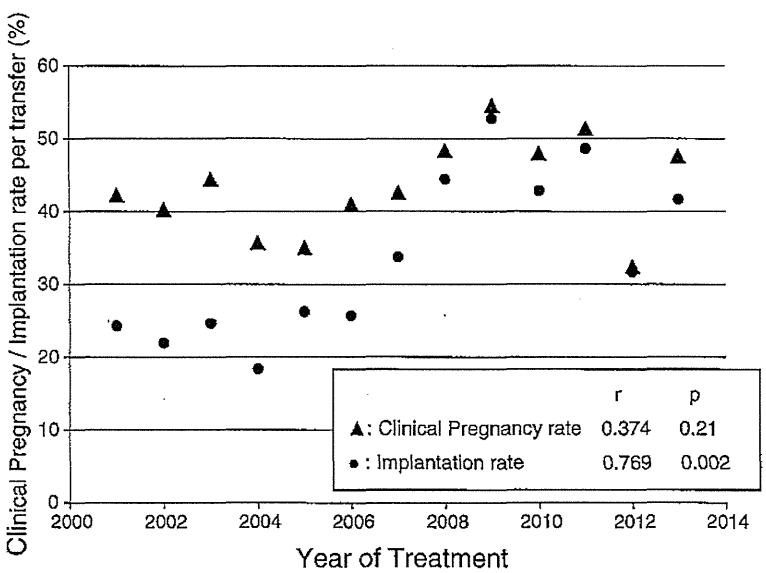

Fig. 2 Clinical pregnancy/ implantation rates in fresh cycles for women aged under 37 in each year

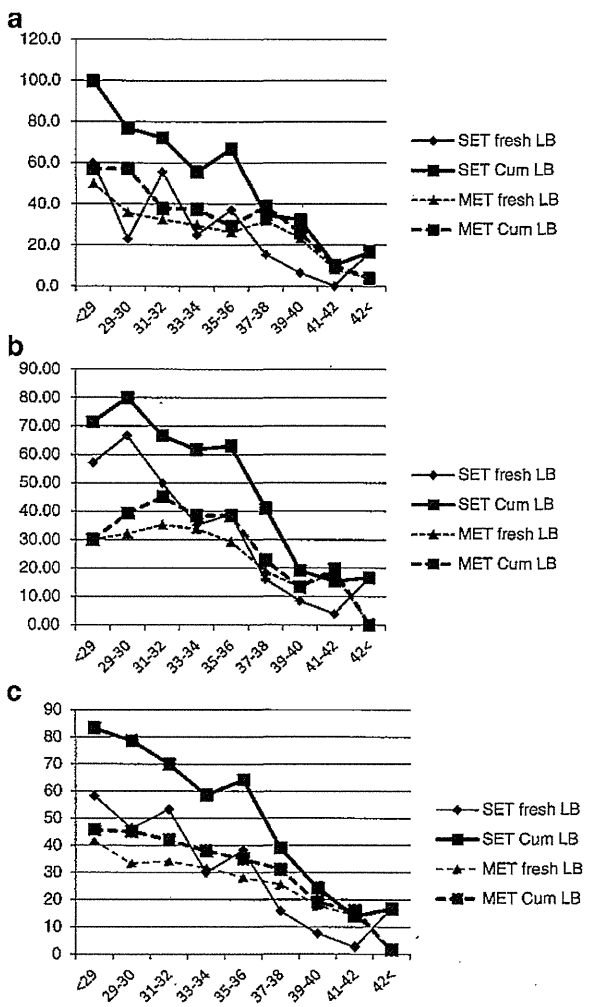

Fig. 3 Live birth rates in fresh transfer cycles and cumulative live birth rates of the two study groups stratified by age. **a** IVF. **b** ICSI. **c** IVF + ICSI

univariate analysis are shown in Table 3. In women aged under 37, number of frozen embryos, presence of good-quality embryos, and eSET were significantly related to cumulative live birth after adjusting for other factors. In women aged between 37 and 40, age and number of frozen embryos were significantly related to cumulative live birth after adjusting for other factors. However, eSET was not significantly related to cumulative live birth (p=0.224). In women aged over 40, none of the factors examined was not related to cumulative live birth.

