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Original Article

Clinical analysis of spontaneous pregnancy reduction in the patients with multiple pregnancies undergoing in vitro fertilization/intracytoplasmic sperm injection-embryo transfer

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Abstract: Objective: To investigate the spontaneous pregnancy reduction (SPR) rate, SPR-related factors and the effects of SPR on pregnancy outcomes in the patients with multiple pregnancies undergoing in vitro fertilization/intracytoplasmic sperm injection-embryo transfer (IVF/ICSI-ET). Methods: Between January 1998 and December 2010, 3957 patients undergoing fresh/frozen-thawed cycles (IVF/ICSI-ET) and their 5106 neonates were enrolled in this study. According to spontaneous pregnancy reduction (SPR), this study included singleton originating from twins [(2→1) group] or from triplets [(3→1) group], and twins originating from triplets [(3→2) group]. According to SPR time, this study included ≤8 week, 8-18 week and ≥18 week's groups. Outcome measures were SPR rate, preterm rate, mean birth weight and the rates of low birth weight and very low birth weight. Results: SPR rate was higher in triplets group than in twins group, in frozen-thawed cycles than in fresh cycles, in the patients ≥35 years than in the patients <35 years (all $P<0.05$). Compared with ≤8 week group, preterm rate was significantly increased in 8-18 week group ($P<0.05$). Pregnancy outcomes were better in (2→1) group than in twins group, in (3→1) group than in triplets group (all $P<0.05$). After multi-fetal pregnancy reduction (MFPR), the mean birth weight was higher and low birth weight was lower in SPR group than in only MFPR group (all $P<0.05$). Conclusion: SPR rate is related to age and the initial number of gestational sacs. Both SPR and MFPR can improve pregnancy outcomes. The later the SPR occurs, the worse the neonatal outcomes are. Due to the possibility of SPR, it is necessary to appropriately delay MFPR until 8 gestational weeks.

Keywords: In vitro fertilization, intracytoplasmic sperm injection, embryo transfer, multiple pregnancy, spontaneous pregnancy reduction, pregnancy outcomes

Introduction

Multiple pregnancies, a kind of abnormal pregnancy, is common in in vitro fertilization/intracytoplasmic sperm injection-embryo transfer (IVF/ICSI-ET) and can lead to pregnancy complications such as premature birth or preeclampsia [1]. With the development of assisted reproductive technology and ultrasonic diagnosis, more and more attention has been paid to spontaneous pregnancy reduction (SPR) [2, 3]. SPR is the phenomenon that during pregnancy, one or several embryos naturally disappear. Rodriguez-Gonzalez et al [4] have reported that SPR incidence is 18.8% in multiple pregnancies; the SPR incidences occurring within 12 weeks after pregnancy are 15.8% in twins, 24.5% in triplets and 38.4% in quadruplets respectively; and 80% SPR occur within 9 weeks after pregnancy. However, how much is the SPR rate? How does SPR affect pregnancy outcomes? Can SPR replace multi-fetal pregnancy reduction (MFPR)? These problems remain to be further studied. In this study, we investigated SPR rate, SPR-related factors and the effects of SPR on pregnancy outcomes in the patients with multiple pregnancies undergoing IVF/ICSI-ET.

Materials and methods

All study methods were approved by Institutional Review Board and Ethics Committee of the First Affiliated Hospital of Zhengzhou University.

Table 1. Effect of different factors on SPR

Group		SPR	χ^2	*P*
Multiple pregnancy	Twin	19.5% (243/1266)	80.02	*P*<0.01
	Triplet	72.9% (35/48)		
Cycle type	Fresh	20.1% (232/1153)	6.047	*P*<0.05
	Frozen-thawed	28.6% (46/161)		
Fertilization way	IVF	18.7% (125/667)	1.877	*P*>0.05
	ICSI	22% (107/486)		
Age (year)	<35	10.2% (233/2281)	13.84	*P*<0.01
	≥35	16.7% (66/395)		

Notes: SPR: spontaneous pregnancy reduction; IVF: in vitro fertilization; ICSI: intracytoplasmic sperm injection.

Written informed consent was obtained from the human participants of this study.

Subjects

Excluding the patients who failed to be followed up, the 3957 patients who underwent IVF/ICSI-ET between January 1998 and December 2010 in our reproductive center and their 5106 neonatus were enrolled in this study. SPR occurred in 278 cycles and MFPR was performed in 90 cycles. This study included fresh and frozen-thawed cycle groups.

Treatment

In fresh cycles, the mid-luteal phase down-regulation was performed. After reaching the standards of down regulation, gonadotropin therapy began until the time of HCG administration. Oocyte retrieval, insemination, embryo transfer and luteal support were performed according to the method in our reproductive center [5].

In frozen-thawed cycles, natural cycles were used in the patients with regular menstrual cycle and hormone replacement treatment in the patients with irregular menstrual cycle.

