Perinatal outcome of twin pregnancies delivered in the University Hospital Centre Zagreb

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Mentor: dr.sc. Vesna Sokol Karadjole, dr.med.

Abbreviations

ART:	Assisted reproductive technology
BHSB:	Beta hemolytic streptococcus group B
CH:	Chronic hypertension
CS:	Caesarean section
DC:	Dichorionic
DCDA:	Dichorionic diamniotic
DMI:	Diabetes mellitus type 1
DMII:	Diabetes mellitus type 2
FET:	Frozen embryo transfer
GDM:	Gestational diabetes mellitus
GH:	Gestational hypertension
GI:	Gastrointestinal
IUD:	Intrauterine death
IUGR:	Intrauterine growth restriction
IVF:	In vitro fertilization
IVH:	Intraventricular hemorrhage
MBR:	Multiple birth rate
MC:	Monochorionic
MCDA:	Monochorionic diamniotic
MCMA:	Monochorionic monoamniotic
NEC:	Necrotizing enterocolitis
PE:	Preeclampsia
pPROM:	Preterm premature rupture of membranes
RDS:	Respiratory distress syndrome
ROP	Retinopathy of prematurity
TAPS:	Twin anemia polycythemia sequence
TRAP	Twin reversed arterial perfusion sequence
TTTS:	Twin-to-twin transfusion syndrome
χ^2	Chi-squared test

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Abstract

Objective: The aim of this retrospective study was to compare the perinatal and neonatal outcome of monochorionic versus dichorionic twins delivered in the year 2019 and 2020 at the University Hospital Centre (UHC) Zagreb.

Methods: This was a retrospective study of 212 twin pregnancies delivered at a tertiary center between 1st of January 2019 and 31st of December 2020. According to chorionicity, pregnancies were classified into three groups: dichorionic-diamniotic (DCDA), monochorionic-diamniotic (MCDA) and monochorionic-monoamniotic (MCMA). The perinatal and neonatal outcome were determined by analyzing maternal diseases, foetal and neonatal complications between the three groups.

Results: The study included 165 (77.9%) DCDA, 45 (21.2%) MCDA and 2 (0.9%) MCMA twins that were delivered in the UHC Zagreb in a 2-year period. The MCDA group showed a significantly higher incidence of adverse foetal and neonatal outcome in comparison with DC twins (p=0.0252, p=0.0104), however no differences in incidence of adverse outcomes were found between MCDA and DC twins and maternal diseases such as gestational diabetes, gestational hypertension and preeclampsia. Mothers of DC twins conceived more often through assisted reproductive technology compared to mothers of MC twins (58.8% vs 8.9%, p<0.0001). Preterm delivery occured in almost 61% of all twin pregnancies and the number of extremely preterm deliveries (22 – 28 weeks) was significantly higher in MC compared to DC twins (p=0.0032). Among the 3 groups, there were no difference in pPROM. Caesarean section occurred in 75.8% of DC vs. 61.7% of MC twin pregnancies. Furthermore, MCDA group was more commonly delivered by emergent CS compared to DC group (69% vs. 42.4%). Analysing foetal complications, MCDA twins had a significantly higher number of growth discordance in comparison to DC twins (24.4% vs. 7.9%, p=0.0316). Significant differences were also detected in the birth weight of the 1st (p<0.0001) and the 2nd twin (p=0.0018) and in the Apgar scores in the 1st and 5th minute (1st minute: p=0.0009, p=0.0019; 5th minute: p=0.0002, p=0.0003). According to complications specific for MC pregnancies, TTTS and TAPS occurred in 6.4% and 8.5% of MC twins respectively. MCDA twins had a significantly higher number of complications of prematurity compared to DC twins (p=0.0108).

Conclusion: The incidence of adverse perinatal and neonatal outcome in MC twins is significantly higher compared to DC twin pregnancies, while the incidence of maternal complications in the two groups was not significantly different. The results of this study support the current knowledge about twin pregnancies and further emphasize the importance of regular antenatal care for twin pregnancies with special emphasis on MC pregnancies.

Key words: monochorionic twin pregnancy, dichorionic twin pregnancy, perinatal outcome, neonatal outcome

Sažetak

Cilj: Cilj ove retrospektivne studije je usporediti perinatalni i neonatalni ishod jednojajčanih u odnosu na dvojajčane blizance porođene u Kliničkom bolničkom centru Zagreb u 2019. i 2020. godini.

Metode: Retrospektivno istraživanje uključilo je 212 blizanačkih trudnoća porođenih u tercijarnom centru u periodu od 01. siječnja 2019. do 31. prosinca 2021. U odnosu na korionicitet blizanaca, ispitanice su podjeljene u tri skupine: bikorijati-biamnijati (BCBA), monokorijati-biamnijati (MCBA) i monokorijati-monoamnijati (MCMA). Perinatalni i neonatalni ishod određivani su analizom razvoja maternalne patologije u trudnoći, i analizom fetalnih i neonatalnih komplikacija između tri skupine.