Discussion

Our results showed that eSET policy with a cleavage stage embryo in women aged under 37 improved CLBR compared with MET, while it did not in women aged 37 and older. In

Table 1 Characteristics and outcomes of treatment cycles in six study groups

	(A) eSET <37	(B) MET <37	(C) eSET 37–40	(D) MET 37–40	(E) eSET >40	(F) MET >40	p value(A vs. B)	p value(C vs. D)	p value(E vs. F)
No. of treatment cycle	221	457	160	320	48	188			
IVF/ICSI	99/122	194/263	57/103	158/162	16/32	75/113	0.56	0.004	0.41
Long/antagonist/others	141/73/7	389/53/15	49/109/2	230/79/11	4/43/1	88/87/13	<0.001	<0.001	<0.001
No. of retrieved oocytes	10.1±5.1	10.6±5.3	7.8±5.0	8.6±4.6	6.1±2.9	7.0±4.4	0.23	0.08	0.08
Cycles with good-quality embryos (%/fresh transfer)	201 (91.0 %)	330 (72.2 %)	141 (88.1 %)	242 (75.6 %)	42 (87.5 %)	122 (64.9 %)	<0.001	0.001	0.002
Clinical pregnancy (%/fresh transfer)	100 (45.2 %)	184 (40.3 %)	33 (20.6 %)	97 (30.3 %)	7 (14.6 %)	34 (18.1 %)	0.22	0.024	0.57
Live birth (%/fresh transfer)	88 (39.8 %)	144 (31.5 %)	19 (11.9 %)	70 (21.9 %)	3 (6.3 %)	19 (10.1 %)	0.033	0.008	0.58
Multiple birth (%/live birth)	0	34 (23.6 %)	0	15 (21.4 %)	0	1 (5.3 %)	<0.001	0.034	0.69
Cycles with frozen embryos (%/fresh transfer)	221	191 (41.8 %)	160	106 (33.1 %)	48	32 (17.0 %)	<0.001	<0.001	<0.001
No. of frozen embryos/freeze cycle	3.4±2.3	2.1±3.2	2.3±1.5	1.4±2.5	1.9±1.1	0.6±1.6	<0.001	<0.001	<0.001
FET clinical pregnancy (%/freeze cycle)	90 (40.7 %)	57 (29.8 %)	48 (30.0 %)	21 (19.8 %)	10 (20.8 %)	5 (15.6 %)	0.022	0.063	0.56
FET live birth(%/freeze cycle)	78 (35.3 %)	45 (23.6 %)	32 (20.0 %)	13 (12.3 %)	4 (8.3 %)	2 (6.3 %)	0.009	0.1	0.73
FET multiple birth	2 (2.6 %)	10 (22.2 %)	2 (6.3 %)	3 (23.1 %)	0 (0 %)	1 (50.0 %)	<0.001	0.136	0.33
Cumulative clinical pregnancy (%/retrieval)	160 (72.3 %)	220 (48.1 %)	78 (48.8 %)	112 (35.0 %)	16 (33.3 %)	39 (20.7 %)	<0.001	0.004	0.066
Cumulative live birth (%/retrieval)	146 (66.1 %)	177 (38.7 %)	51 (31.9 %)	81 (25.3 %)	7 (14.6 %)	21 (11.2 %)	<0.001	0.13	0.51
Cumulative multiple birth (/CLB)	2 (1.4 %)	46 (26.0 %)	2 (3.9 %)	18 (22.2 %)	0 (0 %)	2 (9.5 %)	<0.001	0.004	0.42

Values presented as n (%) or mean±SD

Table 2 Multilevel univariate analysis in the two age groups with cumulative live birth as the dependent variable