Clinical treatment and follow-up

In this study, MFPR was performed to reduce one or two fetuses in the patients with triplet. Telephone follow-up was performed to collect data including maternal health condition and complications during gestational period, and fetal gestational weeks, birth weight and complications.

SPR diagnosis and neonatal outcomes

SPR was diagnosed according to ① B-mode ultrasound showed empty gestational sac; ② One or more embryos disappeared after identifying heart tube beat. Neonatal outcomes were evaluated based on five-year national medical college textbook "*Pediatrics*" including birth weight, preterm rate and low birth weight rate [6].

Grouping

According to the number of initial gestational sac, this study was divided into singleton, twins and triplets groups. According to SPR, this study was divided into singleton originating from twins [(2→1) group] or from triplets [(3→1) group], and twins originating from triplets [(3→2) group]. According to SPR time, this study was divided into ≤8 weeks, 8-18 weeks and ≥18 week's groups. According to SPR occurrence after MFPR in triplets, this study was divided into SPR group and only MFPR group. Outcome measures were SPR rate, preterm rate, mean birth weight and the rates of low birth weight and very low birth weight.

Statistical analysis

Statistical treatment was performed with SPSS16.0 software. Measurement data first underwent normality test, and then were expressed as mean ± standard deviation. *t* test was used in the comparison between groups. Continuous variables (three categories) were compared using analysis of variance (ANOVA) for normally distributed variables. Fisher's exact test was used for categorical variables with small expected numbers. Statistical significance was established at *P*<0.05.

Results

SPR rate and SPR-related factors

Among the 3957 patients, there were 1314 patients with multiple pregnancies excluding 90 patients undergoing MFPR. Of the 1314 patients, 48 patients (containing 35 patients with SPR) had triplet pregnancy and 1266 patients (containing 243 patients with SPR) had twin pregnancy. Total SPR rate was 21.2% (278/1314) and SPR rate was higher in triplet pregnancy (72.9%, 35/48) than in twin preg-

Table 2. Neonatal outcome in the patients with and without SPR

Group	Case (n)	Birth weight (g)	Preterm rate (%)	Low birth weight rate (%)	Very low birth weight rate (%)
Singletons					
Original singleton	2553	3376.5±541.4	8.02 (205/2553)	0.6 (15/2553)	0.5 (12/2553)
(2→1) group	243	3303.4±617.3$^{\Delta,*}$	13.6 (33/243)$^{\Delta,*}$	1.2 (3/243)	2.9 (7/243)$^{\Delta,*}$
(3→1) group	12	3312.5±595.1	0 (0/12)	0 (0/12)	8.3 (1/12)
Twins					
Original twins	1023	2567.0±818.5	41.4 (424/1023)	5.1 (105/2046)	4.1 (84/2046)
(3→2) group	23	2634.8±471.2$^{\Delta}$	52.5 (12/23)$^{\Delta}$	17.4 (8/46)	4.3 (2/46)$^{\Delta}$
Triplets	13	1793.5±509.3	92.3 (12/13)	31.0 (9/29)	37.9 (11/39)

Notes: $^{\Delta}$indicates $P<0.05$, compared with original singleton group. *indicates $P<0.05$, compared with original twins group. SPR: Spontaneous Pregnancy Reduction; (2→1) group: singleton originating from twins after SPR; (3→1) group: singleton originating from triplets after SPR; (3→2) group: twins originating from triplets after SPR.

Table 3. Neonatal outcomes in different time of spontaneous pregnancy reduction

Group	Case (n)	Preterm rate (%)	Low birth weight rate (%)	Very low birth weight rate (%)
≤8 gestational weeks	218	12.8 (28/216)	10.8 (24/223)	1.3% (3/223)
8-18 gestational weeks	59	28.5 (17/59)$^{\Delta}$	16.7 (11/66)	1.5% (1/66)

Notes: $^{\Delta}$indicates $P<0.05$, compared with ≤8 gestational weeks.

nancy (19.5%, 243/1266) (χ^2=80.02, P<0.01) (**Table 1**).

SPR rate was significantly lower in fresh cycles (20.1%, 232/1153) than that in frozen-thawed cycles (28.6%, 46/161) (χ^2=6.047, P<0.05). There was no statistical significance in SPR rate between IVF (18.7%, 125/667) and ICSI (22%, 107/486) (χ^2=1.877, P>0.05) (**Table 1**).

SPR rate was higher in the patients ≥35 years (16.7%, 66/395) than in the patients <35 years (10.2%, 233/2281) (χ^2=13.84, P<0.01, OR= 1.75, 95% CI: 1.29-2.35) (**Table 1**).

