„CAROL DAVILA''UNIVERSITY OF MEDICINE AND PHARMACY BUCHAREST

DOCTORAL SCHOOL

FIELD OF MEDICINE

DOCTORAL THESIS

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EVALUATION OF NEWBORNS CONCEIVED THROUGH ASSISTED HUMAN REPRODUCTION TECHNIQUES BASED ON THE APPLIED PROTOCOL

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List of Published Scientific Papers:

1. Tocariu R, Stan D, Mitroi RF, Căldăraru DE, Dinulescu A, Dobre CE, et al. Incidence of complications among in vitro fertilization pregnancies. J Med Life. 2023 Mar;16(3):399–405. https://pmc.ncbi.nlm.nih.gov/articles/PMC10165528/

2. Tocariu R, Niculae LE, Niculae A Ștefan, Carp-Velișcu A, Brătilă E. Fresh versus Frozen Embryo Transfer in In Vitro Fertilization/Intracytoplasmic Sperm Injection Cycles: A Systematic Review and Meta-Analysis of Neonatal Outcomes. Medicina (Mex). 2024 Aug;60(8):1373. https://www.mdpi.com/1648-9144/60/8/1373

3. Tocariu R, Dinulescu A, Prejmereanu A, Maier C, Coricovac AM, Archir ED, et al. Risk Factors for Prematurity and Congenital Malformations in Assisted Reproductive Technology Pregnancies—A Retrospective Study. J Clin Med. 2024 Jan;13(21):6470. https://www.mdpi.com/2077-0383/13/21/6470

4. Niculae LE, Tocariu R, Archir E-D, Niculae A-Ș, Coricovac A-M, Comandașu D-E, et al. Neonatal Health Following IVF: Own Versus Donor Material in Singleton and Multiple Pregnancies. Life. 2025 Apr 1;15(4):578. https://www.mdpi.com/2075-1729/15/4/578

Introduction

Infertility is a real issue in today's society, with a continuous increase in prevalence, making in vitro fertilization (IVF) a life-saving solution for this condition.

In the United States, 12.7% of women of reproductive age suffer from infertility.(1) In 85% of cases, the underlying pathology is represented by ovulatory dysfunction (70% of ovulatory dysfunctions being attributed to polycystic ovary syndrome), tubal dysfunction, or male-factor infertility.(2,3) In 15% of cases, the cause remains unknown, but factors such as obesity, age, or smoking are presumed to be associated with infertility.(1,2)

In the United States, to date, 5 million babies have been born through IVF, and the annual rate of pregnancies achieved through assisted reproduction accounts for 1.6% of all births.(1,4)

Worldwide, approximately 350,000 babies are born each year through assisted reproductive technology (ART).(5)

In vitro fertilization can be combined with intracytoplasmic sperm injection (ICSI), a fertilization technique in which a single sperm is directly injected into the cytoplasm of the oocyte. This technique, developed in the 1990s, is currently used in approximately 93% of cases of male infertility.(2)

Embryos resulting from IVF or ICSI can be transferred into the uterus during the same stimulation cycle (fresh embryos) or cryopreserved and transferred later after thawing (frozen/thawed embryo transfer) when the patient's hormone levels are closer to physiological conditions.(2,4)

Although most babies conceived through assisted reproductive technology are healthy, some studies indicate an increased risk of obstetric and perinatal complications, such as pregnancy-induced hypertension, preterm birth, low birth weight, congenital anomalies, and neurodevelopmental disorders (cognitive and language disorders, cerebral palsy, autism).(6)

It is believed that these adverse effects may be attributed to the higher incidence of multiple pregnancies associated with ART. However, in recent years, the practice of implanting a single embryo has been increasingly adopted, leading to a significant reduction in twin pregnancies resulting from IVF.(3)

I. Backround

1. Medically Assisted Human Reproduction Techniques

1.1 Indications for Medically Assisted Human Reproduction

The most common indication for medically assisted reproduction (MAR) is infertility. According to the definition of the World Health Organization (WHO), infertility is the inability to achieve a pregnancy after 12 or more months of unprotected sexual intercourse. Infertility can be caused by male factors, female factors, or conditions affecting both partners. However, in approximately 15% of cases, the etiology of infertility remains unexplained.(7,8)

The causes of male infertility include infections, trauma, hormonal dysfunctions, medications, oncological diseases, exposure to toxic substances, or lifestyle-related factors.

