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Risk factors and clinical outcomes of monozygotic multiple pregnancies following in vitro fertilization: a retrospective cohort study

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Abstract

Objective: This study aimed to evaluate the risk factor associated with the incidence of monozygotic multiple pregnancies (MMP) in assisted reproductive technology (ART) and to compare prognostic outcomes across chorionicity types.

Materials and methods: A retrospective analysis of 48,271 clinical pregnancy cycles following embryo transfer between November 2020 and November 2023 at the CITIC-Xiangya Reproductive & Genetic Hospital.

Results: Among 25,524 single-embryo transfer (SET) cycles, 774 MMP cases were identified, resulting in an MMP rate of 3.0%. Multivariate regression analyses revealed fresh embryo transfer (OR = 1.25, 95% CI 1.05–1.49, P < 0.05) and blastocyst transfer (OR = 3.86, 95% CI 1.46–10.20, P < 0.05) as independent risk factors for MMP. For prognostic analysis of twin pregnancies in ART, in the singleton live birth group (initially diagnosed twins/multiples reduced to single live birth due to pregnancy loss or adverse events), the preterm birth rate of monozygotic monochorionic (MZMC) twins was significantly higher than that of dizygotic dichorionic (DZDC) twins (10.7% vs. 22.8%, P = 0.002). In the twin births group, MZMC twins had a significantly shorter gestational age (36.32 ± 2.08 vs. 35.38 ± 2.11 vs. 36.11 ± 2.13 weeks, P < 0.001) and lower birth weight (2.46 ± 0.47 vs. 2.27 ± 0.48 vs. 2.45 ± 0.52 kg, P < 0.001) compared to monozygotic dichorionic (MZDC) and DZDC twins.

Conclusions: Fresh embryo transfer and blastocyst transfer as independent risk factors for MMP in ART. Monochorionic twins had a significantly higher risk of adverse perinatal outcomes compared to dichorionic twins.

Introduction

Assisted reproductive technology (ART) has emerged as a primary treatment for infertility. However, it is associated with a significantly elevated risk of monozygotic multiple pregnancies (MMP) [1]. MMP results from the division of a single fertilized egg during early embryogenesis, yielding genetically identical fetuses. Current research suggests that ART confers a 2- to 10-fold higher incidence of MMP compared to spontaneous conception [2]. The risk of MMP include younger maternal age [3,4] and ART-related techniques, particularly ovarian stimulation [5], embryo cryopreservation and extended embryo culture [6]. Given the association between monozygotic twinning and adverse perinatal outcomes [1,7], minimizing the incidence of MMP in ART is crucial. Nevertheless, the risk factors and mechanisms underlying embryo splitting are still unclear.

The increased risk of MMP after ART has generated significant clinical concern, as monozygotic twins are associated with higher rates of placenta previa, premature rupture of membranes, spontaneous abortion, premature delivery, and low birthweight compared to dizygotic twins [1,7]. MMP may result in severe complications, such as twin-twin transfusion syndrome (TTTS) [8]. In prognostic studies of

monozygotic twins, they are classified as either monochorionic twins (two fetuses share a single placenta), or dichorionic (each fetus has an individual placenta). It is well-established that monochorionic twins carries substantially higher risks of perinatal complications [9,10]. A retrospective study assessing prognostic differences in twin pregnancies revealed that monochorionic twins had significantly increased incidences of premature birth, stillbirth and placenta previa compared to dichorionic twins [11]. Current researches on adverse outcomes in ART-related monozygotic twins frequently fails to distinguish chorionic types, thereby hindering a thorough assessment of their pregnancy-related risks.

This study aims to identify the risk factors associated with MMP in ART, and compare adverse prognostic outcomes differences among monozygotic monochorionic (MZMC) twins, monozygotic dichorionic (MZDC) twins, and dizygotic dichorionic (DZDC) twins.

Material and method

Patient selection and study design

This retrospective cohort study analyzed 48,271 clinical pregnancies following embryo transfer at Reproductive and Genetic Hospital of CITIC-Xiangya between November 2020 and November 2023. For the risk factor analysis of monozygotic multiple pregnancies (MMP), both monozygotic singleton pregnancy (MSP) and MMP derived from single embryo transfer (SET) cycles were included. MMP were defined by the presence of two or more detectable fetal heartbeats, encompassing multi-chorionic pregnancies and monochorionic multiple pregnancies. For the prognostic comparisons of twin pregnancies, the DZDC twins from double embryo transfer cycles (DET), as well as the MZMC and MZDC twins from SET cycles, were included.

