

Maternal and neonatal outcomes following *in vitro* fertilization: A cohort study in Romania

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Abstract. Although in recent years the number of pregnancies obtained through *in vitro* fertilization (IVF) has increased significantly, a higher incidence of complications has been identified in this group. The widespread development and use of IVF has led to an increasing rate of multiple pregnancies and thus their associated complications. However, whether these complications occur due to assisted reproduction or infertility problems remains to be elucidated. In the present study, a comparison was made of IVF pregnancies with spontaneous conception, and the risk of obstetric and neonatal complications was assessed. An ambispective observational cohort study was carried out between January 2017 and October 2021 at Elias University Emergency Hospital, Romania. The exposed cohort included 132 IVF pregnancies and the control cohort included 157 spontaneous pregnancies. The IVF group included 110 pregnancies with fresh embryo-transfer and 22 pregnancies with cryopreserved embryo-transfer. Obstetric, perinatal and neonatal complications were analysed by multivariable logistic analysis. The results showed that, IVF pregnancies had a higher risk of pregnancy-induced hypertension (OR=6.42, 95% CI=1.72-23.92) and placental abnormalities (OR=5.49, 95% CI=1.07-28.17). Neonates obtained through IVF had a higher risk of prematurity (OR=6.52, 95% CI=2.99-14.20), low birth weight (LBW) (OR=17.18, 95% CI=7.06-41.87), small for gestational age (OR=4.10, 95% CI=1.95-8.59) and were more

frequently hospitalized in the Neonatal Intensive Care Unit (NICU) (OR=11.91, 95% CI=5.72-24.81). Nulliparous women were associated with an increased risk of NICU admission (OR=0.46, 95% CI=0.25-0.88) and risk of LBW (OR=0.37, 95% CI=0.19-0.75). Maternal age ≥ 35 years had no influence as a confounding variable. In conclusion, this cohort study showed that IVF pregnancies have a higher risk of obstetric and neonatal complications than pregnancies obtained by spontaneous conception.

Introduction

Recently, the use of assisted reproduction techniques (ARTs) has increased worldwide. In Romania, in 2016, according to ESHRE records, 0.7% of births were obtained through assisted reproduction (1).

The benefits of treating infertility are known. Nevertheless, previous findings have shown that ARTs increase the risk of obstetric and perinatal complications, even after limiting the analysis to singleton pregnancies (2-4).

It is not possible to establish with certainty whether the complications are determined by the techniques used [e.g., *in vitro* fertilization or intracytoplasmic sperm insemination (ICSI)], the culture medium used, cryopreservation techniques, or embryo selection methods. Individual factors such as the etiology of infertility or maternal characteristics (age, parity) should also be taken into consideration (5). Other studies have shown the opposite result, namely, that pregnancies obtained through *in vitro* fertilization (IVF)/intracytoplasmic sperm injection (ICSI) are not correlated with the occurrence of these complications in pregnancy. This hypothesis has been supported by studies conducted on populations of subfertile women who obtained a pregnancy without the use of ART, but who nevertheless presented complications during pregnancy. Marino *et al* demonstrated in a cohort study conducted between 1986 and 2002 that, subfertile women, diagnosed with infertility but untreated, are at risk of perinatal complications such as prematurity, low birth weight (LBW) and very low birth weight (VLBW) (6). After adjusting for confounding factors, Jaques *et al* demonstrated in a cohort study that subfertile women who become pregnant spontaneously are at risk for obstetric and perinatal complications compared to the general population. These complications included

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Abbreviations: ART, assisted reproductive techniques; IVF, *in vitro* fertilization; ICSI, intracytoplasmic sperm injection; LBW, low birth weight; VLBW, very low birth weight; SGA, small for gestational age; IUGR, intrauterine growth restriction; LGA, large for gestational age; NICU, Neonatal Intensive Care Unit; RDS, respiratory distress syndrome; Nasal CPAP, nasal continuous positive airway pressure; e-SET, elective single embryo-transfer

Key words: *in vitro* fertilization, obstetric complications, neonatal complications, elective single embryo-transfer, multiple pregnancies

pregnancy-induced hypertension, preeclampsia, perinatal death, but also an increased risk of prematurity and LBW (7).

Researchers believe that the increased number of multiple pregnancies in the ART group may be another cause for the increased incidence of complications in this population. Simultaneously, it has been shown that complications also occur in singleton pregnancies obtained through IVF (8).

In the present study, a comparison of pregnancies obtained through IVF was made with those obtained spontaneously to identify the risk of obstetric and neonatal complications between the two groups in Romania.

