

## Does assisted reproductive technology increase adverse perinatal outcome in preterm twins? A hospital based comparative study at a single tertiary center in Croatia

*Povećava li pomognuta oplodnja incidenciju loših perinatalnog ishoda kod prijevremeno rođenih blizanaca? Komparativna studija u hrvatskom tercijarnom centru.*

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### Summary

**Objective:** The aim of this study is to investigate the association of assisted reproductive technology (ART) as an independent risk factor for obstetric complications and perinatal outcome in preterm twin pregnancies in 5 years period at a single tertiary center.

**Methods:** We collected the data (from maternal and neonatal medical records) from 88 preterm twin pregnancies conceived after ART and 147 preterm twin pregnancies conceived naturally in a 5-year period.

**Results:** Pregnancy complications in ART mainly included preterm premature rupture of membranes, gestational hypertension and gestational diabetes. More than 85% of ART twins were born by caesarean section, significantly higher than non-ART twins ( $p = 0.005$ ). Neonatal complications in both groups mainly included low Apgar score in 1st minute, perinatal infections, respiratory distress syndrome (RDS) and hyperbilirubinemia and the incidences of these complications were comparable between the groups. According to Weinberg's differential rule, there were 32 monozygotic ART twins and 119 monozygotic non-ART twins. This represents a monozygotic twin rate of 4.7% for ART births and 0.6% for non-ART births.

**Conclusion:** Our results indicated that ART procedures were not associated with adverse perinatal outcome and that ART is not associated with increased obstetric complications in preterm twins followed and born in a single tertiary center.

**Key words:** twin pregnancy, assisted reproductive technology, obstetric complications

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### Sažetak

**Uvod:** Cilj ove studije je istražiti povezanost između potpomognute oplodnje kao nezavisnog čimbenika rizika za opstetričke komplikacije i perinatalnog ishoda u prijevremeno rođenih blizanačkih trudnoća u petogodišnjem razdoblju u jednom tercijarnom centru.

**Metode:** sakupljamo podatke (iz majčinih i novorođenačkih povijesti bolesti) iz 88 prerano rođenih blizanačkih trudnoća začelih potpomognutom oplodnjom i 147 prerano rođenih blizanačkih trudnoća začelih prirodnim putem u petogodišnjem razdoblju.

**Rezultati:** komplikacije kod trudnoća iz potpomognute oplodnje uglavnom su uključivale preuranjeno prijevremeno puknuće plodovih ovoja, hipertenziju u trudnoći, te gestacijski dijabetes. Više od 85% blizanačkih trudnoća začelih potpomognutom oplodnjom dovršeno je carskim rezom, značajno više nego blizanačkih trudnoća začelih prirodnim putem ( $p=0.005$ ). Neonatalne komplikacije u obje skupine uglavnom su uključivale nisku ocjenu po Apgaru u prvoj minuti, perinatalne infekcije, respiratorni distress sindrom (RDS) i hiperbilirubinemiju, te su ove komplikacije bile slične u navedene dvije grupe. Prema Weinbergovom pravilu razlikovanja bilo je 32 monozigota u skupini blizanaca začelih potpomognutom oplodnjom i 119 monozigota u skupini blizanaca začelih prirodnim putem. Navedeno predstavlja monozigotnu stopu od 4.7% za skupinu potpomognute oplodnje i 0.6% za skupinu začetu prirodnim putem.

**Zaključak:** naši rezultati govore da potpomognuta oplodnja nije povezana s komplikacijama perinatalnog ishoda niti s opstetričkim komplikacijama u prijevremeno rođenih blizanaca koji su praćeni u jednom tercijarnom centru.