Rezultati: Istraživanjem je obuhvaćeno 165 (77,9%) BCBA, 45 (21,2%) MCBA blizanaca i 2 (0,9%) MCMA blizanaca koji su rođeni u KBC Zagreb u periodu od dvije godine. MCBA skupina je imala značajno veću učestalost fetalnih komplikacija i nepovoljnih neonatalnih ishoda u usporedbi s BC blizancima (p=0,0252, p=0,0104), međutim nije bilo statistički značajnih razlika između bolesti majki MCBA i BC blizanaca poput gestacijskog dijabetesa, gestacijske hipertenzije i preeklampsije. Majke dvojajčanih blizanaca su češće začele pomoću medicinske potpomognute oplodnje u usporedbi s majkama jednojajčanih blizanaca (58,8% nasuprot 8.9%, p <0,0001). Prijevremeni porodi dogodili su se u gotovo 61% svih blizanačkih trudnoća, a broj izrazito prijevremenih poroda (22 – 28 tjedna) bio je značajno veći kod MC u usporedbi s BC blizancima (p=0,0032). Među 3 skupine nije bilo razlike u prijevremenom prsnuću plodovih ovoja. Carskim rezom je rođeno 75.8% BC nasuprot 61.7% MC blizanačkih trudnoća. Nadalje, MCBA blizanci su češće porođeni hitnim carskim rezom u usporedbi s BC blizancima (69,0% nasuprot 42.4%). Analizirajući fetalne komplikacije, MCBA blizanci su imali značajno veći broj diskordantnog rasta u usporedbi s BC blizancima (24,4% nasuprot 7,9%, p=0,0316*). Također su pronađene značajne razlike u porođajnoj težini prvog (p <0,0001) i drugog blizanca (p = 0,0018) te u Apgar indeksima u 1. i 5. minuti (1. minuta: p = 0.0009, p = 0.0019; 5.minuta: p = 0,0002, p = 0,0003). Prema komplikacijama specifičnim za MC trudnoće, TTTS i TAPS su pronađeni u 6,4%, odnosno 8,5% MC blizanaca. MCBA blizanci su imali značajno veći broj neonatalnih komplikacija u usporedbi s BC blizancima (p = 0,0108*).

Zaključak: Incidencija nepovoljnih perinatalnih i neonatalnih ishoda kod MC blizanaca značajno je veća u usporedbi s BC blizanačkim trudnoćama, dok se učestalost bolesti majki u te dvije skupine nije značajno razlikovala. Rezultati ovog istraživanja podupiru dosadašnja saznanja o blizanačkim trudnoćama i dodatno ističu važnost redovite antenatalne skrbi za blizanačke trudnoće s posebnim naglaskom na MC trudnoće.

Ključne riječi: jednojajčani blizanci, dvojajčani blizanci, perinatalni ishod, neonatalni ishod

Introduction

The rate of multiple births is increasing due to delayed childbearing, and consequently, increased use of assisted reproductive technology (ART), such as in vitro fertilization (IVF) or induction of ovulation. (1–6) In the United States, the multiple birth rate (ratio of MB per 1,000 live births) (5) increased more than 59%, from 19.3 to 30.7 between 1980 and 1999. (4) In Korea, the MBR rose from 10.0 in 1991 to 27.5 in 2008, accounting for a 275% increase in the MBR over the span of 18 years. (5) Similarly, in France the twin pregnancy rate increased by 80% from 1972 to 2006. (7)

A twin pregnancy, also known as a gemellary pregnancy, refers to the production of two offspring in one pregnancy. (1,8) Twin pregnancies can be divided according to zygosity and chorionicity. Zygosity denotes to the type of conception. Dizygotic or fraternal twins account for 70% of twin pregnancies and occur when two ova are fertilized by two sperm cells, due to multiple ovulations. Monozygotic or identical twins account for 30% of twin pregnancies and occur due to the division of a zygote derived from the fertilization of one ovum by one sperm cell. Furthermore, chorionicity denotes the type of placentation. Dizygotic twin pregnancies develop into dichorionic diamniotic (DCDA) placentation, while monozygotic twin pregnancies can be divided into 3 types of placentation depending on the time of division of the zygote. If the zygote divides within 3 days after fertilization, it will produce DCDA twins and if it divides later, between the 3^{rd} and 9^{th} day, it will produce monochorionic diamniotic (MCDA) twins. Furthermore, if the division occurs between the 8^{th} and 12^{th} day after fertilization, it will produce monochorionic monoamniotic (MCMA) twins while the remaining can divide even later resulting in conjoined MCMA twins. (2)

Chorionicity is the main determinant of the perinatal outcome in twin pregnancies. Since the monochorionic (MC) placenta is designed for a singleton fetus, and might not provide adequate physiological support for twins, MC twins are associated with increased risks compared with dichorionic (DC) twins, and these risks necessitate closer surveillance. Therefore, chorionicity determination, which can be established early in the first trimester of a pregnancy, should be an integral part of a prenatal ultrasound assessment of all multiple pregnancies. (9) Certain characteristics of ultrasound can give insight into the chorionicity, including difference in gender (dichorionic), presence of two placentas (dichorionic), a four layered intertwin membrane (dichorionic), and the way the placenta and the intertwin membrane intersect. In dichorionic twins, the placenta projects between the layers of the intertwin membrane forming a triangular “lambda” or “twin peak” sign (Figure 1a). In monochorionic twins, the lack of projection of the placenta between the intertwin membrane creates an appearance of a “T” sign (Figure 1b). (2)