Patients and methods

Data collection. This is an ambispective study (retrospective, between January 2017 and September 2018, and prospective, between October 2018 and March 2021) conducted at Elias University Emergency Hospital, Bucharest (Romania). The pregnancies obtained by IVF were grouped 1:1 with the pregnancies obtained by spontaneous conception. The study included 157 pregnancies obtained through IVF, from which were excluded pregnancies obtained from oocyte donation (n=19), sperm donation (n=1) and pregnancies with incomplete retrospective data (n=5). Thus, the final cohort was composed of 132 pregnancies obtained through IVF and 157 pregnancies obtained spontaneously. The two groups were compared for the risk of obstetric and neonatal complications. Depending on the type of embryo transferred, the IVF group was divided into two subgroups: fresh and frozen embryo.

The present study was approved by the Ethics Committee of Elias University Emergency Hospital (approval no.: 7017). Informed consent was obtained from the pregnant women included in the study. Data were retrospectively obtained from observation sheets and prospectively, anamnestic and from observation sheets during hospitalization. Patients with chronic conditions (chronic arterial hypertension, diabetes mellitus, cardiovascular disease or fetal abnormalities) were excluded because they could have been considered important confounders.

Variables. The maternal variables studied included complications in pregnancy (pregnancy induced-hypertension, gestational diabetes and placental abnormalities such as placenta praevia and placental abruption) and type of pregnancy (singleton/multiple).

Neonatal variables included gestational age (weeks), changes in birth weight (grams) [LBW, small for gestational age (SGA), large for gestational age (LGA), high birth weight, intrauterine growth restriction (IUGR)]. Using their birth weight, newborns can be classified as having a low birth weight which is <2,500 g or as having a high birth weight which is between 4,000 and 4,500 g. Newborns can be also classified by intrauterine growth and gestational age. In this regard, we defined small for gestational age as a birth weight below the 10th percentile for gestational age. Large for gestational age was defined as a birth weight above the 90th percentile for gestational age. Intrauterine growth restriction was defined as a reduction in the expected fetal growth and was usually an ultrasound finding. The incidence of neonatal complications [respiratory distress syndrome (RDS), apnea of prematurity,

neonatal arterial hypotension and congenital anomalies], Apgar score at 1 min, admission in NICU, the need for respiratory support by oxygen therapy, non-invasive ventilation with a T-piece system and nasal CPAP or mechanical ventilation were analyzed. The need for parenteral nutrition, antibiotic therapy, vasoactive medication and blood transfusions was also analyzed.

Statistical analysis. The analysis of qualitative variables was performed using the Chi-square independence test (Pearson). Maternal age as a numerical variable was analyzed using ANOVA parametric test as the values had a Gaussian distribution for each of the three subgroups compared. A significant difference was detected globally thus, the analysis was continued using the Fisher LSD post hoc test to make comparisons between all pairs of groups.

For gestational age and birth weight analysis, the non-parametric Kruskal-Wallis comparison test was used as the distribution of data from the three groups was not Gaussian. Subsequently, a post hoc analysis was performed by the Dunn procedure for multiple pair comparisons.

In addition, a multivariable logistic analysis was performed using maternal age and parity as independent variables to identify the predictive factors for the complications sought and for the association between IVF and spontaneous conception. From this analysis, odd ratios (OR) were calculated with a 95% confidence interval (CI) and probability (P)-value. The limit for statistical significance was $P < 0.05$. Alpha level was 0.05 for all tests. MS Excel 2019 was used for data analysis.

Results

Maternal and neonatal characteristics for the IVF/spontaneous conception group. This study included 132 pregnancies obtained through IVF and 157 pregnancies obtained through spontaneous conception, from January 2017 to March 2021. The IVF group comprised 77 singleton pregnancies and 55 multiple pregnancies (26 twin pregnancies and one triplet pregnancy), and the control group consisted of 157 single pregnancies and did not include multiple pregnancies. The IVF group was divided into two subgroups depending on the type of embryos used for transfer: fresh embryo (n=61 used for singleton pregnancies and n=49 used for multiple pregnancies), and cryopreserved embryo (n=16 used for singleton pregnancies and n=6 used for multiple pregnancies). Tables I and II summarize maternal and neonatal characteristics in the IVF and in the spontaneous conception (control) groups.

In the post hoc analysis of maternal age, highly significant differences were observed between the control group and IVF singleton pregnancy group, but also between the control group and the IVF multiple pregnancy group. However, no statistically significant differences were demonstrated between the two subgroups of IVF. In the present study, women who opted for IVF were older than those in the control group (Table I).

Analyzing gestational age and birth weight, the differences found among the three groups were highly significant ($P < 0.0001$, both for gestational age and birth weight). The

Table I. Maternal and neonatal characteristics for the IVF/spontaneous conception group.

