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Research Article

Perinatal Risks of Twin Pregnancies and Births-Data for the Decision-Making Process Prior to Double Embryo Transfer

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Abstract

Multiple pregnancies are related to a higher maternal and fetal risk than singleton pregnancies. Current data on risk assessment of twins versus single pregnancies are relevant for a rational decision-making process on the number of embryos transferred after assisted reproductive techniques to avoid twin pregnancies. In this retrospective cohort study at a tertiary perinatal center, maternal and neonatal outcomes of singleton and twin deliveries were investigated using matched pair analysis. 15,018 births were analyzed including 738 twin and 19 triplet pregnancies. The mean gestational age at delivery was 38.7 ± 2.9 for singletons and for twins 34.8 ± 3.6 weeks ($p<0.001$). Significantly more multiple pregnancies occurred after assisted reproduction, 27.51 vs. 4.02% after spontaneous conception. The odds ratios for medical treatment in pregnancy, hospitalization, and cesarean section were higher in twin pregnancies ($p<0.001$). Neonatal hospitalization rate, need for intubation and artificial ventilation showed a higher risk for twins ($p<0.001$). It was evident that the main risk in twin pregnancies is preterm delivery itself. Pregnancies and births of monochorionic twins had a significantly lower gestational age, fetal weight at birth and higher risk for respiratory distress syndrome of the neonate compared to dichorionic twins, but no further statistically increased perinatal risks. Twin pregnancies are related to adverse outcome mainly related to preterm delivery. Monochorionic twins do not lead to a further significant increase of adverse outcome than dichorionic pregnancies. These results can be used for the individual decision-making process prior to embryo transfer to avoid twin pregnancies after ART.

Keywords: Multiple pregnancy; Twin; Perinatal outcome; ART; Chorionicity; TTTS

Abbreviations: ANOVA: Analysis Of Variance; aOR: Adjusted Odds Ratio; ART: Assisted Reproduction Techniques; BMI: Body Mass Index; CI: Confidence Interval; CS: Cesarean Section; DC: Dichorionic; GDM: Gestational Diabetes Mellitus; IUG: Intrauterine Growth Restriction; MA: Monoamniotic; MC: Monochorionic; NEC: Necrotizing Enterocolitis; NICU: Neonatal Intensive Care Unit; OR: Odds Ratio; PE: Preeclampsia; RR: Relative Risk; TTTS: Twin-To-Twin Transfusion Syndrome; WHO: World Health Organization

Introduction

The incidence of multiple pregnancies has increased due to Assisted Reproductive Techniques (ART) in the last decades. National data of the German birth registry show that before ART was introduced, the twin birth rate was 10 of 1,000 births [1]. After the first successful assisted reproduction in Germany in 1981, the mean twin rate increased to 13.2/1,000 in the 90ies and up to 17.8/1000 twins and 0.4/1000 higher multiple pregnancies between 2010 and 2020 [1].

In a European study from 2016, data of twin births in several countries were compared [1]. Birth registries showed national differences in the incidence of twins with a range from 9 to 26.5/1000 pregnancies for the year 2010. The reported German twin rate of 18/1,000 births and triplet rate of 0.3/1,000 was about the European average. In relation to the results of an older similar study with data of 2004, increasing rates of multiple pregnancies in all European countries are evident [2].

Twin pregnancies are associated with increased maternal and fetal risks compared to singleton pregnancies. In a recent multi-country survey by the World Health Organization (WHO), current data of the perinatal outcome associated with twin pregnancy were presented [3]. These data show that twin pregnancies are related to a higher risk of preterm delivery, lower Apgar scores than 7, higher risk of low birth weight, stillbirth, and early neonatal death, as well as admission to Neonatal Intensive Care Units (NICU) and any adverse perinatal outcomes. A study of European countries showed a pooled Relative Risk (RR) for very preterm births (<$32^{0/7}$ weeks) in multiple pregnancies of 11.7 (95% CI: 11.1-12.4) and an estimated pooled RR of 2.4 (95% CI: 1.5-3.6) for fetal and neonatal death compared to singletons [1].

The perinatal risks associated with monochorionic twin pregnancies are especially high. In 2008 the German guidelines for prenatal care were adapted and the early ultrasonographic risk evaluation of multiple pregnancies with determination of chorionicity became the standard[1]. Due to the awareness of early ultrasonographic risk evaluation of multiple pregnancies, improved prenatal care, and improved obstetrical procedures, morbidity and mortality of twins are decreasing in developed countries in the last decades, but still remain high [4]. Monochorionic Monoamniotic (MCMA) pregnancies are rare and usually monitored as well as treated in tertiary referral centers. Studies of MCMA pregnancies are often published separately from other twin pregnancies [5].

The optimal gestational week for delivery in twin or multiple pregnancies to reduce maternal and fetal complication rate is still under discussion [6]. The recommendations differ depending on chorionicity and amnionicity. Based on a data-linkage cohort study with 43,311 twin infants born at a gestational age of $34^{0/7}$ weeks or later, a recent study recommends not to deliver twins routinely before $37^{0/7}$ weeks of gestation [7]. Very recently, a national guideline for the risk management of twin pregnancies and at time of birth was published, including recommendations for screening of pregnancy complications and for the mode of the delivery based on chorionicity [8].

It is evident that the incidence of twins is related to ART treatments and national standards concerning the number of embryos transferred [1]. In spite of extensive discussions by German experts in reproductive medicine concerning the single-embryo transfer strategy to avoid twin pregnancies [9], no recently published study of perinatal risks of twin pregnancies are available. These data are needed to be used for the decision-making process of infertile couples with planned ART treatment. One review article on the perinatal risk of multiple pregnancies was published by Dudenhausen, et al. in 2010 [10]. In a case-control study, 89 twin pregnancies were compared to 178 controls after matching for Body Mass Index (BMI), age, parity and ethnicity [11]. Multiple pregnancies showed a higher risk for hypertensive pregnancy disorders and treatment in intensive care units, but not for Gestational Diabetes Mellitus (GDM).