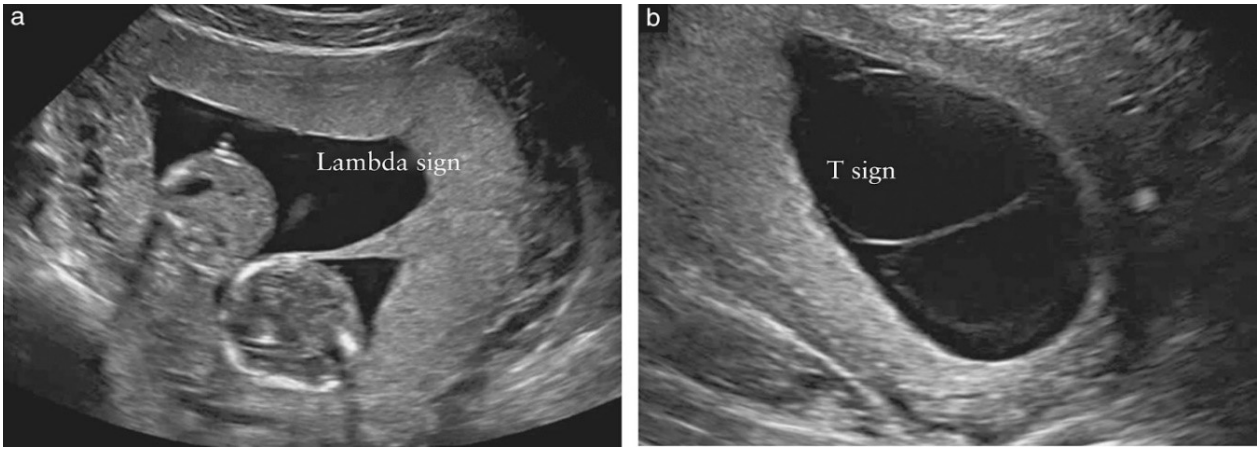

Figure 1 Ultrasound images showing (a) “lambda” or “twin peak” sign denoting a triangular extension of chorionic tissue from the placenta to the intertwin membrane, characteristic of a dichorionic twin pregnancy; (b) “T” sign denoting the formation of a right angle at the thin intertwin membrane and placental junction, characteristic of a monochorionic twin pregnancy.

Furthermore, the pathologic examination is also specific for each type of twin pregnancy. DCDA pregnancy is determined by observing two separate placentas and a four layered intertwin membrane. When it presents with a fused placenta, the distinction can be made by observing the intertwin membrane. MCDA presents with fusion of two placentas into one and a two layered intertwin membrane. Lastly, MCMA presents with absence of an intertwin membrane (Figure 2). (1) It is important to note that MC type specific complications, mentioned later, typically arise from the anatomic characteristics of the placenta, such as peripheral cord insertion, uneven placental sharing and the presence or absence of different types of intertwin vascular anastomoses. (2,10)

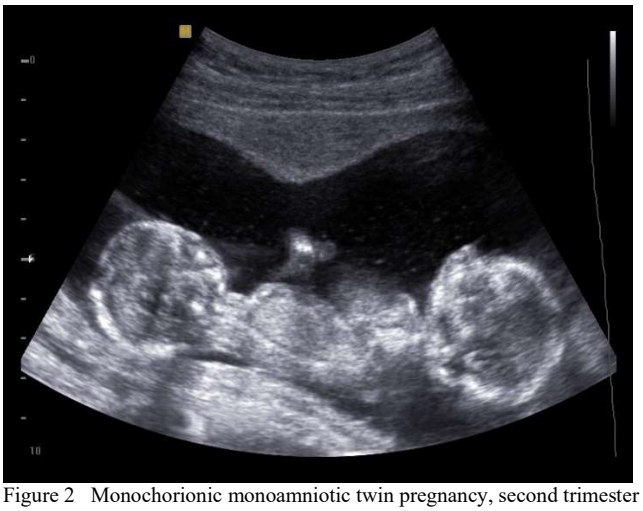
Figure 2 Monochorionic monoamniotic twin pregnancy, second trimester ultrasound image.

Twin pregnancies have a 3 to 7 times higher risk of adverse perinatal and neonatal outcome compared to singleton pregnancies (11). They are associated with higher rates of maternal complications of pregnancy such as hypertensive disorders (gestational hypertension and preeclampsia), gestational diabetes mellitus (GDM), maternal anaemia and thromboembolic disorders. (3) Furthermore, twins have an increased incidence of preterm premature rupture of membranes (pPROM) and preterm birth. Nevertheless, problems in childbirth are more common

than with singletons, and roughly half of all twins are born via caesarean section (CS). (12,13) Perinatal mortality is generally higher among twins due to higher incidence of intrauterine growth restriction (IUGR), malformations and intrauterine death (IUD) of one or both twins. (3,14,15) Significantly, discordant growth is an important factor along with selective intrauterine growth restriction (sIUGR) that negatively influences perinatal mortality and morbidity in twins. (16–19) MC twins are especially at risk for adverse perinatal outcomes. Despite monochorionic twins amounting to 20% of twin pregnancies, they account for 30% of pregnancy-related complications (10, 13, 17). MC twins share one placenta and are therefore prone to hemodynamic complications, such as twin-to-twin transfusion syndrome (TTTS), twin anaemia polycythaemia sequence (TAPS), as well as twin reversed arterial perfusion sequence (TRAP). (2,14,21,22)

Adverse neonatal outcome is generally higher in twin compared to singleton pregnancy and it is characterized with an increased rate of respiratory distress syndrome (RDS), intracranial bleeding and neonatal infection due to a higher number of premature borne babies. (3)

The purpose of this study was to compare the perinatal outcome of dichorionic (DC) versus monochorionic (MC) twin pregnancies delivered in the University Hospital Centre Zagreb, Croatia.

Materials and Methods

This is a retrospective cohort study of twin pregnancies delivered at the Department of Gynaecology and Obstetrics, University Hospital Centre Zagreb in Croatia, in a two-year period, between January 2019 and December 2020. The cases were extracted from the birth registry, and detailed data was collected using the Hospital's Information System (Bolnički Informatički Sustav - BIS). Twelve cases were excluded from further analysis due to missing data. Following exclusion, 212 cases were included in the study. This study was approved by the Ethical Committee of the University Hospital Centre Zagreb.

