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ARTICLE

Comparison of maternal, fetal, obstetric and neonatal outcomes for 234 triplet pregnancies conceived *in vivo* versus IVF and ICSI conceptions

BIOGRAPHY

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KEY MESSAGE

Triplet pregnancies conceived by assisted reproductive technology (ART) have a higher rate of intrauterine growth restriction than triplet pregnancies conceived *in vivo*. Placental examination would be helpful to provide more information regarding the pathogenic mechanisms of these ART-related results.

ABSTRACT

Research question: Is there a difference in maternal, fetal, obstetric and neonatal outcomes for triplet pregnancies when comparing in vivo conceptions with those conceived by assisted reproductive technology (ART)?

Design: This single-centre, retrospective cohort study included all triplet pregnancies followed up at La Paz University Hospital, Madrid between 2000 and 2022. The characteristics of the pregnant women, and maternal, fetal, obstetric and perinatal outcomes were examined. Univariate and multivariate statistical analyses were performed.

Results: In total, 234 triplet pregnancies were analysed: 92 in the natural and assisted insemination conception group (in-vivo conception) and 142 in the in vitro fertilization and intracytoplasmic sperm injection conception group (ART conception). ART triplet pregnancies were more common between 2000 and 2010 ($P = 0.003$). The percentage of monochorionic triamniotic pregnancies was significantly higher ($P = 0.02$) in the in-vivo conception group, and the percentage of dichorionic triamniotic pregnancies was significantly higher ($P = 0.003$) in the ART conception group. After adjusting for confounders, intrauterine growth restriction (IUGR) remained significantly more common in the ART conception group (adjusted odds ratio 8.65, 95% CI 1.66−45.03; $P = 0.01$). Differences in maternal age ($P = 0.61$), threatened preterm labour ($P = 0.10$), Apgar score ≤5 at 5 min ($P = 0.99$), umbilical cord pH <7.20 ($P = 0.99$) and fetal death ($P = 0.99$) disappeared after adjustment for confounders.

Conclusion: ART triplet pregnancies had a higher rate of IUGR than in vivo triplet pregnancies. This could be related to higher maternal age, and higher rates of Apgar score ≤5 at 5 min and umbilical cord pH <7.20 in these pregnancies. In these cases, placental examination could provide valuable information.

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KEY WORDS
Assisted reproductive technology
Multiple gestations
Pregnancy outcomes
Triplet pregnancies

INTRODUCTION

The number of triplet pregnancies has increased over past decades due to the rise in use of assisted reproductive technology (ART) (*Murray et al., 2014*). There is a direct association between a higher number of embryo transfers and a higher number of fetuses (*Morency et al., 2016*), although triplet pregnancies have been reported after single embryo transfers (*Yamashita et al., 2020*). Triplet pregnancies account for 0.15% of all pregnancies in the authors' healthcare region in Spain (*Duyos et al., 2013*).

Triplet pregnancies are considered to be high risk (*Geipel et al., 2005*), and triplets are associated with higher rates of maternal and fetal complications compared with singletons and twins (*Ballabh et al., 2003*; *Wen et al., 2004*). In triplet pregnancies, the outcomes of monochorionic triamniotic (MCTA) and dichorionic triamniotic (DCTA) pregnancies are worse than those of trichorionic triamniotic (TCTA) pregnancies (*Bajoria et al., 2006*; *Geipel et al., 2005*; *Kawaguchi et al., 2013*; *Spencer et al., 2009*), especially those related to twin-to-twin transfusion syndrome (TTTS), twin anaemia polycythaemia sequence (TAPS), premature rupture of membranes (PROM) (*Adegbite et al., 2005*), intrauterine growth restriction (IUGR), risk of stillbirth (*Glinianaia et al., 2021*) and low birth weight (*Lopes Perdigao et al., 2016*). TCTA pregnancies have a lower risk of death and prematurity, and have higher birth weights (*Spencer et al., 2009*).

In general, ART pregnancies [in vitro fertilization (IVF) and intracytoplasmic sperm injection (ICSI)] have been associated with poorer obstetric outcomes (*Fennessy et al., 2015*). However, few previous studies have compared maternal and neonatal outcomes between naturally conceived and ART triplet pregnancies. Some of these studies found no differences between groups (*Fennessy et al., 2015*; *Morency et al., 2016*), and one study suggested high levels of neonatal morbidity in the ART conception group (*Zuppa et al., 2007*). When comparing different types of ART, no differences have been found in obstetric outcomes (*Badreldin et al., 2021*).

As few studies exist on triplet pregnancies, and few patients were included in the studies that exist, this work aims to analyse triplet pregnancies at a referral hospital for the management of these pregnancies. The objective of this study was to compare the prevalence of maternal, fetal, obstetric and perinatal outcomes in spontaneous and ART triplet pregnancies at the study centre.

MATERIALS AND METHODS

La Paz University Hospital is a tertiary hospital with 1200 beds, where 1100 doctors and 2000 nurses serve 1.5 million patients. It is a referral hospital for multiple pregnancies in Spain. Approximately 200 multiple births, mostly twins, take place annually.

Study design and eligibility criteria

This retrospective cohort study compared triplet pregnancies conceived by ART (IVF or ICSI) with triplet pregnancies conceived naturally or through assisted insemination (the outcomes of assisted insemination cases are more similar to natural pregnancies) (*Fennessy et al., 2015*; *Friedler et al., 1994*; *Morency et al., 2016*). Pregnant women were included from January 2000 to April 2022. Clinical information was retrieved from the medical records of triplet pregnancies followed up at the Maternal-Fetal Medicine Unit, La Paz University Hospital following approval by the Research Ethics Committee of La Paz University Hospital (PI-5286 2022.168, date of approval 14 July 2022). Triplet pregnancies were diagnosed, and amnionicity, chorionicity and gestational age were determined at the study hospital by expert sonographic obstetricians during the first and second trimesters. Chorionicity was confirmed after birth by pathological examination of the placenta.

