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The influence of ovulation induction and assisted conception on maternal and perinatal outcomes of twin pregnancies

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Abstract. – OBJECTIVE: To compare obstetrical and neonatal outcomes of twin pregnancies conceived via assisted reproductive technology (ART) with those of naturally conceived ones and to investigate the influence of the ART procedure type on these parameters.

PATIENTS AND METHODS: This observational study included 450 ART and 647 spontaneous twin pregnancies delivered over 15 years at a single university-based hospital of Rome, Italy. Logistic and linear regression models adjusted for confounding factors were used to evaluate the effect of ART and the type of assisted conception (IVF/ICSI, ovulation induction ± intrauterine insemination, egg/embryo donation) on maternal and perinatal outcomes.

RESULTS: The mean gestational age was significantly lower in pregnancies conceived via ART. The occurrence rates of gestational diabetes, antenatal admission, prophylactic administration of corticosteroid, very preterm delivery and neonatal intensive care unit admission were higher in the ART group. Twin pregnancies achieved via egg/embryo donation had a lower risk of maternal thrombocytopenia and cervical incompetence and were at greater risk of receiving corticosteroid prophylaxis and patent ductus arteriosus than pregnancies obtained by IVF/ICSI. Conception by ovulation induction was associated with reduced risk of hyperemesis gravidarum and longer neonatal hospitalization compared to pregnancies obtained by IVF/ICSI.

CONCLUSIONS: Assisted conception was associated with adverse obstetrical outcomes and lower gestational age, but after adjustment for gestational age neonatal immediate outcomes were similar to those observed in the spontaneous group. There were no many important differences in the outcomes of twin pregnancies obtained by a different type of conception.

Key Words:
Assisted reproductive techniques, Ovulation induction, Infertility, Twin pregnancy, Obstetrics, Perinatal outcomes.

Introduction

The multiple birth rate has increased since 1980 in all developed countries. In the United States, it rose from 2.93% in 2000 to 3.37% in 2013[1]. The increase in twin birth rate has been associated with widespread use of fertility treatments and older maternal age at childbearing. The average age of women giving birth in Europe has risen in the past decades. Thus, the motherhood postponement has become an issue related to female reproductive health and is considered to be the main factor of the increasing use of assisted reproductive technology (ART)[2]. Therefore, whereas it is fully documented that ART is a risk factor for the onset of twins, multiple pregnancy has become one of the most undesirable consequences of ART because of the relationship with several maternal and neonatal complications including pregnancy induced hypertension, preeclampsia, gestational diabetes mellitus, preterm delivery, low birth weight, perinatal mortality, respiratory disorders, and cerebral palsy[3]. Also, many studies indicate that ART could be responsible for obstetrical and perinatal complications[2,4-9]. It has been reported that women undergoing infertility treatment are more likely to develop pre-eclampsia, gestational diabetes and undergo premature birth and cesarean section, with a higher risk of low birth weight and perinatal

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mortality[10]. Nevertheless, there are many disputes concerning the influence of assisted conception and several studies have shown the inconsistent effect of ART on maternal and fetal outcomes[11,12]. It has also been suggested that subfertility *per se* makes an additional contribution to some of these poorer outcomes and considering that infertility is a heterogeneous condition, caused by various underlying pathologies, it is possible that some of the mechanisms leading to infertility also play a role in the etiology of these outcomes[13-15].

We already reported data on 345 dichorionic twin pregnancies conceived via ART and spontaneously, showing that the first group was at greater risk of poor outcomes, including placental abruption and patent ductus arteriosus[16]. We have now enlarged our sample, including also monochorionic twin pregnancies, to compare the obstetrical and neonatal outcome of twin pregnancies conceived via ART with those of naturally conceived ones and to determine the influence of the ART procedure type on these parameters.

Patients and Methods

Between January 2001 and June 2015 we collected data on ART or spontaneously conceived twin pregnancies delivered at San Pietro Fatebenefratelli Hospital of Rome, a university-affiliated tertiary obstetrical care center. The study was reviewed and approved by the Institutional Review Board.

We included pregnancies ending by spontaneous delivery after 24 weeks of gestation. We excluded spontaneous gestations with a discrepancy in menstrual and ultrasonographic gestational age more than 7 days, triplet pregnancies reduced to twin ones and pregnancies with insufficient clinical data.

For each pregnancy included in the study, obstetrical and perinatal data were collected from maternal and neonatal medical records. The International Statistical Classification of Diseases and Related Health Problems 10th Revision (ICD-10)-WHO was used to identify pregnancy, childbirth, and puerperium diseases[17].

The type of conception (ART or spontaneous) was recorded. ART procedures were furthermore divided into three groups: *in vitro* fertilization (IVF) or (ICSI); egg or embryo donation; ovarian induction (OI) with or without intrauterine insemination (IUI).