Effects of SPR on neonatal outcomes

Among the 3867 patients excluding 90 patients who underwent MFPR from 3957 patients, 1059 patients underwent multiple births (27.4%, 1059/3867) and 2796 patients containing 243 patients with (2→1) underwent single birth. Neonatal outcomes were poorer in twin group and triplet group than in singleton group (P<0.05, **Table 2**).

Neonatal outcomes were poorer in (2→1) group than in singleton group (P<0.05), but were better in (2→1) group than in twin group (P<0.05) (**Table 2**).

Neonatal outcomes were better in (3→2) group than in triplet group (P<0.05), but were similar between (3→2) gr oup and twin group (P>0.05) (**Table 2**).

SPR time and neonatal outcomes

In this study, 78.4% SPR (218 patients) occurred within 8 weeks after pregnancy, 21.2% SPR (59 patients) within 8-18 weeks and only 0.4% SPR (one patient) after 18 weeks. Preterm rate was higher in 8-18 weeks group than in ≤8 weeks group (P<0.05, OR=2.74, 95% CI: 1.38-5.47). As the extension of SPR time, there was an increased trend in low birth weight and very low birth weight rates (all P>0.05). Only in one patient, SPR occurred after 18 gestational weeks and the neonate had 33^{+3} gestational weeks, a birth weight of 1300 g and fallot tetrad (**Table 3**).

SPR and MFPR

MFPR was used to reduce one fetus from triplet in 90 cycles. However, of the 90 cycles, survival singleton occurred in 13 cycles and survival twins in 77 cycles after MFPR. In the 13 singleton cycles, SPR was identified one week after MFPR in 2 cycles and within 4 weeks in 11 cycles with a SPR rate of 14.42% (13/90) (**Table 4**).

According to SPR occurrence after MFPR in triplets, this study was divided into SPR group and

Table 4. Neonatal outcome in the patients with and without SPR or MFPR

Group	Case (n)	Birth weight (g)	Preterm rate (%)	Low birth weight rate (%)	Very low birth weight rate (%)
Singletons					
Original singleton	2553	3376.5±541.4	8.02 (205/2553)	0.6 (15/2553)	0.47 (12/2553)
2→1	243	3303.4±617.3$^{\Delta,*}$	13.6 (33/243)$^{\Delta,*}$	1.2 (3/243)	2.9 (7/243)$^{\Delta,*}$
3→1	12	3312.5±595.1	0 (0/12)	0 (0/12)	8.3 (1/12)
SPR group	13	3003.8±535.2*	23.1 (3/13)*	15.4 (2/13)$^{\Delta,*}$	7.7 (1/13)
Twins					
Original twins	1023	2567.0±818.5	41.4 (424/1023)	5.1 (105/2046)	4.1 (84/2046)
3→2	23	2634.8±471.2$^{\Delta}$	52.5 (12/23)$^{\Delta}$	17.4 (8/46)	4.3 (2/46)$^{\Delta}$
Only MFPR group	77	2507.6±480.1	41.6 (32/77)	44.2 (68/154)	8.4 (13/154)
Triplets	13	1793.5±509.3	92.3 (12/13)	31.0 (9/29)	37.9 (11/39)

Notes: $^{\Delta}$indicates $P<0.05$, compared with (3→1) group. *indicates $P<0.05$, compared with original singleton group. SPR: Spontaneous pregnancy reduction; MFPR: Multi-fetal reduction technology; (2→1) group: singleton originating from twins after SPR; (3→1) group: singleton originating from triplets after SPR; SPR group: SPR occurrence after MFPR in triplets; (3→2) group: twins originating from triplets after SPR; Only MFPR group: no SPR occurrence after MFPR in triplets.

only MFPR group. Compared with only MFPR group, the birth weight was significantly increased (t=3.563, $P<0.05$), and low birth weight rate was significantly decreased ($P<0.05$) in SPR group (**Table 4**).

There were no significant differences in neonatal outcomes among only MFPR group, twins group and (3→2) group ($P>0.05$) (**Table 4**).

Compared with singleton group and (3→1) group, the birth weight was the lowest, and the preterm rate and low birth weight rate were the highest in SPR group ($P<0.05$, **Table 4**).

Discussion

At present, 2 or more embryos are transferred in the most of Chinese reproductive centers, so the rate of multiple pregnancies after assisted reproduction is remaining obstinately high. Although MFPR can reduce the number of survival fetuses, there are many problems such as an abortion rate of 8%-16%, retained fetal growth restriction and low birth weight after MFPR. More and more attention has been paid to SPR in multiple pregnancies. What population is SPR likely to occur in? Whether can SPR improve neonatal outcomes? Whether can SPR replace MFPR? These problems will be discussed in this study.

What population is SPR likely to occur in?