This study was approved by the Ethics Committee of the Reproductive and Genetic Hospital of CITIC-Xiangya. The study design was outlined in Fig 1.

Outcome measures

Clinical pregnancy was defined as ultrasound confirmation of at least one gestational sac in 28 days after embryo transfer. The gestational sac count refers to the total number of gestational or chorionic sacs (i.e., one chorionic sac indicates monochorionic pregnancy, while two chorionic sacs indicate dichorionic pregnancies). Obstetric and neonatal outcomes included ectopic pregnancy, early and late miscarriage, preterm birth (< 37 weeks), live birth rate, gestational age, mode of delivery, birth weight, congenital anomalies, and pregnancy complications such as hypertension, diabetes, anemia, edema, placenta previa, and placental abruption. All terminology aligned with standardized definitions from the International Glossary of Infertility and Fertility Care [12] and comparable peer-reviewed publications [13,14].

Statistical Analysis

All statistical analyses were performed using IBM SPSS Statistics (v25.0). Continuous variables were described as mean ± standard deviation and compared with the independent Student's t-test or one-way ANOVA. Categorical variables were presented as percentages and analyzed using the Chi-square or Fisher's exact test. Binary logistic regression was applied to adjust for variables. Statistical significance was defined as $P < 0.05$.

Result

Among 48,271 total pregnancy cycles, 25,524 cycles underwent SET, resulting in 774 cases of MMP (comprising 488 monochorionic and 286 dichorionic pregnancies). Cycles with sex-discordant newborns (potentially resulting from ART-derived singleton pregnancies combined with natural conception) were excluded in MMP. The overall incidence of MMP in SET cycles was 3.0%.

Risk factors for MMP in ART

Table 1 presents the results of the univariate regression analysis assessing risk factors for MSP and MMP in SET cycles. No significant differences were observed in general characteristics between MSP (n = 24,750) and MMP (n = 774), including the age and ethnicity of female and male, female BMI, duration of infertility, infertility type, and infertility diagnosis. Additionally, ART-related factors, including quality of transfer blastocysts, endometrial thickness, assisted hatching and blastocyst biopsy showed no statistically significant differences. MMP were significantly higher in fresh embryo transfer (21.3% vs. 25.3%, P = 0.008) and blastocyst-stage transfer (98.2% vs. 99.4%, P = 0.016).

Table 1
Univariate regression analysis of risk factors for MSP and MMP in SET cycles.

Cycles	MSP	MMP	P
	24,750	**774**	
Female age	32.38 ± 4.29	32.27 ± 4.26	0.507
Male age	34.42 ± 5.11	34.21 ± 4.90	0.242
Female BMI	21.96 ± 2.74	21.98 ± 2.63	0.824
Duration of infertility	3.24 ± 2.65	3.12 ± 2.46	0.203
Infertility type			0.461
Primary	23.9%	22.7%	
Secondary	76.1%	77.3%	
Infertility diagnosis			
Tube factor	73.3%	72.6%	0.661
Ovulatory disorders	14.8%	13.7%	0.407
Endometrial factor	20.7%	20.3%	0.790
Female ethnicity			0.374
Han	83.5%	82.3%	
Non-Han	16.5%	17.7%	
Male ethnicity			0.666
Han	85.0%	85.5%	
Non-Han	15.0%	14.5%	
The type of embryo transfer cycle			0.008
Fresh embryo transfer	21.3%	25.3%	
Frozen-thawed embryo transfer	78.7%	74.7%	
The stage of the embryo transfer			0.016
Cleavage stage	1.8%	0.6%	
Blastocyst	98.2%	99.4%	
Quality of transfer blastocysts			0.102
High quality	72.0%	74.6%	

Cycles	MSP	MMP	P
	24,750	**774**	
Low quality	28.0%	25.4%	
Endometrial thickness	11.75 ± 2.00	11.82 ± 1.89	0.354
Assisted hatching			0.580
Yes	0.2%	0.3%	
No	99.8%	99.7%	
Blastocyst biopsy			0.112
Yes	27.3%	24.7%	
No	72.7%	75.3%	

Furthermore, multivariate logistic regression was performed to account for potential confounding factors associated with MMP. As shown in Table 2, after adjusting for factors including female age, BMI, assisted hatching and blastocyst biopsy, both fresh embryo transfer (OR = 1.25, 95% CI 1.05–1.49, P = 0.014) and blastocyst transfer (OR = 3.86, 95% CI 1.46–10.20, P = 0.006) remained as independent risk factors for MMP.