The gestational age was calculated basing on the last menstrual period (confirmed by first trimester ultrasound estimate) for spontaneous pregnancies and by adding two weeks to the date of embryo transfer for ART pregnancies. Preterm delivery and very preterm delivery were defined as delivery before 37 and 32 weeks respectively. Birth weights below 2500 g, 1500 g, and 1000 g were classified as low birth weight (LBW), very low birth weight (VLBW) and extremely low birth weight (ELBW) respectively. Newborns with birth weight below the 10th percentile for gestational age were considered small for gestational age (SGA).

Statistical Analysis

Mean plus minus Standard Deviation (SD) for quantitative variables and number and percentages for categorical variables are reported to describe the study population.

To study the effect of the use of ART or specific ART procedures on obstetrical and neonatal outcomes we used multivariable logistic regression models for analysis of categorical outcomes and linear regression models for continuous ones.

Analysis of obstetrical outcomes was performed by adjusting the model for the age of the mother at delivery, active smoking, nulliparity, pre-existing chronic diseases (hypertension, diabetes mellitus, hypothyroidism, thrombophilia), chorionicity. To analyze perinatal outcomes, two models were built: model A was adjusted for age of the mother at delivery, active smoking, nulliparity, pre-existing chronic diseases, chorionicity, newborn sex; model B was adjusted for the same factor but also for gestational age. Model B was used to calculate ART type effect on perinatal outcomes.

A Bayesian regression technique based on an un-informative Cauchy prior distribution was used, in order to better manage analyses with rare outcomes and complete or quasi-complete predictor outcome separation that would result in artificially extreme estimates[18].

Non-parametric bootstrap with 10,000 repetitions was applied to the regression analysis to compute the empirical variability of the estimates, especially useful in the case of non-normal distribution of the effects and with rare outcomes[19]. Effect sizes are reported as adjusted Odds Ratio (aOR) for logistic regressions and as adjusted mean difference (adjM) for linear regressions. Bias-corrected and accelerated bootstrap 95% confidence intervals (BCa 95% CIs) are reported for inference purposes[20]. An effect size is reported as significant when its BCa 95% CI do not contain the null effect value. We also

reported standardized effect sizes (sES), defined as the ratio between Mean and SD of the bootstrap distributions of an effect size, as a tool to assess the relative inferential strength of the association between predictors on the outcomes (the further from zero is the absolute value, the more significant the association).

In the analysis, we preferred to report effect sizes and confidence intervals instead of *p*-values, because they provide an estimation of the importance of an effect and the degree of uncertainty around such estimation. In this choice, we tried to conform to a growing tendency in the current literature which is promoted by many scientific associations and journals[21,22].

Results

In the study period, 1,241 twin pregnancies (2.3% of all deliveries) were delivered at our hospital. After application of the exclusion criteria, 1,097 twin pregnancies were considered.

According to conception type, pregnancies were divided into two groups:

1. 450 (41%) conceived via ART procedures: 377 (83.8%) IVF/ICSI; 37 (8.2%) OI ± IUI; 36 (8.0%) egg/embryo donation)

2. 647 (59%) conceived spontaneously.

Table I. Maternal characteristics in assisted reproductive technique and spontaneous twin conception.

	ART (n = 450)	Spontaneous (n = 647)
Age mean ± SD (years)	37.5 ± 5.6	33.4 ± 4.9
Nulliparity (%)a	421 (93.6)	337 (52.1)
Previous miscarriage (%)	116 (25.8)	148 (22.9)
Smoke (%)	34 (7.6)	98 (15.2)
Systemic disease (%)	106 (23.6)	75 (11.6)
Monochorionicity (%)	21 (4.7)	189 (29.2)

Women who conceived via ART were older (37.5 ± 5.6 *vs.* 33.4 ± 4.9 years), more often nulliparous (93.6% *vs.* 52.1%), more likely to have previous abortions (25.8% *vs.* 22.9%) and to be affected by pre-existing systemic diseases (23.6% *vs.* 11.6%), less likely to smoke during pregnancy (7.6% *vs.* 15.2%) and to have monochorionicity (4.7% *vs.* 29.2%) than women who conceived spontaneously (Table I).

The results of the logistic regression model concerning obstetrical outcomes are presented in Table II. The mean gestational age was significantly lower in the ART group compared to controls (adjM -5.25, BCa 95% CIs -8 to -2.66). The incidence rates of gestational diabetes (aOR 2.28, BCa 95% CIs 1.27-4.21), antenatal admission (aOR 1.6, BCa 95% CIs 1.09-2.3), prophylactic

Table II. Comparison of pregnancy outcome between ART and sponeaneous groups.

