„CAROL DAVILA" UNIVERSITY OF MEDICINE AND PHARMACY
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DOMAIN
MEDICINE

IMPLICATION OF IN VITRO FERTILIZATION ON PREGNANCY AND NEONATAL PROGNOSIS

SUMMARY OF DOCTORAL THESIS

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Content

1. Introduction

Recently, infertility has become a major public health problem with a significant impact on the quality of life of couples diagnosed with this condition. The causes are multiple and can affect both female and male patients. Given the public importance of this consequence associated with various pathologies, much has been invested in research to identify a solution to treat and improve the health of this group of patients.

Assisted reproduction methods have gained an impressive technological advance over time. Currently, there are various techniques that can be adapted individually, depending on the pre-existing pathology that led to infertility. The most commonly described and used are in vitro fertilization (IVF), intracytoplasmic insemination of the sperm, intrafallopian transfer of gametes and even pregnancy through donated oocytes.

After prolonged use of assisted reproduction, studies in these patient groups have found that these pregnancies have a higher risk of complications than spontaneously obtained pregnancies. The researchers attempted to identify the risk factors that are associated with the reported complications, but found that no exact causal factor could be established.

Initially, it was considered that assisted reproduction techniques cause complications in pregnancy, but later, it was discussed whether the etiology of infertility does not have a synergistic effect with the treatment methods used. Simultaneously, the advances in research have led to the identification of additional risk factors, individual to the patient, which seem to influence the final prognosis of pregnancies obtained through assisted reproduction.

In this regard, maternal age was considered an important risk factor because it has been shown, that older women tend to opt for these methods of treatment because they have low ovarian reserve, but also pre-existing comorbidities. The other risk factors identified are nulliparity, increased body mass index, smoking status, the pre-existing chronic pathologies and the associated medication.

Complications reported in the literature associated with assisted reproduction methods can be subdivided into maternal and neonatal complications. Of the maternal ones, the most frequently described were hypertensive diseases such as pregnancy-induced hypertension, complicated by preeclampsia or eclampsia, as well as placental abnormalities, gestational diabetes and biliary diseases such as pregnancy cholestasis.

Regarding the reported neonatal complications, these include an increased rate of prematurity, changes in normal birth weight, the presence of congenital anomalies identifiable

at birth, increased hospitalization in neonatal intensive care units – NICU, for appropriate monitoring and supportive care, depending on the complications reported.

Two topics that have gained interest in the recent period time are the embryonic transfer protocol used and the type of oocyte chosen to obtain a pregnancy.

The increased rate of prematurity among newborns obtained by assisted reproduction has led to careful research into the causes that may explain this effect. Thus, it was found that, a large part of the pregnancies obtained through assisted reproduction are multiple pregnancies, which increases the risk of prematurity regardless of the presence of infertility, respectively, its treatment. For this reason, it was considered necessary to implement protocols that reduce the number of embryos transferred to improve the prognosis of pregnant women and newborns. Currently, the most common protocol used in several European countries is the transfer of a single embryo. Although it has been observed that this does not increase the chance of obtaining a pregnancy, it reduces the risks associated with the transfer of a number of more than 3 embryos.

Regarding the type of oocyte used to obtain a pregnancy, significant differences were identified between the use of fresh oocytes and cryopreservation techniques.

Frozen oocytes have important benefits for pregnant women in that they reduce the incidence of ovarian hyper-stimulation syndrome associated with the transfer of fresh oocytes, but, on the other hand, they increase the risk of pregnancy induced-hypertension.

In terms of neonatal outcome, frozen oocytes reduce the incidence of prematurity and low birth weight, but some of these newborns are at an increased risk of high birth weight. For this reason, the population of women who comply with the cryopreservation protocol should be carefully chosen, taking into consideration the maximum benefits, but also, diminishing the total risk.

In Romania, since 2015, the National Assisted Reproduction Program has been operating, which financially supports infertile couples to obtain a pregnancy. This program includes in vitro fertilization techniques, intrauterine insemination, intracytoplasmic sperm insemination, screening and preimplantation genetic diagnosis. The relatively recent introduction of this program may explain the small number of studies on this population group of patients.

The motivation for choosing this subject for doctoral thesis, stems from the lack of national statistics on the prognosis of newborns obtained through assisted reproduction. These statistics would improve the management of pregnancies knowing the impact of each assisted reproduction procedure on the outcome of pregnant women and newborns. These protocols are necessary to identify neonatal complications and congenital malformations reported at distance

from birth. The dynamic follow-up of these patients allows the early identification of complications and appropriate management of pediatric patients obtained through in vitro fertilization.