Table 2
Multivariate regression analysis of risk factors for MMP in SET cycles

Female age	**OR (95%CI)**	**P**
	1.00 (0.98–1.02)	**0.842**
Female BMI	1.00 (0.98–1.03)	0.907
The type of embryo transfer cycle	1.25 (1.05–1.49)	0.014
Fresh embryo transfer		
Frozen-thawed embryo transfer		
The stage of the embryo transfer	3.86 (1.46–10.20)	0.006
Cleavage stage		
Blastocyst		
Assisted hatching	3.80 (0.79–18.32)	0.096
Yes		
No		
Blastocyst biopsy	0.92 (0.77–1.10)	0.355
Yes		
No		

Following the risk factor analysis, subgroup comparisons were conducted between MSP and MMP across different types of transfer cycles. As shown in Table 3, within the fresh embryo transfer subgroup, no statistically significant differences were observed between the MSP and MMP groups in basic FSH, E2 levels, ovarian stimulation protocols, insemination methods, sperm source, or sperm parameters. In the frozen–thawed embryo transfer subgroup, use of natural cycle protocols was significantly associated with a elevated incidence of MMP (P = 0.007).

Table 3
Risk factors for MMP in fresh and frozen–thawed embryo transfer cycles

Fresh embryo cycles	**MSP**	**MMP**	**P**
	5,282	**196**	
Basic FSH level (mIU/mL)	6.01 ± 2.82	5.83 ± 2.29	0.100
E2 (pL/mL)	4034.1 ± 1330.2	4107.8 ± 1189.4	0.533
Ovarian stimulation protocol			0.330
Downregulation	92.5%	94.9%	
Antagonists	6.1%	3.6%	
Others	1.3%	1.5%	
Insemination methods			0.693
IVF	72.1%	74.5%	
ICSI	16.5%	14.3%	
Half ICSI	11.3%	11.2%	
Sperm source			0.457
Fresh semen	88.5%	89.3%	
Epididymal puncture	1.1%	0%	
Testicular biopsy	0.9%	0.5%	
Frozen semen	9.4%	10.2%	
Sperm concentration	49.1 ± 28.9	48.6 ± 24.3	0.975
Sperm motility	29.9 ± 12.3	29.9 ± 12.1	0.839
Frozen–thawed embryo cycles	19,468	578	
Ovarian stimulation protocol			0.007
Natural cycle	47.3%	53.1%	
Artificial cycle	5.9%	6.7%	
Downregulation	33.0%	26.5%	
Other	13.8%	13.7%	

Prognostic analysis of monozygotic and dizygotic twins with different chorionicity

To compare the differences in pregnancy outcomes among the three groups, we analyzed the pregnancy outcomes based on the number of cycles. A comparison of monozygotic and dizygotic twin pregnancies in ART revealed that (Table 4) the early miscarriage rate was significantly higher in monozygotic twins (21.7% in MZMC and 24.1% in MZDC) than in dizygotic twins (3.1%). Moreover, the live birth rate was significantly lower in monozygotic twins (68.9% in MZMC and 71.0% in MZDC) than in dizygotic twins (92.4%). The MZMC group exhibited a significantly higher late miscarriage rates than the DZDC group(4.3% vs. 9.4%, $P < 0.001$).

We evaluated the prognostic differences between monozygotic and dizygotic twins according to chorionicity in obstetric and neonatal outcomes. The outcomes were classified based on final live births into either the singleton birth group (initially diagnosed twins/multiples reduced to single live birth due to pregnancy loss or adverse events) or the twin births group. To minimize potential confounding from natural conception, data from natural cycle protocols in frozen embryo transfer cycles were excluded from this analysis. The analysis comprised 324 cases of MZMC twins and 103 cases of MZDC twins from single-embryo transfer cycles, along with 8,686 cases of DZDC twins from double-embryo transfer cycles (Table 5). The results indicated no statistically significant differences in pregnancy complications, including gestational hypertension, gestational diabetes, and cesarean section rates among the three groups ($P > 0.05$).