Inclusion criteria were patients with a triplet pregnancy gestation since the beginning of the pregnancy (n = 239 pregnancies), diagnosed at the study hospital or at another centre with subsequent confirmation of a triplet pregnancy by the sonographic obstetricians at the study hospital, who were monitored and whose babies were delivered (n = 234 pregnancies) at the study hospital. Exclusion criteria were patients who did not deliver at the study hospital and patients lost to follow-up (n = 5 pregnancies) (**FIGURE 1**). All types of ART (ICSI and IVF) were clustered for analysis. Patients using donor gametes (oocytes or sperm) were eligible for inclusion. Where any fetal deaths occurred, the patients were not excluded from the analysis.

Study variables

The following data were collected: maternal age at time of conception; pre-pregnancy maternal body mass index (BMI); year of conception; pre-existing medical disorders related to subfertility; parity; and type of conception [in-vivo conception (natural conception and assisted insemination) versus ART (IVF and ICSI)]. The maternal complications analysed were: pre-eclampsia; gestational hypertension; gestational diabetes; intrahepatic cholestasis; pregnancy-induced hypothyroidism; and iron-deficiency anaemia. The fetal complications evaluated were: IUGR and the different types of IUGR (I, II, III); TTTS and its stages; TAPS; biometry discordance; amniotic fluid discordance; and malformations. Threatened preterm labour (TPL), PROM, cervical cerclage, type of caesarean section and prematurity were also registered. The following perinatal complications were recorded: neonatal death; neonatal weight; umbilical cord pH; and Apgar score at 5 min. Not all data were available for all patients, and missing data were excluded from the statistical analysis. BMI data were missing for 58 pregnancies, birthweight data were missing for 169 fetuses, umbilical cord pH was missing for 149 fetuses, and Apgar score at 5 min was missing for 236 fetuses.

Statistical analysis

Quantitative variables have been expressed as mean ± SD for normally distributed variables, and as median [interquartile range (IQR)] for non-normally distributed variables. Qualitative variables have been expressed as frequency and percentage. The Kolmogorov–Smirnov test was used to determine if the quantitative variables were distributed normally. An analysis of the qualitative variables was performed using chi-squared test or Fisher's exact test (if the expected values in any of the cells of the contingency table were <5), Student's t-test for parametric quantitative variables, and Mann–Whitney U-test for non-parametric quantitative variables. The selected association measure for qualitative variables was the odds ratio (OR) with 95% CI. To identify factors associated with complications (confounders), a logistic regression model was constructed, using spontaneous versus ART triplet pregnancies as the dependent variable, and the possible prognostic

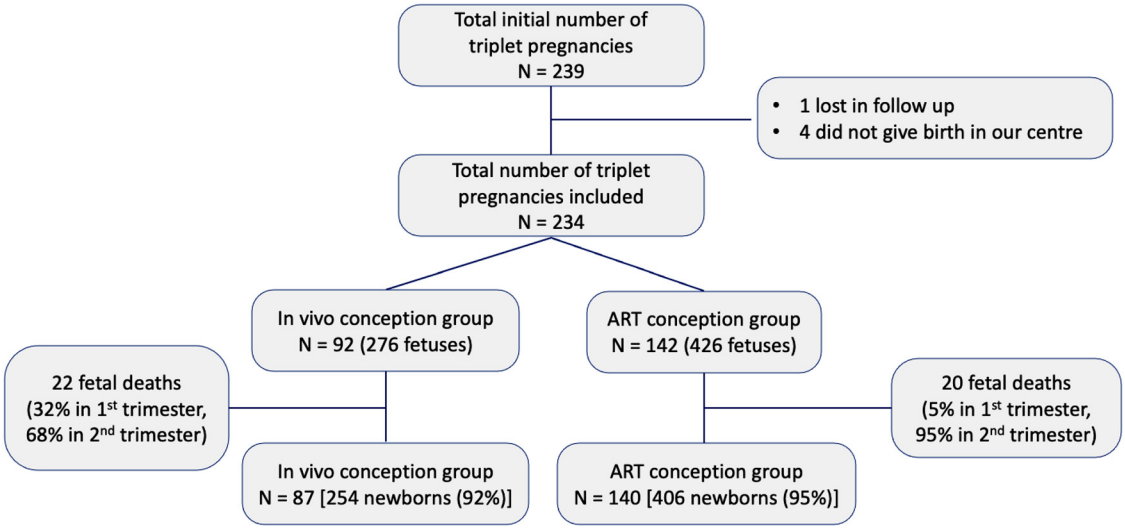

FIGURE 1 Inclusion and exclusion criteria for number of analysed fetuses. *In-vivo* conception group, natural and assisted insemination; assisted reproductive technology (ART) conception group, in vitro fertilization and intracytoplasmic sperm injection.

factors as independent variables. These were selected as the variables that showed a significant difference on first analysis (IUGR, TPL, fetal death, Apgar score at 5 min, umbilical cord pH, year of conception, maternal age, MCTA pregnancy and DCTA pregnancy). All tests were bilateral, and $P < 0.05$ was considered to indicate significance. Statistical Package for Social Science Version 25 (IBM Corp.) was used for statistical analysis.

RESULTS

In total, 239 pregnant women carrying triplets were identified at the beginning of the study. Five patients were lost to follow-up. One case was lost during pregnancy monitoring because the patient moved to another country, and four cases did not deliver at the study hospital. Finally, 234 patients were analysed in this study: 92 in the in-vivo conception group and 142 in the ART conception group (FIGURE 1). After initial analysis between groups, a logistic regression multivariate analysis including variables with a significant *P*-value was undertaken to determine the impact of ART.

Regarding the type of conception, 32 triplet pregnancies were conceived by assisted insemination (24 with partner's sperm and 8 with donor's sperm), 119 by IVF (94 with autologous oocytes and 25 with oocyte donation), and 23 by ICSI (11 with autologous oocytes and 12 with oocyte donation). All cases were transferred at the blastocyst stage. Two embryos were transferred in 121 cases, and three embryos were transferred in 21 cases. Fresh embryos were transferred in 111 cases, and frozen embryos were transferred in 31 cases. None of the embryos underwent assisted hatching or biopsy prior to transfer.