2. Study objectives

Infertility is increasingly gaining the attention of clinicians worldwide because of the significant reduction in quality of life of patients due to the psycho-socio-emotional impact determined by the inability to conceive a child.

For this reason, more and more investment has been made in research in recent decades to identify risk factors that could explain the complications associated with assisted reproduction procedures. These risk factors may be, on the one hand, individual factors, related to the patient, but they may also be factors related to the treatment used to reduce infertility in order to achieve a pregnancy.

Most of the information currently known on this subject comes from studies conducted internationally, but in Romania, research on the complications caused by assisted reproduction and especially the impact on the newborn, are at the beginning of the road. The urgent need to have national statistics related to the already known information laid the foundation of this paper.

The main objective of this study was to demonstrate the correlations between the use of in vitro fertilization technique and the increased risk of maternal and neonatal complications in a cohort of patients in Romania.

3. Materials and methods

This study was conducted in 2017-2021 and has an observational, analytical, cohort design, the data used being obtained both retrospectively and prospectively.

The research was performed at the Elias University Emergency Hospital, within the Department of Obstetrics-Gynecology and Neonatology.

The data used in this study were obtained mainly from the observation sheets of hospitalized patients in the above-mentioned period in the departments of Obstetrics-Gynecology and Neonatology, as well as anamnestic.

Patients who met the inclusion criteria consisted of pregnant women who obtained pregnancies (single or multiple) through in vitro fertilization, pregnant women who obtained a spontaneous pregnancy, newborns obtained by in vitro fertilization and those conceived spontaneously.

We excluded from this study pregnancies obtained using assisted reproduction methods other than in vitro fertilization, those obtained from donated oocytes or sperm and pregnancies that met some of the inclusion criteria but had incomplete data that would not have allowed statistical analysis.

The two types of groups - IVF versus control, were subdivided into single versus multiple pregnancies and were grouped in a 1: 1 ratio before excluding those who did not meet the criteria for this study.

The variables analyzed included data on the pregnant women and data on the newborns. For the group of pregnant women, demographic information was collected, as well as the complications that occurred during pregnancy.

For the newborn, demographic information included gestational age, birth weight and its changes, the risk of prematurity and complications associated with premature birth, as also the necessary treatment for these newborns.

Microsoft Excel and IBM SPSS Statistics 20.0 were used for statistical analysis.

4. Results

4.1 Descriptive analysis of the patient group

The database of this study included 177 newborns included in the IVF group and 200 newborns included in the control group. Both groups analyzed included both single and multiple pregnancies (Table 4.1).

Table 4.1 Maternal and neonatal characteristics for the IVF/spontaneous conception group

Variable + study group	No	Minimum	Maximum	Media	Standard deviation	p Kruskal-Wallis
Maternal age | Multiple Study	77	25	50	34.70	5.73	**< 0.0001**
Maternal age | Single Study	100	26	50	35.75	5.46	
Maternal age | Control	200	19	44	30.63	5.11	
GA | Multiple Study	77	26	38	35.86	2.07	**< 0.0001**
GA | Single Study	100	33	40	37.94	1.73	
GA | Control	200	33	42	38.85	1.22	
BW | Multiple Study	77	800	3080	2314.16	412.06	**< 0.0001**
BW | Single Study	100	1580	4550	3056.30	562.33	
BW | Control	200	1530	4460	3266.75	418.52	

Analyzing maternal age, gestational age and birth weight using the Kruskal-Wallis test, we identified significant differences between the analyzed groups ($p < 0.0001$).

4.2 Analysis of maternal characteristics and complications in the IVF group versus spontaneous conception

We included in the study variables that correspond to maternal characteristics and that can be considered additional, individual risk factors such as maternal age and parity. Statistical analysis showed that there are statistically significant differences in terms of maternal age <35 years between the study group and the control group ($p < 0.0001$), but no differences between the two IVF subgroups ($p = 0.276$). We also analyzed patients included in the study for the risk of associated maternal complications and identified statistically significant differences for pregnancy-induced hypertension and placental abnormalities ($p = 0.0011$ and $p = 0.0113$, respectively). These differences were not demonstrated for single IVF pregnancies versus multiple IVF pregnancies (Table 4.2).