Variable	Group	No.	Mean	SD	Min	Quartile	1st Median	Quartile	3rd Max	P-value
Maternal age (years)	Control group	157	30.55	5.20	19	27	31	34	44	
	IVF Singleton	77	35.90	5.69	26	31	36	39	50	<0.0001 ^a
	IVF Multiple	55	34.80	6.33	25	31	35	39	50	<0.0001 ^a
GA (weeks)	Control group	157	38.88	1.24	33	39	39	40	42	
	IVF Singleton	77	37.97	1.63	33	38	38	39	40	<0.0001 ^b
	IVF Multiple	55	35.87	2.29	26	35	36	37	38	<0.0001 ^b
BW (g)	Control group	157	3,260.45	416.62	1,530	3,080	3,250	3,500	4,460	
	IVF Singleton	77	3,002.60	658.87	240	2,670	3,090	3,340	4,550	<0.0001 ^b
	IVF Multiple	55	2,289.82	446.74	800	2,095	2,340	2,570	3,080	<0.0001 ^b

IVF, *in vitro* fertilization; GA, gestational age; BW, birth weight; SD, standard deviation. P<0.0001, highly significant. P-values according to ^aANOVA and ^bKruskal Wallis.

Table II. Maternal characteristics for the IVF/spontaneous conception group.

Variable	IVF			P-value ^a for all	P-value ^a IVF Singleton/Multiple
	Control group (157) (%)	Singleton (77) (%)	Multiple (55) (%)		
Age ≤35 years	125 (79.62)	33 (42.86)	27 (49.09)	<0.0001	0.4782
Age ≥35 years	32 (20.38)	44 (57.14)	28 (50.91)		
Multiparous	71 (45.22)	10 (12.99)	32 (58.18)	<0.0001	<0.0001
Nulliparous	86 (54.78)	67 (87.01)	23 (41.82)		
Embryos: not used (control group)	157 (100.00)	0 (0.00)	0 (0.00)	<0.0001	0.1336
Fresh embryos	0 (0.00)	61 (79.22)	49 (89.09)		
Cryopreserved embryos	0 (0.00)	16 (20.78)	6 (10.91)		
Embryo transfer, 0	157 (100.00)	0 (0.00)	0 (0.00)	<0.0001	<0.0001
Embryo transfer, 1	0 (0.00)	53 (68.83)	2 (3.64)		
Embryo transfer, ≥2	0 (0.00)	24 (31.17)	53 (96.36)		

All data are expressed as n (%). P<0.0001, highly significant. P-values according to ^aChi-square test. IVF, *in vitro* fertilization.

mean GSA and average birth weight were significantly lower in the IVF group (in singleton pregnancies ~37.97 weeks and ~3,002 g), especially for multiple pregnancies (35.87 weeks and ~2,289 g respectively), compared to spontaneously obtained pregnancies (38.88 weeks and ~3,260 g) (Table I).

Table II shows that ~50% of the pregnant women in the IVF group were >35 years of age (57.14% in singleton pregnancies group and 50.91% in multiple pregnancies group) compared with the control group (20.38%, P<0.0001). Additionally, pregnant women who obtained a singleton pregnancy through IVF tended to be nulliparous (87.01%), compared to those who obtained a multiple pregnancy (41.82%, P<0.0001).

The pregnancy distribution according to the type of embryo used, i.e., fresh vs. cryopreserved was analyzed (Table II). The results showed that IVF pregnancies were obtained predominantly by using fresh embryos, with a higher frequency in multiple pregnancies than singleton ones (89.09 vs. 79.22%,

P=0.1336). The use of cryopreserved embryos was more frequently reported in singleton pregnancies than multiple pregnancies (20.78 vs. 10.91%, P=0.1336).

In >30% of IVF singleton pregnancies, 2 or more embryos were initially transferred, resulting in a single pregnancy (Table II). For this reason, whether or not the loss or underdevelopment of an embryo was an independent risk factor for complications, was assessed. However, no significant difference for any complication analyzed was identified.

Furthermore, the possibility that the loss of an embryo was caused by risk factors associated with the IVF procedure, such as maternal age (≥35 years), nulliparity, type of embryo used (fresh or cryopreserved) was assessed; however, no conclusive evidence for this hypothesis was obtained. The results were not statistically significant for maternal age (≥35 years; P=0.2558), nulliparous women (P=0.9318), or type of embryo used, i.e., fresh/frozen (P=0.2222) (Table III).

Table III. Analysis of the risk factors that can influence the loss of an embryo in IVF pregnancies.