Maternal demographics included maternal age at delivery, parity, mode of conception (spontaneous vs. assisted conception), and type of chorionicity. The assisted mode of conception included pregnancies performed through in vitro fertilization (IVF) or frozen embryo transfer (FET). The type of chorionicity was determined by characteristic findings on ultrasonography, and confirmed by gross and pathohistological examination of the placenta.

Maternal pregnancy complications included diabetes in pregnancy (gestational diabetes mellitus, diabetes mellitus type 1 and type 2) and hypertensive disorders (gestational hypertension, chronic hypertension, and preeclampsia).

Perinatal outcome included gestational age at delivery, delivery type, length of hospitalization, presence of preterm premature rupture of membranes (pPROM) and foetal complications, such as discordant growth, spontaneous abortion, intrauterine death (IUD), congenital malformations and MC type specific complications such as selective intrauterine growth restriction (sIUGR), twin-to-twin transfusion syndrome (TTTS), and twin anaemia polycythaemia sequence (TAPS). Gestational age at delivery was divided into 5 groups; 41 – 38 weeks, 37 – 35 weeks, 34 – 29 weeks, 28 – 22 weeks, and < 22 weeks. Delivery type was also divided into 5 groups; vaginal, elective caesarean section (CS), emergent CS, spontaneous abortion, and combined delivery. The combined delivery was defined as the 1st twin delivered vaginally and the 2nd twin by an emergent CS. The length of hospitalization was calculated in days from the admission day to the day of discharge. The presence of pPROM (rupture of membranes prior to 37 weeks of gestation) was analysed according to gestational age; 37 – 35 weeks, 34 – 29 weeks, 28 – 22 weeks, and < 22 weeks. Discordant growth (applies to DCDA) and sIUGR (applies to MCDA and MCMA) was defined as a neonatal weight difference ≥ 25%. TTTS was determined by characteristic ultrasound findings (Figure 2) of polyhydramnios around the recipient twin (deepest vertical pocket of amniotic fluid ≥8cm), and oligohydramnios around the donor twin (deepest vertical pocket of amniotic fluid ≤2cm). Further evaluation of TTTS was determined according to the Quintero staging. (23) Posnatal TTTS diagnosis was confirmed with a Hb difference >5g/dl between the twins. TAPS was founded on the discordance of middle cerebral artery peak systolic velocity measurements assessed by a Doppler ultrasound (MCA-PSV >1.5 multiples of the median [MoM] in donor twin and <1.0 in recipient twin). Spontaneous abortion was defined as loss of a pregnancy < 22 weeks of gestation. IUD was defined as death of the fetus in the uterus ≥ 22 weeks of gestation. Congenital malformations were defined as structural or functional anomalies of prenatal origin and included: congenital malformations of the nervous system (spina bifida, hydrocephalus

etc.), musculoskeletal system (pes equinovarus), digestive system (omphalocele, gastroschisis), circulatory system (heart malformations) and malformations of the eye, ear, face and neck.

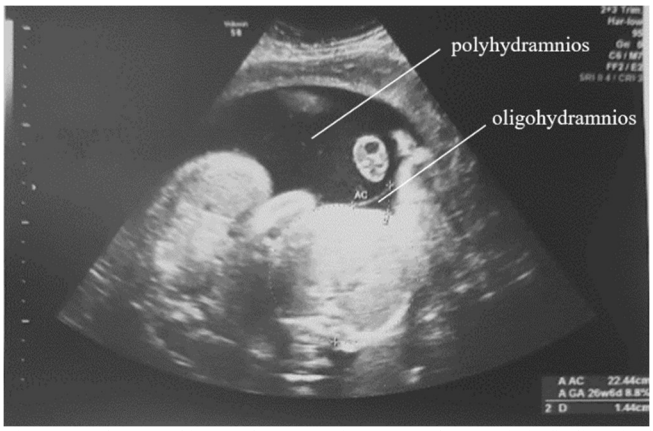

Figure 3 Ultrasound image demonstrating TTTS characteristic findings including polyhydramnios around the recipient twin, and oligohydramnios around the donor twin.

Neonatal outcomes included birth weight, Apgar score in the 1st and 5th minute, the presence of complications of prematurity, the presence of congenital neonatal malformations, neonatal death and neonatal infection. The complications of prematurity included respiratory distress syndrome (RDS), intraventricular hemorrhage (IVH), retinopathy of prematurity (ROP), and necrotizing enterocolitis (NEC). Neonatal malformations included all previously noted congenital malformations as well as unrecognized foetal disorders such as heart defects, neurological malformations, GI malformations etc. Neonatal death was defined as a death within the first 28 days of life. Postnatal infection included infection with various pathogens (BHSB, E. coli. pseudomonas aeruginosa, etc.) that were clinically and laboratory confirmed.