Table 4.2 Maternal characteristics and complications in the IVF group versus spontaneous conception

Variable	p Chi2 total	p Chi2 Multiple vs Single Study
Pregnancy type	< 0.0001	< 0.0001
Maternal age <35 years	< 0.0001	0.276
Gesta	0.758	0.331
Para	< 0.0001	< 0.0001
Gestational diabetes	0.272	0.181
Pregnancy induced hypertension	0.0011	0.180
Placental anomalies	0.0113	0.959
Preeclampsia		0.704

4.3 Analysis of neonatal characteristics and complications in the IVF group versus spontaneous conception

Regarding the analysis of neonatal characteristics and complications in the present study (Table 4.3), we identified statistically significant differences for the mode of birth. Thus, we demonstrated that IVF pregnancies tended to be frequently obtained by cesarean section compared to the control group ($p < 0.0001$).

Also, statistically significant differences were identified for the risk of hospitalization in neonatal intensive care ($p < 0.0001$), the risk of prematurity ($p < 0.0001$), low birth weight ($p < 0.0001$) and high birth weight ($p = 0.034$) between the IVF group versus the control group. Regarding the neonatal complications that appeared immediately postnatal, we identified statistically significant differences for respiratory distress syndrome (RDS, $p < 0.0001$), apnea of prematurity ($p = 0.004$), arterial hypotension ($p < 0.0001$) and variations in serum glucose concentration ($p < 0.0001$).

The analysis of neonatal complications between single IVF and multiple IVF pregnancies (Table 4.3) demonstrated statistical significance differences for the risk of hospitalization in NICU ($p < 0.0001$), the risk of prematurity ($p < 0.0001$) and low birth weight ($p < 0.0001$), the risk of intrauterine growth restriction (IUGR, $p = 0.047$), the risk of developing RDS ($p < 0.0001$), arterial hypotension ($p < 0.010$) and changes in serum blood glucose concentration ($p = 0.00$).

Table 4.3 Neonatal characteristics and complications in the IVF group versus spontaneous conception

Variable	p Chi2 total	p Chi2 Multiple vs Single Study
Mode of delivery	< 0.0001	0.379
Neonatal sex	0.869	0.737
NICU	< 0.0001	< 0.0001
Apgar <7 (1 min)	0.826	0.791
Preterm	< 0.0001	< 0.0001
LBW	< 0.0001	< 0.0001
SGA	< 0.0001	< 0.0001
LGA	0.034	0.539
Macrosomia	0.227	0.076
IUGR	0.085	0.047
Congenital anomalies	0.120	0.457
RDS	< 0.0001	< 0.0001
Arterial hypotension	< 0.0001	0.010
Apnea of prematurity	0.004	0.283
Serum glucose variations	< 0.0001	0

4.4 Analysis of neonatal investigations and treatment in the IVF group versus spontaneous conception

Given that a significant number of newborns required hospitalization in NICU, we considered it appropriate to perform a statistical analysis for the risk of requiring intensive care in the neonatal period in both the IVF group and the control group (Table 4.4).

Thus, performing the Chi square test we identified statistically significant differences for the risk of requiring respiratory support with oxygen therapy ($p < 0.0001$), non-invasive ventilation with Neo-puff ($p < 0.0001$), respectively nasal CPAP ($p = 0.001$). We also identified that newborns included in the IVF group have a higher risk of requiring hydro-electrolytic rebalancing infusion ($p < 0.0001$), vasoactive medication ($p < 0.0001$) and antibiotic therapy ($p < 0.0001$), compared to the control group. The analysis of the imagistic tests identified that patients in the IVF group associated a higher risk of brain malformations than patients included in the control group ($p = 0.0176$). Between the IVF group of single versus multiple pregnancies we identified significant differences for the need for oxygen therapy ($p < 0.0001$), non-invasive ventilation with Neo-puff ($p = 0.017$), hydro-electrolytic rebalancing infusion ($p = 0.00$), vasoactive drugs ($p = 0.028$) and antibiotic therapy ($p < 0.0001$).

Table 4.4 Analysis of neonatal investigations and treatment in the study groups

Variable	p Chi2 total	p Chi2 Multiple vs Single study
Oxygen therapy	< 0.0001	< 0.0001
Neo-puff	< 0.0001	0.017
Nasal CPAP	0.001	0.983
Mechanical ventilation	0.063	0.953
Cerebral anomalies	0.0176	0.02885
Cardiac anomalies	0.105	0.57
Exogenous surfactant	0.172	0.972
Endogenous perfusion	< 0.0001	0
Antibiotic therapy	< 0.0001	< 0.0001
Vasoactive drugs	< 0.0001	0.028
Blood transfusion	0.643	0.454

4.5 Analysis of IVF characteristics in single versus multiple pregnancies

Because we included only one assisted reproduction procedure in the present study, we obtained information about some of the stages of in vitro fertilization, namely about the type of oocyte used and the number of embryos transferred to obtain a pregnancy.