	ART (n = 450)	Spontaneous (n = 647)	aOR[1]
Gestational hypertension (%)	88 (19.6)	88 (13.6)	0.92
Gestational diabetes mellitus (%)	38 (8.4)	18 (2.8)	2.28
Cholestasis (%)	48 (10.7)	44 (6.8)	1.22
Hyperemesis (%)	8 (1.8)	27 (4.2)	0.47
Cerclage (%)	8 (1.8)	17 (2.6)	1.04
Placental abruption (%)	24 (5.3)	17 (2.6)	2.4
Placenta previa (%)	12 (2.7)	5 (0.8)	2.97
Premature rupture of membranes (%)	107 (23.8)	137 (21.2)	1.18
Intrauterine growth restriction (%)	62 (13.8)	82 (12.7)	1.18
Thrombocytopenia (%)	12 (2.7)	15 (2.3)	0.97
Twin-twin transfusion syndrome (%)	1 (0.2)	21 (3.2)	0.27
Amniotic fluid alterations (%)[2]	38 (8.4)	54 (8.3)	0.92
Antenatal admission (%)	103 (22.9)	128 (19.8)	1.6
Corticosteroid prophylaxis (%)	195 (43.3)	223 (34.5)	1.67
Very preterm delivery (%)	40 (8.9)	45 (7)	2.24
Cesarean section (%)	425 (94.4)	559 (86.4)	1.32
Postpartum hemorrhage (%)	29 (6.4)	26 (4)	1.29
Intensive care unit admission (%)	11 (2.4)	5 (0.8)	2.11
			adjM[1]
Gestational age mean ± SD (days)	247.7 ± 18.7	250.9 ± 17.4	-5.25
Length of hospitalization mean ± SD (days)	9.1 ± 7.7	7.9 ± 6.4	0.79

[1]Adjusted for maternal age, smoke, nulliparity, systemic diseases, chorionicity.[2]Included oligohygramnios and polyhydramnios.

administration of corticosteroid (aOR 1.67, BCa 95% CIs 1.2-2.23), and very preterm delivery (aOR 2.24, BCa 95% CIs 1.24-4.16) were higher in women who conceived via ART.

Table III depicts the influence of ART conception on perinatal outcomes of 2194 neonates included in the study (900 ART conceived and 1294 spontaneous twins). Model A showed that ART neonates were at greater risk to be LBW (aOR 1.47, BCa 95% CIs 1.17-1.83), VLBW (aOR 1.84, BCa 95% CIs 1.19-2.81) and ELBW (aOR 3.1, BCa 95% CIs 1.38-7.06) and had lower mean birth weight (adjM -116, BCa 95% CIs -169 to -60.7) and length (adjM -0.44, BCa 95% CIs -0.83 to -0.05). ART twins had also higher risk of low Apgar at 5 minutes (aOR 1.82, BCa 95% CIs 1.09-3.01), jaundice (aOR 1.33, BCa 95% CIs 1.03-1.71), respiratory complications (aOR 1.74, BCa 95% CIs 1.31-2.32), neurological complications (aOR 2.76, BCa 95% CIs 1.41-5.31), and patent *ductus arteriosus* (aOR 2.03, BCa 95% CIs 1.01-4.1) compared to spontaneous twins. In addition, ART group showed an increased rate of NICU admission (aOR 1.95, BCa 95% CIs 1.51-2.49). Model B showed that assisted conception, independently from gestational age, was only associated with increased risk of NICU admission (aOR 1.5, BCa 95% CIs 1.07-2.1).

ART type effect on obstetrical and perinatal outcomes are reported in Tables IV and V. Twin pregnancies achieved via egg/embryo donation were at lower risk of thrombocytopenia (aOR 0.26, BCa 95% CIs 0.12-0.42) and cervical incompetence (aOR 0.56, BCa 95% CIs 0.33-0.77) than pregnancies obtained by IVF/ICSI, while had an increased risk of receiving corticosteroid prophylaxis (aOR 2.13, BCa 95% CIs 1.01-4.64) and patent *ductus arteriosus* (aOR 4.13, BCa 95% CIs 1.32-16.7). Conception by OI ± IUI was associated with reduced risk of *hyperemesis gravidarum*

Table III. Comparison of birth outcome and neonatal complications between ART and spontaneous twins.