Normality of distribution for continuous variables was checked using Shapiro-Wilk test. Continuous data was compared using Student's t-test for independent samples or Mann-Whitney U test according to the distribution or size of the data. Frequencies were compared using $\chi 2$ test. Post-hoc corrections were made by the step down method using Sidak adjustments. All tests were performed using Python 3.7. programming language. A statistically significant difference was determined between DC and MC twin pregnancies and the mode of conception including spontaneous ($p<0.0001$) and ART ($p<0.0001$). Also, a statistically significant difference was found between DC and MC twins and the birth weight of the 1st twin ($H=2391.00000$, $p<0.0001$) and 2nd twin ($H=2799.50000$, $p=0.0018$). Additionally, analysis of the data showed a significant difference between DCDA and MCDA twin pregnancies and the 1st minute and 5th minute Apgar scores in the 1st and 2nd twin (1st min., 1st twin [$H=2874.00000$, $p=0.0009$]; 1st min., 2nd twin [$H=2889.00000$, $p=0.0019$]; 5th min., 1st twin [$H=2807.00000$, $p=0.0002$]; and 5th min., 2nd twin [$H=2765.50000$, $p=0.0003$]). Furthermore, a statistically significant difference was found between DC and MC twins and the following: the gestational age at delivery at 22 – 28 weeks ($p=0.0032$*); the combined (vaginal + emergent CS) delivery type ($p=0.0396$*); the growth discordance ($p=0.0316$*); and the complications of prematurity found in both twins ($p=0.0108$*). Adjusted p values are marked with an asterisk (*).

Results

This retrospective study included 212 twin pregnancies delivered at the University Hospital Centre Zagreb in a 2-year period. According to chorionicity, the twins were divided into three groups: dichorionic diamniotic (DCDA) which included 165 (77.9%) twins, monochorionic diamniotic (MCDA) which included 45 (21.2%) twins and monochorionic monoamniotic (MCMA) which included 2 (0.9%) twins. The comparison between the three groups showed no statistically significant difference in maternal age and parity. The number of spontaneously conceived pregnancies was significantly higher in the MC group compared to the DC group, (91.4% vs 41.2%, $p<0.0001$). Whereas, nearly 60% of mothers in the DC group were conceived using assisted reproductive technology (ART). (Table 1)

This study analyzed the presence of diabetes (DMI, DMII, GDM) and hypertensive disorders (GH, CH, PE) in DC and MC pregnancies. According to the results, there were no significant differences between the DC and MCDA groups. GDM made up for the highest portion of diabetes in pregnancy in both DC (97%) and MCDA (100%) twins. In addition, gestational hypertension accounted for the highest portion of hypertensive disorders in DC twins (50.0%) and preeclampsia in MCDA twins (66.7%). Since MCMA twins had no maternal diseases and accounted for a very small sample (N = 2), they were excluded from the analysis.

Table 1 Demographics

Demographics	*Type of chorionicity*			*Total (N, %)*	*p value (DC vs MC)*
	Dichorionic (DC)	**Monochorionic (MC)**			
	DCDA (N, %)	**MCDA (N, %)**	**MCMA (N, %)**		
	165 (77.9)	**45 (21.2)**	**2 (0.9)**	**212 (100.0)**	
Maternal age (mean ± STD)	33.7 ± 4.5	32.8 ± 4.5	38.0 ± 2.8	33.6 ± 4.5	0.39
Parity					
Nullipara	105 (63.6)	24 (53.33)	1 (50.00)	130 (61.3)	0.41
Primapara	36 (21.8)	13 (28.89)	1 (50.00)	50 (23.6)	0.32
Multipara	24 (14.6)	8 (17.78)	0 (0.0)	32 (15.1)	0.69
Mode of conception					
Spontaneous	68 (41.2)	41 (91.1)	2 (100.0)	111 (52.4)	<0.0001
ART	97 (58.8)	4 (8.9)	0 (0.0)	101 (47.6)	<0.0001
Diseases in pregnancy					
Diabetes in pregnancy	36 (21.8)	5 (11.1)	0 (0.0)	41 (19.3)	
GDM	35 (97.2)	5 (100.0)	0 (0.0)	40 (18.9)	0.72 *
DM type I	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	NA *
DM type II	1 (2.8)	0 (0.0)	0 (0.0)	1 (0.5)	0.97 *
Hypertension in pregnancy	26 (15.8)	6 (13.3)	0 (0.0)	32 (15.1)	
GH	13 (50.0)	2 (33.3)	0 (0.0)	15 (7.1)	0.97 *
CH	3 (11.5)	0 (0.0)	0 (0.0)	3 (1.4)	0.99 *
PE	10 (38.5)	4 (66.7)	0 (0.0)	14 (6.6)	0.97 *

DCDA: dichorionic diamniotic; MCDA: monochorionic diamniotic; MCMA: monochorionic monoamniotic; ART: assisted reproductive technology; GDM: gestational diabetes mellitus; DM: diabetes mellitus; GH: gestational hypertension; CH: chronic hypertension; PE: preeclampsia; NA: not applicable. * p value adjusted for multiple comparisons using the Holm-Bonferroni method (DCDA vs MCDA).

Preterm delivery occured in 129 (60.8%) twin pregnancies. The majority of DC twins (43.6%) were delivered at term (38 to 41 weeks), while a vast number of MCDA twins (35.6%) were born preterm, between 35 to 37 weeks. MCMA twins did not survive past 28 weeks. According to the gestational age, the number of extremely preterm deliveries (22 – 28 weeks) was significantly

higher in the group of MC twins compared to the DC group (p=0.0032*). The comparison of types of delivery including vaginal and caesarean section between the three groups showed no significant differences. Most of the twins were delivered via caesarean section (72.6%), one fourth were delivered vaginally (21.2%) and the remaining few (6.2%) resulted in either a combined birth (vaginal and emergent CS) or a spontaneous abortion. DC twins were more commonly delivered by an elective (57.6%), while MCDA by emergent CS (69.0%). MC twins had a significantly higher number of combined deliveries compared to DC twins (p=0.0396*). In DC and MCDA group of twins, pPROM most commonly occurred between the 35th to 37th week of gestation, while it did not occur in MCMA group. According to the gestational age of pPROM, there were no statistically significant differences between the three groups of twins. Mothers of DC twins stayed in the hospital longer than MC twins, but there was no significant difference in the length of hospitalization days. (Table 2)