The cause of subfertility was not identified after completion of the study in 65 cases. Maternal origin was determined in 27 cases, with polycystic ovary syndrome ($n = 16$) and endometriosis ($n = 10$) being the most common causes; paternal origin was determined in 11 cases, with asthenozoospermia being the most common cause ($n = 9$); and both maternal and paternal origins were determined in eight cases. In 33 cases, this was the first embryo transfer in the ART process, while there were previous failed attempts in 29 cases.

Maternal age did not follow a normal distribution (Kolmogorov–Smirnov test $P = 0.002$). The global median age was 35 (IQR 33–37) years. Maternal age was higher in the ART conception group (median 35 versus 34 years; $P = 0.01$). Pre-pregnancy maternal BMI did not follow a normal distribution (Kolmogorov–Smirnov test $P = 0.003$). Global median BMI was 24.1 (IQR 22.6–25.6) kg/m^2. No differences were found between the groups ($P = 0.49$). Seven patients (4.0%) had BMI ≥30 kg/m^2, but the difference was not significant ($P = 0.24$) (TABLE 1).

Regarding the year of conception, the odds of ART triplet pregnancy was lower post 2011 [adjusted OR (aOR) 0.27, 95% CI 0.13–0.56]. In total, 140 cases were conceived between 2000 and 2010 (45 in-vivo conception versus 95 ART conception), and 94 cases were conceived between 2011 and 2022 (47 in-vivo conception versus 47 ART conception) (TABLE 1).

Regarding parity, the triplet pregnancy was the first gestation in 123 cases (52.6%), and the second (or more) gestation in 111 cases (47.7%). There were no cases with more than one triplet pregnancy. The triplet pregnancy was the first gestation in 43 cases (46.7%) in the in-vivo conception group, and in 80 cases (56.3%) in the ART conception group. This difference was not significant ($P = 0.18$) (TABLE 1).

Of the triplet pregnancies, 156 (66.7%) were TCTA pregnancies, 63 (26.9%) were DCTA pregnancies and 15 (6.4%) were MCTA pregnancies. The difference in the number of TCTA pregnancies between the groups was not significant ($P = 0.78$). The odds of an MCTA pregnancy was lower in the ART conception group (aOR 0.14, 95% CI 0.28–0.79), and the odds of a DCTA pregnancy was lower in the in-vivo conception group (aOR 3.05, 95% CI 1.37–6.79) (TABLE 2).

Regarding maternal complications, no differences were found between the in-vivo conception group and the ART conception group: pre-eclampsia (eight versus nine cases), gestational hypertension (three versus four cases), gestational diabetes (six versus ten cases), intrahepatic cholestasis (four versus thirteen cases), pregnancy-induced hypothyroidism (ten versus nine cases) and iron-deficiency anaemia (seventeen versus twenty six cases) (TABLE 1).

TABLE 1 DESCRIPTION OF MATERNAL CHARACTERISTICS AND MATERNAL COMPLICATIONS

Variable	Total, *n* = 234	In-vivo conception, *n* = 92	ART conception, *n* = 142	*P*-value	OR	95% CI	Adjusted *P*-value[a]	Adjusted OR[a]	95% CI
Maternal characteristics									
Maternal age (years)	35 (32–37)	34 (32–36)	35 (33–37)	0.01[b]	-	-	0.61	1.08	0.99–1.18
Pre-pregnancy body mass index (kg/m^2)	24.1 (22.6–25.6)	23.8 (22.5–25.5)	24.4 (22.6–25.6)	0.49[b] 0.24[d]	 -4.56	 -0.53–38.73	0.72 0.34	1.21 5.56	0.45–1.78 0.67–45.21
<30	169 (96.0)	73 (98.6)	96 (94.1)						
≥30	7 (4.0)	1 (1.4)	6 (5.9)						
Year of conception				0.005[c]	0.47	0.27–0.81	0.003	0.27	0.13–0.56
2000–2010	140 (59.8)	45 (48.9)	95 (66.9)						
2011–2022	94 (40.2)	47 (51.1)	47 (33.1)						
Parity				0.18[c]	0.68	0.40–1.15	0.34	0.57	0.42–4.35
First gestation	123 (52.6)	43 (46.7)	80 (56.3)						
Second or more gestation	111 (47.4)	49 (53.3)	62 (43.7)						
Maternal complications									
Pre-eclampsia	17 (7.3)	8 (3.4)	9 (6.3)	0.60[c]	0.71	0.26–1.91	0.14	0.83	0.49–12.34
Gestational hypertension	7 (3.0)	3 (1.3)	4 (1.7)	1.00[d]	0.86	0.18–3.93	0.59	0.93	0.32–15.67
Gestational diabetes	16 (6.9)	6 (6.5)	10 (7.0)	1.00[c]	1.08	0.38– 3.09	1.00	1.14	0.78–3.53
Intrahepatic cholestasis	17 (7.3)	4 (4.3)	13 (5.6)	0.20[d]	2.21	0.70– 7.02	0.45	1.89	0.89–11.23
Pregnancy-induced hypothyroidism	19 (8.2)	10 (11.0)	9 (6.3)	0.22[c]	0.54	0.21–1.41	0.34	0.67	0.78–2.56
Iron-deficiency anaemia	43 (20.3)	17 (18.5)	26 (18.3)	1.00[c]	0.98	0.50–1.94	1.00	0.97	0.42–7.68

Data are presented as median (interquartile range) or *n* (%).

[a] Variables included in logistic regression model: intrauterine growth restriction, threatened preterm labour, fetal death, Apgar score at 5 min, umbilical cord pH, year of conception, maternal age, monochorionic triamniotic pregnancy and dichorionic triamniotic pregnancy.

[b] Data compared by Mann–Whitney *U*-test.