	ART twins (n = 900)[1]	Spontaneous twins (n= 1294)[1]	aOR[2]	BCa 95% CIs	sES	aOR[3]	BCa 95% CIs	sES
Stillbirth (%)	7 (0.8)	14 (1.1)	0.99	0.36, 2.44	-0.05	0.49	0.17, 1.45	-1.39
Apgar 5' < 7 (%)	48 (5.3)	54 (4.2)	1.82	1.09, 3.01	2.35	0.76	0.4, 1.43	-0.84
Low birth weight (%)	601 (66.8)	749 (57.9)	1.47	1.17, 1.83	3.49	1.19	0.92, 1.54	1.35
Very low birth weight (%)	73 (16.2)	78 (6)	1.84	1.19, 2.81	2.8	0.85	0.49, 1.47	-0.54
Extremely low birth weight (%)	27 (3)	23 (1.8)	3.1	1.38, 7.06	2.77	1.09	0.42, 2.89	0.19
Small for gestational age (%)	148 (16.4)	230 (17.8)	0.9	0.68, 1.16	-0.78	0.99	0.75, 1.31	-0.01
Congenital anomalies (%)	55 (6.1)	83 (6.4)	0.97	0.59, 1.6	-0.12	0.89	0.54, 1.49	-0.47
Respiratory disorders (%)[4]	197 (21.9)	199 (15.4)	1.74	1.31, 2.32	3.84	1.17	0.82, 1.66	0.9
Neurological complications (%)[5]	42 (4.7)	28 (2.2)	2.76	1.41, 5.31	3.07	1.84	0.97, 3.42	1.88
Infectious diseases (%)	40 (4.4)	31 (2.4)	1.56	0.91, 2.66	1.64	1.25	0.72, 2.18	0.82
Jaundice (%)	217 (24.1)	263 (20.3)	1.33	1.03, 1.71	2.17	1.05	0.8, 1.38	0.36
Necrotizing enterocolitis (%)	10 (1.1)	10 (0.8)	1.56	0.61, 3.92	0.96	1.01	0.37, 2.51	0.01
Haematological disorders (%)[6]	58 (6.4)	84 (6.5)	1.59	0.98, 2.57	1.88	0.97	0.6, 1.63	-0.15
Hypoglycemia (%)	49 (5.4)	87 (6.7)	0.75	0.47, 1.2	-1.2	0.69	0.43, 1.1	-1.58
Patent ductus arteriosus (%)	28 (3.1)	21 (1.6)	2.03	1.01, 4.1	2.03	0.93	0.43, 2.07	-0.2
Patent ovale foramen (%)	27 (3)	24 (1.9)	1.62	0.81, 3.69	1.28	1.15	0.58, 2.4	0.4
NICU admission (%)	239 (26.6)	225 (17.4)	1.95	1.51, 2.49	5.25	1.5	1.07, 2.1	2.41
Neonatal mortality (%)[7]	8 (0.9)	8 (0.6)	1.9	0.62, 6.16	1.13	0.73	0.21, 2.44	-0.56
			adjM[2]	BCa 95% CIs	sES	adjM[3]	BCa 95% CIs	sES
Birth weight mean ± SD (grams)	2249.2 ± 515	2333.4 ± 494	-116	-169, -60.7	-4.21	- 5.08	-38.6, 29	-0.32
Birth length mean ± SD (cm)	45.4 ± 3.5	45.6 ± 3.3	-0.44	-0.83, -0.05	-2.28	0.22	-0.02, 0.46	1.78
Length of hospitalization mean ± SD (days)	11.7 ± 13.7	9.9 ± 12.1	2.21	0.79, 3.87	2.81	0.2	-0.77, 1.13	0.41

[1]7 ART stillbirth neonates and 14 spontaneous stillbirth neonates were excluded from the comparison of neonatal outcomes. [2]Adjusted for maternal age, smoke, nulliparity, systemic diseases, chorionicity and neonatal sex. [3]Adjusted for maternal age, smoke, nulliparity, systemic diseases, chorionicity, neonatal sex and gestational age. [4]Included respiratory distress syndrome, transient tachypnea of the newborn and neonatal apnea, pneumotorax, bronchopulmonary dysplasia, pulmonary haemorrhage. [5]Included intracerebral haemorrhage, intraventricular haemorrhage, hydrocephalus. [6]Included anemia, polycythemia, thrombocytopenia, piastrinosis, neutropenia. [7]66 ART and 76 spontaneous neonates who were transferred to other hospital were excluded from the comparison of neonatal mortality.

Table IV. Adjusted effect of ART type on obstetrical outcomes.