This presented study from a single University Hospital adds data to the perinatal risk evaluation of twin pregnancies and the association with chorionicity.

Material and Methods

This is a retrospective single center study in a tertiary perinatal center at the University Hospital in Germany. After obtaining the approval of the local ethics committee (EK456112018), clinical data of multiple births with at least 22 weeks of gestation were included in the study. The study period was defined from 1/2014 until 12/2019.

Clinical data were extracted from the clinical charts and analyzed in a digital data sheet. No prior personal informed consent was needed for the retrospective analysis of the data and chart review. Prenatal, obstetrical and neonatal documentation were used for the study. Data of the prenatal care of the pregnancy from the standardized document were transferred into a digital document and included into the analysis. Figure 1 shows the number of deliveries which are included into the study.

1 Gemeinsamer Bundesausschuss, Unterausschuss Familienplanung 2020. URL:https://www.g-ba.de/downloads/40-268-696/2008-03-13-Mutter-Chorionizität_Abschluss.pdf, Assessed 05 December 2021.

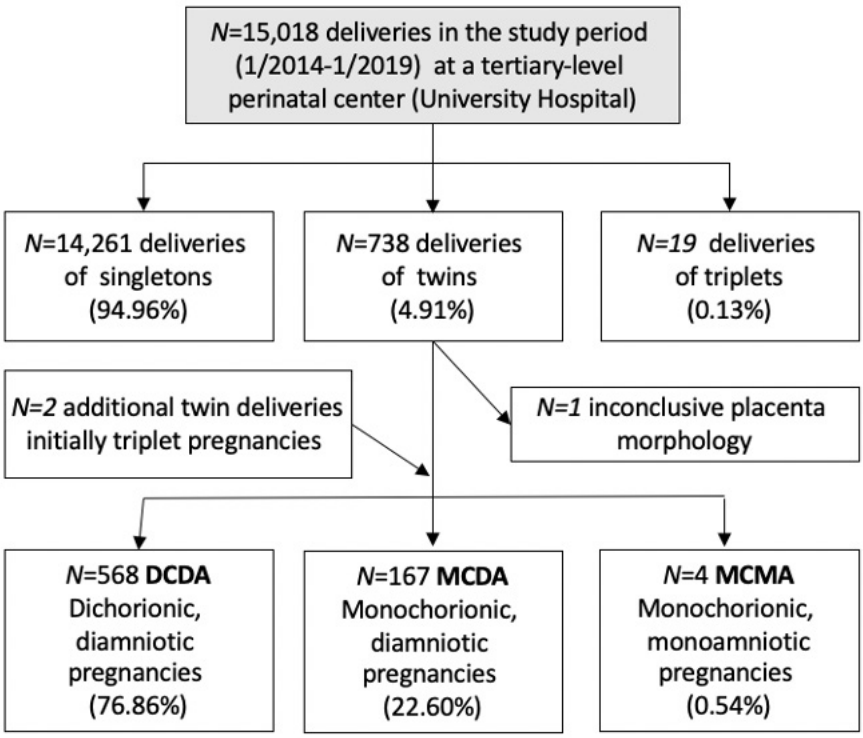

Figure 1: Study groups with classification of singleton and multiple deliveries and subgroups with chorionicity and amnionicity.

Complications of pregnancy and delivery as well as neonatal data were attributed to the mother. Neonatal outcome data after transfer to the neonatal unit of the same University Hospital were documented for each child. The clinical parameters and definitions of clinical diagnoses as gestational diabetes which are included into the analysis are shown in detail in the online resource.

Data are presented as means ± standard deviation of the mean, percentages and Odds Ratios (OR). Categorical variables are presented as percentages compared via the chi-squared test or Fisher´s exact test. Continuous variables in two groups were tested with the Mann-Whitney-U test. For univariate analysis of three independent groups the Analysis Of Variance (ANOVA) with the non-parametric test of Kruskal-Wallis with 95% confidence interval was chosen. For nominal data chi-square test with Cramer V-test for significance was applied. Group differences and risk calculation were considered to be significant at a p-value of <0.001 due to multiple testing. The statistical analysis was performed with Statistical Package for Social Science (IBM Statistics for Windows, Version 27.0, Released 2020. Armonk, NY).

To outweigh the lack of randomization in the observational study a case control analysis with matching for the potentially confounding factors of age, BMI, and parity was performed for twin births vs. singleton births (N = 735 pairs) after propensity score matching (0.00001 confirmation of score, exact and by chance). The OR was calculated after chi-square test with Mantel-Haenszel test. A propensity score matching for gestational week did not lead to a sufficient matching (matched pairs >75% of initial twins, non-significant different in matched factors). Therefore, binary logistic regression was performed in a model with 3 subgroups of preterm and term delivery to calculate the effect of preterm delivery on identified risk factors in the twin and singleton pregnancies and deliveries. GraphPad Software version 8.2 for Windows, La Jolla California USA, www.graphpad.com was used for plotting the data.

Results

Between January 2014 and December 2019, a total of 15,018 deliveries were recorded in the tertiary perinatal center. Within these six years of the study period, the average number of annual deliveries was 2503.17 ± 106.95 (2.392-2.668). The age of all women at birth was 31.01 ± 5.14 (13-53). The BMI at the first control in pregnancy was 25.29 ± 5.65 (14.10 – 73.09 kg/m^2) and the mean gestational age at delivery was 38.54 ±3.10 weeks (21.57-42.71). For 5.23% of all pregnancies, the mode of conception was documented as conception after ART.

Table 1 shows the clinical data of all births of single newborns, in comparison to twins and triplets. No multiple pregnancies with higher grades were treated during the study period.