**Ključne riječi:** blizanačka trudnoća, potpomognuta oplodnja, opstetričke komplikacije

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## Introduction

Nowadays, infertility affects 13-15% of couples worldwide, out of which 30% are diagnosed with unexplained or idiopathic infertility.<sup>1</sup> The rapid progress and wide application of assisted reproductive technology (ART), especially in vitro fertilization and embryo transfer (IVF-ET), make it possible for infertile couples to conceive a baby successfully. The introduction of assisted reproductive technology increased the rate of twin birth.<sup>2</sup> In the United States the twin birth rate has increased by 76% from 1980 to 2009 and in Australia by 15% from 1992 to 2012.<sup>2</sup> In Croatia, the twin rate increased by 55% from 1986 to 2018.<sup>3</sup> The rate of twin birth is high among ART conceived children and have a greater risk for preterm birth and adverse perinatal outcomes in comparison with singletons.<sup>4,5</sup> Studies comparing natural conception and assisted reproductive technology determined that ART pregnancies unfavorably affect mothers and their newborns.<sup>6,7</sup> Although twin pregnancies are known to be related to higher risks of maternal and fetal complications than singleton pregnancies in natural conception, it is not yet clear whether twin pregnancies following ART are associated with higher risks of obstetric and neonatal complications when compared with naturally conceived twin pregnancies.<sup>8,9</sup> The conflicting results from some studies<sup>4,10</sup> are derived from the fact that the monochorionicity rate among ART-conceived twin pregnancies is quite rare, when compared with those conceived naturally, where more than 20% pregnancies present as monochorial. Moreover, monochorionic pregnancies present with a significantly higher proportion of adverse perinatal outcome.<sup>11</sup> As a result, the adverse impact of assisted reproductive technology on the outcomes of ART-conceived twins may be compensated by a lower incidence of monochorionicity. It is important to emphasize that ART pregnancies, whether it is a twin or singleton one, pose additional obstetric risk factors such as advanced maternal age and nulliparity.<sup>12</sup>

Recent literature findings demonstrate significantly higher rates of preterm birth, caesarean section and small for gestational age in the twins conceived by ART procedures compared with spontaneously conceived twin pregnancies.<sup>12,13</sup> In the past decade, growing interest has been paid to the role of ART in maternal and neonatal outcomes of twin pregnancies, but the results remain inconsistent.<sup>4,10,14</sup>

The aim of this study is to investigate the association of ART as an independent risk factor for obstetric complications and perinatal outcome in preterm twin pregnancies in a 5-year period at single tertiary center in Croatia.

## Materials and Methods

Our group retrospectively analyzed the data from 88 preterm ART conceived twin pregnancies and 147 preterm twin pregnancies conceived spontaneously in a 5-year period at the Department of Obstetrics and Gynecology, University Hospital Centre Zagreb from January 2014-December 2018. ART group included patients who had undergone in vitro fertilization-embryo transfer (IVF/ET) or frozen-embryo transfer.

Preterm delivery was defined as delivery at less than 37 weeks of gestation according to the crown-rump length (CRL) ultrasound dating twin pregnancies at 11-14 weeks of gestation.

Maternal complications included confirmed preterm premature rupture of membranes (defined as rupture of membranes prior to the 37th week of gestation), gestational diabetes (plasma glucose level with 75 g oral glucose tolerance test; fasting > 5.1, after 60 min > 10.0, after 120 min > 8.5 mmol/L), gestational hypertension (blood pressure elevation > 140/90 mmHg after 20 weeks of gestation in the absence of proteinuria or other signs of preeclampsia), preeclampsia (hypertension > 140/90 mmHg in association with proteinuria > 0.3g/24h urine, or with thrombocytopenia, impaired liver function, renal insufficiency, pulmonary edema, cerebral or visual disturbances), placenta praevia (recorded as after 28 weeks of gestation, the placenta attaches to the lower segment of the uterus, and even the lower margin of the placenta reaches or covers the cervical orifice, and its position is lower than the first exposed part of the fetus), placental abruption (defined as after 20 weeks of gestation or during childbirth, the placenta in its normal position is partially or completely removed from the wall of the uterus before the fetus is delivered), intrauterine fetal demise (verified by an ultrasound examination that documented absence of fetal cardiac activity) and caesarean section.

Neonatal outcomes included very low birth weight (birth weight < 1500 g), Apgar score < 7 in 1<sup>st</sup> and 5<sup>th</sup> minutes respectively, respiratory distress syndrome (a syndrome of respiratory difficulty in newborn infants caused by a deficiency of a surfactant presented with tachypnea with a respiratory rate of more than 60 respirations per minute, grunting, retractions, nasal flaring and cyanosis, desaturation and decreased air entry), neonatal death (within 28 days after birth), perinatal asphyxia (pH < 7.0, 1<sup>st</sup> minute Apgar score < 7 and base deficit  $\geq$  12 mmol/L), perinatal infection (infection occurred in utero at and around the time of delivery includes neonatal bacteraemia and sepsis (of early or late onset), meningitis, pneumonia and other respiratory infections such as bronchiolitis, caused by

bacteria, parasites, viruses or fungi), hyperbilirubinemia (bilirubin level > 17 micromol/L), congenital heart disease (heart abnormality presented at birth) and Down syndrome. Ultrasound EFW discordance was calculated as  $100 \times (\text{larger EFW} - \text{smaller EFW}) / \text{larger EFW}$ .