	Ovodonation (n=37) vs IVF/ICSI (n=377) [1]			Ovarian stimulation ± IUI (n=36) vs IVF/ICSI (n=377)[1]		
	Groups' stratification [2]	aOR (BCa 95% CIs)	sES	Groups' stratification [2]	aOR (BCa 95% CIs)	sES
Gestational hypertension	15 (40.5%) vs 70 (18.6%)	1.65 (0.73, 3.6)	1.25	3 (8.3%) vs 70 (18.6%)	0.5 (0.15, 1.54)	-1.22
Gestational diabetes	4 (10.8%) vs 33 (8.8%)	0.72 (0.21, 2.57)	-0.63	1 (2.8%) vs 33 (8.8%)	0.42 (0.1, 2.37)	-1.37
Cholestasis	2 (5.4%) vs 43 (11.4%)	0.44 (0.07, 1.85)	-1.25	3 (8.3%) vs 43 (11.4%)	0.79 (0.23, 2.59)	-0.52
Hyperemesis	2 (5.4%) vs 6 (1.6%)	2.5 (0.36, 25.6)	0.78	0 (0%) vs 6 (1.6%)	0.39 (0.18, 0.6)	-3.32
Cerclage	0 (0%) vs 7 (1.9%)	0.56 (0.33, 0.77)	-2.76	1 (2.8%) vs 7 (1.9%)	1.08 (0.18, 18.1)	-0.16
Placental abruption	2 (5.4%) vs 20 (5.3%)	1.66 (0.26, 11.3)	0.34	2 (5.6%) vs 20 (5.3%)	0.86 (0.12, 3.89)	-0.38
Placenta previa	1 (2.7%) vs 10 (2.7%)	0.83 (0.17, 9.52)	-0.4	1 (2.8%) vs 10 (2.7%)	1.21 (0.26, 11.2)	-0.07
Premature rupture of membranes	8 (21.6%) vs 88 (23.3%)	1.1 (0.42, 2.68)	0.14	11 (30.6%) vs 88 (23.3%)	1.32 (0.57, 2.92)	0.66
Intrauterine growth restriction	5 (13.5%) vs 50 (13.3%)	1.05 (0.36, 2.98)	-0.05	7 (19.4%) vs 50 (13.3%)	1.49 (0.6, 3.53)	0.76
Thrombocytopenia	0 (0%) vs 9 (2.4%)	0.26 (0.12, 0.42)	-4.35	3 (8.3%) vs 9 (2.4%)	3.63 (0.55, 18.5)	1.36
Twin-twin transfusion syndrome	0 (0%) vs 1 (0.3%)	0.79 (0.48, 1)	-1.16	0 (0%) vs 1 (0.3%)	0.72 (0.34, 1)	-1.13
Amniotic fluid alterations	4 (10.8%) vs 30 (8%)	1.04 (0.3, 3.68)	-0.08	4 (11.1%) vs 30 (8%)	1.49 (0.47, 4.73)	0.45
Antenatal admission	5 (13.5%) vs 90 (23.9%)	0.63 (0.22, 1.74)	-0.94	8 (22.2%) vs 90 (23.9%)	0.88 (0.36, 2.14)	-0.37
Corticosteroid prophylaxis	23 (62.1%) vs 154 (40.8%)	2.13 (1.01, 4.64)	1.99	18 (50%) vs 154 (40.8%)	1.43 (0.66, 2.96)	0.97
Very preterm delivery	4 (10.8%) vs 31 (8.2%)	1.74 (0.45, 6.19)	0.69	5 (13.9%) vs 31 (8.2%)	1.46 (0.43, 4.22)	0.55
Cesarean section	36 (97.3%) vs 354 (93.9%)	1.02 (0.12, 5)	0.28	35 (97.2%) vs 354 (93.9%)	2.52 (0.45, 12.3)	1.36
Postpartum hemorrhage	3 (8.1%) vs 25 (6.6%)	0.83 (0.18, 3.06)	-0.42	1 (2.8%) vs 25 (6.6%)	0.48 (0.11, 2.76)	-1.15
Intensive care unit admission	1 (2.7%) vs 9 (2.4%)	0.82 (0.18, 7.49)	-0.46	1 (2.8%) vs 9 (2.4%)	1.26 (0.27, 11.8)	-0.01
	Groups' means [3]	adjM (BCa 95% CIs)	sES	Groups' means [3]	adjM (BCa 95% CIs)	sES
Gestational age (days)	244 ± 20.2 vs 248.5 ± 17.6	-5.85 (-13.8, 0.61)	-1.61	243.7 ± 26.5 vs 248.5 ± 17.6	-4.16 (-14.2, 3.63)	-0.93
Length of hospitalization (days)	8.3 ± 4.8 vs 9 ± 7.7	-1.39 (-3.29, 0.24)	-1.41	11.2 ± 9.4 vs 9 ± 7.7	2.3 (-0.13, 6.28)	1.33

[1]IVF/ICSI is the baseline. Analysis adjusted for maternal age, smoke, nulliparity, systemic diseases, chorionicity. [2]Number of cases (%) in the ovodonation or ovarian stimulation ± IUI group having the condition vs number of cases (%) in the IVF/ICSI group. [3]Mean ± SD of the continuous variables in the ovodonation or ovarian stimulation ± IUI group vs the IVF/ICSI group.