	Birth of a singleton n=14,261 (94.96% of all births)	**Twin birth n=738 (4.91%)**	**Birth of triplets n=19 (0.13%)**	**p-Wert (Kruskal-Wallis, chi-square t-test, Cramer V)**
Maternal age at birth (years), *N*=15,018	31 [28, 35]	32 [29, 35]	32 [29, 36]	**p=0.001**
BMI (kg/m²) at first appointment in pregnancy, *N*=14,958	24.09 [21.36; 28.17]	23.89 [21.32, 27.77]	25,15 [22.68, 28.73]	p=0.649
Increase in maternal weight until birth (kg), *N*=12,478	13 [10, 17]	15 [11, 19]	13 [12, 15]	**p<0.001**
Weight gain [kg] per week	0.33 [0.25, 0.42]	0.43 [0.32, 0.54]	0.41 [0.37, 0.49]	
Obstetrical history *N*=14,080				
Previous pregnancies	1 [0, 2]	1 [0, 1]	1 [0, 2]	**p<0.001**
Previous births	1 [0, 1]	0 [0, 1]	1 [0, 1]	
Previous CS	2068 (14.50%)	67 (9.08%)	2 (10.5%)	
Antenatal care visits *N*=14,902	12 [10, 13]	10 [8, 14]	8 [6, 12]	**p<0.001**
Antenatal care visits/weeks of gestation	0,29 [0.25, 0.34]	0,29 [0.24, 0.34]	0,23 [0.19-0.38]	p=0.087
Antenatal visits with ultrasound *N*=12,325	4 [3, 5]	4 [3,6]	6 [3, 10]	**p<0.001**
Number of ultrasound/ weeks of gestation	0,10 [0.08, 1,27]	0,11 [0.08, 1,16]	0,17 [0.11-0.30]	**p<0.001**
First documented antenatal control (week), *N*=7,417	10 [8, 11]	9 [8, 10]	10 [8, 11]	p=0.054
Pregnancy after ART	574 (4.02%)	203 (27.51%)	8 (42.10%)	**p<0.001**
Maternal diseases before and in pregnancy	2327 (16.31%)	77 (10.43%)	0	**p<0.001**
Maternal outcome				
Treatment in pregnancy Glucocorticoids for prevention of RDS, *N*=7,792	1573 (19.89%)	352 (47.63%)	19 (100%)	**p<0.001**
Tocolysis i.v., *N*=9,949	384 (4.06%)	95 (19.67%)	4 (28.57%)	**p<0.001**
Cerclage, *N*=7,457	72 (1.01%)	8 (2.20%)	0	p=0.095
Maternal hospitalization before birth [days], *N*=10,868	3.89 ± 11.05 (0-199)	9.20 ± 14.65 (0-69)	10.27 ± 15.64 (0-57)	**p<0.001**
Maternal complications in pregnancy, *N*=15,018				
Preterm labour	1737 (12.18%)	339 (45.93%)	12 (63.16%)	**p<0.001**
Placenta praevia	142 (1.00%)	7 (0.95%)	0	p=0.004
Intrauterine growth restriction	958 (6.72%)	74 (10.03%)	3 (15.79%)	p=0.001
Pregnancy induced hypertension or preeclampsia	1206 (8.46%)	88 (11.92%)	1 (5.26%)	p=0.004

Diabetes or GDM	1957 (13.72%)	90 (12.20%)	5 (26.32%)	p=0.137
Gestational age at delivery [weeks]				
Preterm deliveries	39,43 [38.43, 40.43]	36,14 [33.00, 37.43]	32,57 [32.39, 34.14]	
<$37^{0/7}$ weeks of gestation	1935 (13.57%)	444 (60.16%)	19 (100%)	
< $28^{0/7}$ weeks of gestation	288 (2.05%)	50 (7.05%)	0	**p<0.001**
$28^{0/7}$ - $33^{6/7}$ weeks	654 (4.59%)	177 (23.98%)	12 (63.16%)	
$34^{0/7}$ - $36^{6/7}$ weeks	993 (6.96%)	217 (29.40%)	7 (36.84%)	
Delivery mode Cesarean section	4290 (30,08%)	545 (73.75%)	20 (100.00%)	**p<0.001**
Primary CS	1376 (9.65%)	226 (30.58%)	9 (45.00%)	**p<0.001**
Secondary CS	2143 (15.03%)	283 (38.35%)	9 (45.00%)	**p<0.001**
Repeat CS	771 (5.40%)	36 (4.88%)	2 (10.00%)	p=0.573
Emergency CS	285 (6.68%)	14 (2.46%)	1 (5.26%)	**p<0.001**
Hysterectomy	13 (0.11%)	5 (0.82%)	0	**p<0.001**
Vaginal delivery	9 233 (64.75%)	183 (24.80%)	0	**p<0.001**
Vacuum extraction, forceps	668 (4.68%)	10 (1.36%)	0	p=0.002
Documented **postpartal complications** (*N*=15,018)	390 (2.73%)	54 (7.32%)	0	**p<0.001**
Placental complications (N=7,457)	292 (4.12%)	10 (2.75%)	0	p=0.338
Cardiovascular complications	51 (0.36%)	12 (1.63%)	0	p=0.003
Maternal death	0	0	0	
Fetal and neonatal outcome				
Arterial pH of umbilical cord artery, *N*=12,788 (first child)	7.28 [7.21, 7.33]	7.33 [7.30, 7.36]	7.34 [7.31, 7]	
Birth weight [g]	3340 [2950, 3680]	2360 [1860, 2710]	1640 [1470, 2140]	**p<0.001**
Birth weight<1 500 g	574 (4.02%)	116 (15.72%)	6 (31.58%)	
Neonatal outcome				
APGAR 5 min	9 [9, 10]	9 [9, 10]	8 [8, 9]	
AGPAR <8 (first child)	927 (6.50%)	112 (15.18%)	1 (5.3%)	**p<0.001**
Transfer to pediatric clinic	4 001 (28.06%)	581 (78.73%)	19 (95.00%)	
Hospitalization [days]	4 [3,4]	5 [4, 8]	24 [6, 43]	
Severe malformations	383 (2.69 %)	16 (2.17 %)	0	p=0.536
Death of a child/birth, *N*=15,018				
Intrauterine death	106 (0.69%)	10 (1.35%)	1 (5%)	**P=0.001**
Perinatal mortality (within 7 days)	28 (0.20%)	6 (0.82%)	0	P=0.003

Data are presented as median, interquartile range or in percentage. p<0.001 was defined as significant for group differences with multiple comparisons. *For twin deliveries the main code is shown. Abbreviations: BMI: Body Mass Index; CS: Cesarean Section.