SPR define standards differ, so SPR rate (3.51%-38%) is very different [7-15]. It is reported that SPR rate is about 12%-30% in assisted reproduction, even as high as 38% in twin pregnancy [8]. However, about 80% SPR occur about 8 gestational weeks [9]. In this study, SPR was diagnosed according to ① B-mode ultrasound showed empty gestational sac; ② One or more embryos disappeared after identifying heart tube beat. In this study, SPR rate was 21.2% and 78.4% SPR occurred before 8 gestational weeks, which is consistent with the results reported above.

Dickey et al [14] have believed that SPR rate is positively correlated with the number of gestation sac, and SPR is associated with small uterine space and the relative lack of blood supply of the gestation sac caused by multiple pregnancies. In this study, SPR rate was higher in triplet pregnancy than in twin pregnancy, demonstrating that with the increase in the number of the gestation sac, the possibility of SPR is increased. Our study also indicated that there was no a statistical difference in SPR between IVF and ICSI, which is consistent with the results reported by La et al [16]. In this study, SPR rate was higher in the patients ≥35 years than in the patients <35 years, which is consistent with the results described by Ross et al [17] and La et al [18]. In this study, SPR was higher in frozen-thawed cycle than in fresh cycles, which may be associated with freezing and thawing [15, 16]. We believe that SPR is not related to the mode of assisted reproduction (IVF or ICSI), but associated with age and the number of gestational sac.

Effects of SPR on neonatal outcomes

In multiple pregnancies, SPR reduces intrauterine pressure, improving neonatal outcomes. However, fetal death may affect other living fetus in multiple pregnancies.

Dickey et al [14] have reported that SPR can increase neonatal body weight and body weight is associated with the number of gestational sac. Pinborg et al [7] have found that the birth weight is significantly lower in (2→1) group than in singleton group; and with the time delay of SPR, the birth weight is decreased. Shebl et al [19] have described that the risks of low birth weight and inadequate gestational age are higher in other living fetus after SPR. Chasen et al [11] have believed that the birth weight is slightly lower in (2→1) group than in singleton group; and the gestational age is longer in (2→1) group than in twin group, but is similar to that in singleton group. Rodriguez et al [10] have reported that the gestational age is similar in (2→1) group and singleton group. In this study, the preterm rate and the rates of low birth weight and very low birth weight were higher in (2→1) group than in singleton group ($P<0.05$), but were lower in (2→1) group than in twin group ($P<0.05$). The birth weight was slightly lower in (3→1) group than in singleton group, but was similar in (3→2) group and twin group. The preterm rate and low birth weight rate were higher in (3→2) group and twin group. SPR can increase neonatal body weight.

Consistent with other results [7, 20], this study suggests that the later SPR occurs, the worse neonatal outcomes are. Preterm rate and low birth weight rate were higher in 8-18 weeks group than in ≤8 weeks group. Only in one patient, SPR occurred after 18 gestational weeks and the neonate had 33^{+3} gestational weeks, birth weight of 1300 g and fallot tetrad.

Whether can SPR replace MFPR?

Early MFPR can effectively decrease complications and improve neonatal outcomes [21]. In this study, compared with triplet group, the mean birth weight and gestational weeks were significantly increased, and the preterm rate and low birth weight rate were significantly decreased in only MFPR group. The neonatal outcomes were similar in only MFPR group and (3→2) group. Our results are consistent with the results that MFPR can improve neonatal outcomes which are similar to that caused by SPR [22]. However, the sample size of triplets in this study was limited, so the conclusions above are for reference only.

In this study, SPR rate was 14.4% after MFPR. According to SPR occurrence after MFPR in triplets, this study was divided into SPR group and only MFPR group. Compared with only MFPR group, the birth weight was significantly increased ($P<0.05$), low birth weight rate and preterm rate were significantly decreased in SPR group (all $P<0.05$). SPR after MFPR can further improve neonatal outcomes in triplets. Compared with (3→1) group, the birth weight was similar, and the preterm rate and low birth weight rate were decreased in SPR group. Most SPR occurred within 8 gestational weeks in (3→1) group, but SPR occurrence was late in SPR group, affecting neonatal outcomes. SPR rate was high within 8 gestational weeks, so it is necessary to appropriately delay MFPR until 8 gestational weeks in order to reduce complications caused by MFPR.

Multiple pregnancies are a common complication in IVF/ ICSI-ET and SPR is common in multiple pregnancies [23, 24]. SPR rate is related to patients' age and the initial number of gestational sacs. SPR can improve pregnancy outcomes. The later SPR occurs, the worse neonatal outcomes are. Both SPR and MFPR can improve pregnancy outcomes. Due to SPR possibility, it is necessary to appropriately delay MFPR until 8 gestational weeks in order to reduce complications caused by MFPR, but SPR cannot completely replace MFPR. Improving embryo quality and reducing the number of embryo transfer are important for the safety of assisted reproductive technology.

Disclosure of conflict of interest

None.

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