No. of embryo transfer	Age 35 years (%)	Age \geq 35 years (%)	P-value ^a
1	25 (47.17)	28 (52.83)	0.2558
2	8 (33.33)	16 (66.67)	
No. of embryo transfer	Multiparous (%)	Nuliparous (%)	P-value ^a
1	7 (13.21)	46 (86.79)	0.9318
2	3 (12.50)	21 (87.50)	
No. of embryo transfer	Fresh embryos (%)	Cryopreserved embryos (%)	P-value ^a
1	44 (83.02)	9 (16.98)	0.2222
2	17 (70.83)	7 (29.17)	

All data are expressed as n (%). P<0.05, significant; P<0.001, highly significant. P-values according to ^aChi-square test. IVF, *in vitro* fertilization.

Obstetric and neonatal complications. Obstetric and neonatal complications in the IVF group compared with the spontaneous conception group are described in Table IV.

Variables showing obstetric and neonatal complications among the three groups were analyzed to identify for which there was a possible influence determined either by the use of IVF or by the type of pregnancy, i.e., single and multiple, respectively.

Regarding obstetric complications, women who obtained a pregnancy through IVF, compared with spontaneous conception, had an increased rate of pregnancy-induced hypertension (P<0.0001). In the IVF group, this complication was more common in multiple pregnancies compared with singleton ones (18.18 vs. 6.49%). No statistically significant difference was demonstrated between the groups for gestational diabetes (P=0.6139) and placental abnormalities (P=0.0695).

Regarding neonatal complications, the proportion of prematurity, LBW, SGA and LGA was significantly higher in the IVF group compared with the spontaneous conception group, except for LGA, which occurred only in singleton IVF pregnancies. No differences were demonstrated between the groups for IUGR (P=0.0507) and high birth weight (P=0.2559).

Although none of the pregnancies obtained by spontaneous conception had an Apgar score <7 each 1 min, it cannot be said that pregnancies obtained by IVF have a risk of hypoxia at birth, as no statistically significant difference was demonstrated (P=0.0702).

Neonates obtained through IVF were more frequently admitted in NICU, compared with those obtained spontaneously (P<0.0001). Admission of the neonate in NICU was more common in the case of multiple pregnancies, compared with singleton pregnancies (67.27 vs. 33.77%).

Given that the rate of prematurity was higher in the IVF group, this was closely correlated with the frequency of respiratory distress syndrome in these neonates (P<0.0001). As for the other complications analysed, the RDS rate was higher for multiple pregnancies compared with singleton

ones (60.00 vs. 18.18%). For this reason, the need for oxygen therapy was higher in the IVF vs. control group (P<0.0001), but the need for exogenous surfactant administration was not statistically significant among the groups (P=0.3816).

Regarding the need of respiratory support, it was observed that neonates obtained by IVF required more frequently non-invasive ventilation with T-piece system compared to those obtained spontaneously (P<0.0001). Nasal CPAP and invasive ventilation were used with similar frequency within the two groups (P=0.0812, respectively P=0.0695).

The incidence of apnea of prematurity did not show statistically significant differences between the groups (P=0.2079), but IVF neonates showed more frequent arterial hypotension compared to those obtained spontaneously (P<0.0001). This complication occurred more frequently in multiple pregnancies, compared with singletons in the IVF group (30.91 vs. 12.99%).

Analysis of neonates with congenital anomalies, including congenital heart abnormalities did not show statistically significant differences between IVF pregnancies compared with the control group (P=0.1727 and P=0.6608).

The results showed that neonates obtained through IVF, especially those from multiple pregnancies, required more frequently parenteral nutrition (P<0.0001), antibiotic therapy (P<0.0001) and vasoactive drugs (P<0.0001). The use of blood transfusions did not show significant differences between the study groups (P=0.0568).

Analysis of obstetric and neonatal complications in the IVF group vs. spontaneous conception stratified by the type of embryo used: fresh vs. cryopreserved. An analysis of whether the type of embryo used could have any influence on the occurrence of obstetric and neonatal complications was performed using the Fisher test. The results were not statistically significant for most of the complications studied (P>0.05) (Table V).

Analysis of obstetric and neonatal complications in the IVF group vs. spontaneous conception stratified by parity and

Table IV. Analysis of obstetric and neonatal complications in the IVF group vs. spontaneous conception by birth plurality.