The statistical analysis performed on the IVF group of single and multiple pregnancies (table 4.5) showed significant differences in the number of embryos transferred ($p < 0.0001$). We found that 21% of single pregnancies benefited from the transfer of 2 embryos, without maintaining the viability of both embryos until the end of pregnancy. In this case, if the theory of the "vanishing twin" syndrome was taken into account, it could explain some of the neonatal outcomes for single IVF pregnancies (Fig. 4.1).

Table 4.5 Oocyte type and number of embryos transferred in IVF pregnancies

Variable	p Chi2 Multiple vs Single Study
Fresh/frozen oocyte	0.219
1 embryo transfer - SET	< 0.0001
2 embryo transfer - DET	< 0.0001
>2 embryo transfer	0.972

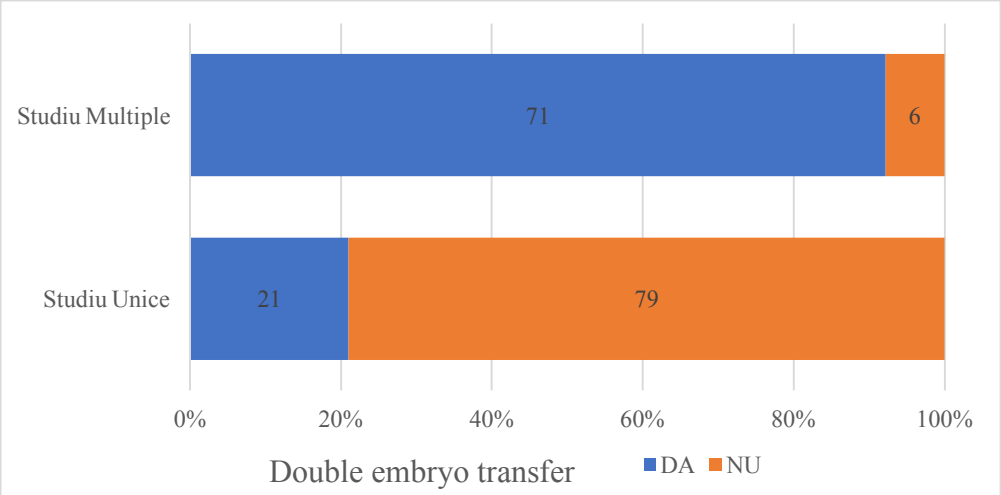

Fig. 4.1 Comparison of batch distribution according to the transfer of two embryos

4.6 Analysis of the relationship between the type of oocyte used and maternal characteristics/complications

According to the literature, the type of oocyte used in the assisted reproduction procedure to obtain a pregnancy can influence the prognosis of that pregnancy by increasing or reducing the risk of maternal and neonatal complications.

We performed a statistical analysis to demonstrate an association between the type of oocyte used and maternal complications in the group of patients included in this study.

In table 4.6 we can see that the type of oocyte used, preferably the frozen one, increases the risk of gestational diabetes ($p = 0.006$) and placental abnormalities ($p = 0.032$) for pregnant women in the IVF group.

Table 4.6 The relationship between the type of oocytes used and maternal characteristics/complications

Variable	p Chi^2	Interpretation
Maternal age <35 years	0.256	NS
Gestational diabetes	0.006	S
Pregnancy induced hypertension	0.172	NS
Placental anomalies	0.032	S
Preeclampsia	0.198	NS

4.7 Analysis of the relationship between the type of oocyte used and neonatal characteristics/complications

We also wanted to identify whether the type of oocyte used influences the risk of neonatal complications in the group of patients obtained by IVF (Table 4.7).

Statistical analysis using the Chi square test identified significant differences for the risk of having an Apgar score <7 at 1 minute of life (p = 0.005), the risk of prematurity (p = 0.033), RDS (p = 0.00), apnea prematurity (p = 0.001) and arterial hypotension (p = 0.016) in association with frozen oocytes.