Table 1: Baseline characteristics of the pregnancy with singleton, twin and triplet births.

Maternal age and increase in weight during pregnancy were significantly higher in the subgroups of twin and triplet births than singletons (p<0.001). Women with twin pregnancies had significantly less previous pregnancies and births than women with singletons and triplets (p<0.001). The total number of prenatal care visits of women with twins and triplets was smaller than in singleton pregnancies. This can be explained by shorter pregnancy duration and hospitalization. After adjustment for the duration of the pregnancy this was not significant anymore. Nevertheless, the total number of ultrasound controls in multiple pregnancy per week was significantly higher than in singleton pregnancies (p<0.001). The first prenatal control was documented earlier in twin pregnancies. Pregnancies documented as conception with ART were found in 4.02% of singletons, but 27.61% of twins and 42.10% of triplets (p<0.001). Significantly more women with singleton pregnancies had documented chronic diseases in the medical history (p<0.001).

Twin and triplet pregnancies were associated with a significantly higher maternal risk for medical treatment, hospitalization and complications. The percentages of pregnancies with Intrauterine Growth Restriction (IUGR), as well as preterm labor and delivery were significantly higher in the group of twins and triplets. No statistical differences were found for GDM. The delivery mode showed statistically significant differences: vaginal delivery was less likely in twin pregnancies and did not occur in triplets (p<0.001). The risks for Cesarean Section (CS), emergency CS and CS with hysterectomy differed in the group of singleton and twin pregnancies. Twin pregnancies showed a significantly higher risk of postpartal complications (p<0.001), but not placental complications. No statistically significant differences were found for placenta praevia. In the 6-year study period no maternal death occurred. One 31-year-old woman with a severe complex cardiac disease died three weeks after delivery in an intensive care unit.

The perinatal outcome showed statistically significant differences for the Apgar score at 5 min, transfer to neonatal unit (28.06% of singletons, 78.73% of twins and 95.00% of triplets) and the duration of hospitalization (4.44, 8.29, and 29.59 days) (p < 0.001). The risk of intrauterine death of a child was statistically increased in twins compared to singletons (p < 0.001).

To control the neonatal outcome parameters for potential documentation bias, neonatal data documented by the pediatric unit of the perinatal center were analyzed additionally. 4,713 children born in the same university hospital were transferred after birth to the neonatal unit. The mean gestational age at birth of those children was 35.66 ± 4.07 (22.29 - 41.86) weeks and the mean duration of hospitalization was 19.79 ± 24.94 (1 – 268) days. Of those 3,529 newborns (74.88%) were singletons, 1,127 (23.91%) were born as twins and 57 as triplets (1.21%).

Significant differences in gestational age at delivery were seen in the subgroups, as well as for birth weight (p < 0.001). The subgroups showed also statistically significant differences for medical treatment of the neonates concerning intubation, artificial and tracheal ventilation, and hospitalization. Perinatal asphyxia and malformations occurred more frequently in the singleton groups (p < 0.001). No statistically significant differences were seen in the three groups considering neonatal diagnosis (Table 2).

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Increase in maternal weight until birth (kg), *N*=12,478	13 [10, 17]	15 [11, 19]	13 [12, 15]	**p<0.001**
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Fetal and neonatal outcome				
Arterial pH of umbilical cord artery, *N*=12,788 (first child)	7.28 [7.21, 7.33]	7.33 [7.30, 7.36]	7.34 [7.31, 7]	**p<0.001**
Birth weight [g]	3340 [2950, 3680]	2360 [1860, 2710]	1640 [1470, 2140]	
Birth weight<1 500 g	574 (4.02%)	116 (15.72%)	6 (31.58%)	
Neonatal outcome				
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Severe malformations	383 (2.69 %)	16 (2.17 %)	0	p=0.536
Death of a child/birth, *N*=15,018				
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Data are presented as median, interquartile range or in percentage. p<0.001 was defined as significant for group differences with multiple comparisons. *For twin deliveries the main code is shown. Abbreviations: BMI: Body Mass Index; CS: Cesarean Section.

Table 2: Fetal and neonatal outcome of the 4,713 children transferred to the neonatal ward.

Preterm delivery was significantly more often in twins and triplets compared to singletons, even exclusively analyzing the neonates after transfer to the pediatric ward.

To control for possible maternal factors with impact on the risk of preterm delivery, the risk analysis of singleton and twin pregnancies and births was calculated after propensity score matching for maternal age, BMI, and parity (online resource, table 1). After matching, the mean gestation age at birth and birth weight was lower in twin pregnancies with 34.90 vs. 37.63 gestational weeks and 2,2234 vs. 3,223 g ($p < 0.001$). Preterm delivery occurred in all categories (<28, 28-34, 34-37 gestational weeks) significantly more often. Twin pregnancies showed significantly higher risk of preeclampsia (PE) and IUGR, compared to singletons with an adjusted OR (aOR) of 1.63, 95% CI 1.13-2.34 and aOR1.65, 95% CI 1.11-2.45 respectively (Figure 2). Twins were delivered significantly more often via cesarean section ($p < 0.001$), The aOR for postpartal complications after twin deliveries was 2.92, 95% CI of 1.69-5.06 ($p < 0.001$). Considering neonatal risks, hospitalization of the newborn was significantly more often (aOR 3.96, 95%CI 3.56-5.48, $p < 0.001$), but neonatal mortality did not differ significantly between twins and singletons.