If growth discordance occurred then it was defined as a birthweight difference greater than 25%.

The statistics computed included mean, standard deviation and a number of available observations of continuous variables that are presented as median  $\pm$  SD. We performed the Student's t-test (for continuous variables) and Pearson's chi-square test (for categorical variables) to determine the association between the various factors under investigation. All p-values of less than 0.05 were considered significant. Weinberg's differential rule was used to estimate monozygotic twin rate.<sup>15</sup> The statistical software package SPSS 17.0 for Windows (SPSS Inc., Chicago, IL, U.S.) was used for data analyses.

### Results

Both groups were comparable in demographic, social, and past obstetric history characteristics. The maternal age and the proportion of primiparity in the

ART group were significantly higher than in the non-ART group ( $31.26 \pm 0.362$  versus  $29.23 \pm 0.292$  years old,  $p < 0.05$ , and 81.82% versus 44.88%,  $p < 0.05$ , respectively). We found a higher rate of > 35 years old mothers in the ART group, but not significantly (32 versus 28,  $p = 0.016$ ). There was no preexisting hypertension and no difference in gestational age between the groups ( $34.5 \pm 0.32$  weeks versus  $33.32 \pm 0.42$  weeks,  $p = 0.439$ ) (Table 1).

Pregnancy complications in the ART group mainly included preterm premature rupture of membranes (pPROM), gestational hypertension and gestational diabetes mellitus (GDM), without significant difference between the groups. In contrast, the proportion of caesarean section was significantly higher in the ART group ( $p = 0.005$ ) (Table 2).

Table 3 summarises neonatal outcomes between the groups. Out of 470 newborns, 461 (98.09%) were born alive. Perinatal mortality in this study consisted from 9 cases of early neonatal death related to birth asphyxia, congenital heart disease and severe respiratory distress syndrome, respectively. There was no documented case of stillbirth in our cohort. Thus, we report a rate of 1.9 early neonatal deaths for 100 livebirths without significant statistical difference between the groups.

Table 1 Maternal characteristics

Tablica 1. Majčinske karakteristike

	Non-ART group (N = 147)	ART group (N = 88)	P-value P-vrijednost
Maternal age (years) / Starost majke	$29.23 \pm 0.292$	$31.26 \pm 0.362$	$< 0.05$
> 35 years / godina	32 (21.77%)	28 (31.82%)	$> 0.05$
Mean gestational age (weeks) Srednja gestacijska dob (tjedni)	$34.5 \pm 0.318$	$33.32 \pm 0.420$	$> 0.05$
Primiparae (%) / Prvorotke	66 (44.88%)	72 (81.82%)	$< 0.05$
Preexisting diabetes mellitus Ranije postojeći diabetes mellitus	1 (0.68%)	1 (1.14%)	$> 0.05$

Table 2 Pregnancy complications

Tablica 2. Komplikacije u trudnoći

	Non-ART group (N = 147)	ART group (N = 88)	P-value P-vrijednost
Intrauterine demise	5	2	0.625
Placenta praevia	0	3	0.025
Abruptio placentae	1	2	0.294
GDM	9	7	0.596
pPROM	13	12	0.263
Gestational hypertension / Gestacijska hipertenzija	15	8	0.787
Preeclampsia	5	3	0.997
Caesarean section / Carski rez	110	75	0.005

GDM: gestational diabetes mellitus, pPROM: preterm premature rupture of membranes