Table 4.7 The relationship between the type of oocytes used and neonatal characteristics/complications

Variable	p Chi^2	Interpretation
Neonatal sex	0.593	NS
NICU	0.070	NS
Apgar < 7 (1 min)	0.005	S
Preterm	0.033	S
LBW	0.547	NS
SGA	0.967	NS
LGA	0.503	NS
Macrosomia	0.315	NS
IUGR	0.249	NS
Congenital anomalies	0.846	NS
RDS	0.000	HS
Arterial hypotension	0.016	S
Apnea of prematurity	0.001	HS
Glucose variations	0.120	NS

4.8 Analysis of the relationship between the type of oocyte used and the need for neonatal investigations and treatment

In Table 4.8 we can see that the use of frozen oocytes increases the risk that newborns in the IVF group require non-invasive ventilation with Neo-puff (p = 0.000) and nasal CPAP (p = 0.000), the risk of requiring administration of exogenous surfactant (p = 0.000), but also hydro-electrolytic rebalancing infusion (p = 0.004) and antibiotic therapy (p = 0.007). Also, the use of frozen oocytes increases the risk of identifying brain malformations in newborns in the IVF group (p = 0.034).

Table 4.8 Relationship between the type of oocyte used and the need for neonatal investigations and treatment

Variable	p Chi^2	Interpretation
Oxygen therapy	0.127	NS
Neo-puff	0.000	HS
Nasal CPAP	0.000	HS
Mechanical ventilation	0.056	NS
Cerebral anomalies	0.034	S
Cardiac anomalies	0.055	NS
Exogenous surfactant	0.000	HS
Endogenous perfusion	0.004	S
Antibiotic therapy	0.007	S
Vasoactive drugs	0.055	NS
Blood transfusions	0.294	NS

4.9 Multivariable logistical analysis of IVF, nulliparity and maternal age > 35 years to estimate the risk of maternal and neonatal complications

Given that the prognosis of IVF pregnancies is influenced by other risk factors independent of the reproductive procedure itself, we performed a multivariable analysis to identify whether the three independent variables influence the risk of maternal and neonatal complications.

Thus, in table 4.9 we can see that the simultaneous presence of the three prediction variables - IVF, nulliparity and maternal age > 35 years significantly increases the risk of pregnancy induced-hypertension (p = 0.0343), placental abnormalities (p = 0.0064) and gestational diabetes (p = 0.0470).

Table 4.9 The influence of the three independent variables on the risk of maternal complications

Variable	p Log (Likelihood Ratio)	p Score	p Wald	Interpretation
Pregnancy induced hypertension	0.0398	0.0343	0.0490	S
Placental anomalies	0.0097	0.0064	0.0170	S
Gestational diabetes	0.0337	0.0470	0.0774	S

Also, in table 4.10 we demonstrated that the simultaneous presence of nulliparity, advanced maternal age (> 35 years) and in vitro fertilization procedure have a significant

influence on the risk of hospitalization in NICU (p <0.0001), prematurity (p= 0.0004) and low birth weight (p = 0.0001), risk of RDS (p = 0.0002) and arterial hypotension (p = 0.0043).
We observed that the three variables also have statistical significance on the risk of requiring oxygen therapy (p <0.0001) and ventilation with a non-invasive system (Neo-puff - p = 0.0035, nasal CPAP - p = 0.0815), hydro-electrolytic rebalancing infusion (p = 0.0011), antibiotic therapy (p = 0.0014) and vasoactive medication (p = 0.0012).

Table 4.10 The influence of the three independent variables on the risk of neonatal complications

Variable	**p Log (Likelihood Ratio)**	**p Score**	**p Wald**	**Interpretation**
NICU	< 0.0001	< 0.0001	< 0.0001	HS
Apgar <7 (1 min)	0.2222	0.2090	0.2732	NS
Preterm	0.0001	0.0002	0.0004	HS
LBW	< 0.0001	< 0.0001	0.0001	HS
SGA	0.5883	0.5911	0.5950	NS
LGA	0.2895	0.3003	0.3158	NS
Macrosomia	0.1474	0.1437	0.1762	NS
IUGR	0.2789	0.3549	0.4302	NS
RDS	< 0.0001	< 0.0001	0.0002	HS
Arterial hypotension	< 0.0001	< 0.0001	0.0043	HS
Apnea of prematurity	0.1222	0.1604	0.2477	NS
Oxygen therapy	< 0.0001	< 0.0001	< 0.0001	HS
Neo-puff	0.0002	0.0005	0.0035	HS
Nasal CPAP	0.0210	0.0317	0.0815	S
Exogenous surfactant	0.0652	0.1769	0.8782	NS
Mechanical ventilation	0.1377	0.1442	0.2038	NS
Endovenous perfusion	0.0003	0.0006	0.0011	HS
Antibiotic therapy	0.0002	0.0006	0.0014	HS
Vasoactive drugs	0.0006	0.0005	0.0012	HS
Blood transfusions	0.6315	0.6305	0.6356	NS

5. Discussion

Following the statistical analysis of the three groups of patients (IVF - single pregnancies, multiple pregnancies, control group), we found that the use of in vitro fertilization increases the risk of maternal and neonatal complications in this small cohort of patients.