(aOR 0.39, BCa 95% CIs 0.18-0.6) and longer neonatal hospitalization (aOR 3.17, BCa 95% CIs 1.2-5.4) compared to IVF/ICSI group. Both egg/embryo donation and OI ± IUI groups had a trend of lower risk of twin-twin transfusion syndrome than the baseline group (aOR 0.79, BCa 95% CIs 0.48-1 and aOR 0.72, BCa 95% CIs 0.34-1, respectively) but the estimates are unstable due to the extreme rarity of this condition, since there was only one case in IVF/ICSI group. In addition, OI

Table V. Adjusted effect of ART type on perinatal outcomes.

	Ovodonation (n=74) vs IVF/ICSI (n=754)[1]			Ovarian stimulation ± IUI (n=72) vs IVF/ICSI (n=754)[1]		
	Groups' stratification [2]	aOR (BCa 95% CIs)	sES	Groups' stratification [2]	aOR (BCa 95% CIs)	sES
Stillbirth	1 (1.4%) vs 4 (0.5%)	1.11 (0.09, 58.8)	-0.01	2 (2.8%) vs 4 (0.5%)	1.56 (0.37, 5.46)	0.49
Apgar 5' < 7	5 (6.8%) vs 35 (4.6%)	0.83 (0.13, 3.59)	-0.29	8 (11.1%) vs 35 (4.6%)	1.23 (0.53, 3.15)	0.21
Low birth weight	53 (71.6%) vs 501 (66.5%	0.97 (0.53, 1.84)	-0.09	47 (65.3%) vs 501 (66.5%)	1.08 (0.58, 2.09)	0.24
Very low birth weight	9 (12.2%) vs 53 (7%)	1.19 (0.46, 4.01)	0.23	11 (15.3%) vs 53 (7%)	1.81 (0.61, 6.54)	0.95
Extremely low birth weight	3 (4.1%) vs 18 (2.4%)	0.83 (0.2, 3.34)	-0.39	6 (8.3%) vs 18 (2.4%)	1.71 (0.73, 4.79)	1.03
Small for gestational age	8 (10.8%) vs 123 (16.3%)	0.72 (0.32, 1.55)	-0.91	17 (23.6%) vs 123 (16.3%)	1.72 (0.95, 3.21)	1.67
Congenital anomalies	2 (2.7%) vs 39 (5.2%)	0.63 (0.1, 2.39)	-0.84	6 (8.3%) vs 39 (5.2%)	1.45 (0.57, 3.63)	0.6
Respiratory disorders	21 (28.4%) vs 160 (21.2%	1 (0.46, 2.04)	-0.03	16 (22.2%) vs 160 (21.2%)	0.81 (0.42, 1.51)	-0.74
Neurological complications	4 (5.4%) vs 29 (3.8%)	1.43 (0.44, 4.38)	0.46	9 (12.5%) vs 29 (3.8%)	2.67 (0.996, 6.94)	1.93
Infectious diseases	3 (4.1%) vs 32 (4.2%)	0.56 (0.16, 1.91)	-1.01	5 (6.9%) vs 32 (4.2%)	1.58 (0.52, 4.49)	0.66
Jaundice	17 (23%) vs 78 (23.6%)	0.67 (0.37, 1.22)	-1.43	22 (30.6%) vs 78 (23.6%)	1.41 (0.76, 2.53)	1.07
Necrotizing enterocolitis	2 (2.7%) vs 6 (0.8%)	1.9 (0.22, 24.9)	0.53	2 (2.8%) vs 6 (0.8%)	2.43 (0.33, 25.7)	0.72
Haematological disorders	7 (9.5%) vs 44 (5.8%)	1.16 (0.4, 3.59)	0.2	7 (9.7%) vs 44 (5.8%)	1.63 (0.44, 4.85)	0.67
Hypoglicemia	4 (5.4%) vs 39 (5.2%)	0.78 (0.22, 2.62)	-0.5	6 (8.3%) vs 39 (5.2%)	1.82 (0.65, 4.68)	0.99
Patent ductus arteriosus	7 (9.5%) vs 15 (2%)	4.13 (1.32, 16.7)	2.26	6 (8.3%) vs 15 (2%)	2.18 (0.75, 7.04)	1.36
Patent ovale foramen	5 (6.8%) vs 18 (2.4%)	1.55 (0.37, 5.84)	0.56	4 (5.6%) vs 18 (2.4%)	1.52 (0.48, 4.5)	0.53
NICU admission	34 (45.9%) vs 246 (32.6%	1.39 (0.68, 2.83)	0.89	25 (34.7%) vs 246 (32.6%)	1.1 (0.55, 2.41)	0.18
Neonatal mortality	1 (1.4%) vs 4 (0.5%)	1.19 (0.34, 42.5)	0.1	3 (4.2%) vs 4 (0.5%)	5.26 (0.48, 262)	1.29
	Groups' means [3]	adjM (BCa 95% CIs)	sES	Groups' means [3]	adjM (BCa 95% CIs)	sES
Birth weight (grams)	2166.8 ± 547.3 vs 2266.4	-4.67 (-72.5, 61.3)	-0.13	2150.3 ± 625.8 vs 2266.4 ± 499.2	-53.2 (-133, 28.4)	-1.27
Birth length (cm)	44.6 ± 3.7 vs.45 ± 3.4	-0.41 (-0.99, 0.15)	-1.4	44.9 ± 4 vs 45.5 ± 3.4	-0.25 (-0.82, 0.3)	-0.89
Length of hospitalization (days)	14 ± 16.7 vs 11.1 ± 12.9	0.57 (-1.61, 2.98)	0.52	15.4 ± 17.5 vs 11.1 ± 12.9	3.17 (1.2, 5.4)	2.97