[c] Data compared by chi-squared test.

[d] Data compared by Fisher's exact test.

Variables with missing data: pre-pregnancy body mass index.

In-vivo conception, natural pregnancies and assisted insemination cycles; assisted reproductive technology (ART) conception, in vitro fertlization and intracytoplasmic sperm injection cycles; OR, odds ratio; CI, confidence interval.

TABLE 2 DESCRIPTION OF CHORIONICITY AND FETAL COMPLICATIONS

Variable	Total, *n* = 234	In-vivo conception, *n* = 92	ART conception *n* = 142	*P*-value	OR	95% CI	Adjusted *P*-value[a]	Adjusted OR[a]	95% CI
Chorionicity									
MCTA	15 (6.4)	13 (14.1)	2 (1.4)	0.003[c]	0.09	0.02–0.39	0.02	0.14	0.28–0.79
DCTA	63 (26.9)	17 (18.5)	46 (32.4)	0.02[b]	2.11	1.12–3.98	0.003	3.05	1.37–6.79
TCTA	156 (66.7)	62 (67.4)	94 (66.2)	0.88b	0.94	0.54–1.65	0.78	0.87	0.45–1.56
Fetal complications									
IUGR	19 (8.1)	2 (2.2)	17 (12.0)	0.002[c]	6.12	1.37–27.15	0.01	8.65	1.66–45.03
TTTS	5 (2.1)	1 (1.1)	4 (2.8)	0.65[c]	2.63	0.29–23.97	0.43	3.45	0.92–21.32
TAPS	1 (0.4)	1 (1.1)	0 (0)	0.39[c]	-	-	0.45	-	-
Amniotic fluid discordance	12 (5.1)	4 (4.3)	8 (5.6)	0.76[c]	1.31	0.38–4.49	0.75	1.45	0.64–5.68
Biometry discordance	11 (4.7)	5 (5.4)	6 (4.2)	0.75[b]	0.76	0.22–2.59	0.78	0.67	0.59–3.34
Fetal complications	Total, *n* = 702	In-vivo conception, *n* = 276	ART conception, *n* = 426	*P*-value	OR	95% CI	Adjusted *P*-value	Adjusted OR	95% CI
Fetal malformations	7 (1.0)	3 (1.1)	4 (0.9)	1.00[c]	0.86	0.19–3.88	1.00	0.76	0.31–3.90
Fetal death	42 (6.0)	22 (8.0)	20 (4.7)	0.004[b]	0.29	0.15–0.54	0.99	0.56	0.45–1.35

Data are presented as median (interquartile range), *n* (%) or mean ± standard deviation.

[a] Variables included in logistic regression model: IUGR, threatened preterm labour, fetal death, Apgar score at 5 min, umbilical cord pH, year of conception, maternal age, MCTA pregnancy and DCTA pregnancy.

[b] Data compared by chi-squared test.

[c] Data compared by Fisher's exact test.

In-vivo conception, natural pregnancies and assisted insemination cycles; assisted reproductive technology (ART) conception, in vitro fertlization and intracytoplasmic sperm injection cycles. MCTA, monochorionic triamniotic; DCTA, dichorionic triamniotic; TCTA, trichorionic triamniotic; IUGR, intrauterine growth restriction; TTTS, twin-to-twin transfusion syndrome; TAPS, twin anaemia polycythaemia sequence; OR, odds ratio; CI, confidence interval.

In terms of fetal complications, 19 cases (8.1%) presented with IUGR (two in the in-vivo conception group and seventeen in the ART conception group), with a significantly higher risk of IUGR in the ART conception group (aOR 8.65, 95% CI 1.66–45.03). Overall, two cases were type I, nine cases were type II and eight cases were type III. No differences were found between the groups for any of the other fetal complications: amniotic discordance (four versus eight cases), biometry discordance (five versus six cases) and TTTS (one versus four cases; two cases were stage I and three cases were stage II). Only one case in the in-vivo conception group developed TAPS.

Malformations appeared in seven of the 702 fetuses (1%). These malformations were choroid plexus cysts (*n* = 2), an aberrant right subclavian artery (*n* = 2), Cantrell pentalogy (*n* = 1), Down syndrome (*n* = 1) and tetralogy of Fallot (*n* = 1). No differences were found between the groups. Fetal death occurred in 42 fetuses (5.7%; 12.1% in the in-vivo conception group and 3.8% in the ART conception group), with increased risk in in-vivo conception group (*P* = 0.004); however, this difference was not significant after adjustment (aOR 0.56, 95% CI 0.45–1.35) (TABLE 2). Fetal deaths occurred during the first (19%) and second (81%) trimesters, between weeks 9 and 27, through selective occlusion of the umbilical cord due to malformations or severe IUGR in 21 fetuses (seven in the in-vivo conception group and fourteen in the ART conception group), and there was spontaneous loss of all three fetuses in seven patients (five in the in-vivo conception group and two in the ART conception group) (FIGURE 1).

Cervical cerclage was used in 51 pregnancies (21.8%) and 53 cases (22.6%) presented with PROM. No differences were found in these obstetric complications between groups. One hundred and four cases (44.4%) presented with TPL (thirty three in the in-vivo conception group and seventy one in the ART conception group), with higher risk in the ART conception group (*P* = 0.04); however, there was no significant difference between the groups following adjustment (aOR 1.70, 95% CI 0.89–3.23) (TABLE 3).

Regarding delivery, caesarean section was performed in the 227 cases having at least one living fetus. Caesarean section was elective in 106 cases (46.7%). An emergency caesarean section was performed in 47 cases (20.7%) due to PROM, 29 cases (12.8%) due to risk of loss of fetal well-being, 27 cases (11.9%) due to labour, and 18 cases (7.9%) due to maternal pathology. No differences were found between the groups for any of these causes (TABLE 3).