Variable	Control group (157) (%)	IVF		P-value ^a for all	P-value ^a IVF Singleton/ Multiple
		Singleton (77) (%)	Multiple (55) (%)		
Maternal complications					
Pregnancy-induced hypertension	3 (1.91)	5 (6.49)	10 (18.18)	<0.0001	0.0370
Gestational diabetes	6 (3.82)	5 (6.49)	2 (3.64)	0.6139	0.4702
Placental anomalies	2 (1.27)	4 (5.19)	4 (7.27)	0.0695	0.6218
Neonatal complications					
Prematurity	10 (6.37)	13 (16.88)	29 (52.73)	<0.0001	<0.0001
LBW	7 (4.46)	14 (18.18)	38 (69.09)	<0.0001	<0.0001
SGA	13 (8.28)	11 (14.29)	21 (38.18)	<0.0001	0.0016
IUGR	3 (1.91)	5 (6.49)	0 (0.00)	0.0507	0.0540
LGA	12 (7.64)	9 (11.69)	0 (0.00)	0.0373	0.0086
High birth weight	6 (3.82)	4 (5.19)	0 (0.00)	0.2559	0.0861
Apgar score <7 per 1 min	0 (0.00)	1 (1.30)	2 (3.64)	0.0702	0.374
NICU	12 (7.64)	26 (33.77)	37 (67.27)	<0.0001	0.00014
RDS	10 (6.37)	14 (18.18)	33 (60.00)	<0.0001	<0.0001
Apnea of prematurity	2 (1.27)	4 (5.19)	2 (3.64)	0.2079	0.6717
Arterial hypotension	7 (4.46)	10 (12.99)	17 (30.91)	<0.0001	0.0118
Cardiac anomalies	14 (8.92)	9 (11.69)	7 (12.73)	0.6608	0.8569
Congenital anomalies	1 (0.64)	3 (3.90)	2 (3.64)	0.1727	0.9386
Oxygen therapy	10 (6.37)	20 (25.97)	35 (63.64)	<0.0001	<0.0001
Exogenous surfactant	2 (1.27)	3 (3.90)	2 (3.64)	0.3816	0.9386
T-piece system	3 (1.91)	8 (10.39)	12 (21.82)	<0.0001	0.0710
Nasal CPAP	2 (1.27)	5 (6.49)	3 (5.45)	0.0812	0.8052
Mechanical ventilation	2 (1.27)	4 (5.19)	4 (7.27)	0.0695	0.6218
Parenteral nutrition	12 (7.64)	17 (22.08)	27 (49.09)	<0.0001	0.0012
Antibiotic therapy	9 (5.73)	12 (15.58)	26 (47.27)	<0.0001	<0.0001
Vasoactive drugs	6 (3.82)	7 (9.09)	14 (25.45)	<0.0001	<0.0001
Blood transfusions	3 (1.91)	3 (3.90)	5 (9.09)	0.0568	0.2175

All data are expressed as n (%). P<0.05, significant; P<0.001, highly significant. P-values according to ^aChi-square test. IVF, *in vitro* fertilization; LBW, low birth weight; SGA, small for gestational age; IUGR, intrauterine growth restriction; LGA, large for gestational age; NICU, Neonatal Intensive Care Unit; RDS, respiratory distress syndrome; Nasal CPAP, nasal continuous positive airway pressure.

maternal age. The impact of IVF, nulliparity and advanced maternal age on the occurrence of obstetric and neonatal complications was assessed by performing multivariable logistical analysis. The result of the analysis was expressed, for each variable, as the odds Ratio (OR) + 95% confidence interval of OR and P-value (Table VI).

Nulliparity, as a confounding variable, influenced NICU admission (OR=0.46, 95% CI=0.25-0.88), LBW (OR=0.37, 95% CI=0.19-0.75), the need of parenteral nutrition (OR=0.49, 95% CI=0.26-0.94) and antibiotic therapy (OR=0.48, 95% CI=0.25-0.97), but age ≥ 35 years had no influence as a confounding variable.

During our analysis, it was found that women who underwent IVF treatment were 6.42-fold more likely to develop pregnancy-induced hypertension (OR=6.42, 95% CI=1.72-23.92) and 5.49-fold more likely to develop placental abnormalities (OR=5.49, 95% CI=1.07-28.17).

Neonates obtained by IVF procedures were 6.52-fold more predisposed to prematurity (OR=6.52, 95% CI=2.99-14.20), 17.18-fold more likely to have LBW (OR=17.18, 95% CI=7.06-41.87) and 4.10-fold more likely to be SGA (OR=4.10, 95% CI=1.95-8.59). In addition, these neonates were 11.91-fold more likely to be admitted in NICU (OR=11.91, 95% CI=5.72-24.81). RDS was 8.71-fold more likely to occur in the IVF group (OR=8.71, 95% CI=4.00-18.95), that required oxygen therapy (OR=11.19, 95% CI=5.16-24.26), non-invasive with a T-piece system (OR=7.88, 95% CI=2.17-28.57) and nasal CPAP (OR=5.79, 95% CI=1.14-29.43).

IVF neonates were 6.57 time more likely to require parenteral nutrition (OR=6.57, 95% CI=3.14-13.76) and antibiotic therapy (OR=6.94, 95% CI=3.06-15.72). As IVF patients are 4.16-fold more likely to develop neonatal arterial hypotension (OR=4.16, 95% CI=1.66-10.46), they are 3.44-fold more

Table V. Analysis of obstetric and neonatal complications in the IVF group vs. spontaneous conception stratified by the type of embryo used: fresh vs. cryopreserved.