In the singleton birth group, the preterm birth rate of MZMC was significantly higher than that of DZDC (10.7% vs. 22.8%, $P = 0.002$). In the twin births group, MZMC had the significantly shortest gestational age (36.32 ± 2.08 vs. 35.38 ± 2.11 vs. 36.11 ± 2.13 weeks, $P < 0.001$), lowest birth weight (2.46 ± 0.47 vs. 2.27 ± 0.48 vs. 2.45 ± 0.52 kg, $P < 0.001$), and highest preterm birth rate (73.1% vs. 43.7% vs. 48.6%, $P < 0.001$) among the three groups. Additionally, no significant differences were found in birth malformations ($P > 0.05$).

Table 4
Pregnancy outcomes of twin pregnancies with different chorionicity

Number	**DZDC**	**MZMC**	**MZDC**	**P**
	9,403	**470**	**145**	
Ectopic pregnancy	1.0%[a]	0%[a]	1.4%[a]	0.081
Early miscarriage	3.1%[a]	21.7%[b]	24.1%[b]	< 0.001
Late miscarriage	4.3%[a]	9.4%[b]	4.8%[a,b]	< 0.001
Live birth	92.4%[a]	68.9%[b]	71.0%[b]	< 0.001
Note: Different superscript letters indicate a statistically significant difference.				

Table 5
Obstetric and neonatal outcomes of twin pregnancies with different chorionicity

Number of total	**DZDC**	**MZMC**	**MZDC**	**P**
	8,686	**324**	**103**	
Number of singleton birth group	2,197	79	66	
Gestational hypertension	6.1%[a]	8.9%[a]	4.5%[a]	0.419
Gestational diabetes	14.9%[a]	15.2%[a]	16.7%[a]	0.978
Anemia during pregnancy	4.2%[a]	3.8%[a]	4.5%[a]	0.973
Edema during pregnancy	8.8%[a]	5.1%[a]	6.1%[a]	0.226
Placenta previa	0.8%[a]	1.3%[a]	1.5%[a]	0.837
Placental abruption	0%[a]	0%[a]	0%[a]	0.965
Preterm birth	10.7%[a]	22.8%[b]	15.1%[a,b]	0.002
Gestational age, weeks	38.52 ± 2.05[a]	37.83 ± 2.48[a]	38.50 ± 1.99[a]	0.069
Cesarean section	72%[a]	72.2%[a]	78.8%[a]	0.473
Birth weight, kg	3.16 ± 0.58[a]	3.08 ± 0.66[a]	3.22 ± 0.57[a]	0.736
Birth malformations	0.6%[a]	2.5%[a]	0%[a]	0.106
Number of twin births group	6,489	245	37	
Gestational hypertension	8.3%[a]	9.8%[a]	5.4%[a]	0.076
Gestational diabetes	13.8%[a]	16.7%[a]	8.1%[a]	0.463
Anemia during pregnancy	5.6%[a]	5.3%[a]	2.7%[a]	0.719
Edema during pregnancy	12.8%[a]	12.7%[a]	10.8%[a]	0.868
Placenta previa	0.9%[a]	0%[a]	0%[a]	0.248
Placental abruption	0.2%[a]	0%[a]	0%[a]	0.759
Preterm birth	43.7%[a]	73.1%[b]	48.6%[a]	< 0.001
Gestational age, weeks	36.32 ± 2.08[a]	35.38 ± 2.11[b]	36.11 ± 2.13[a]	< 0.001

Note: Different subscript letters indicate a statistically significant difference.

Number of total	DZDC	MZMC	MZDC	P
	8,686	324	103	
Cesarean section	96.2%[a]	96.3%[a]	94.6%[a]	0.810
Birth weight, kg	2.46 ± 0.47[a]	2.27 ± 0.48[b]	2.45 ± 0.52[a]	< 0.001
Birth malformations	1.4%[a]	2.4%[a]	0%[a]	0.326
Note: Different subscript letters indicate a statistically significant difference.				

Discussion

This study reports a 3.0% incidence of MMP following SET in 25,524 clinical pregnancy cycles, aligning with the previously reported ART-associated range of 1.3%–4.9% [2]. Fresh embryo transfer and blastocyst transfer were identified as independent risk factors for MMP in ART.