Gestational age at delivery did not follow a normal distribution (Kolmogorov–Smirnov test *P* = 0.002). No differences were found between the groups (*P* = 0.17). The median gestational age at delivery was 33 (IQR 31–35) weeks. After group stratification, no differences were found (TABLE 3).

Of the 660 newborns, no differences were found regarding neonatal death (*P* = 0.67). Birth weight followed a normal distribution (Kolmogorov–Smirnov test *P* = 0.053). The mean ± SD birth weight was 1828 ± 411g. No differences existed between the groups (*P* = 0.28). After group stratification, no differences were found. Umbilical cord pH did not follow a normal distribution (Kolmogorov–Smirnov test *P* = 0.001). The median umbilical cord pH was 7.30 (IQR 7.28–7.34). After stratification, the unadjusted analysis showed increased odds of umbilical cord pH <7.20 for the ART conception group

TABLE 3 DESCRIPTION OF OBSTETRIC COMPLICATIONS, CAESAREAN SECTION, PREMATURITY AND NEONATAL COMPLICATIONS

Variable	Total, *n* = 234	In-vivo conception, *n* = 92	ART conception, *n* = 142	*P*-value	OR	95% CI	Adjusted *P*-value[a]	Adjusted OR[a]	95% CI
Obstetric complications									
PROM	53 (22.6)	18 (19.6)	35 (24.6)	0.42[d]	1.34	0.71–2.55	0.56	1.56	0.67–3.45
Threatened preterm labour	104 (44.4)	33 (35.9)	71 (50.0)	0.04[d]	1.78	1.04–3.06	0.10	1.70	0.89–3.23
Cervical cerclage	51 (21.8)	21 (22.8)	30 (21.1)	0.74[d]	0.90	0.48–1.70	0.77	0.92	0.78–2.23
Birth	Total, *n* = 227	In-vivo conception, *n* = 87	ART conception, *n* = 140	*P*-value	OR	95% CI	Adjusted *P*-value	Adjusted OR	95% CI
Type of caesarean section									
Elective	106 (46.7)	42 (48.3)	64 (45.7)	0.78[d]	0.90	0.52–1.54	0.80	0.94	0.67–2.34
Maternal pathology	18 (7.9)	9 (10.3)	9 (6.4)	0.31[d]	0.59	0.22–1.56	0.42	0.64	0.45–2.45
Risk of loss of fetal well-being	29 (12.8)	12 (13.8)	17 (12.1)	0.83[d]	0.86	0.39–1.91	0.85	0.83	0.23–3.01
PROM	47 (20.7)	14 (16.1)	33 (23.6)	0.23[d]	1.61	0.80–3.21	0.43	1.53	0.67–4.56
Labour	27 (11.9)	10 (11.5)	17 (12.1)	1.00[d]	1.06	0.46–3.01	1.00	1.12	0.35–3. 53
Prematurity (weeks of gestation)	33 (31–35)	34 (32–35)	33 (P25=31, P75=34.75)	0.17[b]	-	-	0.45	0.67	0.32–2.21
<32	59 (26.0)	19 (21.8)	40 (28.6)	0.28[d]	1.43	0.75–2.68	0.43	1.56	0.63–3.56
32–37	159 (70.0)	63 (72.4)	96 (68.6)	0.55[d]	0.83	0.46–1.50	0.67	0.67	0.52–2.04
>37	9 (4.0)	5 (5.7)	4 (2.9)	0.30[e]	0.48	0.12–1.84	0.21	0.56	0.43–3.32
Neonatal complications	Total, *n* = 660	In-vivo conception, *n* = 254	ART conception, *n* = 406	*P*-value	OR	95% CI	Adjusted *P*-value	Adjusted OR	95% CI
Neonatal death	6 (0.9)	3 (1.2)	3 (0.7)	0.67[e]	0.61	0.12-3.05	0.54	0.53	0.32–4.56
Birth weight (g)	1828 ± 411	1874 ± 382	1791 ± 431	0.28[c]	-	-	0.43	0.56	0.54–3.57
<1500	97 (19.8)	39 (17.6)	58 (21.6)	0.30[d]	1.29	0.82 - 2.02	0.45	1.45	0.67–2.23
1500–2500	367 (74.7)	168 (75.7)	199 (74.0)	0.67[d]	0.91	0.60–1.37	0.63	0.56	0.45–3.31

(*continued on next page*)

TABLE 3 (*Continued*)

Variable	Total, *n* = 234		In-vivo conception, *n* = 92	ART conception, *n* = 142	*P*-value	OR	95% CI		Adjusted *P*-value[a]	Adjusted OR[a]	95% CI
>2500	27 (5.5)	15 (6.8)	12 (4.5)		0.32[d]	0.64	0.29–1.40	0.34		0.43	0.23–1.56
Umbilical cord pH	7.30 (7.28–7.34)	7.29 (7.27–7.34)	7.30 (7.28–7.34)		0.96[b]	-	-				
<7.20	34 (6.7)	4 (1.8)	30 (10.2)		0.01[e]	3.38	1.19–4.79	0.99		2.45	0.37–24.56
≥7.20	477 (93.3)	213 (98.2)	264 (89.8)								
Apgar score at 5 min					0.002[e]	6.05	2.09–	0.99		7.89	0.67–23.24
≤5	34 (8.0)	4 (3.4)	30 (9.8)								
>5	390 (92.0)	114 (96.6)	276 (90.2)				17.44				

Data are presented as median (interquartile range) or *n* (%).

[a] Variables included in logistic regression model: intrauterine growth restriction, threatened preterm labour, fetal death, Apgar score at 5 min, umbilical cord pH, year of conception, maternal age, monochorionic triamniotic pregnancy and dichorionic triamniotic pregnancy.

[b] Data analysed by Mann-Whitney *U*-test.

[c] Data compared by *t*-test.

[d] Data compared by chi-squared test.

[e] Data compared by Fisher's exact test.

Variables with missing data: birth weight, umbilical cord pH and Apgar score 5 at min.