Variable	Fresh embryos (110) (%)	Cryopreserved embryos (22) (%)	P-value ^a
Maternal complications			
Pregnancy-induced hypertension	12 (10.91)	3 (13.64)	0.4937
Gestational diabetes	4 (3.64)	3 (13.64)	0.0754
Placental anomalies	6 (5.45)	2 (9.09)	0.4912
Neonatal complications			
Prematurity	35 (31.82)	7 (31.82)	0.6062
LBW	45 (40.91)	7 (31.82)	0.2917
SGA	28 (25.45)	4 (18.18)	0.3349
IUGR	3 (2.73)	2 (9.09)	0.1612
LGA	8 (7.27)	1 (4.55)	0.5383
High birth weight	4 (3.64)	0 (0.00)	0.4778
Apgar score <7 per 1 min	1 (0.91)	2 (9.09)	0.0719
NICU	53 (48.18)	10 (45.45)	0.5010
RDS	39 (35.45)	8 (36.36)	0.6325
Apnea of prematurity	3 (2.73)	3 (13.64)	0.0579
Arterial hypotension	22 (20.00)	5 (22.73)	0.524
Cardiac anomalies	12 (10.91)	4 (18.18)	0.2077
Congenital anomalies	4 (3.64)	1 (4.55)	0.8063
Oxygen therapy	47 (42.73)	8 (36.36)	0.3795
Exogenous surfactant	2 (1.82)	3 (13.64)	0.0325
T-piece system	15 (13.64)	5 (22.73)	0.2432
Nasal CPAP	4 (3.64)	4 (18.18)	0.0263
Mechanical ventilation	5 (4.55)	3 (13.64)	0.1024
Parenteral nutrition	36 (32.73)	8 (36.36)	0.5281
Antibiotic therapy	31 (28.18)	7 (31.82)	0.5290
Vasoactive drugs	17 (15.45)	4 (18.18)	0.5001
Blood transfusions	6 (5.45)	2 (9.09)	0.4912

All data are expressed as n (%). P<0.05, significant; P<0.001, highly significant. P-values according to ^aFisher test. IVF, *in vitro* fertilization; LBW, low birth weight; SGA, small for gestational age; IUGR, intrauterine growth restriction; LGA, large for gestational age; NICU, Neonatal Intensive Care Unit; RDS, respiratory distress syndrome; Nasal CPAP, nasal continuous positive airway pressure.

likely to require vasoactive medication (OR=3.44, 95% CI=1.26-9.41).

The results also showed that, although a simple analysis between groups did not reach the 95% significance threshold (P<0.05) for placental anomalies and the need of nasal CPAP (in which case P>0.05, but P<0.1-90% significance threshold), ART significantly influenced the risk of these complications when other factors were taken into account in a multivariable analysis.

Discussion

In the present study, we demonstrated that pregnancies obtained through *in vitro* fertilization (IVF) have a higher risk of complications compared with pregnancies obtained spontaneously, which correlates with data from the literature.

Over time, research has shown that some pregnancies obtained through assisted reproduction techniques (ARTs) have a higher risk of pregnancy-induced hypertension (9-11),

gestational diabetes (8,12,13) and placental anomalies (14,15). Complications can also affect the neonates, who are at risk of prematurity (2,3), low birth weight (LBW) (2,4), small for gestational age (SGA) (2), large for gestational age (LGA) (16-18) and congenital anomalies (2-4).

The results of the present study showed that women who become pregnant by IVF have a higher risk of developing pregnancy-induced hypertension and placental abnormalities. In a population-based cohort study, Opdahl *et al* compared pregnancies obtained by ART with pregnancies obtained spontaneously and showed an increased risk for hypertensive disorders in pregnancy in the ART group (9). In the present study, this effect could have been explained by the increased proportion of women over the age of 35 years, but the multivariable analysis revealed that advanced maternal age had no influence on the risk of pregnancy complications. In addition, given that an increased proportion of women with multiple IVF pregnancies developed pregnancy-induced hypertension, we consider that multiple pregnancies are an

Table VI. Multivariable analysis of obstetric and neonatal complications in the nulliparous group.