[1]IVF/ICSI is the baseline. Analysis adjusted for maternal age, smoke, nulliparity, systemic diseases, chorionicity, neonatal sex and gestational age.[2]Number of cases (%) in the ovodonation or ovarian stimulation ± IUI group having the condition vs number of cases (%) in the IVF/ICSI group.[3]Mean ± SD of the continuous variables in the ovodonation or ovarian stimulation ± IUI group vs the IVF/ICSI group.

± IUI twins showed a trend of increased risk of neurological complications compared to the IVF/ICSI group (adjM 2.67, BCa 95% CIs 0.996-6.94).

Discussion

The large number of multiple pregnancies occurring after infertility treatment has been a major obstacle from the early days of ART, because they are associated with an increased risk of maternal and perinatal complications as well as higher health care costs than singleton ones. The major concern is whether the twinning itself is the main risk parameter or if IVF techniques (i.e. laboratory procedures, ovarian stimulation, embryo culture media and time) and subfertility (including the parental characteristics *per se*) could have negative impacts on the ART twins.

We already reported data about 345 dichorion di-amniotic twin pregnancies conceived spontaneously and via ART showing lower gestational age and birth weight and higher prevalence of placental abruption and patent *ductus arteriosus* in the group of assisted conception[16]. We have now broaded the sample including also monochorionic pregnancies to apply the results to the general population of twin pregnancies.

Our results confirmed that ART use was more frequent in older and nulliparous women with history of previous miscarriages and underlying chronic diseases. These patients carry additional age- and parity-related risks, which are recognized predictor parameters of pregnancy complications. Different studies have reported that women with untreated subfertility who became pregnant had a greater frequency of adverse outcomes than the general population, suggesting that the underlying conditions of the infertile couples may be associated with pathogenetic mechanisms which interfere with both fertility and pregnancy course [8, 14, 23].

In the present study ART twin pregnancies had a higher risk of adverse obstetrical outcomes even after adjustment for maternal characteristics, such as age, parity, and systemic diseases. ART conception was associated with lower gestational age and increased risk of very preterm delivery, gestational diabetes mellitus and placental abruption compared to spontaneous pregnancies. We also found that women with induced pregnancy were at greater risk to be admitted to the hospital before delivery and they were more likely to receive corticosteroid prophylaxis.

The reason for the increased risk of preterm birth following assisted reproduction technique is still unclear. Whereas no arguments exist as to the role of infertility treatment in increasing the rate of preterm birth by increasing the multiple birth rate, some data suggest an infertility technology-related cause, showing higher rate of preterm birth in couples with female factor infertility (mainly treated with IVF) compared with couples with male factor infertility, mainly treated with ICSI[8]. Other data indicate that infertility rather than its treatment is the cause, suggesting a patient-related increased risk in preterm birth of infertile women in need of ART[24]. At the same time, a potential treatment bias, such as iatrogenic preterm birth in IVF gestations considered by many as 'premium' pregnancies, cannot be overlooked[24].

The 2.3 times higher rate of gestational diabetes mellitus we found among ART mothers may be related to several factors, including the underlying infertility mechanisms (i.e. ovulatory disorders, polycystic ovary syndrome, obesity or unknown infertility), the change in hormone levels (i.e. estrogen, progesterone and insulin growth factor) during ovarian stimulation in ART treatment, the drug type used for ovulation induction or even the presence of underlying metabolic and vascular factors exacerbated during ovulation induction, and the IVF/ICSI procedure[25, 26].