Age	Under 37			Between 37 and 40			Over 40		
	Odds ratio	95 % CI	*p* value	Odds ratio	95 % CI	*p* value	Odds ratio	95 % CI	*p* value
Year	1.11	1.06–1.16	<0.001	1.02	0.96–1.07	0.535	1.08	0.97–1.21	0.169
Age	0.93	0.88–0.99	0.022	0.73	0.61–0.89	0.001	0.70	0.47–1.03	0.067
ICSI	1.01	0.75–1.37	0.928	0.61	0.41–0.91	0.015	1.66	0.70–3.94	0.251
Long protocol	1.09	0.76–1.57	0.641	1.44	0.95–2.17	0.087	1.20	0.54–2.67	0.655
Antagonist protocol	1.12	0.76–1.65	0.557	0.74	0.49–1.13	0.161	1.10	0.50–2.44	0.816
No. of retrieved oocytes	1.06	1.03–1.09	<0.001	1.06	1.02–1.10	0.008	1.08	1.00–1.18	0.063
No. of frozen embryos	1.21	1.14–1.28	<0.001	1.29	1.18–1.41	<0.001	1.14	0.93–1.40	0.208
Good-quality embryo	3.32	2.21–4.99	<0.001	2.64	1.44–4.84	<0.001	1.70	0.66–4.40	0.271
eSET	3.08	2.20–4.31	<0.001	1.38	0.91–2.10	0.130	1.36	0.54–3.41	0.515

women of advanced reproductive age, eSET policy had a modest effect, being associated with decreased multiple birth rate and comparable cumulative outcome compared with MET.

Table 3 Multivariate logistic regression analysis of possible related factors for cumulative live birth in the three age groups: (A) women under 37 years of age, (B) women aged between 37 and 40, and (C) women aged over 40

A			
	Under 37		
	Adjusted odds ratio	95 % CI	*p* value
Year	1.05	0.97–1.13	0.222
Age	0.94	0.88–1.00	0.065
No. of retrieved oocytes	1.02	0.99–1.06	0.204
No. of frozen embryos	1.12	1.05–1.20	<0.001
Good-quality embryo	2.14	1.38–3.32	<0.001
eSET	1.93	1.10–3.36	0.021
B			
	Between 37 and 40		
	Adjusted odds ratio	95 % CI	*p* value
Age	0.78	0.64–0.96	0.018
ICSI	0.65	0.42–1.00	0.051
Long protocol	1.18	0.24–5.86	0.836
Antagonist protocol	0.95	0.19–4.73	0.950
No. of retrieved oocytes	1.00	0.95–1.05	0.908
No. of frozen embryos	1.24	1.12–1.38	<0.001
Good-quality embryo	1.58	0.83–3.02	0.166
eSET	1.37	0.82–2.28	0.224
C			
	Over 40		
	Adjusted odds ratio	95 % CI	*p* value
Year	1.10	0.98–1.24	0.110
Age	0.73	0.49–1.08	0.118
No. of retrieved oocytes	1.09	0.99–1.19	0.075

There have been a few reports describing the efficacy of eSET in older patients [6, 7]. In those retrospective studies, patients in the study period were divided into eSET group and DET group on the decision by clinicians or patients. These methods could cause selection bias, and the patients with poor prognosis tend to choose or be recommended for DET. In the present study, many patients with recurrent implantation failure or who had only low-quality embryos were recommended for MET rather than SET after eSET has become mandatory in selected patients in Japan. Therefore, we included the patients who underwent MET in the period when MET was exclusively performed and excluded the patients who experienced three or more previous OR cycles from the analysis to exclude the selection bias as much as possible.

There have been a lot of randomized studies comparing the effectiveness of eSET versus DET in young women. In a systematic review, eSET strategy resulted in a lower live birth rate in fresh cycles than DET but achieved a comparable outcome after an additional frozen embryo transfer [5]. In the present study, eSET group in young women achieved a significantly higher live birth rate both in fresh cycles and in cumulative results than MET group. One of the reasons for the difference of live birth rate between the two groups in fresh cycles is that approximately 72 % of the patients in MET group had good-quality embryos, while 91 % of the patients in eSET group did, as shown in Table 1. However, multivariate logistic regression analysis showed that eSET was an independent factor even after adjusting for other indices of embryo quality such as embryo score and number of frozen embryos. Another possible factor associated with superiority of eSET over MET for cumulative live birth is increased number of frozen embryo transfer cycles. Several advancements in ART including COS protocols and culture conditions resulted in increased implantation rate and comparable clinical pregnancy rate with a decreased number of transferred embryos, as shown in Fig. 1. Actually, introduction of eSET strategy