Male infertility is classified based on its cause into pre-testicular, testicular, and post-testicular infertility.

Female infertility is most commonly caused by ovulation disorders but can also result from uterine or fallopian tube conditions, endocrine dysfunctions, infections, oncological diseases, or surgical interventions.

1.2 Complications of Medically Assisted Reproduction (MAR)

Medically assisted reproduction (MAR) is associated with several complications, affecting both the mother and the fetus.

Most complications arise from multiple pregnancies, which has led to the encouragement of single embryo transfer (SET).(9,10) Multiple pregnancy is one of the most significant risk factors for complications associated with MAR, most often occurring as a result of transferring multiple embryos.

One of the strategies to reduce the high incidence of multiple pregnancies in IVF has been to minimize the number of embryos transferred.

Over time, attempts were made to transfer two embryos, however, the rate of twin pregnancies remained high, accounting for approximately 30% of all pregnancies. In 1999, guidelines recommended transferring no more than two high-quality embryos per IVF cycle, with the remaining embryos being cryopreserved.

However, elective single embryo transfer (eSET) has proven to be the only effective method to reduce the incidence of multiple pregnancies.

Initially, this technique yielded unsatisfactory results, with pregnancy rates not exceeding 10% per embryo transfer. However, over time, outcomes have significantly improved, reaching success rates comparable to those initially achieved with the transfer of two embryos.(11,12)

1.2.1 Prematurity

Preterm birth can occur spontaneously or be medically indicated for specific reasons. In singleton pregnancies conceived through IVF with or without ICSI, medical indications for induced preterm birth include pregnancy-induced hypertension, intrauterine growth restriction, antepartum hemorrhage, congenital anomalies, or extreme maternal anxiety.(13)

Regarding the incidence of iatrogenic preterm birth, studies have identified a 40% higher risk in pregnancies conceived through conventional IVF compared to those conceived via ICSI.(14)

IVF with or without ICSI is associated with an increased risk of preterm birth compared to naturally conceived pregnancies. Singleton pregnancies conceived through IVF/ICSI have up to an 80% higher risk of prematurity compared to those conceived spontaneously.(13)

Risk factors for preterm birth in IVF pregnancies include advanced maternal age (over 39 years) or very young maternal age (under 25 years), multiple pregnancies, gestational hypertension, placenta previa, and embryo reduction.

However, most risk factors are not specific to pregnancies achieved through MAR, as they also represent general risk factors for preterm birth in the overall population.(15)

The primary cause of prematurity associated with MAR is multiple gestation. However, even singleton pregnancies conceived through IVF with or without ICSI carry a higher risk of prematurity compared to naturally conceived singleton pregnancies.(16)

1.2.2 Low Birth Weight

IVF is a risk factor for low birth weight through several mechanisms.

Ovarian stimulation in IVF has been associated with a higher risk of low birth weight compared to spontaneous conception, with possible causes including poorer oocyte quality and reduced endometrial receptivity. Additionally, the in vitro culture of embryos may contribute to low birth weight.

Birth weight is also influenced by the type of embryo transferred, whether fresh or frozen. Some authors consider that underlying infertility and its causes play a more significant role in determining adverse perinatal outcomes, including low birth weight.(17)

1.2.3 Retinopathy of Prematurity

Retinopathy of prematurity (ROP) is a vasoproliferative vitreoretinal disease that primarily affects premature infants with a gestational age of less than 28 weeks.