Regarding the influence of assisted reproduction techniques on newborns' outcomes, we found a higher prevalence of LBW, VLBW and ELBW in the assisted conception group. Our results also showed that ART twins, when compared to spontaneous ones, had increased risk of low Apgar at 5 minutes, jaundice, respiratory and neurological disorders, PDA, NICU admission and higher length of neonatal hospitalization. However, after controlling for the gestational age, those complications were no longer apparent. Since we found that assisted conception had a role in reducing gestational age, we can assume that ART procedures do produce an increased neonatal risk not by itself but through the induction of a premature delivery. Nevertheless, a significantly increased risk of NICU hospitalization remained in induced-pregnancy twins when compared with spontaneous ones. As ART pregnancies are highly valued by women and their caregivers, it may be plausible that assisted conception newborns were more likely to receive a higher protective care for relatively minor complications.

Concerning the effect of ART type on pregnancy outcomes, our findings suggested that egg/embryo donation reduced the risk of cervical incompetence and thrombocytopenia and it had increased risk of steroid prophylaxis for fetal lung maturation. However, these outcomes are very rare and in the current literature there are not studies reporting an adequate number of cases to be sure whether these findings are stable or just accidental. We also found that twins conceived via egg/embryo donation were more likely to have patent *ductus arteriosus*. LBW, reduced gestational age, respiratory disorders and maternal corticosteroid administration during pregnancy are important risk factors for PDA. As this condition represents a relatively frequent anomaly in preterm births[27], it seems appropriate to associate our result with the prematurity condition that, as demonstrated by our analysis, was more frequent in the ART group. However, this anomaly may be also due to maternal characteristics including underlying causes of infertility and age, which is usually more advanced in women conceiving via heterologous fertilization. OI ± IUI procedure was shown to be associated to lower risk of hyperemesis gravidarum. Unfortunately, the frequency of this complication was too small to draw any conclusion about the mechanism determining our results, which may be related to the underlying subfertility or drugs used for ovarian stimulation. Ovarian induction was also associated with a longer neonatal hospitalization; however no other study investigated this relationship, therefore further research is needed to investigate this relationship.

In the current literature, there are contradictory results about the difference in the rate of adverse obstetric and perinatal outcomes between twins conceived naturally and by assisted conception. However, many studies did not consider chorionicity as a confounding factor. Since monochorionic pregnancies are at greater risk of complications, the evaluation of ART influence in these studies may be compromised. Nevertheless it should be considered that the bichorionicity is strongly related to the ART procedure itself, acting therefore as a mediator of the total effect of the ART on the outcomes. By adjusting for the chorionicity we tried to investigate the direct effect of ART on the outcome separated from the effect elicited through chorionicity, and this approach could be at the origin of the some differences between other studies and ours. In most studies, only IVF and/or ICSI are evaluated, and the effect of maternal age, parity, smoke, chronic diseases, considered confounding factors for predicting the perinatal outcomes of twin pregnancies, has not been evaluated. The heterogeneous results reported can also depend on differences in studied populations and/or in the management approach to twin pregnancy[28]. The power of this study included the large sample and the analysis of the influence of several confounding variables on our results. We also included all types of induced pregnancies (ovarian stimulation, IVF/ICSI, and egg donation) and evaluated their impact in the global results.

The major limitation of the study is that, as this is a cohort study, it suffers from difficult of interpretation in the case of rare outcomes for which it is difficult to ascertain the degree of type 1 or 2 error, especially in the cases in which there is not any case of the considered outcome in the exposed group (such as the relation between ART type and twin to twin transfusion syndrome). Another limitation is that we investigated the relationship between ART procedure and ART type with a series of outcomes which can be causally connected between to each other (such as premature rupture of membranes and preterm delivery). Finally, while investigating the neonates, we analyzed twins which by definition are not statistically independent. Although more complex statistical procedures would have allowed to adjust for this lack of independency, we decided not to use them because dyad interdependence, in the context of around two thousand data points, should not cause perceptible deformations in the results.

Conclusions

The assisted conception was associated with adverse obstetrical outcomes, lower gestational age and higher rate of NICU admission. As prematurity is the main factor related to neonatal morbidity, these findings suggest that maximum efforts aimed to develop effective interventions to prevent preterm birth in multiple pregnancies are needed. Couples undergoing ART, which often consider multiple births as "premium pregnancies", have to be informed about the risks of twin pregnancies associated with infertility treatment for both mothers and neonates. The current strategies of selective embryo transfer together with a careful and well-considered management of non-IVF ovarian induction should be implemented.

Conflict of Interests
The authors have no conflict of interest to declare. This article has not been funded or sponsored by any source.

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