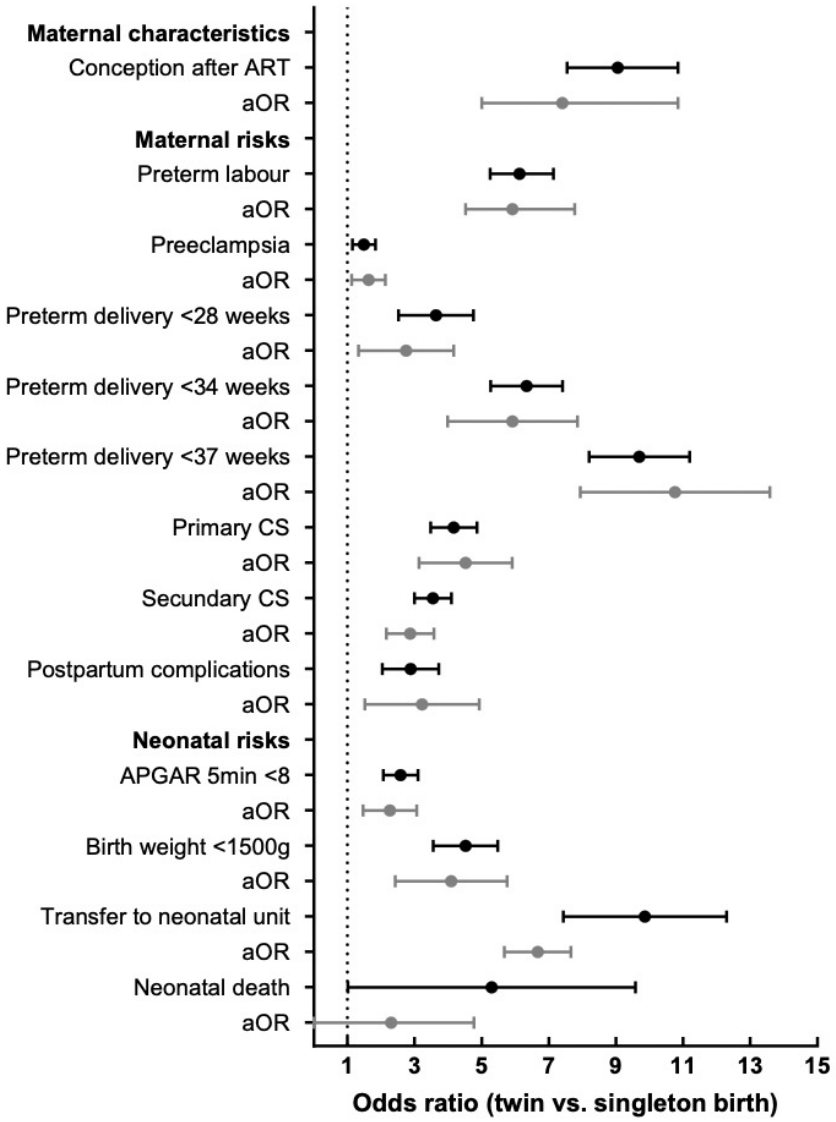

Figure 2: OR of perinatal outcome with 14.999 deliveries and after matched pair analysis. N=735 twins, N=735 singleton deliveries. *In grey: OR after adjustment for maternal age, BMI, parity.*

Perinatal risks were compared in subgroups of preterm delivery within twins and in comparison to singletons. IUGR, secondary cesarean section, and various antenatal complications were significantly increased after preterm birth ($p < 0.001$). Maternal risks as placenta praevia, PE, GDM showed no association with gestational age within the groups of twins and compared to singletons.

Maternal characteristics as pregnancy after ART and prior CS do not show differences for preterm birth within the groups of twins. But in comparison to singleton deliveries the higher ratio of ART conception in twins is highly significant in all preterm groups ($p < 0.001$).

Considering delivery mode, the significant differences seen in twin pregnancies compared to singletons depending on gestational age are confirmed in all groups of preterm delivery ($p < 0.001$). Twins in the group after early preterm delivery ($< 34^{0/7}$ gestational weeks) had a significantly higher OR for intubation than term twin pregnancies. But compared to singletons, the risk was even lower ($p < 0.001$). Enterocolitis, ventricular hemorrhage and neonatal surgery were significantly increased in twins after preterm delivery before 34 gestational weeks compared to term twin deliveries. Nevertheless, for all neonatal risks related to preterm birth the data show no significant differences between twins and singletons of the same gestational age groups.

The twin pregnancies were compared in two subgroups based on chorionicity (Figure 3). Perinatal outcome parameters, such as gestational week at birth, preterm delivery < 37 weeks of pregnancy, primary cesarean section, birth weight of both twins and Apgar score of the twin with the lower birth weight, showed significantly higher risks in the monochorionic group, which included MCMA and MCDA (monochorial diamniotic) twins ($p < 0.001$). Neonates from MCDA twin pregnancies have a higher risk of Respiratory Distress Syndrome (RDS), but not of other complications compared to DCDA twins. Severe maternal complications after birth did not occur more frequently in the MCDA group.