Variable	Main variable: control vs. IVF group			Confounding factor: Nulliparous		
	Odds ratio	95% CI	P-value Chi ² Wald	Odds ratio	95% CI	P-value Chi ² Wald
Maternal complications						
Pregnancy-induced hypertension	6.42	1.72-23.92	0.0056	0.60	0.22-1.64	0.3261
Gestational diabetes	1.35	0.41-4.50	0.6223	0.37	0.12-1.20	0.0986
Placental anomalies	5.49	1.07-28.17	0.0410	1.25	0.31-5.01	0.7556
Neonatal complications						
Prematurity	6.52	2.99-14.20	<0.0001	0.70	0.36-1.36	0.2940
LBW	17.18	7.06-41.87	<0.0001	0.37	0.19-0.75	0.0052
SGA	4.10	1.95-8.59	0.0002	0.69	0.36-1.37	0.2949
IUGR	2.39	0.51-11.26	0.2712	4.16	0.50-34.53	0.1865
LGA	1.18	0.46-3.04	0.7362	0.53	0.22-1.31	0.1683
High birth weight	1.17	0.31-4.48	0.8147	0.24	0.06-0.99	0.0480
Apgar score <7 per 1 min	-	-	0.9954	0.81	0.07-9.52	0.8714
NICU	11.91	5.72-24.81	<0.0001	0.46	0.25-0.88	0.0186
RDS	8.71	4.00-18.95	<0.0001	0.54	0.28-1.04	0.0664
Apnea of prematurity	4.00	0.73-21.95	0.1111	1.65	0.32-8.48	0.5432
Arterial hypotension	4.16	1.66-10.46	0.0024	1.15	0.52-2.56	0.7292
Cardiac anomalies	1.47	0.65-3.33	0.3524	0.49	0.23-1.07	0.0769
Congenital anomalies	4.55	0.46-44.61	0.1936	1.03	0.18-5.96	0.9682
Oxygen therapy	11.19	5.16-24.26	<0.0001	0.52	0.28-1.01	0.0519
Exogenous surfactant	3.93	0.70-21.99	0.1189	0.20	0.04-1.10	0.0639
T-piece system	7.88	2.17-28.57	0.0017	0.59	0.24-1.49	0.2691
Nasal CPAP	5.79	1.14-29.43	0.0344	0.77	0.21-2.88	0.7088
Mechanical ventilation	4.29	0.82-22.39	0.0845	0.77	0.21-2.91	0.7106
Parenteral nutrition	6.57	3.14-13.76	<0.0001	0.49	0.26-0.94	0.0318
Antibiotic therapy	6.94	3.06-15.72	<0.0001	0.48	0.25-0.97	0.0414
Vasoactive drugs	3.44	1.26-9.41	0.0162	0.91	0.39-2.18	0.8438
Blood transfusions	3.08	0.74-12.89	0.1234	0.64	0.19-2.23	0.4890

IVF, *in vitro* fertilization; LBW, low birth weight; SGA, small for gestational age; IUGR, intrauterine growth restriction; LGA, large for gestational age; NICU, Neonatal Intensive Care Unit; RDS, respiratory distress syndrome; Nasal CPAP, nasal continuous positive airway pressure; CI, confidence interval. P<0.05, significant; P<0.001, highly significant.

independent risk factor, especially if the mother is >35 years of age (19).

Two recent meta-analyses compared singleton IVF pregnancies with spontaneous conception and demonstrated an increased risk of placental anomalies associated with IVF (14,20). Karami *et al* demonstrated that the risk of placental abnormalities was identified for multiple pregnancies obtained by IVF (15), but this aspect was not demonstrated in the present study.

Bosdou *et al* demonstrated in a recent meta-analysis that using IVF compared with intracytoplasmic sperm insemination (ICSI) increases the risk of developing gestational diabetes (GD) (21). In this study, the results were inconsistent with the data reported in the literature (8,12,13). Even after adjustment for confounding factors (advanced maternal age, nulliparity), having an IVF pregnancy did not increase the risk of GD.

Consistent with the results of previous studies (2,3), the prematurity rate was higher in the IVF group, compared with the control group. A recent meta-analysis, which included 52 cohort studies, with a total of 181,741 single pregnancies obtained by IVF/ICSI and approximately 4.6 million spontaneous neonates worldwide showed that pregnancies obtained by ART had increased risk of prematurity, LBW, congenital anomalies and perinatal mortality (2). Given that 41.6% of all IVF pregnancies were multiple pregnancies, we consider that the increased incidence of prematurity can be a side effect of these high-risk pregnancies. To verify this hypothesis, we performed a stratified analysis of data according to the type of pregnancy (singleton/multiple). Thus, after the analysis was restricted to singleton pregnancies, we observed a reduction in the incidence of complications for most of the variables studied. This conclusion has been demonstrated in previous studies (22-25).

The increased incidence of prematurity in the present study was also correlated with changes in birth weight. Thus, we observed that neonates obtained by IVF have a higher risk of LBW and SGA, compared to those obtained spontaneously. This may be associated with the increased rate of multiple pregnancies in the IVF population, but Schieve *et al* demonstrated that the risk was also observed for singleton ART pregnancies (4). However, the associated maternal complications such as pregnancy-induced hypertension and placental abnormalities, as well as the culture medium used for embryo development, could have influenced birth weight.