Current research remains controversial regarding the association between embryo transfer cycle types (fresh vs. frozen-thawed) and the incidence of MMP in ART. In a retrospective cohort study, *Liu et al* [15,16] found no significant association between transfer cycles and MMP risk. Whereas *Ikemoto et al* [17] suggested that frozen-thawed embryo transfer is associated with MMP in ART. Conversely, a study [18] reported that frozen-thawed embryo transfer acts as a protective factor, reducing the incidence of MMP in ART. However, these inconsistencies are likely attributable to methodological limitations in prior studies, including small sample sizes and potential confounding factors. Our findings indicate that fresh embryo transfer increases the risk of MMP in ART. We hypothesize that the body remains influenced by ART-induced ovulation-stimulating hormones during fresh embryo transfer, potentially altering the uterine environment [3] and hormone levels [5], and thereby increasing the risk of MMP.

In the frozen embryo transfer subgroup, natural cycle protocols were associated with an elevated incidence of MMP. We speculate that this may arise from the concurrence of spontaneous conception during natural cycles.

Our findings suggest that blastocyst transfer is an independent risk factor for MMP [19]. Although the underlying mechanisms remain unclear, it is widely hypothesized that the extended in vitro culture duration in blastocyst transfer may induce zona pellucida (ZP) hardening and oxidative stress [18,20], potentially culminating in ZP microperforations and premature embryo hatching. Furthermore, elevated glucose concentrations in blastocyst-stage culture media might contribute to MMP through reactive oxygen species (ROS)-mediated apoptosis within the inner cell mass (ICM), thereby compromising intercellular adhesion and predisposing to ICM fission [21]. In addition, our results revealed that assisted hatching, blastocyst biopsy, and other zona pellucida manipulations are not associated with MMP, which is consistent with previous research reports [22,23].

Finally, we further explored the association between the prognostic outcomes of twins in SET and double embryo transfer cycles. Monozygotic twin pregnancies in ART have a poorer prognosis than dizygotic twin pregnancies, primarily characterized by a higher incidence of pregnancy complications, increased miscarriage rates, and diminished live birth rates. However, our findings support previous reports that monozygotic twins exhibit higher miscarriage rates and reduced live birth success compared to dizygotic twin pregnancies [11]. The specific etiology remains incompletely elucidated, though some studies suggest that monozygotic multiple pregnancy loss may be linked to selective fetal demise in utero [24].

Chorionicity serves as a critical prognostic determinant in twin pregnancy outcomes. Our analysis confirms that monochorionic twin gestations conceived through ART are associated with significantly elevated risks of adverse perinatal outcomes, including preterm birth and low birth weight. Specifically, MZMC twins demonstrated shorter gestational ages and lower fetal birth weights compared to MZDC twins [25]. We hypothesize that the poorer prognosis of MZMC twins in ART primarily stems from placental sharing. Placental sharing in monochorionic twins may precipitate discordant fetal growth, amplifying the risk of complications such as respiratory distress syndrome, congenital heart disease [26], and TTTS [27], thereby endangering both maternal and fetal health. *Lopriore et al* reported that the incidence of severe complications, including TTTS, exceeds 25% in monochorionic twin pregnancies [28]. Therefore, rigorous prenatal surveillance and timely interventions can substantially mitigate these chorionicity-associated risks [29].

This study has several methodological limitations. First, in double-embryo transfer cycles, when two gestational sacs and pregnancies are present, it is difficult to differentiate between dizygotic and monozygotic twins, potentially introducing misclassification bias in prognostic evaluations of the two groups. Second, in natural cycle protocols in frozen embryo transfer cycles, spontaneous conception may occur simultaneously. Third, the absence of postnatal genetic testing represents a further limitation, which was not performed to confirm whether the fetuses originated from the same embryo. These limitations may collectively contribute to overestimation of MMP incidence. Conversely, our exclusion of natural cycle protocols in frozen embryo transfer cycles for prognostic analysis might underestimate the true incidence of ART-associated monozygotic twinning.

This study identifies fresh embryo transfer and blastocyst transfer as independent risk factors for MMP in ART. Monochorionic twins exhibit a significantly higher risk of adverse perinatal outcomes compared to dichorionic twins. To address these risks, the implementation of specialized monitoring protocols for monochorionic ART pregnancies should be prioritized to mitigate perinatal risks.

Statements and Declarations

Author Contribution

Shuoping Zhang and Qinwei Zhou contributed to conceptualizing the manuscript and collecting data. Qinwei Zhou drafted the original manuscript. Fei Gong contributed to patient management. Lizhi Leng revised the manuscript. All authors were involved in the critical revision of subsequent drafts and the approval of the manuscript.

