IS THERE DIFFERENCE IN PERINATAL OUTCOME OF SINGLETON AND TWIN PREGNANCIES AFTER ASSISTED CONCEPTION: TWO-YEAR EXPERIENCE

Marko Vulić[1], Damir Roje[1], Zoran Meštrović[1], Tomislav Strinić[1], Ivica Stipić[1] and Ivona Vrkić[2]

[1]Clinical Department of Gynecology and Obstetrics, School of Medicine, University of Split; [2]School of Medicine, University of Split, Split, Croatia

SUMMARY – The aim of the study was to compare perinatal outcome of singleton and twin pregnancies conceived after assisted reproductive technologies (ART). This retrospective study included singleton and twin pregnancies conceived after ART in the period from January 1, 2007 until December 31, 2008. The study variables were maternal age (years), parity, body mass index (BMI; kg/m^2), week ($\leq 36^{6/7}$ and ≥ 37) and mode of delivery (vaginal and cesarean section), birth weight (grams) and APGAR score (≤ 7; 8-10). During the study period, there were 195 pregnancies after ART that fulfilled inclusion criteria. We found no between-group difference in parity (χ^2=0.0133; P=0.9081), but such difference was found in mean age (t=2.0486; P=0.0419) and BMI (χ^2=31.038; P=0.001). A statistically significant difference was recorded in preterm delivery rate (χ^2=25.539; P=0.001), average duration of pregnancy (t=12.8591; P=0.001), average birth weight (t=10.5446; P=0.001) and mode of delivery (χ^2=13,691; P=0.001). A statistically significant difference was found in low birth weight babies (χ^2=102.02; P=0.001) and APGAR score (χ^2=19.96; P=0.001), but there was no difference in the prevalence of small for gestational age babies (χ^2=0.90629; P=0.635). In conclusion, this study indicated the perinatal outcome after ART to be considerably poorer in twins than in singletons.

Key words: *Reproductive techniques, assisted; Pregnancy outcome*

Introduction

More than a million babies have been born in the last three decades since the first birth after assisted reproduction[1]. The incidence of birth after assisted reproduction technologies (ART) is rising across the world, particularly in developed countries. Today, one of 50 births is the result of ART[2,3]. The risks for pregnancies after ART have been mostly related to twin and higher order pregnancies, and include preterm delivery and neonatal complications associated with prematurity[4-6]. It is generally accepted that, unlike twin pregnancies, singleton pregnancies after ART have worse perinatal outcome compared to spontaneously conceived pregnancies[4,6,7]. The probable explanation for the worse perinatal outcome of singleton pregnancies is micromanipulation of the gametes, while transfer of two embryos is the most logical explanation for better outcome in twin pregnancies[8].

Perinatal risks associated with singleton pregnancies after ART are spontaneous abortion, preterm delivery, low birth weight (LBW), very low birth weight (VLBW) and small for gestational age (SGA) babies, increased perinatal mortality and cesarean section rate, higher incidence of congenital anomaly and higher admission rate in neonatal intensive care unit (NICU)[4-7,9].

The aim of the study was to compare perinatal outcome of singleton and twin pregnancies conceived after ART at Split University Hospital Center during a two-year period.

Materials and Methods

We conducted a retrospective study that included singleton and twin pregnancies after ART during the period from January 1, 2007 until December 31, 2008. Study variables were maternal age (years), parity, body mass index (BMI; kg/m²), week (≤36⁶/⁷ and ≥37) and mode of delivery (vaginal and cesarean section), birth weight (grams) and APGAR score (≤7, 8-10)[5,7,8]. Stillbirths, aneuploidy and congenital anomaly were excluded from the study. SGA babies were calculated from the tables of birth weights, which were adjusted for local specificity[10]. Gestational age at birth was calculated by adding 14 days to the day of ovum pick-up as the week of amenorrhea for the *in vitro* fertilization (IVF) group and by adding 14 days to the presumed day of ovulation for the ovulation induction group.

Statistical analysis was performed with Statistica SPSS version 13.0 (SPSS, Chicago, IL) using the Student's t-test and χ^2-test. The level of statistical significance was set at $P<0.05$.