Table 3 Neonatal outcome  
 Tablica 3. Neonatalni ishod

	Non-ART group (N = 294 newborns) <i>novorođenčad</i>	ART group (N = 176 newborns) <i>novorođenčad</i>	P-value <i>P-vrijednost</i>
Apgar < 7 in 1st minute / <i>u 1. minuti</i>	74	38	0.333
Apgar < 7 in 5th minute / <i>u 5. minuti</i>	30	17	0.300
Weight/težina < 1500 g	66	38	0.828
Neonatal death / <i>Neonatalna smrt</i>	6	3	0.797
Perinatal infection / <i>Perinatalna infekcija</i>	34	24	0.509
Perinatal asphyxia / <i>Perinatalno zagušenje</i>	18	8	0.469
Hyperbilirubinemia	25	10	0.259
RDS	27	16	0.973
Congenital heart disease / <i>Prirodna bolest srca</i>	7	5	0.760
Sy Down	2	1	0.883
NICU admission / <i>NICU prijem</i>	91	52	0.692

RDS: respiratory distress syndrome, NICU: Neonatal Intensive Care Unit  
*RDS: respiratorni distres sindrom, NICU Neonatalna jedinica intenzivne njege*

Table 4 Monozygotic and dizygotic ART and non-ART preterm twins in a 5-year period  
 Tablica 4. Monozigotični i dizigotični ART prijevremeni blizanci u petogodišnjem razdoblju

	Non-ART group 294 N (%)	ART group 176 N (%)
Opposite sex twins / <i>Blizanci suprotnog spola</i>	88 (29.94%)	72 (40.91%)
Same sex twins / <i>Blizanci istog spola</i>	206 (70.06%)	104 (59.09%)
Weinberg's differential rule / <i>Wienberg diferencijalno pravilo</i>	174	144
Dizygotic twins / <i>Dizigotski blizanci</i>	120	32
Monozygotic twins / <i>Monozigotski blizanci</i>	19.905	679
All births where sex is available / <i>Sva rođenja gdje je spol dostupan</i>	294	176
Twins where sex is available / <i>Blizanci gdje je spol dostupan</i>		
Dizygotic twin rate / <i>Dizigotska stopa blizanaca</i>	0.9%	21.2%
Monozygotic twin rate / <i>Monozigotska stopa blizanaca</i>	0.6%	4.7%

There were no significant differences in terms of the incidence of very low birth weight (birth weight < 1500 g), Apgar scores in 1<sup>st</sup> and 5<sup>th</sup> minute respectively, neonatal asphyxia, perinatal infections, respiratory distress syndrome and admission for NICU ( $p > 0.05$ ). There was 1 case of twin-to-twin transfusion syndrome in the control group.

Table 4 shows the monozygotic and dizygotic rate among ART and non-ART twins in the observed period. Of twins where sex is available for both babies, the same sex twins made up 59.09% of ART twins compared to 70.06% of non-ART twins. According to Weinberg's differential rule, there were 32 monozygotic ART twins and 119 monozygotic non-ART twins. This represents a monozygotic twin rate of 4.7% for ART births and 0.6% for non-ART births.

## Discussion

Since it was previously reported that the rate of preterm birth is significantly higher in ART conceived twins than spontaneously conceived twins,<sup>16</sup> it was interesting to determine whether preterm birth affects the perinatal outcome in comparing groups. Our results indicated that ART procedures were not associated with adverse perinatal outcome and that ART did not affect the overall rate of neonatal outcome in preterm twins followed and born in a single tertiary center. Although twin pregnancies are frequently observed in IVF-ET treatments, it is still not well known if they have additional maternal and fetal risks compared with spontaneously conceived twin pregnancies. Various studies have been performed to

address this issue, but their results are inconsistent primarily due to different types of obstetric care and delivery management that can affect perinatal outcomes and potential bias caused by unmeasured confounders. Our reported preterm birth rate between groups is consistent and comparable with other published studies<sup>6,17,18</sup> and with one meta-analysis suggesting ART as a predictor for preterm birth as much as it is a history of preterm birth.<sup>19</sup> However, most of the studies comparing ART versus spontaneously conceived twin pregnancies report comparable perinatal outcomes regardless of preterm birth rate. This underlines the overall progress in ART twin pregnancy surveillance despite an anticipated higher proportion of monochorionicity and maternal age.