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Ethics Approval

This study has been approved by the Ethics Committee of CITIC-Xiangya Reproductive & Genetic Hospital (approval number: LL-SC-2025-014), all patient data were completely anonymized before access, and the Ethics Committee waived the requirement for written informed consent for the use of their medical record data for research.

Competing Interests

There were no conflicts of interest or competing interests by any of the authors in this study.

Clinical trial number: Not applicable

Data Availability Statement

All data used or analyzed in this manuscript are available from the corresponding author upon reasonable request.

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Figures

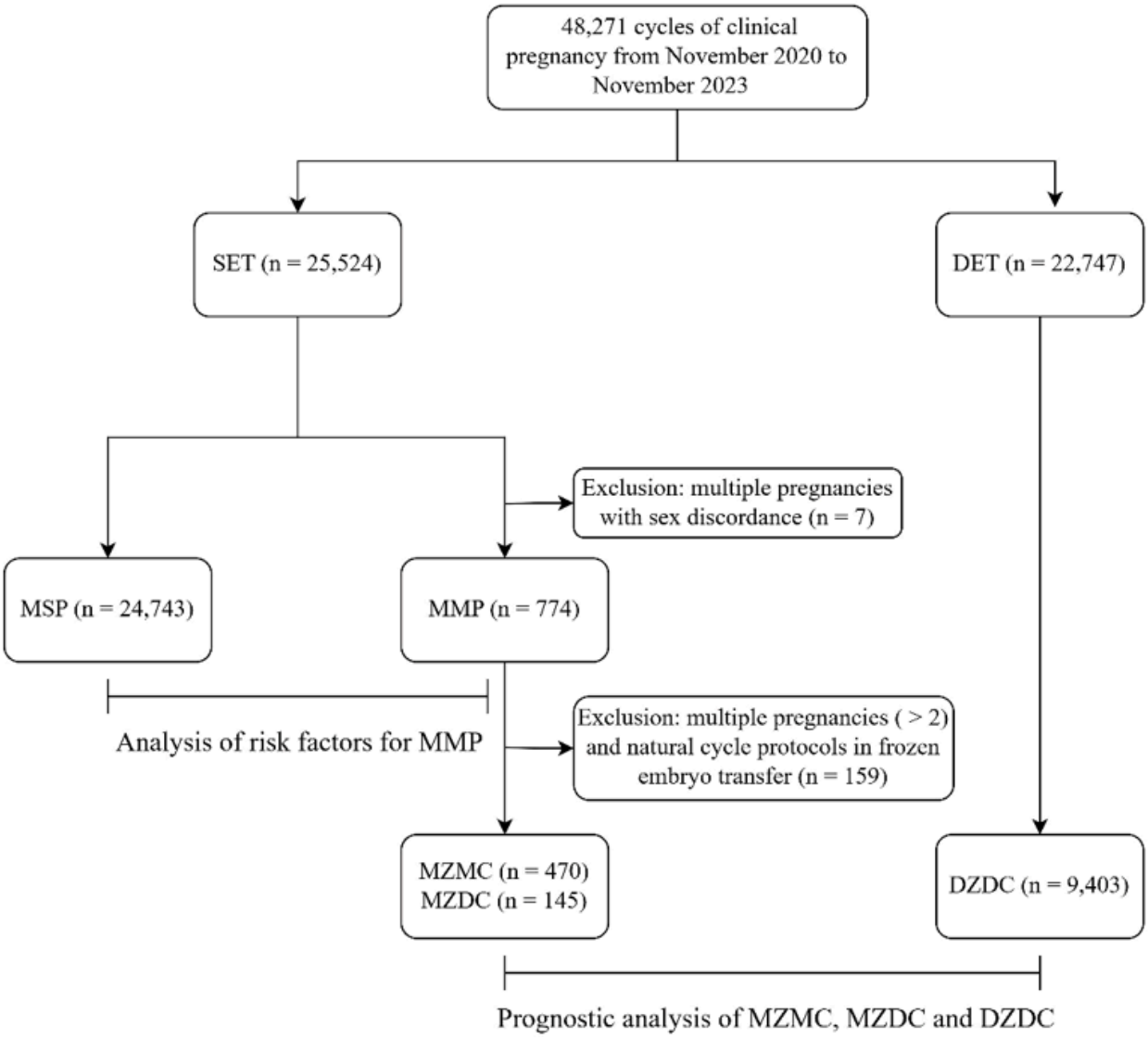


Figure 1

Flowchart of study design.