Table 2 Perinatal outcome

Perinatal outcome	*Type of chorionicity*			*Total (N, %)*	*p value (DC vs MC)*
	Dichorionic (DC)	**Monochorionic (MC)**			
	DCDA (N, %)	**MCDA (N, %)**	**MCMA (N, %)**		
	165 (77.9)	**45 (21.2)**	**2 (0.9)**	**212 (100.0)**	
Gestational age at delivery (weeks)					
41 – 38	72 (43.6)	11 (24.4)	0 (0.0)	83 (39.2)	0.18 *
37 – 35	59 (35.8)	16 (35.6)	0 (0.0)	75 (35.4)	0.98 *
34 – 29	21 (12.7)	10 (22.2)	0 (0.0)	31 (14.6)	0.44 *
28 – 22	5 (3.0)	7 (15.6)	1 (50.0)	13 (6.1)	0.0032 *
<22	8 (4.9)	1 (2.2)	1 (50.0)	10 (4.7)	0.98 *
Delivery type					
vaginal	32 (19.4)	12 (26.7)	1 (50.0)	45 (21.2)	0.62 *
CS	125 (75.8)	29 (64.5)	0 (0.0)	154 (72.6)	
elective	72 (57.6)	9 (31.0)	0 (0.0)	81 (52.6)	0.06 *
emergent	53 (42.4)	20 (69.0)	0 (0.0)	73 (47.4)	0.62 *
Combined	0 (0.0)	2 (4.4)	0 (0.0)	2 (1.0)	0.0396 *
Spon. Ab.	8 (4.8)	2 (4.4)	1 (50.0)	11 (5.2)	0.68 *
pPROM (weeks)					
37 – 35	24 (14.5)	5 (11.1)	0 (0.0)	29 (13.7)	0.94 *
34 – 29	14 (8.5)	3 (6.7)	0 (0.0)	17 (8.0)	0.95 *
28 – 22	2 (1.2)	1 (2.2)	0 (0.0)	3 (1.4)	0.95 *
<22	2 (1.2)	0 (0.0)	0 (0.0)	2 (1.0)	0.94 *
Hospitalization days (mean ± STD)	14.8 ± 11.8	13.6 ± 10.5	7.0 ± 5.6	14.5 ± 11.5	0.32

DCDA: dichorionic diamniotic; MCDA: monochorionic diamniotic; MCMA: monochorionic monoamniotic; pPROM: preterm premature rupture of membranes; CS: caesarean section; Spon. Ab.: spontaneous abortion. * p value adjusted for multiple comparisons using the Holm-Bonferroni method.

According to foetal complications of DC and MC twins detected antenatally, MCDA twins (24.4%) had a statistically higher number of growth discordance in comparison to DC twins (7.9%), p=0.0316*. (Table 3) Due to the small sample size (N = 2), MCMA twins were excluded from the analysis. The complications specific to MC twins, TTTS and TAPS, were observed in 3

(6.7%) and 4 (8.9%) MCDA pregnancies respectively. Furthermore, in both DC and MC pregnancies, 11 foetuses died in utero after 22 weeks of gestation, of which more than half were MCDA (54.5%). Also, 21 foetuses were spontaneously aborted, of which three fourths were DCDA (76.2%). Congenital malformations were found in 10 foetuses which included a spinal mass, single umbilical artery (SUA) syndrome, a cystic mass in the mediastinum, Tetralogy of Fallot (TOF), acardiac twinning or twin reversed arterial perfusion sequence (TRAP), cystic hygroma, vitium cordis (organic heart defect), hydrocephalus, hydrops fetalis, omphalocele and polycystic kidneys. Furthermore, one pair of MCMA twins was spontaneously aborted at week 19, while the other pair died in utero at week 28. (Table 3)

Table 3 Perinatal outcome: foetal complications

Foetal complications	*Type of chorionicity*			*p value (DCDA vs MCDA)*
	Dichorionic (DC)	**Monochorionic (MC)**		
	DCDA (N, %)	**MCDA (N, %)**	**MCMA (N, %)**	
	165 (77.9)	**45 (21.2)**	**2 (0.9)**	
Discordant growth/sIUGR	13 (7.9)	11 (24.4)	0 (0.0)	0.0316 *
Complications of MC twins				
TTTS	NA	3 (6.7)	0 (0.0)	NA
TAPS	NA	4 (8.9)	0 (0.0)	NA
IUD				
one twin	1 (0.6)	0 (0.00)	0 (0.0)	0.93 *
both twins	1 (0.6)	3 (6.7)	1 (50.0)	0.06 *
Missed abortion				
one twin	0 (0.0)	1 (2.2)	0 (0.0)	0.32 *
both twins	8 (4.9)	1 (2.2)	1 (50.0)	0.90 *
Congenital malformations [a]				
one twin	6 (3.6)	4 (8.9)	0 (0.0)	0.56 *
both twins	0 (0.0)	0 (0.0)	0 (0.0)	NA

DCDA: dichorionic diamniotic; MCDA: monochorionic diamniotic; MCMA: monochorionic monoamniotic; sIUGR: selective intrauterine growth restriction; TTTS: twin-to-twin transfusion syndrome; TAPS: twin anemia polycythemia sequence; IUD: intrauterine death; NA: not applicable. [a] structural or functional anomalies of the nervous system (spina bifida, hydrocephalus etc.), musculoskeletal system (pes equinovarus), digestive system (omphalocele, gastroschisis), circulatory system (hearth malformations) and malformations of the eye, ear, face and neck. * p value adjusted for multiple comparisons using the Holm-Bonferroni method.