For LGA, and high birth weight, there were no differences observed between the groups. Maheshwari *et al* demonstrated that the transfer of cryopreserved embryos reduced the risk of prematurity, LBW and SGA, but increased the incidence of LGA and high birth weight (16). A retrospective study showed that transferring cryopreserved embryos increased the birth weight with an average of 142.3 g. The authors of that study concluded that the cryopreservation group had a 1.7-fold higher risk of high birth weight (17). The analysis of our data did not show an increased risk of LGA ($P=0.5383$), or high birth weight ($P=0.4778$), when using cryopreservation techniques (Table V).

Results of the present study did not demonstrate an increased risk for congenital anomalies associated with IVF. This is consistent with literature data, as previous findings have shown that the use of ICSI increases the risk of congenital anomalies compared to IVF (26).

Given the increased incidence of prematurity in the IVF group, the neonates included in our study had general prematurity complications. Thus, they were more likely to be hospitalized in NICU for respiratory complications such as respiratory distress syndrome (RDS), for which they needed oxygen therapy and non-invasive respiratory support. The risk of arterial hypotension may be secondary to both prematurity and placental abnormalities that may be associated with maternal bleeding and the risk of neonatal hypovolemia.

The risk of pregnancy complications is directly proportional to maternal age (27). It is considered that women >35 years of age may associate other comorbidities in addition to the etiology of infertility. This can influence the evolution of IVF pregnancies, including placing the pregnant woman in a risk category (28). For this reason, a data analysis was performed according to the presence of nulliparity and advanced maternal age, but the results were not significant for most of the complications analysed. However, advanced maternal age and nulliparity are risk factors that predispose to the use of IVF anyway and this may be the reason that the complications analyzed were not influenced. Wennberg *et al* demonstrated in a retrospective study that advanced maternal age increased the risk of complications in pregnancy, regardless of the type of conception, i.e., spontaneous vs. assisted reproduction (29).

Given the increased rate of multiple pregnancies in the IVF group, it is necessary to implement single embryo transfer protocols. It is currently considered the most effective method to avoid multiple pregnancies and their associated complications (30-33). According to ESHRE records, the transfer of a single embryo in Romania increased from 16.9% in 2015 to 22.5% in 2016 (1,34).

The use of cryopreservation allows the selection of high-quality embryos and the transfer of a single embryo,

which helps reduce the incidence of multiple pregnancies and thus the associated complications. Given the benefits reported after the transfer of cryopreserved embryos, the implementation of the freeze-all strategy is being discussed (35-37). The reported benefits include a reduction in the overall incidence of ovarian hyperstimulation syndrome and the incidence of prematurity, LBW and VLBW. Simultaneously, recent findings have shown an increased risk of pregnancy-induced hypertensive conditions, including pre-eclampsia (38). The risk of complications calls into question the widespread use of the freeze-all transfer policy in the pregnancies obtained by ART (16,39).

The present study has some limitations. First, its partial retrospective design, a single-center study, and the small cohort of patients are factors that may limit the generalization of our findings. Additionally, the lack of multiple pregnancies in the control group reduces the possibility to determine exactly whether they influence the prognosis of pregnancies obtained through IVF. Multicenter, prospective studies on large population groups from Romania are needed to demonstrate that IVF use increases the risk of pregnancy complications.

Second, data on the etiology of infertility were limited and could not be included. Further studies are needed to include information on the cause of infertility, on the ovarian stimulation protocol used, serum hormone levels during ovarian stimulation, as well as the embryonic stage at the time of transfer (cleavage or blastocyst stage).

Third, no information was obtained on the diagnosis of subfertility in the control population, which, according to the above-mentioned studies (6,7), can influence the results.

In conclusion, women who become pregnant through *in vitro* fertilization have a higher risk of complications in pregnancy. Multiple pregnancies can be considered a risk factor, but literature data show that singleton pregnancies obtained through IVF also present complications. Additional factors that could not be controlled in the present study (ovarian stimulation protocol, culture medium used, type of embryo transferred, population of subfertile women) may influence this risk.

Given the results of the present study, we recommend the implementation of single embryo transfer protocols to reduce the complications associated with multiple pregnancies.

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Availability of data and materials

All data generated or analyzed during this study are included in this published article.

Authors' contributions

AMB, SDP and SV designed the study. AMB and SDP consulted the literature and collected bibliographical data.

AMB and SDP wrote the paper. SV reviewed and edited the manuscript. AMB and SV confirm the authenticity of all the raw data. All authors have read and approved the final manuscript for publication.

Ethics approval and consent to participate

The present study was approved by the Ethics Committee of Elias University Emergency Hospital (approval no. 7017). Written informed consent was obtained from each mother.

Patient consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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