In-vivo conception, natural pregnancies and assisted insemination cycles; assisted reproductive technology (ART) conception, in vitro fertilization and intracytoplasmic sperm injection cycles; PROM, premature rupture of membranes; OR, odds ratio; CI, confidence interval.

(OR 3.38, 95% CI 1.19–4.79), with 34 cases (6.7%) having umbilical cord pH <7.20 (four in the in-vivo conception group and thirty in the ART conception group); however, after adjustment, no significant differences were seen (aOR 2.45, 95% CI 0.37–24.56). Apgar score at 5 min was >5 in 390 cases (92%) and ≤5 in 34 cases (8%) (four cases in the in-vivo conception group and thirty cases in the ART conception group). Before adjustment, the odds of an Apgar score ≤5 at 5 min was higher in the ART conception group (OR 7.89, 95% CI 0.67–23.24), but no difference existed between the groups following adjustment (aOR 7.89, 95% CI 0.67–23.24) (TABLE 3).

DISCUSSION

This study found that triplet pregnancies conceived by ART (IVF and ICSI) will have slightly worse obstetric outcomes compared with spontaneous triplet pregnancies, with notably higher IUGR rates.

This study analysed 234 triplet pregnancies and 702 fetuses. To the best of the authors' knowledge, this is the largest cohort reported in a triplet study (*Fennessy et al., 2015*; *Morency et al., 2016*; *Rajan et al., 2018*; *Shah et al., 2018*; *Zuppa et al., 2007*). In total, 61% of the triplet pregnancies were conceived by ART, as was the case in previous studies (*Al-Sunaidi et al., 2011*; *Morency et al., 2016*; *Rajan et al., 2018*), by transfer of two or three embryos, as in the present series. Over the last decade, this number has decreased due to the tendency to transfer a single embryo. In the ART conception group, most gestations were first-time attempts, in line with previous studies (*Morency et al., 2016*; *Shah et al., 2018*). MCTA pregnancies are associated with a higher number of fetal complications (*Geipel et al., 2005*), but only represented 6.4% of all triplet pregnancies in the present study. Upon adjusting for confounders, it was found that these differences did not affect the complications outcomes. At least 121 cases were monozygotic triplet pregnancies (cases with a monozygotic twin pair and a single embryo transferred during ART).

Given that fewer embryos have been transferred in recent years, a higher percentage of ART triplet pregnancy conceptions was observed between 2000 and 2010. At this time, the monitoring of triplet pregnancies was not as standardized as it is today and, therefore, these pregnancies may have had poorer outcomes, explaining the higher IUGR rates in this series.

Regarding maternal characteristics, maternal age was higher in the ART conception group, although this difference disappeared after adjusting for confounders. Other studies did not find differences (*Fennessy et al., 2015*; *Morency et al., 2016*), although *Shah et al. (2018)* found a higher maternal age in the ART conception group. No differences were found in pre-pregnancy maternal BMI in the study groups, in contrast to other series (*Fennessy et al., 2015*; *Morency et al., 2016*). *Shah et al. (2018)* found a higher BMI in the natural pregnancy group. In the present series in a Spanish population, no differences were found in maternal complications between the groups. There were fewer cases of gestational diabetes in the study population compared with other series (*Badreldin et al., 2021*; *Morency et al., 2016*); possible explanations for this may include strict weight and diet control, and the Mediterranean diet consumed in Spain.

After adjusting for confounders, IUGR remained significantly more common in triplet pregnancies conceived by ART. Some studies have suggested that pregnancies conceived by ART have more placental abnormalities, possibly explaining the higher rate of IUGR (*Cochrane et al., 2020*; *Petersen et al., 2020*). No differences were found in other fetal complications, in line with previous studies (*Fennessy et al., 2015*; *Morency et al., 2016*; *Shah et al., 2018*).

Regarding obstetric outcomes, some studies found that TPL was among the most common complications (*Al-Sunaidi et al., 2011*; *Chibber et al., 2013*; *Mazhar et al., 2008*), as was the case in the present series in which 44.4% of triplet pregnancies presented with TPL. After adjusting for confounders, the difference in TPL between the groups disappeared. TPL is related to various factors. Placental abnormalities associated with ART may explain, in part, the higher TPL rates in triplet pregnancies (*Perni et al., 2005*; *Sills et al., 2004*).

Triplet pregnancies conceived by ART have been found to be associated with higher rates of abnormal umbilical cord insertion (velamentous and marginal), placenta previa, placental abruption, increased thickness, and a higher incidence of haematoma (*Cochrane et al., 2020*; *Joy et al., 2012*; *Petersen et al., 2020*).

Despite increases in TPL and PROM, most gestations reached the expected term for triplet pregnancies (34–35 weeks). The use of cervical cerclage was similar to that reported by *Morency et al. (2016)*, but was less than half that reported by *Rajan et al. (2018)*. PROM results were similar to those of previous series (*Morency et al., 2016*; *Rajan et al., 2018*).

Only one previous study with 24 triplet pregnancies, the results of which were not adjusted for confounders, found considerable neonatal morbidities in the ART conception group, especially malformations (*Zuppa et al., 2007*). The present study found no difference in neonatal deaths between the groups, in line with the findings of *Fennessy et al. (2015)*.

Caesarean deliveries were performed in all cases in the present study, in common with two previous studies (*Shah et al., 2018*; *Zuppa et al., 2007*). In three prior series (*Fennessy et al., 2015*; *Morency et al., 2016*; *Rajan et al., 2018*), vaginal delivery occurred in a few cases. In the present study, triplet deliveries were performed as elective procedures at 34–35 weeks of gestation, in accordance with the study centre's protocol, and PROM was the most common reason for an emergency caesarean section. In other studies, TPL (*Fennessy et al., 2015*; *Morency et al., 2016*), PROM (*Morency et al., 2016*) and pre-eclampsia (*Morency et al., 2016*; *Shah et al., 2018*) were the most common reasons for emergency caesarean delivery.