In accordance with the increased incidence of growth discordance among MCDA group (Table 3), they had a significantly lower birthweight compared to DC twins (1904g vs 2355g, $p<0.0001$). (Table 4) Furthermore, a significant difference was observed between DC and MC twins and the birth weight of the 1st twin ($p<0.0001$) and the 2nd twin ($p=0.0018$). In regards to the Apgar score, the results demonstrated lower 1st and 5th minute scores in both twins in the MCDA group compared to both twins in the DC group. A statistically significant difference was observed between DC and MCDA twins and the Apgar scores in the 1st and 5th minute (1st min., 1st twin; $p=0.0009$ vs 1st min., 2nd twin; $p=0.0019$; 5th min., 1st twin; $p=0.0002$ vs 5th min., 2nd twin; $p=0.0003$). In compliance with the higher incidence of preterm birth (Table 2), MCDA twins had a significantly higher number of prematurity complications compared to DC twins ($p=0.0108$*). Furthermore, 21 (10.0%) neonates presented with malformations which included all the congenital malformations, as well as malformations undetected prenatally. Moreover, there were 8 neonatal deaths, of which 4 (50.0%) died between 0 and 6 days after birth (not presented in table).

Comparing DC and MCDA groups, there were no significant differences in the number of postnatal infections among the twins. (Table 4)

Table 4 Neonatal outcome

Neonatal outcome	*Type of chorionicity*			*p value (DC vs MC)*
	Dichorionic (DC)	**Monochorionic (MC)**		
	DCDA (N, %)	**MCDA (N, %)**	**MCMA (N, %)**	
	165 (77.9)	**45 (21.2)**	**2 (0.9)**	
Birth weight (mean ± STD)				
1st twin [a]	2355.5 ± 776.7	1904.5 ± 779.6	220.0 ± 28.2	<0.0001
2nd twin [b]	2284.8 ± 795.3	1897.1 ± 873.6	645.0 ±431.3	0.0018
Apgar Score (mean ± STD)				
1st min.				
1st twin	8.9 ± 2.4	7.8 ± 3.2	NA	0.0009
2nd twin	8.7 ± 2.5	7.4 ± 3.4	NA	0.0019
5th min.				
1st twin	9.1 ± 2.3	8.2 ± 3.0	NA	0.0002
2nd twin	8.9 ± 2.4	7.8 ± 3.2	NA	0.0003
Neonatal complications				
Complications of prematurity [c]				
one twin	16 (9.7%)	5 (11.1%)	NA	0.95 *
both twins	13 (7.9%)	12 (26.7%)	NA	0.0108 *
Congenital malformations [d]				
one twin	12 (7.3%)	7 (15.6%)	NA	0.41 *
both twins	0 (0.0%)	1 (2.2%)	NA	0.29 *
Neonatal death				
one twin	1 (0.6%)	3 (6.6%)	NA	0.06 *
both twins	1 (0.6%)	1 (2.2%)	NA	0.79 *
Postnatal infection				
one twin	16 (9.7%)	3 (6.7%)	NA	0.90 *
both twins	7 (4.2%)	6 (13.3%)	NA	0.19 *

DCDA: dichorionic diamniotic; MCDA: monochorionic diamniotic; MCMA: monochorionic monoamniotic; NA: not applicable. [a] 1st twin was born first. [b] 2nd twin was born second. [c] Respiratory distress syndrome (RDS), intraventricular hemorrhage (IVH), retinopathy of prematurity (ROP), and necrotizing enterocolitis (NEC). [d] Foetal congenital malformations and neonatal malformations prenatally undetected. * p value adjusted for multiple comparisons using the Holm-Bonferroni method (DCDA vs MCDA).

According to this study, MCDA twins had a significantly higher incidence of adverse perinatal and neonatal outcome in comparison with DC twins (p=0.0252, p=0.0104). (Table 5)

Table 5 Perinatal and neonatal adverse outcome in DC and MC pregnancies

Adverse outcome	*Type of chorionicity*			*Total (N, %) 212 (100.0)*	*p value*
	Dichorionic (DC)	**Monochorionic (MC)**			
	DCDA (N, %) 165 (77.9)	**MCDA (N, %) 45 (21.2)**	**MCMA (N, %) 2 (0.9)**		
Perinatal					
Maternal	52 (31.5)	11 (24.4)	0 (0.0)	63 (29.7)	0.44
Foetal	62 (37.6)	28 (62.2)	2 (100.0)	92 (43.4)	0.0252
Neonatal	39 (23.6)	21 (46.7)	NA	60 (28.6)	0.0104

DCDA: dichorionic diamniotic; MCDA: monochorionic diamniotic; MCMA: monochorionic monoamniotic. * p value adjusted for multiple comparisons using the Holm-Bonferroni method (DCDA vs MCDA).

Discussion

This retrospective cohort study aimed to compare the perinatal and neonatal outcome between dichorionic and monochorionic twin pregnancies. The three groups, DCDA, MCDA, and MCMA, were compared to evaluate differences in perinatal and neonatal outcome. DC and MCDA twin pregnancies, after exclusion of MCMA twins, were compared for maternal, foetal and neonatal complications.