Results

During the two-year period, 195 pregnancies met the inclusion criteria. The mean age of women with

Table 1. Maternal characteristics in singleton and twin pregnancies conceived after assisted reproductive technologies

Variable	Singleton, n (%) (n=150)	Twins, n (%) (n=45)	P-value
Age (yrs, χ±SD)	33.72±4.52	32.13±4.72	*P=0.0419 t=2.0486
Parity			
Primiparae	120 (80)	37 (82.2)	**P=0.9081
Multiparae	30 (20)	8 (17.8)	χ^2=0.0133
BMI (kg/m²)			
18.5-24.9	23 (15.3)	7 (15.4)	**P=0.001
25-29.9	122 (81.3)	24 (53.8)	χ^2=31.038
≥30	5 (3,4)	14 (30.8)	

*Student's t-test; **χ^2-test; BMI = body mass index

singleton pregnancies after ART was 33.72±4.52, while in twin pregnancy group it was 32.13±4.72. Difference in the mean age was statistically significant (t=2.0486; P=0.0419).

In the singleton pregnancy group, there were 120 primiparae and 30 pluriparae. In the twin pregnancy group, 37 women were primiparae and eight pluriparae. This difference was not statistically significant (χ^2=0.0133; P=0.9081). There was a difference in BMI between the groups (χ^2=31.038; P=0.0001) (Table 1).

In the singleton pregnancy group, ten of 150 women had preterm delivery. In the twin pregnancy group, 17 of 45 women had preterm delivery. Statistical analysis revealed a difference in the preterm delivery rate (χ^2=25.539; P=0.0001).

Variable	Singleton, n (%) (N=150)	Twins, n (%) (N=45)	P-value
Week of delivery			
term delivery (≥37)	140 (93.33)	28 (62.2)	*P=0.001
preterm delivery (≤36⁶/⁷)	10 (6.67)	17 (37.8)	χ^2=25.539
Birth weight (g, χ±SD)	3423.40±527.41	2435.5±625.12	**P=0.001 t=10.5446
Pregnancy duration (wk, χ±SD)	38.68±1.67	34.76±2.16	**P=0.001 t=12.8591
Delivery mode			
vaginal	86 (57.3)	11 (24.4)	*P=0.001
cesarean section	64 (42.7)	34 (75.6)	χ^2=13.691

*χ^2-test; **Student's t-test

Table 2. Perinatal outcome in singleton and twin pregnancies after assisted reproductive technologies

Variable	Singleton n (%) (n=150)	Twins n (%) (n=90)	P-value*
SGA	7 (4.7)	6 (6.7)	*P=0.635
AGA	135 (90)	81 (90)	χ^2=0.90629
LGA	8 (5.3)	3 (3.3)	
Birth weight (g)			
≤2499	6 (4)	58 (64.44)	*P=0.001
>2500	144 (96)	32 (35.56)	χ^2=102.02
APGAR score			
8-10	143 (95.3)	72 (80)	*P=0.001
≤7	7 (4.7)	18 (20)	χ^2=19.96

*χ^2-test; SGA = small for gestational age; AGA = appropriate for gestational age; LGA = large for gestational age

Table 3. Prevalence of small for gestational age, large for gestational age, low birth weight babies and APGAR score in singleton and twin pregnancies after assisted reproductive technologies

Differences were recorded in the mean birth weight (singleton 3423.4±52.41 *vs.* twins 2435.5±625.12; t=10.5446; *P*=0.0001) and mean pregnancy duration (singleton 38.68±1.67 *vs.* twins 34.768±2.16; t=12.8592; *P*=0.001).

In the singleton pregnancy group, 64 women had cesarean section, while in the twin group 34 had cesarean section. Statistical analysis yielded a difference in the cesarean section rate (χ^2=13.691, *P*=0.001) (Table 2).

In the singleton pregnancy group, seven of 150 babies were SGA, while in the twin group six of 90 were SGA. This difference was not statistically significant (χ^2=0.90629; *P*=0.635). We found difference in LBW babies (χ^2=102.02; *P*=0.001) and APGAR scores (χ^2=19.96; *P*=0.001) between the study groups (Table 3).