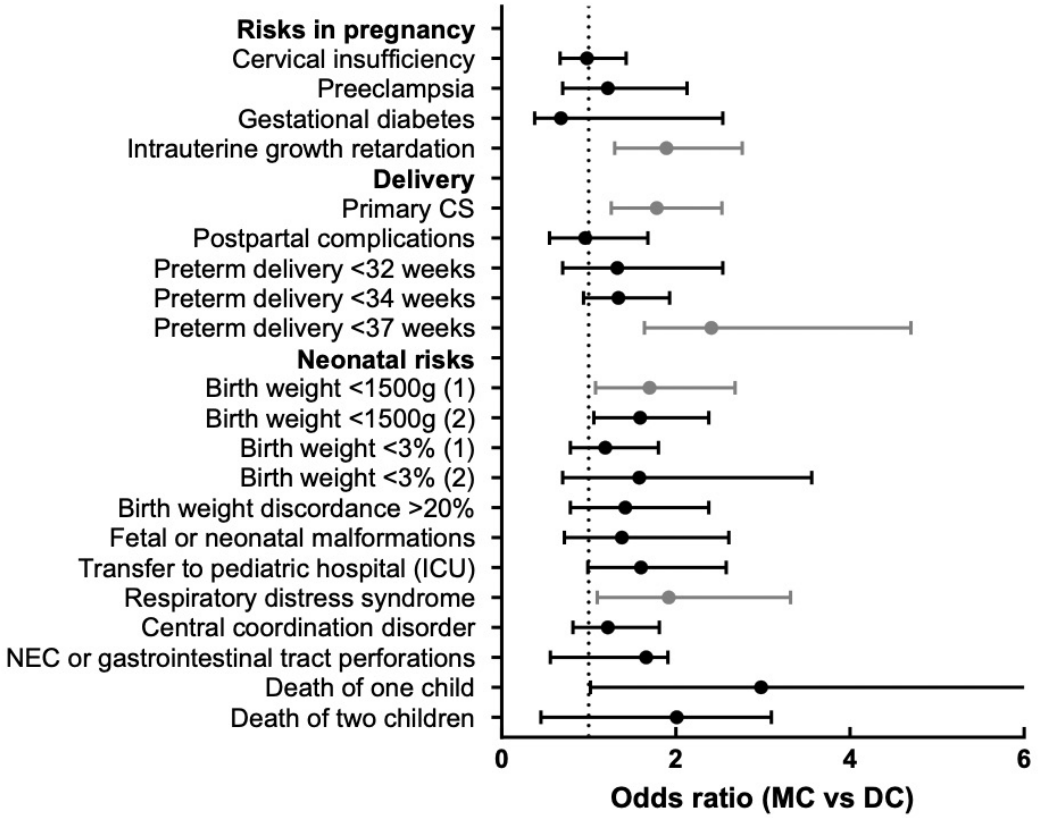

Figure 3: OR of perinatal outcome in comparison MC-DC-twins (*N*=171 vs. 568). *In grey: OR with significant differences between MC vs. DC twins.*

In 34 of 167 births with MCDA placentation, a Twin to Twin Transfusion Syndrome (TTTS) was documented (20.36%). The mean age of these women did not differ from pregnant women with MCDA twins without TTTS. But, gestational age at birth as well as birth weight of the twins showed significant differences if TTTS was diagnosed. The gestational age at birth was significantly lower than in the group without TTTS (31.03 ± 4.14 vs. 35.02 ± 2.96 weeks, $p < 0.001$).

The CS rate after TTTS was 61.76% vs. 34.10% without TTTS ($p = 0.003$). Nine women with a MCDA twin pregnancy had intrauterine laser treatment and three women had therapeutic amnioreduction.

Discussion

The perinatal outcome data of a single tertiary perinatal center show a significant difference in maternal characteristics and almost in all perinatal outcome parameters in multiple versus singleton deliveries. Although the findings are already described previously, the context of medical treatment influences the relative risk. Internationally as well as over the period of the last 25 years differences of perinatal risks in multiple pregnancies and births vary largely [3].

Women in our study with multiple pregnancies were significantly older and pregnancies occurred in a significant higher proportion after ART. Medical interventions as antenatal RDS prophylaxis and intravenous tocolysis in pregnancy as well as maternal hospitalization differ significantly between the singleton group and multiple pregnancy groups. The most profound difference in perinatal outcome was the lower gestational age at delivery (38.74 weeks vs. 34.80 weeks for twins and 32.60 weeks for triplets, $p < 0.001$).

The number of higher multiple pregnancies was low with only 19 triplet pregnancies occurred in the study period. In spite of the small number of births after triplet pregnancies, this study shows an intensified need for antenatal medical controls and hospitalization. Triplet pregnancies were conceived in 42.10% after ART, compared to 4.02 % for singletons. Diabetes or GDM were documented more frequently in triplets with 26.32% vs. 13.72% for singletons and 12.20% for twins. Glucose intolerance may be aggravated in triplets more than in twins [12]. All triplets were delivered by cesarean section and the perinatal outcome of the 57 neonates was appropriate for preterm delivery: 77.19 % needed artificial ventilation, but only three neonates had intubation and tracheal ventilation. The mean duration of hospitalization of triplets was significantly higher than of singletons with 23.82 vs. 4.44 days ($p < 0.001$).

The detailed risk analysis was performed for singleton vs. twin pregnancies. Twin pregnancies are related to a higher risk of severe maternal life-threatening events, such as PE, postpartal complications and higher cesarean section rates. This corresponds to the results of other studies [13,14]. The higher fetal and neonatal risk related to twin pregnancies and births is mainly associated to preterm delivery [4]. Figure 4 shows the mean gestational age at birth and quartiles of all singleton and twin pregnancies as well as birth weight in both groups of our study.

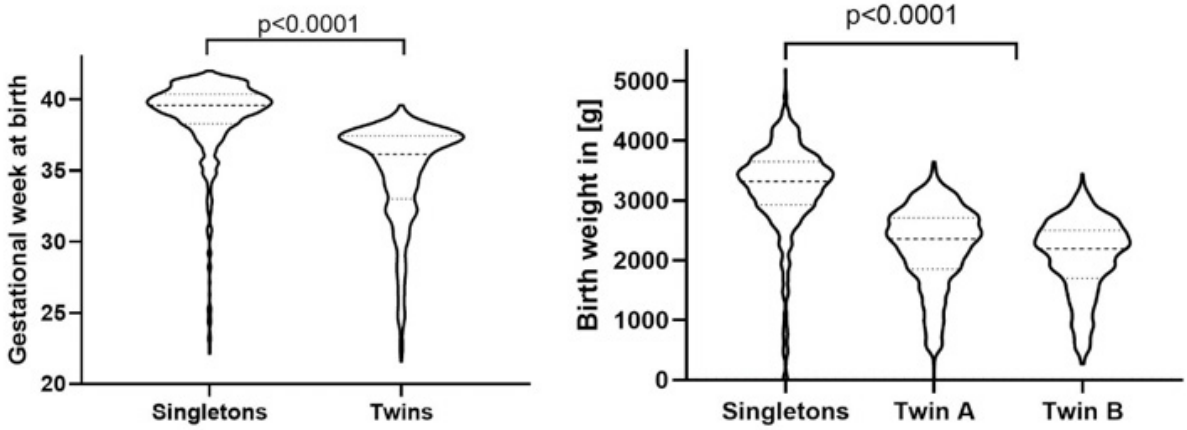

Figure 4: Median and interquartile range of gestational age at birth and birth weight of twin and singleton pregnancies and births (N=679 pairs after propensity score matching for maternal age, BMI and parity).