resulted in larger number of frozen embryos with higher quality and more chances of pregnancy with subsequent embryo transfer cycles. In a previous paper, COS has been reported to decrease endometrial receptivity [11] and implantation rate in fresh embryo cycles has been shown to be lower than that of frozen embryo cycles [12]. In most of previous randomized studies, outcomes of eSET with additional one frozen embryo transfer cycles were compared with those of one fresh DET cycles. In the present study, cumulative outcomes of eSET were compared with those of MET. It means that, if there are four embryos suitable for transfer, women in eSET group have four chances of pregnancy—one fresh and three frozen transfer cycles—while those in MET group have only two chances—one fresh and one frozen transfer cycles. Multiple frozen embryo transfer cycles could maximize CLBR in eSET group.

While eSET policy has a marked efficacy in younger women, it reduced live birth rate significantly in fresh cycles in women aged between 37 and 40. It is interesting that, instead of possible selection bias especially in and after 2008, eSET did not improve cumulative outcomes in them. However, CLBR in eSET group was slightly higher than that in MET group of the same age (31.9 vs. 25.3 %, respectively), though not statistically significant. eSET also significantly reduced cumulative multiple birth rate compared with MET (3.9 vs. 22.2 %). These results support the efficacy of eSET in women of this age group and suggest that the embryos from old women are more sensitive to the detrimental effect of COS on the endometrium, while the embryos from younger women might be able to overcome the influences of COS. In multivariate analysis, not eSET but number of frozen embryos was associated with cumulative live birth in women aged 37–40. Recently, a review has been published regarding the efficacy of the freeze-all policy, that is, elective cryopreservation of all viable embryos in fresh cycles [13]. "Freeze-all policy" might be applied for patients of advanced reproductive age in order to avoid the influence of COS and increase number of frozen embryos.

In women aged over 40, introduction of eSET policy resulted in comparable CLBR and decreased multiple birth rate, though not significant. However, none of the examined factors including eSET was significantly related to cumulative live birth. eSET policy in unselected patients of this age group might be difficult, and further examination for better outcomes may be necessary.

In summary, eSET policy in younger women resulted in increased CLBR compared with MET. In women aged 37 and over, CLBR in eSET group was similar with that in MET group. In all age groups, eSET reduced multiple birth rates. The significance of eSET in women of advanced reproductive age is limited presently, and further research on the strategy to improve cumulative outcomes in them, including all-freeze policy, may be necessary.

Compliance with ethical standards

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Conflict of interest The authors declare that they have no conflict of interest.

Ethical approval This study was approved by the institutional review board of our institution.

Informed consent Informed consent was obtained from all individual participants included in the study.

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知っておきたい 流産と切迫流産のこと

「流産」「切迫流産（せっぱくりゅうざん）」という言葉を聞くだけで、
心配な気持ちになる妊婦さんも少なくありません。
むやみに怖がるのではなく、
正しい知識と対処法を知っておきましょう。

イラスト／おたざわゆみ　デザイン／米山和子

まずは知っておきたいこと

流産は何かをしたから、何かをしなかったから、起こることではありません

妊娠中のママならば、赤ちゃんの元気を願うことでしょう。でも残念ながら流産は一定の確率で起こるもの。その大半はたまたま受精卵に異常があったためで、だれの責任でもありません。

…だから、妊娠しても心配しすぎず普通の生活を送ってください

監修
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1982年東京大学医学部卒業。同年、同大学医学部産婦人科学教室入局。85年同大学医学部附属病院に習慣流産専門外来を開設、責任者として運営を担当。2012年より現職。

知っておきたいこと

1 流産と切迫流産 どちらも症状は一緒です

妊娠初期の妊婦さんの最も気になるトラブルといえば、流産、そして切迫流産でしょう。おなかの赤ちゃんの命にかかわるこの2つのトラブル、どちらも「出血」と「腹痛（おなかの張り・痛み）」という同じ症状が、異常に気づくサインとなります。