Discussion

In the present study, we demonstrated that twin pregnancies conceived after ART are at increased perinatal risks in terms of preterm delivery, mean birth weight, average pregnancy duration, cesarean section, LBW babies and APGAR score. We did not find increased prevalence of SGA babies in twins compared to singletons after ART. These results are consistent with the existing literature and show a satisfactory quality of perinatal care in our Department. Increased maternal age and higher BMI are well known risk factors for prematurity, LBW, VLBW and delivery mode[11,12]. Therefore, the main disadvan-

tage of our study was difference in the mean age and BMI between the study groups. In addition, data were missing on medical and social comorbidity, which are also associated with adverse perinatal outcome[11,12]. The lack of data on pregnancy induced hypertension, pregnancy aggravated hypertension, and those on glucose metabolism in pregnancy should also be noted, as all these are more common in pregnancies after ART[3,5,13]. Pregnancy induced hypertension in pregnancies after ART has an odds ratio (OR) 1.5 (95% CI 1.23-1.95). It seems that the risk of preeclampsia depends on the technique used for conception and is something lower after intracytoplasmic sperm injection (ICSI), with OR 1.30 (95% CI 1.11-1.52) compared to other techniques[14]. In most pregnancies, these entities have little or no effect on pregnancy outcome *per se*, but they increase the risk of other complications[5,15].

It is important to note that women in both groups were older than 30 years, which is consistent with data showing that couples who undergo assisted reproduction are on average about 5 years older than those who conceive spontaneously[14]. The average age of childbearing in recent decades has been increasing in western societies and never before had parents had their child as late as in recent decades. The reasons are complex and are beyond the scope of this study[16]. Data from our study confirm the same trends in our country.

As a rough estimate, IVF twins are born at the average gestational age three weeks lower than IVF singletons[9]. Our results showed an increased incidence of preterm delivery in twins after ART compared with singleton pregnancies, which is consistent with literature data[2,4,9]. In singleton group, the preterm delivery rate was 6.67%, which is comparable to the general population[11,12]. Other studies have shown that the risk

of preterm delivery in singleton pregnancy after ART is three times higher compared to those conceived spontaneously[17]. A possible explanation of these differences could be the higher mean age of this subpopulation (ART), more vigilant antenatal care and/or more detailed screening for risk factors for preterm delivery. Of special importance is iatrogenic prematurity in this subpopulation. It is still not clear whether clinical decisions regarding iatrogenic prematurity reflect overdiagnosing and/or fear from the possible medicolegal consequences.

Results regarding the mean pregnancy duration and birth weight were expected and reflected clear biological differences between singleton and twin pregnancies irrespective of the way of conceiving[9].

We expected a higher overall cesarean section rate. In our study, it was 50.26% (98/195) and was higher than reported in the literature. It is well known that cesarean section rates are considerably higher in IVF twins than in IVF singleton pregnancies, with the relative risk ranging from 1.5 to 3[4,9,18]. Our unpublished data showed that the incidence of cesarean section in singleton pregnancies after ART was 2.9 times higher compared to spontaneously conceived pregnancies. In this study, the incidence of cesarean section in twin pregnancies after ART was 75.6% and was higher than reported in the literature[19,20].

The possible explanations for the higher rate of cesarean section are advanced maternal age, higher BMI, increased rate of preterm delivery, and fear from medicolegal consequences, all of which have been previously published in the literature[19]. These women are older and it has been shown that with advancing maternal age, the overall cesarean rate has doubled from 21.0% at age 20 to 42% in women aged 35 or older[21,22]. BMI is a well-known risk factor for cesarean section[20-22]. In our investigation, more than 80% of women had BMI >25 in both groups. In the USA, one in three nulliparous women was delivered by cesarean section, and increased BMI also contributed to this high rate[22]. The possible explanation for the overall higher cesarean section rate could be the increased preterm delivery rate in the twin group[13]. In the USA, 65.9% of multifetal gestations were delivered by cesarean section regardless of the way of conceiving[22,23]. Nevertheless, vaginal delivery was not even attempted in the majority of multifetal gesta-

tions. The cesarean section rate was higher at earlier gestational ages of delivery. In the USA, the cesarean delivery rate at 28 weeks of gestation was over 60% and declined gradually with advancing gestation[20,22]. It is important to note that evidence based medicine failed to show protective impact of cesarean section on perinatal outcome in preterm delivery[23]. The most logical explanation for the increased cesarean section rate is that the combination of fear from the possible medicolegal consequences, pressure of the surroundings, and the way of conceiving influence the decision for cesarean section.