The comparison of maternal characteristics showed that twin mothers had a significantly higher maternal age during pregnancy compared to singletons (30.97 vs. 31.80 years). After adjustment for maternal age, the odds ratio of several perinatal maternal and neonatal adverse outcome parameters remained significantly increased for twin deliveries (Table 1). A population-based study which evaluated neonatal outcomes for twin pregnancies found that the maternal age did not increase the risk of fetal or infant death, but higher maternal age over 40 years was associated with a higher risk of preterm birth [15]. Another prospective, population-based study of twin deliveries from France showed a lack of association between severe acute maternal morbidity and maternal age [16].

The maternal weight gain of 15.39 kg or 439 g per week in twin pregnancies was significantly higher in comparison to singleton pregnancies with a weight gain of 13.20 kg or 339 g per week ($p<0.001$). The effect of maternal age, weight and weight gain during pregnancy is discussed as a predictor of PE. A recent study could show that excessive weight increase in pregnant women without prior chronic hypertension increases the risk of PE in twin pregnancies [17]. Maternal age and BMI are used to calculate the *a priori risk* for complications like PE in the first trimester of pregnancy [3]. The generally increased risk for PE in twin pregnancies is reported in several previous studies [3,17]. Our data also support the increased risk for PE with 11.92% in twins vs. 8.92% in singleton pregnancies (OR 1.47, 95% CI 1.16-1.85 ($p=0.004$), but the risk was not increased after adjusting for maternal age, BMI and parity. The study of Ram, et al. showed that, the association of maternal obesity with adverse pregnancy outcomes like PE is weaker in twins than that observed in singletons. This could explain our finding after adjusting for BMI [18].

The rate of PE in singleton pregnancies was 8.46 % in our study population. In comparison, the incidence of PE is reported as being 2% in Europe [19]. The high rate of PE in our unit could be explained by the high-risk profile of patients treated in our tertiary referral center.

Former studies found conflicting results considering an association of twin pregnancies and GDM [3,11,16]. Our data show a generally high risk for GDM in both subgroups of singleton and twin pregnancies (8.22% and 6.53%), but not an increased risk in twins. The rate of diagnosed GDM is higher than the German overall prevalence, which was described with 5.9% in 2017 ($N = 44{,}907$ women) [20]. Nevertheless, it must be considered that after the establishment of a 50 g screening test in prenatal care since 2013, the rate of diagnosed GDM is far behind the estimated rates of about 13% [20]. Additionally, the high-risk profile of patients treated in the perinatal tertiary center contributes to the higher rates of GDM in singletons in the study.

Twin pregnancies occurred significantly more frequently after conception with ART than in singletons ($p < 0.001$). This is mostly due to multiple embryo transfer. This strategy in reproductive medicine is responsible for generally high rates of multiple pregnancies after ART across Europe and in Germany [9]. Multiple embryo transfers lead to twin and higher-grade multiple pregnancies with dichorionic placentation. Due to the increased usage of prolonged embryo culture with blastocyst selection and embryo transfer, the incidence of monochorionic twin pregnancies may increase in the future. Up to now, the rate of monochorionic twin pregnancies after ART is significantly lower than of DC twins (8.4% vs. 41.4%) in our collective.

The number of ultrasound controls performed in twin and triplet pregnancies was significantly higher compared to singletons in our cohort with 0.11 ±0.50 for singletons versus 0.13 ±0.06 for twins and 0.20± 0.11 for triplets ($p < .001$). The Clinical Standards Committee (CSC) of the International Society of Ultrasound in Obstetrics and Gynecology (ISUOG) recommends performing a first trimester scan to date the pregnancy according to the crown-rump length of the larger twin [21]. The chorionicity should also be defined during this scan. This recommendation is implemented in Germany in the guidelines for prenatal care of twin pregnancies [8]. After a second trimester scan, the uncomplicated dichorionic twin pregnancies should be scanned every four weeks. The uncomplicated monochorionic twins should be scanned every two weeks after 16 weeks of pregnancy, especially to exclude twin to twin transfusion syndrome or selective IUGR [22]. The German pregnancy guidelines generally categorize the twin pregnancies as high-risk pregnancies which need to be monitored closely[2].

In our study, the most prominent adverse perinatal outcome was preterm birth, which differed significantly in the subgroups of singleton, twins and triplets ($p < 0.001$). The OR of preterm labor in twins compared to singleton was 5.92 with a 95% CI of 4.52-7.77 even after adjusting for maternal age, BMI and parity. The adjusted risk for preterm delivery < $28^{0/7}$ weeks of gestation showed an OR of 2.51 (1.47-4.27), for preterm birth <$34^{0/7}$ weeks 5.71 (4.10-7.95) and for < $37^{0/7}$ weeks 10.51 (8.07-13.70), respectively with p-values < 0.001.

The strong association between multiple pregnancy and preterm labor is already well-known and is estimated to account for almost 50% of the complications observed [22]. The mechanism of preterm birth in twin pregnancies seems to be different than in singletons. Physiological stimuli to the onset of parturition, including stretch, placental corticotrophin-releasing hormone and lung maturity factors, may be stronger in multiple pregnancies due to the increased fetal and placental mass [23]. These

2 Mutterschaftsrichtlinien: https://www.g-ba.de/downloads/62-492-2130/Mu-RL_2020-02-20_iK_2020-04-28.pdf. Assessed 05 December 2021

pathophysiologic characteristics of twin pregnancies also serve as an explanation for the ongoing discussion about the clinical interventions to reduce the risk of preterm delivery in multiple pregnancies. As an example, cervical cerclage reduces preterm birth rates in singletons, but has conflicting results in twins with some studies showing more harm than benefit [23]. In our analysis, cerclage was not performed significantly more often in twin than in singleton pregnancies (1.01 vs. 2.20%, p=0.095). Nevertheless, we saw a significant higher need of other medical interventions like antenatal glucocorticoids, intravenous tocolysis or maternal hospitalization in twin pregnancies compared to singletons in our study.