症状が同じでも、その人の状況によって対処のしかたは違います。早めの処置が必要になる場合もあるので、異常に気がついたらどのような時間でも、まず産院に連絡して指示をあおぎましょう。

注意したい症状はこの2つ

産院に連絡するとき Dr.が知りたいこと

医師から的確な指示をもらうには、必要な情報を伝えることが大切です。まずは深呼吸をして落ち着いて。正確に状況を説明しましょう。

1. 妊娠の週数
2. 子宮の中に胎嚢が確認されているか
3. 胎児の心拍が確認されているか
4. 出血の量、何かかたまりが出たか
5. 腹痛の強さ
6. 症状の経過（どんどん強くなっている、強い痛みが治まってきた、など）

← とにかく産院に連絡して指示をあおぎましょう!

知っておきたいこと

2 流産と切迫流産はまったく違うものです

流産と切迫流産は、名前こそ似ていますが、実はママの体の中で起きている問題はまったく異なります。いちばん大きな違いは、赤ちゃんが生きているかどうか。流産とは赤ちゃんが死亡していて、超音波検査でも心臓の動き（胎児心拍）が確認できない状態で、自覚症状がとくになくても起こっている場合もあります。切迫流産は出血や腹痛などの自覚症状はあっても、超音波検査で胎児心拍が確認できる状態です。

切迫流産

□ 妊娠に伴って出血・腹痛といった流産と同じ症状が出ていること

□ 流産と同じ症状が出ているが、妊娠が継続していること

切迫流産は、極端な言い方をすれば妊娠初期ならばだれもが診断されても不思議ではないものといえるでしょう。なぜなら妊娠していて、出血や腹痛という症状が見られれば、切迫流産ということになるからです。胎児心拍が確認できる場合はもちろん、妊娠の早い時期で胎嚢は確認できても、まだ胎児心拍が確認できないときに出血や腹痛があった場合も、切迫流産と診断されます。

↕

出血・腹痛があれば正常妊娠でも「切迫流産」と診断されます

妊娠初期は、受精卵が子宮内膜に入り込んで胎盤を形成する過程でも出血や腹痛が見られます。ただそれによるものか否かの判断は難しいので、症状があれば切迫流産と診断されるのです。

流　産

□ 妊娠22週未満に妊娠が終了してしまうこと

流産は妊娠22週未満（21週6日まで）に、妊娠が終了してしまうことをいい、その多くは胎児が死亡してから体外に排出されます。妊娠12週未満に起こるものを「早期流産」、それ以降に起こるものを「後期流産」と分けて考えます。それは早期流産と後期流産では、原因が異なることが多く、また、発生する頻度も大きく異なるため。妊娠して流産する確率は10〜15%といわれていますが、そのほとんどは早期流産です。

流産する確率は妊娠全体の10〜15%くらい

知っておきたいこと 3

～妊娠12週未満 早期流産の原因はほとんど胎児側にあります

流産は妊婦さんの7～10人に1人の割合で起こるもので、決してめずらしいものではありません。その9割以上を占める早期流産の原因のほとんどは、胎児側に理由があって発生します。ママが何かをしたからとか、何かをしなかったから流産をしたということはありません。早期流産は運命的なもので、残念ながらなってしまったら防ぐ方法はありません。万一流産を経験したとしても、ママは決して自分を責める必要はないのです。

ママ側の原因

感染症によるもの

妊娠初期に、風疹やサイトメガロウイルスなどの一部のウイルスに感染した場合、流産の原因になったり、赤ちゃんの発育に影響することがあります。

妊娠初期はできるだけ人込みを避ける、外出後は手洗いやうがいをしっかり行うなど、感染症に注意することが、ママが流産を予防するためにできる数少ない方法の一つといえるでしょう。

子宮の異常

子宮に子宮腺筋症や子宮頸管無力症などの病気があると、流産に至る原因になることがあります。子宮筋腫の場合は筋腫が子宮の内側にあり子宮内腔が変形するような状態だと流産の原因になることも。この場合は切迫流産にもなりやすい傾向が。ただし子宮筋腫自体は深刻な病気ではないので、赤ちゃんの心拍が確認できれば問題ないでしょう。