Birth weight is generally associated with gestational age. Twins have an increased risk of preterm delivery[11]. As a rough estimate, IVF twins are born with a mean birth weight ranging between 800 and 1000 g lower than singletons after ART[9]. Our study failed to show an increased incidence of SGA babies after ART in either singleton or twin pregnancies. The more so, we demonstrated the protective impact of ART (relative risk (RR) 2.12 for singletons and 1.49 for twins). In this subpopulation of women, there should be a higher incidence of women with polycystic ovary syndrome[24]. It is characterized by insulin resistance and an increased risk of impaired glucose metabolism in pregnancy, and consequently macrosomic and large for gestational age (LGA) babies[24]. So, we expected a higher incidence of LGA in both groups. Our data showed that RR for LGA babies after ART in both groups was lower than expected (1.88 for singletons and 3 for twins). These data should be taken with caution until further researches.

As we expected, there was a higher incidence of LBW babies in the twin group. This is a logical consequence of the increased incidence of preterm delivery in this group and shorter mean pregnancy duration. A recent meta-analysis showed an increased RR for LBW and VLBW babies (1.7 and 2.7-3, respectively)[4,18].

To sum up, regardless of the objective shortcomings of our study, it indicated the perinatal outcome after ART in twins to be considerably poorer than in singletons. This reflects biologic differences between singleton and twin pregnancies irrespective of the way of conceiving. ART has not been shown as a risk factor for the worse perinatal outcome. Future studies should also focus on the incidence of congenital anomalies in these pregnancies.

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Sažetak

POSTOJI LI RAZLIKA U PERINATALNOM ISHODU JEDNOPLODNIH I BLIZANAČKIH TRUDNOĆA NAKON MEDICINSKI POTPOMOGNUTE OPLODNJE

M. Vulić, D. Roje, Z. Meštrović, T. Strinić, I. Stipić i I. Vrkić

Cilj ove studije bio je istražiti razliku u perinatalnom ishodu jednoplodnih i blizanačkih trudnoća nakon medicinski potpomognute oplodnje (MPO). Ispitana je medicinska dokumentacija jednoplodnih i blizanačkih trudnoća u žena koje su rodile živorođenu djecu nakon MPO u dvogodišnjem razdoblju u Klinici za ženske bolesti i porode, KBC Split, od 1. siječnja 2007. do 31. prosinca 2008. godine. Istraživane varijable bile su dob, paritet, indeks tjelesne mase (kg/m^2), navršeni tjedan poroda (prijevremeni ≤36$^{6/7}$ i terminski ≥37), način dovršetka porođaja (vaginalno i carski rez), porođajna masa djeteta (grami), trofičnost djeteta (hipertrofično, eutrofično i hipotrofično) i APGAR zbroj novorođenčeta (≤7, 8-10). U promatranom razdoblju 195 trudnica je rodilo nakon neke od metoda MPO. Između rodilja s jednoplodnom i blizanačkom trudnoćom koje su zanijele nekom metodom MPO nije bilo razlike u paritetu (χ^2=0,0133; *P*=0,9081), dok su razlike pronađene u prosječnoj dobi (t=2,0486; *P*=0,0419) i indeksu tjelesne mase (χ^2=31,038; *P*=0,001). Statistički značajna razlika nađena je u učestalosti prijevremenog poroda (χ^2=25,539; *P*=0,001), prosječnom trajanju trudnoće (t=12,8591; *P*=0,001) i prosječnoj porodnoj masi djece (t=10,5446; *P*=0,001). Blizanačke trudnoće u usporedbi s jednoplodnim trudnoćama nakon MPO češće se dovršavaju carskim rezom (χ^2=13,691; *P*=0,001). Pronađena je statistički značajna razlika između istraživanih skupina u rađanju djece niske porodne mase (<2500 g) (χ^2=102,02; *P*=0,001) i APGAR zbroju novorođenčadi (χ^2=19,96; *P*=0,001). Nije bilo statistički značajne razlike u učestalosti rađanja hipotrofične djece između istraživanih skupina (χ^2=0,90629; *P*=0,635). U zaključku, perinatalni ishod nakon MPO je lošiji u blizanačkih nego u jednoplodnih trudnoća.

Ključne riječi: *Trudnoća, ishod; Začeće, potpomognuto, metode*