From a demographic perspective, we showed that pregnant women in the control group tended to be <35 years old, compared with the study group. Newborns obtained by IVF tend to be rather premature, with an average gestational age of 35 weeks for multiple pregnancies and 37 weeks for single pregnancies. Also, patients in the IVF group tended to have a low birth weight - <2500 grams.

Analyzing the data, we observed that women in the IVF group tended to transfer an average number of 2 embryos to obtain a pregnancy, which significantly increases the percentage of multiple pregnancies in the study group. The number of multiple pregnancies represents about half of the total number of pregnancies obtained through IVF, which may explain the average gestational age that is included in the definition of prematurity.

Analyzing the individual risk factors, such as the parity of the women included in the study, we observed statistically significant differences between the IVF group and the control group. Thus, we observed that women suffering from infertility tend to be nulliparous compared to those who manage to obtain a pregnancy through spontaneous conception. These results have also been identified in the literature. [1]

Regarding the maternal complications reported in this study, we identified significant differences between the IVF group and the control group for the risk of pregnancy induced-hypertension. The results have been confirmed by studies in the literature, in which the use of IVF increases the risk of hypertensive complications in pregnancy. [2,3] The risk of developing hypertension is correlated with the impact that this pathology has on placental vascularity, significantly increasing the risk of prematurity.[4] Also, in general, the development of hypertensive complications occurs with age, and the results of our study show that IVF patients are more likely to be elderly and more likely to develop pregnancy induced-hypertension. Another risk factor associated with an increased incidence of hypertension in IVF pregnancies is the use of cryopreservation techniques [5], however, our study did not demonstrate this risk.

Another maternal complication frequently reported in pregnant women in the IVF group of this study is the presence of placental abnormalities. Studies in the literature have

reported that assisted reproduction methods can alter the normal development of the placenta, which impact normal growth and development of the fetus. [6,7]

Given the difficulty of obtaining a pregnancy among infertile couples, there is a general tendency for pregnancies obtained by assisted reproduction to be extracted by cesarean section. [8] This was also demonstrated in our study, in which 176/177 IVF pregnancies were extracted by cesarean section.

Because most newborns in the IVF group were premature, we analyzed their risk of complications associated with prematurity, the risk of birth defects and the need for intensive treatment in the immediate postnatal period.

We identified that a significant number of newborns required hospitalization in the neonatal intensive care unit most likely due to prematurity.

Premature birth in the IVF group can be explained, on the one hand, by the increased number of multiple pregnancies, as well as, by maternal characteristics such as age > 35 years and nuliparity. Pregnancy induced-hypertension and placental abnormalities can also speed up premature birth, having an impact on the final prognosis of these newborns. All these factors have also been identified in studies conducted internationally [9], which have further shown that the use of other methods of assisted reproduction can reduce the number of premature births associated with IVF. [10]

Also, IVF infants could have shown signs of fetal distress due to maternal pathologies that could have altered placental vascularity.

For the indirect analysis of fetal distress, we wanted to identify whether newborns obtained by IVF show a more frequent Apgar score <7 at 1 minute of life compared to those obtained spontaneously, but the results were not statistically significant.

In the study group, we have noticed that the birth weight is significantly influenced by the use of IVF, thus, these newborns have an increased risk of low weight, respectively, high birth weight, compared to the control group. The reduction in neonatal weight can be determined by factors with intrauterine impact on placental vascularity and the consequent reduction in fetal nutrition. This weight change can also be "normal" for the gestational age if the newborn is premature. Determining specific causes for the low birth weight of these patients should be carefully analyzed, considering all risk factors present during that pregnancy.