胎児側の原因

自然界の選択

どうして精子や卵子の異常が起きてしまうのか、原因はまだ完全には解明されていません。またたとえ正常な精子と卵子が受精したとしても、その後の過程で異常が生じることも。受精卵の異常は偶然起きてしまうことで、だれにでも起こり得ることなのです。受精卵に重い異常がある場合、育つことができないため、流産という形で自然淘汰されてしまうのです。

受精卵の異常

流産の原因で最も多いのが、受精卵の染色体異常によるもの。精子と卵子のどちらかが、たまたま異常を持っていた場合、育つことができない受精卵が発生します。とくに卵子の異常は加齢によって増える傾向があるため、妊娠する女性の年齢が高くなればなるほど、流産してしまう確率や染色体異常の赤ちゃんが生まれる確率は高くなります。

仕事や家事、日常的な運動や妊娠判明前の一般的な薬の使用はまず影響しません

もっと知りたい Q&A

Q 流産になりやすい体質や習慣はありますか?

A 流産の多くは胎児側が原因の偶発的なものなので、だれにでも起こり得ます。母体側の原因としては、高年出産（35才以上）の人、過去の流産回数が多い人、なんらかの理由で子宮頸管が短い人（子宮円錐切除術を受けたなど）、子宮に病気がある人や子宮内腔が変形するような形の子宮筋腫がある人が、流産しやすい傾向にあるといえます。

Q 早期切迫流産になった人は、切迫早産や早産になりやすい?

A 早期切迫流産になったからといって、その後に切迫早産や早産になりやすいということはありません。ただし、流産を繰り返す場合は、母体側にも原因があることが多いので、それが切迫早産や早産を引き起こす可能性も。医師の指示に従ってください。

知っておきたいこと 4

早期切迫流産の場合は安静にすれば治まることも

切迫流産も、流産同様に妊娠12週未満を早期切迫流産、それ以降を後期切迫流産と分けます。早期切迫流産の場合、出血や腹痛があっても正常妊娠である場合も多いので、胎児心拍や子宮頸管の開き具合、感染の有無などで予後を判断します。赤ちゃんが元気で、子宮頸管が閉じているなら、安静にしていれば症状が治まり、妊娠が継続する可能性が高いので、この場合、あまり心配しすぎないで、医師の指示を守って安静に過ごすことが大切です。

自宅安静の目安は医師に確認しましょう

自宅安静の目安は“簡単な家事や食事、トイレ以外は横になって休む”。入浴も控え、短時間のシャワー程度に。その人の状態により、必要な安静度はさまざまです。自宅安静を指示されたら、気になることは遠慮せず医師に確認を。また自宅安静は家族の協力が不可欠。上の子がいるなどの場合は、実家に帰ったほうが安静を守れるかもしれません。

薬を処方されることはある?

流産の予防効果が確立された薬はありませんが、医師の判断で、ビペリドレート塩酸塩、黄体ホルモン製剤、ヒト絨毛性性腺刺激ホルモン（hCG）製剤などが処方されることも。

知っておきたいこと

5 流産は種類によって対処の方法が変わります

流産はその状況により、「完全流産」「不全流産」「稽留流産」「進行流産」というように分類されます。症状や超音波検査によってわかる子宮内や子宮頸管の様子で、その後のケア方法も違ってきます。また、出血や腹痛などの自覚症状がなくても流産している場合もあります。受精卵が着床したころの早い段階で流産した場合は月経と区別できないことがほとんどで、これは「化学的妊娠」と呼ばれています。