This study found no difference in prematurity between the groups, as was the case in most other reported series (*Fennessy et al., 2015*; *Morency et al., 2016*), although *Shah et al. (2018)* found that naturally conceived pregnancies were premature more often than ART pregnancies, and *Friedler et al. (1994)* found that ART triplet pregnancies were premature more often than naturally conceived triplet pregnancies. Triplet pregnancies tend to be premature because, according to the current protocol, these pregnancies are delivered at 34–35 weeks of gestation. In this series, patients were followed up from 2000, and

TABLE 4 PREVIOUS STUDIES AND THEIR FINDINGS COMPARED WITH THE FINDINGS OF THE PRESENT STUDY

Study	Friedler et al.	Zuppa et al.	Fennessy et al.	Morency et al.	Rajan et al.	Shah et al.	Pena-Burgos et al. (present study)
Year	**1994**	**2007**	**2015**	**2016**	**2018**	**2018**	**2023**
Number of triplets included	151 (13 natural versus 55 ovulation induction by gonadotropins versus 27 ovulation induction by clomiphene citrate versus 56 ART)	24 [6 natural versus 18 assisted (1 ovarian stimulation with clomiphene, 6 with gonadotropins, 8 IVF, 2 ICSI and 1 gamete intrafallopian transfer)]	53 [28 natural versus 25 ART (ovulation induction with either gonadotropins or clomiphene citrate; 8 IVF)]	230 [49 natural versus 181 ART (109 IVF, 35 IUI, 31 ovulation induction drugs and 6 unrecorded specific type of ART)]	82	127 (38 natural versus 89 ART)	234 [92 in-vivo conception (60 natural, 24 AI with partner's sperm, 8 AI with donor's sperm) versus 142 ART conception (94 IVF with autologous oocytes, 25 IVF with oocyte donation, 11 ICSI with autologous oocytes, 12 ICSI with oocyte donation)]
Study type	Retrospective cohort study	Retrospective cohort study	Retrospective cohort study	Retrospective review	Retrospective observational study	Secondary analysis of a multicentre randomized trial for the prevention of preterm birth in multiple gestations	Retrospective unicentric cohort study
Statistical analysis	Univariate	Univariate	Univariate	Univariate	-	Univariate and multivariate	Univariate and multivariate
Most common causes of subfertility	Not mentioned	Not mentioned	Polycystic ovary syndrome and endometriosis	Not mentioned	-	Not mentioned	Polycystic ovary syndrome and endometriosis
Most common type of chorionicity	Not mentioned	TCTA	TCTA	TCTA	TCTA	Not mentioned	TCTA
Maternal age	Not studied	Not studied	No differences Natural 31.6 ± 4.6 versus ART 32.9 ± 3.7	No differences Natural 31.4 ± 5.2 versus ART 33.0 ± 4.9	-	Higher maternal age in the ART conception group Natural 28.5 ± 5.3 versus ART 32.1 ± 4.7	No differences In-vivo conception 34 (IQR 32–36) versus ART conception 35 (IQR 33–37)
Maternal BMI	Not studied	Not studied	No differences Obesity or elevated BMI >40 kg/m^2: natural 4% versus ART 8%	No differences Natural 24.5 ± 5.1 versus ART 25.4 ± 5.3	-	Higher BMI in the natural group Natural 28.0 ± 6.7 versus ART 26.3 ± 7.2	No differences In-vivo conception 23.8 (IQR 22.5–25.5) versus ART conception 24.4 (IQR 22.6–25.6)
Maternal complications	Not studied	Not studied	No differences Pre-eclampsia: natural 0 versus ART 1; intrahepatic cholestasis: natural 1 versus ART 0; iron-deficiency anaemia: natural 4% versus ART 8%; pregnancy-induced hypertension: natural 14% versus ART 24%; gestational diabetes: natural 11% versus ART 8%	No differences Pre-eclampsia and/or HELLP: natural 8.2% versus ART 21.5%; gestational diabetes: natural 8.2% versus ART 10.5%	-	No differences Pre-eclampsia: natural 18.8% versus ART 32.8%; pregnancy-induced hypertension: natural 0% versus ART 2.2%; gestational diabetes: natural 2.6% versus ART 12.4%	No differences All shown as in-vivo conception versus ART conception Pre-eclampsia: 3.4% versus 6.3%; intrahepatic cholestasis: 4.3% versus 5.6%; iron-deficiency anaemia: 18.5% versus 18.3%; gestational hypertension: 1.3% versus 1.7%; gestational diabetes: 6.5% versus 7.0%; pregnancy-induced hypothyroidism (11.0%) versus (6.3%)
Most common maternal complication	Not studied	Not mentioned	Anaemia	Not mentioned	Anaemia	Not mentioned	Anaemia
Fetal complications	Not studied	No differences Biometry discordance >25%: natural 50% versus assisted 33.3%; TTTS: natural 0% versus assisted 1.9%	No differences IUGR: natural 3 versus ART 3; TTTS: natural 2 versus ART 0; fetal death: natural 5% versus ART 7%	No differences Small for gestational age >24 weeks: natural 30.5% versus ART 29.5%; TTTS: 6 cases, no group specified	-	No differences IUGR: natural 18.8% versus ART 17.0%; TTTS: natural 0% versus ART 2.1%	More common IUGR in IVF and ICSI triplet pregnancies All shown as in-vivo conception versus ART conception IUGR: 2.2% versus 12.0%; TTTS: 1.1% versus 2.8%;

(continued on next page)

TABLE 4 (*Continued*)