In the opposite sense, the presence of high birth weight neonates could have been explained by two major causes analyzed on this cohort of patients. On the one hand, there is the gestational diabetes in the IVF group, which could have had a direct impact on fetal growth through

hyperinsulinism, as a growth factor. However, the statistical analysis did not show an association between IVF and the increased risk of acquired diabetes during pregnancy.
The other hypothesis is the correlation of the high birth weight newborn with the use of frozen oocytes to obtain a pregnancy. Studies have shown that, in addition to the multiple benefits associated with cryopreservation of oocytes, they influence the risk of high birth weight for IVF newborns. [11,12]. Unfortunately, these data could not be demonstrated in our study because a statistically significant association between the two variables was not demonstrated.

Regarding respiratory complications in newborns obtained by IVF, we have shown that they are associated with an increased risk of respiratory distress syndrome and apnea of prematurity. The forms of neonatal respiratory distress syndrome were mostly mild-moderate. Severe forms have been diagnosed in few cases, and this may be due to the use of triple therapy for the prevention and management of neonatal RDS. This consists of antenatal administration of corticosteroid therapy for lung maturation, the early use of non-invasive ventilation with Neo-puff or nasal CPAP and the administration of exogenous surfactant, in selected cases.
In our study, we did not demonstrate statistically significant differences between the IVF group and the control group regarding surfactant administration, and these results correlate with the small number of severe RDS patients.

The use of oxygen therapy showed significant differences between the two analyzed groups, and the need for non-invasive ventilation was significantly higher in the IVF group compared with the control group. Mechanical ventilation was reported in a few cases and was not influenced by the use of IVF to obtain a pregnancy, which also correlated with the small number of newborns diagnosed with severe RDS.

From a hemodynamic perspective, arterial hypotension was more frequently associated with IVF, and we consider that this may be correlated with maternal and placental hypertensive pathology reported in pregnant women in the IVF group. The treatment of hypotension showed statistically significant differences; so the use of vasoactive medication was more frequently associated with IVF than with the control group.

Although gestational diabetes did not show statistically significant differences between the groups, IVF neonates showed more frequent variations in serum blood glucose concentration compared with control newborns. These variations, predominantly neonatal hypoglycemia, can be explained by prematurity and low birth weight, under <2500 grams.
These patients more frequently required hydro-electrolytic rebalancing infusion, with statistically significant differences from the control group.

Studies have shown that the use of assisted reproduction increases the risk of birth defects, mainly heart and brain abnormalities.[13,14] We analyzed the incidence of these abnormalities in the IVF group and found significant differences in the risk of brain and heart abnormalities compared with the control group. The most serious brain abnormality identified is agenesis of corpus callosum, without identifying a specific cause for it.

Although malformations have been reported in the literature also in the digestive, renal, musculoskeletal, ocular systems [15,16], in this study we could not demonstrate statistically significant differences for them in the IVF group compared with the control group.

Last but not least, premature birth could have been caused by infectious causes, and this may explain the increased need for antibiotic therapy in the IVF group of newborns.

Analysis of the type of oocyte used to obtain pregnancy through IVF showed that most women tend to use fresh oocytes more frequently to the detriment of frozen oocytes. Analysis of the type of oocyte and its impact on the risk of neonatal complications showed that frozen oocytes increase the risk of prematurity, respiratory and hemodynamic complications that require specific neonatal treatment. These results are inconsistent with data reported in the literature that has shown a reduction in the incidence of prematurity and its associated complications. [11] To identify the cause of this discrepancy between the results of our study and those reported in international studies, multivariate analysis performed on additional risk factors associated with the type of oocyte used is required.

The other additional risk factors included in this study, not directly related to the type of oocyte used are nulliparity, advanced maternal age and IVF use. The simultaneous presence of these factors increases the risk of maternal complications, but also of neonatal complications. This supports the need to identify other confounders in order to be able to predict the potential prognosis of an IVF pregnancy and the appropriate management of obstetric and neonatal complications.

6. Conclusions and perspectives

The final results of this study showed that pregnancies obtained by in vitro fertilization have a higher risk of maternal and neonatal complications in this cohort of patients analyzed, compared with pregnancies obtained spontaneously.

As studies on this topic, conducted mainly on neonatal populations, are currently limited in Romania, we believe that this study may have a significant impact on clinical practice toward obstetricians and neonatologists on the management of pregnant women and newborns in this risk category.

The conclusions between the international studies may often be different and this may be determined, on one hand, by the study design. On the other hand, the confounding factors may play a role, influencing the conclusions because of the lack of adjustment of the final results according to their presence. Individual factors such as maternal age, smoking status, body mass index, parity, cause of infertility, pre-existing pathologies and their treatment must be considered. Couples suffering from infertility should receive counseling before choosing for one of the assisted reproduction procedures, and the treatment and stages of the procedure should be adjusted on a case-by-case basis, according to national statistics for that patient population.