We demonstrated a higher proportion of women older than 35 years in the ART group. In contrast with the results from three other studies,<sup>2,5,6</sup> we did not find that ART mothers had a higher rate of gestational hypertension and gestational diabetes compared with non-ART mothers. Although we had a significantly higher proportion of primiparae in the ART group ( $p < 0.05$ ), the rates of preeclampsia between the groups were comparable. Our results illustrated that the rate of very low birth weight twins did not differ between the groups as in some reports,<sup>2,6,20,21</sup> which can be explained with no significant difference in gestational age between the groups found in this study.

Caesarean sections have been practiced as an obstetric surgical procedure that contributes to reducing fetal complications and incidence has considerably increased over the years worldwide.<sup>22</sup> In our study, the rate of caesarean section was significantly higher in the ART group than in the spontaneous group (85.23% versus 74.83%,  $p < 0.05$ ) and this was equally divided between elective and emergency caesarean section. This result is consistent with other similar studies.<sup>5,9,21</sup> However, some studies have found no significant difference in the rate of caesarean delivery, indicating that institutional differences in management are likely to have a role in the variation of results.<sup>23,24</sup> Obstetricians' lower threshold and anxiety may also contribute to the higher rate of caesarean sections in the ART group.

During the study period, the twin delivery rates following ART decreased from 19.5% to 14% confirming that the number of embryos transferred have been reduced and new criteria for evaluating the success of ART have been developed, concentrated on successful singleton live birth delivery which had an impact on the fall in the proportion of ART multiple births. Multiple pregnancy is the greatest avoidable risk of IVF. The reduction in the multiple pregnancy

rate has been achieved through a combination of statutory regulation, cooperation of clinicians and appropriate patient education to develop a successful strategy to reduce multiple pregnancies without a concomitant reduction in the pregnancy rate. It is also clear from the experience of some countries, such as Sweden and Belgium, that further reduction in the multiple pregnancy rate to single figures is feasible. In his study, Luke concluded that transferring high quality and fewer embryos is responsible for reducing the risk of multiple births from ART treatments and ultimately reducing the perinatal adverse outcomes.<sup>25</sup> Opposite sex twins are all dizygotic as a result from the fertilisation of two separate oocytes by two separate sperms forming two separate zygotes in non-ART pregnancy, or from multiple embryo transfers in ART pregnancy. All dizygotic twins develop their own membranes and placenta and they are dichorionic diamniotic twins. Same sex twins can be monozygotic or dizygotic. All monozygotic are the same sex twins and about half of dizygotic twins are the same sex.<sup>26</sup> In ART pregnancy, monozygotic twins result from splitting from the single transferred embryo on day 3 or after, so monozygotic monochorionic twins are more likely to develop.<sup>27</sup> The possibility to estimate the rate of monozygotic twins is to use the sex of twin pairs by applying Weinberg's differential rule<sup>15,27</sup> or to perform genetic testing. Our results showed that the proportion of same sex twins was lower in the ART group (59.09%), than in non-ART group (70.75%) which is slightly different from Wang et al.,<sup>2</sup> but lower than Pinborg et al.<sup>28</sup> Twin pregnancies after ART procedures are highly associated with multiple embryo transfers.<sup>29</sup> Since the number of transferred embryos was not listed in the maternal medical record, we were unable to investigate the ART group according to the number (single or multiple) of embryo transfers and that was the limitation of the study. Some studies demonstrate that perinatal outcome may vary according to the type of ART treatment (fresh versus frozen embryo transfer, cleavage versus blastocyte transfer),<sup>30-32</sup> but that was not captured in our maternal medical records.

It remains unclear whether the increased risk of adverse obstetric outcomes after ART is a direct effect of the procedure and the technology or whether it reflects some other factor related to the underlying infertility of the couple. If the problem lies in the ART technology itself, more adverse outcomes would be documented for ART twins.<sup>20,33</sup>

The major strength of our study includes the large numbers of twin pregnancies followed and delivered in a single tertiary center with a standardised care protocol for all twin pregnancies.

The limitations of the study are the retrospective nature of data collection and the omission of maternal weight due to the lack of information in the available medical data.

Preterm twin pregnancies after ART are only associated with a higher rate of caesarean delivery which is consistent with the published recommendations that IVF twin pregnancies may be treated as an independent indication for caesarean section.<sup>34</sup> Our study emphasizes that the method of conception seems not to have a negative impact on the course of the pregnancy, obstetric and neonatal outcome. Nevertheless, the influence of ART on preterm birth remains controversial and requires additional studies.

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