Study	Friedler et al.	Zuppa et al.	Fennessy et al.	Morency et al.	Rajan et al.	Shah et al.	Pena-Burgos et al. (present study)
Year	**1994**	**2007**	**2015**	**2016**	**2018**	**2018**	**2023**
							TAPS: 1.1% versus 0%; amniotic fluid discordance: 4.3% versus 5.6%; biometry discordance: 5.4% versus 4.2%
Obstetric outcomes	Not studied	Not studied	No differences Preterm labour: natural 8 versus ART 11; premature rupture of membranes: natural 21% versus ART 16%	No differences Preterm labour: natural 41.4% versus ART 35.9%; premature rupture of membranes: natural 31.0% versus ART 27.5%; cervical cerclage: natural 12.2% versus ART 18.8%	-	Not mentioned	No differences All shown as in-vivo conception versus ART conception Preterm labour: 35.9% versus 50.0%; premature rupture of membranes: 19.6% versus 24.6%; cervical cerclage: 22.8% versus 21.1%
Malformations	Not studied	More frequent in assisted group Natural 0% versus assisted 3.8%	Not studied	No differences Natural 4.8% versus ART 2.4%	-	Not studied	No differences In-vivo conception 1.1% versus ART 0.9%
Caesarean section 100% cases	Not mentioned	Yes	No	No	No	Yes	Yes
Mean gestational delivery age	ART triplet pregnancies were more premature Natural 35.3 weeks versus ovulation induction by gonadotropins 33.4 weeks versus ovulation induction by clomiphene citrate 34.2 weeks versus ART 33.2 weeks	No differences Natural 33 ± 1 weeks versus assisted 33 ± 2 weeks	No differences Natural 31.3 ± 3.9 weeks versus ART 31.5 ± 2.9 weeks	No differences Natural 32.0 ± 4.0 weeks versus ART 32.0 ± 3.7 weeks	32 ± 2.8 weeks	Natural deliveries were more premature Natural 31.2 ± 3.5 weeks versus ART 32.8 ± 2.7 weeks	No differences In-vivo conception 34 (IQR 32–35) versus ART 33 (IQR 31–34.75)
Neonatal death	No differences	No differences Natural 0% versus assisted 3.8%	No differences Natural 6% versus ART 3%	No differences Natural 7.5% versus ART 6.8%	-	No differences Natural 5.3% versus ART 0.7%	No differences In-vivo conception 1.2% versus ART 0.7%
Mean birth weight	ART births had lower birth weights Natural 1963 g versus ovulation induction by gonadotropins 1683 g versus ovulation induction by clomiphene citrate 1863 g versus ART 1743 g	No differences Natural 1760 ± 256 g versus assisted 1907 ± 452 g	No differences Natural 1523 ± 563 g versus assisted 1521 ± 610 g	No differences Natural 1656 ± 569 g versus assisted 1654 ± 545 g	-	Natural births had lower birth weights Natural 1538.9±569.2 g versus assisted 1769.8 ± 495.5 g	No differences In-vivo conception 1874 ± 382 versus ART 1791 ± 431
Other neonatal complications	Not studied	Not studied	Not studied	Not studied	-	No differences pH <7.0: natural 0.9% versus ART 0.7%; Apgar score <7 at 5 min: natural 9.6% versus ART 3.0%	No differences pH <7.20: in-vivo conception 1.8% versus ART 10.2%; Apgar score ≤5 at 5 min: in-vivo conception 1.8% versus ART 10.2%

In-vivo conception, natural pregnancies and assisted insemination cycles; assisted reproductive technology (ART) conception, in vitro fertilization (IVF) and intracytoplasmic sperm injection (ICSI) cycles; AI, assisted insemination; HELLP, haemolysis, elevated liver enzymes and low platelet count; IUI, intrauterine insemination; TCTA, trichorionic triamniotic pregnancy; TTTS, twin-to-twin transfusion syndrome; IUGR, intrauterine growth restriction.

some cases were delivered at 37 weeks. There were also cases of extreme prematurity (<23 weeks), as cerclage was not performed in earlier years and tocolytic treatment was administered at a much lower frequency.

The mean ± SD birth weight was 1828 ± 411 g, and no differences were found between triplet pregnancies conceived *in vivo* or by ART, as was the case in other studies (*Fennessy et al., 2015*; *Morency et al., 2016*). *Shah et al. (2018)* found that natural pregnancy births had lower birth weights, although *Friedler et al. (1994)* found the opposite. The present study found significant differences in Apgar score at 5 min and umbilical cord pH, which disappeared upon adjustment for confounders. However, regardless of these results, all newborns were sent to the neonatal intensive care unit due to low birth weight and prematurity.

Some of the strengths of this study include a very large cohort, thorough data collection, and the use of univariate and multivariate analyses. *Shah et al. (2018)* applied a multivariate analysis, but *Fennessy et al. (2015)* and *Morency et al. (2016)* made no adjustments for confounders. One of the main biases of the present study is its retrospective nature and the associated limitations. Gestational control protocols and medication recommendations have varied over the years. The authors were unable to collect relevant information on all maternal characteristics that may have influenced the results, such as economic status, or tobacco and alcohol consumption. The only pre-existing maternal disorders included were those related to subfertility. Some data were unavailable in medical reports, including the cause of subfertility and neonatal information. Another major bias of this study is that all types of ART were analysed collectively. Some previous studies found differences in complications between oocyte donors and non-oocyte donors (*Gundogan et al., 2010*; *Perni et al., 2005*), although another study found no differences in ART in triplet pregnancies (*Badreldin et al., 2021*). The final main bias of this study is that pregnancy medical care has improved over the last 20 years, and this work includes cases from all of these years. However, *Weissman et al. (2013)* did not find an improvement in triplet pregnancy outcomes over the years.

Previous published studies and their findings are compared with the findings of the present study in **TABLE 4**.

CONCLUSION

In conclusion, triplet pregnancies conceived by ART are more common than in pregnancies conceived naturally, and have a higher rate of IUGR. No differences were found in maternal, obstetric and neonatal complications. More studies are needed to further examine the pathogenic mechanisms of these ART-related results based on placental examination.

AUTHOR CONTRIBUTIONS

- Conception and design of the study and acquisition and analysis of data: EM-PB, ID-M, MDLC.
- Statistical design and analysis: VQ-G.
- Drafting the manuscript: EM-PB, ID-M, JJP-K, RMR-Z, VQ-G, MDLC.

DATA AVAILABILITY

Data will be made available on request.

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