完全流産

子宮内にある胎嚢などが完全に娩出された状態。

症状

下腹部に軽い痛みがあるけれど、しだいに消失していきます。ごく少量の出血がある、または出血は完全に止まっている状態。

超音波でわかること

胎嚢や胎児心拍の動きは確認できません。子宮頸管は閉じています。

対処

流産の手術（子宮の内容物を取り除く手術）は行わないで、しばらく経過観察をします。

不全流産

子宮内の胎嚢などが、完全には娩出されずに残っている状態。

症状

下腹部の痛みが強くなったり弱くなったり、陣痛に似た痛みを感じることもありますが、しだいに軽い痛みに変わります。中等量〜少量の出血が続きます。

超音波でわかること

胎児心拍の動きは確認できません。血液や胎嚢の一部などのかたまりが見えます。子宮頸管は開いているか閉じています。

対処

流産が確定したら、血液検査を行い、子宮の内容物を取り除く手術をします。

稽留流産

自覚症状はないけれども、子宮内で赤ちゃんが死亡してしまっているか、赤ちゃんはできておらず胎嚢だけがそのままとどまっている状態。

症状

自覚症状はなし。

超音波でわかること

胎児心拍の動きは確認できません。赤ちゃんの姿は確認できる場合もあれば、できない場合も。胎嚢の大きさは正常または小さめ。胎嚢の周辺はゆがみが見える場合と見えない場合があり、子宮頸管は閉じています。

対処

流産が確定したら、血液検査を行い、流産手術（子宮の内容物を取り除く手術）をすることが原則ですが、経過観察をすることもあります。

進行流産

子宮が収縮を開始して、流産が進行している状態。残念ながら、進行流産をとどめる手だてはありません。

症状

陣痛に似た規則的な強い痛みが続いて、多めの鮮やかな色の出血が見られます。

超音波でわかること

赤ちゃんや胎児心拍の動きは通常確認できないでしょう。胎嚢は縮小して変形、胎嚢の周辺はぼんやりとした感じに見えます。子宮頸管は開いています。

対処

流産が確定したら、血液検査を行って、流産の手術（子宮の内容物を取り除く手術）をすることが原則ですが、経過観察をすることもあります。

こんなケースも

胞状奇胎

受精卵から派生してやがて胎盤になるはずだった絨毛という組織が異常増殖してぶどうの粒のようになってしまう病気です。受精卵の染色体の異常が原因といわれています。胞状奇胎の場合は、細胞が子宮内に残ろうとする性質があるため、原則2回の手術が必要になり、しばらく厳重な経過観察を要します。ただし、その後の妊娠は可能です。

異所性妊娠（子宮外妊娠）

本来、子宮内に到達して着床するはずの受精卵が、その前にそれ以外の場所に着床して妊娠すること。その多くが細い卵管内で起こり、赤ちゃんや胎盤の成長に耐えきれず卵管破裂や大出血になる可能性が。そのため、異所性妊娠と診断されたら、早急に腹腔鏡下手術もしくは開腹手術で妊娠部位を除去します。もし卵管を除去しても、反対側の卵管があるので、次の妊娠は可能です。

知っておきたいこと

6 流産の手術の流れ

流産にはその後に手術が必要な場合と必要でない場合があります。子宮の中に赤ちゃんや胎盤などの組織が残っている場合は、出血を止める、感染症を予防するなどの目的で、早い時期に手術が必要となります。ただし、完全流産で子宮の内容物がすべて出てしまっている場合は、手術は不要です。

手術は必要な検査を事前に行って、全身麻酔を施し10分程度で終了します。

手術前の検査

流産の診断が確定したら、血液検査など一般的な検査を行います。必要に応じて、出血凝固検査や胸部X線検査などを行う場合もあります。検査内容はそのときの流産の状態によって異なります。

入院

入院の日程は、産院と相談して決定します。一般的には午前中に入院し、午後に手術を受けて、翌日退院になるケースが多いようです。中には日帰り入院となるケースも。術後の出血量や体調によっては、入院が長引く場合も。

手術

手術そのものは10分程度で終わるものです。麻酔はたいてい全身麻酔になるでしょう。妊娠12週以降の場合は、お産と同様に陣痛を薬で誘発してから、子宮の内容物を取るようにします。

退院

手術当日から翌日は安静に過ごします。基本的には麻酔の影響がなくなれば退院できますが、出血量や体調なども考慮した上で判断されます。術後の出血は1週間ほど続き、その期間は子宮収縮剤や感染症予防薬を服用します。