An interesting finding was that in our cohort the rate of fetal malformations analyzed in live-born neonates was not higher in twin pregnancies. The rate of severe or lethal malformations in singleton and twins was 2.69 vs. 2.17% respectively (p = 0.536). The comparison with the perinatal statistics of Saxony showed that for all births a malformation rate of 0.5% was documented for 2017, whereas the malformation rate in tertiary perinatal centers was higher with 2.1%[3]. Even after controlling the malformation rate using the data of neonatal hospitalization, no higher risk for twins considering congenital malformations could be detected. Surprisingly, major but not lethal and minor malformations occurred in our study significantly more often in singletons. This is most likely to be explained by the status of a referral medical center as tertiary perinatal center with a large regional coverage. Especially pregnant women with severe feto-maternal complications are preferably referred to University Hospitals. This selection bias may be seen especially in singletons than in twins.

The mode of delivery differed significantly between twin and singleton pregnancies. The rate of cesarean sections performed was higher in twin pregnancies compared to singletons (30.57% vs. 73.84%). The nationwide cesarean section rate in Germany in 2016 was 30.5%[4]. Internationally large variations of CS rates can be seen due to different obstetrical standards. Even within Europe the CS rates differ between 14.8 to 52.2% [24].

The increased rate of CS in twins is mostly due to iatrogenic indications, e.g. breech presentation of the first twin, preterm delivery before $32^{0/7}$ weeks of gestation and MCMA placentation as proposed by national guidelines [8].

The results of the risk analysis of 739 twin pregnancies and births according to chorionicity in our study group showed 171 births (23.14% of all twin pregnancies) with confirmed monochorionicity, of those 4 (0.54%) were diagnosed as MCMA. The rate of monochorionic twin pregnancies was higher compared to a recent study which showed a rate of 5% [25]. A prospective cohort study of the perinatal outcome parameters in 228 monochorionic twins vs. 598 dichorionic twins showed significant differences in preterm premature rupture of membranes (PPROM) (85.5 vs. 75.1%) and IUGR (19.7 vs. 10.5%), as well as significantly lower gestational age at delivery and subsequently lower birthweights ($p < 0.01$) in monochorionic twins [26]. TTTS was seen in 7.9% in monochorionic pregnancies.

In our study, the rate of TTTS of 19.77% is slightly higher than the prevalence reported in another study [8]. The greater proportion of MCMA pregnancies and the higher number of pregnancies with TTTS can be explained by the referral strategy to tertiary perinatal centers. Our hospital has an especially high expertise in the treatment of twins. The chorionicity-based comparison of maternal and prenatal complications even after adjustment for gestational age and maternal age did not show significant differences apart from the risk for TTTS in monochorionic twins in our study group. Only the risk of preterm birth before 37 weeks of gestation was significantly higher among the monochorionic pregnancies as well as the rate of primary CS.

Most of other studies have shown an increased risk of a major malformations in twin pregnancies, especially in monozygotic twins [21,27]. But this was not confirmed in our study, the OR for MC twins vs. DC twins for lethal and major anomalies was 1.52, 95% CI 0.49-2.93, p=0.146.

There are several limitations of this study, which have to be considered apart from its retrospective character. The analysis is performed with data of a single center. The proportion of complications in pregnancy and with delivery is high in a referral perinatal center and this can be seen in the high preterm delivery rate in singleton pregnancies. Another study with perinatal statistics of a region in Germany shows a preterm delivery rate of 1.3 % in 2017 <34 weeks of gestation [22], whereas in our study the rate of preterm delivery <34 weeks was 7.86%. After trying to match twins and singleton for gestational week, the selection bias became obvious. The risk selection especially of singletons in lower gestational weeks led to a tendency of higher overall risks in singleton pregnancies.

A potential bias of selection is especially seen in the group of monochorionic twins as discussed above. The data were retrospectively collected by extracting information from the clinical records. Although data of the prenatal documents, documentation of birth and obstetrical as well as neonatal

3 Perinatal Statistic, SLAEK 2017, https://www.slaek.de/de/01/Qualitaetssicherung/ExterneQS/80Downloads/landesstatistiken/2016/16n1_Gesamt_2016.pdf, Assessed 05 December 2021

4 Destatis 2017: https://www.destatis.de/DE/Presse/Pressemitteilungen/2017/10/PD17_348_231.html, Assessed 05 December 2021

clinical records were used, a potential loss of information due to inadequate documentation must be considered. This loss of data may especially impair the analysis of a maternal diagnosis as PE or GDM or even conception after ART.

A prospective, interdisciplinary multi-center study with follow-up of the neonates is needed to give an overall impression of multiple pregnancies on clinical outcomes. A national or even international study could clarify many of the aspects discussed above.

Conclusions

The present study shows that with modern obstetrical management multiple pregnancies are still associated with a significantly increased risk for adverse perinatal outcome especially related to preterm delivery. Even though especially monochorionic twins are historically related to a higher perinatal risk, this study of perinatal risks does not prove the increased risks related to monochorionic twins considering the current medical standard of surveillance in pregnancy as well as perinatal and neonatal treatments. The intensified prenatal care with early detection of twin pregnancies and risk-adapted prenatal care may have contributed to the improved risk profile of monochorionic twins in this study.

The data presented in this study can be used for the decision-making process on the number of embryos to be transferred in infertility clinics. Twin pregnancies after ART are avoidable and should be avoided if possible. Study are available from the corresponding author on reasonable request.

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