Our results emphasize that monochorionicity increases the risk for adverse perinatal and neonatal outcome, which is in accordance with the findings reported from other twin studies. (3,9,11,13,14) Preterm birth occurred more often in MC twins than in DC, especially regarding extremely preterm deliveries, which is in accordance with previously mentioned studies. (3,11,24,25) MCDA twins had a significantly greater incidence of growth discordance compared to DC twins. Furthermore, MC twins had a significantly lower birthweight than DC twins. In addition, the Apgar scores in the 1st and 5th minute were significantly lower in MCDA compared to DC twins. Our results are in accordance with another Croatian twin study conducted in the Clinical Hospital "Sveti Duh" in Zagreb. (25) According to our results, TTTS, a complication specific to MC pregnancies, occurred in 6.7% of MCDA twins which is lower than observed in Nunes et al. and Lewi et al. studies (3,26). This could be due to the postnatal exclusion of certain TTTS cases based on the neonatal criteria such as a haemoglobin difference >5g/dl. In their studies, Nunes and co. reported 8 (7.9%) TTTS cases among 228 MCDA twins, while Lewi and co. reported 18 (9%) TTTS cases among 202 MCDA twins. (3,26)

No differences were found in the development of gestational diabetes mellitus, gestational hypertension, and preeclampsia. Similarly, no differences were observed in the occurrence of preterm premature rupture of membranes between the three groups. In MCDA twins, intrauterine death of both twins was higher than in both DC twins but not statistically significant, which is not in accordance with other twin studies (3,27).

As expected, the incidence of assisted reproductive technology was higher in DC than in MC twins. According to our study, one fourth of twin pregnancies (21.2%) were delivered vaginally. Indication for a CS included antepartum haemorrhage (placenta previa, placenta abruptio), complications during labour (imminent asphyxia, labour arrest), malpresentation, cervical dystocia, previous caesarean section, maternal diseases (PE) or MC specific complications. MCDA twins had a significantly higher number of combined deliveries than DC twins. No difference was found in the incidence of missed abortions. Furthermore, mothers of DC twins spent more time in the hospital than MC twins, however no significant statistical difference was found.

Neonatal complications of prematurity including RDS, IVH, ROP and NEC were significantly higher in MCDA twins compared to DC twins. This is in concurrence to more MCDA twins being born preterm. Neonatal death in one of the twins did occur more often in MCDA than DC twins, but no significant difference was observed. Furthermore, the presence of congenital malformations in MCDA and DC twins were similar. Postnatal infections were also found to be similar in both groups, as the twins underwent a similar care protocol.

The limitations of this study warrant consideration and include the insufficient sample size of MCMA twins needed for statistical measurement, which accounted for the exclusion of this group from statistical analysis. Also, the average perinatal outcome of twin pregnancies treated at the UHC Zagreb is not a reflection of the average Croatian perinatal outcome of twin pregnancies, given that the tertiary center treats complex pathologies from the entire territory of Croatia. The strength of our research is the added value to the results of twin pregnancies already published so far. This study substantiates existing literature and brings additional awareness to the higher prevalence of adverse perinatal outcomes that MCDA twin pregnancies face in comparison to DC twins. Determination of chorionicity early in the first trimester and closer surveillance should be an integral part of antenatal care of all multiple pregnancies.

Conclusion

In MCDA twins, the risk of foetal and neonatal adverse outcome is higher than in DC twin pregnancies. To expand, MCDA twins have a significantly higher number of growth discordance and significantly lower birthweight and Apgar scores in comparison to DC twins. Furthermore, the number of extremely preterm deliveries is significantly higher in MC twins. Thus, the neonatal complications of prematurity are also higher among the MCDA group compared to DC group.

The findings in this study support the existing literature and emphasize the importance of early determination of the chorionicity and regular follow-up for prevention of the possible complications of twin pregnancies.

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Figures

Biography

Ana Maria Grubišić was born on the 4th of November, 1997 in Zagreb, Croatia. She lived in many countries throughout the world including Croatia, Belgium, Ireland and Canada. Through this experience, she altered between six schools and was exposed to many languages such as English, Croatian, French, German, Latin, Greek, and Gaelic. While attending secondary school in Canada, she volunteered for the Children's Wish Foundation, explored a captivating section of microbiology at Igor Štagljar's laboratory at the University of Toronto and observed a heart bypass surgery at the Ottawa Heart Institute Foundation. She realized that medicine is a fascinating profession and that helping others, extending lives and curing people would fulfill her as an individual.

In 2015, she decided to enroll into the School of Medicine, University of Zagreb not only to fulfill her wish for a medical career path, but also to regain her Croatian roots. She was able to perform with high grades due to her diligence, organizational skills and ability to withstand pressures. Throughout her studies, she volunteered in a few locations including the Special Hospital for Chronic Childhood Diseases in Gornja Bistra, the Special Hospital Radiochirurgia Zagreb and in a general practice in Utrine. In addition, she volunteered at a general practioner's office in Waldburg, Germany.

She gained lifelong friendships and truly connected with her homeland. She had the opportunity to travel though Croatia, visiting the coastline, the beautiful islands as well as the treasured national parks. Overall, she was able to incorporate her medical studies with an active social life.

In the last year of her medical studies in 2021, she decided to familiarize herself with the world of scientific research. As her graduate thesis, she chose to perform a retrospective study which analyzed the perinatal outcome of twin pregnancies in the University Hospital Centre Zagreb. Furthermore, she gained a lot of practical knowledge during her clinical rotations in Zagreb. She immersed herself into the vast possibilities that this career path offers. In becoming a physician, she believes that she will be able to contribute to the profession in practice and research, while accomplishing her goals.