知っておきたいこと

7 体は1カ月くらいで回復します

流産の手術を受けたあとは約1週間後に検診があり、子宮の回復具合を診察します。流産後、子宮の中は子宮内膜に無数の傷ができてしまい荒れた状態です。出血の量には個人差がありますが、出血は通常1～2週間程度で治まるでしょう。もしもそれ以降も出血が続いて、血のかたまりが出たり、腹痛や発熱が見られる場合は、受診してください。退院後の生活では、シャワーは翌日からOKですが、入浴は医師の許可が出てからにしましょう。セックスは次の月経が来るまでは避けてください。月経は流産後1カ月くらいに再開します。月経が来た時点で、子宮が元の状態に戻って回復したと判断できます。

流産しても、順調に子宮が回復すれば、次の妊娠に影響を及ぼす心配はありませんので安心してください。妊娠は一回一回がそれぞれ独立しているものなので、一度流産したら、次からの妊娠も流産を繰り返しやすくなるということはありません。次の妊娠はできれば月経が2～3回来てからのほうが、子宮の状態も安定してより安心と考えられています。前回の流産のことをいつまでも気にせず、次の妊娠に向けて心と体の準備を整えておきましょう。

次の妊娠はできれば月経が2～3回来たあとまで子宮の回復を待ちましょう

知っておきたいこと

8 2回流産を繰り返したら不育症の専門医を受診して

流産した経験があるママは、だれもが「もしかしたら今度も…」と心配してしまうようですが、1回だけの流産経験だったら、気にする必要はありません。ただ、2回続けて流産した場合は、習慣流産（3回以上続けて流産すること）の可能性があるので、専門の医師を受診しましょう。また1回の流産でも、それが後期流産だった場合は、相談してみるといいでしょう。習慣流産などの不育症の場合、ストレスを取り除くことで改善する例も見られます。難しいかもしれませんが、努めて気にしすぎないようにすることも大切です。

※不育症、習慣流産の専門医はFuiku-Laboで検索できます。(http://Fuiku.jp)

もっと知りたいQ&A

Q 前回の妊娠では胎嚢が確認できたけれど、赤ちゃんが確認できないままで流産しました。今回の妊娠では、茶色い出血がずっとありますが、主治医には「異常ない」と言われました。妊娠3カ月に入ってもそんな状態なので気になります。
(K・Kさん・妊娠4カ月)

A 流産は確率的に本当にたまたま起こるものです。今回の症状は切迫流産ということになりますが、医師が妊娠8週を過ぎて胎児心拍を確認している、かたまりのような出血はないことからも、心配ないでしょう。体を横にして安静にしていると、出血は止まりやすくなります。

Q 流産経験があるため、今回の妊娠には必要以上に慎重になってしまいます。走ったり、ダンスなどの運動をするのも控え、冷やさないよう腹巻きをつけていますが…。
(H・Mさん・妊娠4カ月)

A 実は、精神的なストレスが流産につながるという説もあります。慎重になることで安心できるのであれば、それは悪いことではありません。ただ、運動を控えたり冷えを防止したりすること自体に、流産を予防する効果があったという報告は今のところないので、期待できません。

Q 前回は胎嚢は確認できたけれど赤ちゃんが見えず、仕事で長時間立ち仕事をしたあとに流産。今回の妊娠では胎児心拍も確認できたので、その後「もう安心」とゴルフの練習に励んだら、出血。切迫流産になり、自宅安静を指示されてしまいましたが…。
(S・Oさん・妊娠4カ月)

A 前回の流産は、たまたま起きたもので、立ち仕事は関係ないでしょう。妊娠経過が順調でも、激しい運動をすると出血することがあります。これは赤ちゃん側の絨毛という組織が、胎盤を形成するために子宮内膜に入り、血管を破って根をはろうとする際に出た血が、ママが運動することで子宮口から出てくるのが原因と考えられています。だからといって流産する心配はありません。安静にしていれば出血はやがて止まるでしょう。とはいえ妊娠中の運動は体に負担がかからない程度のものにしましょう。