The statistical analysis in our study revealed that pregnant women in the IVF group, compared with those in the spontaneous conception group, have a higher risk of developing pregnancy induced-hypertension and placental abnormalities. These results correspond to those described in the literature. It also increases the risk of gestational diabetes when nuliparity, maternal age> 35 years and the use of IVF for pregnancy are simultaneously present.

Regarding the cohort of newborns, we have noticed that half of the number of IVF pregnancies were multiple pregnancies, which may explain the increased incidence of prematurity in the study group. Analyzing the distribution of the number of embryos transferred, we found that women who opt for a pregnancy through assisted reproduction tended to transfer at least two embryos, which explains the number of multiple pregnancies. It is important to support the implementation of single embryo transfer protocols in our country, as is already being practiced in several European countries at this time. [17] This is quite important because, although we can consider that it reduces the chance of obtaining a pregnancy in total, a unique pregnancy obtained through IVF is still associated with a better neonatal prognosis than multiple pregnancies.

Neonatal complications reported in the IVF group can be determined, largely by premature birth, in association with hypertensive and placental pathology in pregnancy.
Thus, we demonstrated that newborns in the IVF group are rather premature and require a more frequent hospitalization in the NICU for respiratory symptoms (respiratory distress syndrome, apnea of prematurity), hemodynamic manifestations such as arterial hypotension, but also metabolic imbalances such as variations in serum blood glucose concentration, with a predominance of neonatal hypoglycemia. The presence of these manifestations justifies the more frequent use of respiratory support (oxygen therapy, non-invasive ventilation), hemodynamic support with positive inotropic medication, metabolic support with hydro-electrolytic rebalancing infusion and infectious control with antibiotic therapy.

A restricted analysis of the type of oocyte used and the associated risk of neonatal complications showed that frozen oocytes increase the risk of adverse clinical outcome in neonates. This is not in line with international studies and in order to demonstrate causality, further studies and multivariate analysis are needed.

For the three confounding factors - independent predictive variables - present in our group of patients, we performed a multivariable analysis and demonstrated that their simultaneous presence increases the risk of maternal and neonatal complications.
Thus, nuliparity, advanced maternal age and IVF use increase the risk of pregnancy induced-hypertension, placental abnormalities and gestational diabetes, and in newborns, it increases the risk of prematurity and its associated complications, as well as more frequent use of specific treatment for their management.

The limitations of the study are represented by the partial retrospective nature of the study, which limited the possibility to obtain data that could have influenced the final results of the study, but which also led to the exclusion of patients whose data on the analyzed variables were incomplete. Also, the cohort of patients included in the study was quite small, although the study was conducted over a period of 4 years. We could not obtain information about individual, additional risk factors from parents, as well as data about the in vitro fertilization procedure used - ovarian stimulation protocol, culture medium, embryonic stage at the time of transfer, previous use of others assisted reproduction methods.

Although the results of our study are largely in line with those published in international studies, we consider it necessary to conduct multicenter, prospective studies on large groups of patients, including data on confounders factors, causes of infertility, population of subfertile women in order to obtain national statistics needed to guide physicians in clinical practice.

Another perspective is to conduct studies that include long-term follow-up of these newborns in order to identify complications or birth defects that could not have been diagnosed in the immediate postnatal period. The results of these studies can help create standardized protocols for monitoring these patients.

In conclusion, we consider that it is difficult to establish a causal relationship between IVF and maternal and neonatal complications, as there was a lack of important information that could have changed the final statistics. There are probably factors that together increase the risk of complications, and assisted reproduction and infertility can have a synergistic effect in this regard.

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List of published articles

1. Articles from doctoral research published in journals listed in the ISI system

1.1 Bănică AM, Popescu SD, Vlădăreanu S. Maternal and neonatal outcomes following *in vitro* fertilization: A cohort study in Romania. *Exp Ther Med*. 2022;23(1):34. doi:10.3892/etm.2021.10956 **Impact factor: 2.447**
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2. Articles from doctoral research published in journals indexed in PubMed

2.1 Bănică AM, Popescu SD, Vlădăreanu S. Obstetric and Perinatal Complications Associated with Assisted Reproductive Techniques - Review. *Maedica (Bucur)*. 2021;16(3):493-498. doi:10.26574/maedica.2020.16.3.493
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