The incidence of ROP decreases with increasing gestational age and birth weight. Other risk factors for ROP include oxygen therapy, surfactant administration, chorioamnionitis, sepsis, and neonatal hypotension.(18–20)

Given that some complications associated with medically assisted reproduction (MAR) are also risk factors for ROP, studies have been conducted to investigate whether MAR itself is an independent risk factor for ROP.

1.2.4 Respiratory Pathology

Preterm newborns with a gestational age between 34 weeks and 36 weeks and 6 days conceived through IVF have an increased risk of respiratory morbidity, a risk that decreases with advancing gestational age.

These newborns have a higher risk of respiratory distress syndrome, transient tachypnea of the newborn, apnea, pulmonary hypertension, and pneumothorax compared to term newborns.(21)

Additionally, this category of newborns has an increased risk of hospitalization in the Neonatal Intensive Care Unit (NICU). Medically assisted reproduction (MAR) is an independent predictor of the need for respiratory support, including continuous positive airway pressure (CPAP), invasive mechanical ventilation (IMV), and surfactant administration.(22)

1.2.5 Congenital Malformations

Congenital malformations are structural anomalies that develop during intrauterine life and are found in approximately 6% of newborns.(23)

Several studies have evaluated the association between medically assisted reproduction (MAR) and the risk of congenital heart defects, with contradictory results. Some authors have reported findings that confirm this association, identifying a 37% higher risk of congenital heart malformations in newborns conceived through MAR. However, no significant differences were observed in the incidence of these malformations between pregnancies achieved through conventional IVF and ICSI.(24)

2. Types of Genetic Material and Techniques Used in Medically Assisted Reproduction (MAR)

2.1 Comparison Between Donated and Own Genetic Material

MAR using donated oocytes is becoming increasingly common and represents a way to overcome barriers related to ovarian reserve depletion, age-related fertility decline, or genetic disorders.(25)

Singleton pregnancies achieved with donated oocytes are associated with a higher risk of perinatal complications compared to those achieved through IVF using the patient's own genetic material.

Two recent meta-analyses, from 2017 and 2019, highlighted higher rates of prematurity and low birth weight (<2500g) in newborns conceived with donated oocytes compared to those conceived through IVF with autologous oocytes.(26,27)

Most studies also report a higher incidence of adverse perinatal outcomes in multiple pregnancies resulting from donated oocytes compared to multiple pregnancies achieved with the patient's own material. Regarding congenital malformations, there are few studies comparing their incidence in pregnancies achieved through IVF with donated versus own oocytes, and the results remain contradictory.(28)

2.2 Comparison Between Fresh and Frozen Embryos

With the introduction of controlled ovarian stimulation, the cryopreservation of obtained embryos has become a way to maximize the potential of an IVF cycle, especially in the context of single embryo transfer.

Initially, frozen embryos were preserved through a slow freezing process, but with the introduction of embryo vitrification, superior results were achieved in terms of post-implantation survival.(29)

Currently, there is a growing trend toward the use of cryopreserved embryos. The indications for cryopreservation are not limited to storing surplus embryos that are not used in fresh embryo transfer cycles.

The "freeze-all" strategy involves the cryopreservation of all embryos and their transfer in a later cycle, separate from ovarian stimulation. This approach has several indications, including the prevention of ovarian hyperstimulation syndrome, the use of gonadotropin-releasing hormone (GnRH) agonists for ovulation induction, poor ovarian response, elevated progesterone levels at the end of ovarian stimulation, endometriosis, and preimplantation genetic testing.(30)

Additionally, high levels of estrogen and progesterone associated with ovarian stimulation may have a negative impact on the endometrium and the implanted embryo, effects that can be mitigated by adopting the "freeze-all" strategy.(31)

II. Special Section – Personal Contributions

3. Working Hypothesis and General Objectives

In vitro fertilization (IVF) represents a significant advancement in modern reproductive medicine, offering a solution for numerous couples facing infertility. The introduction of this technology has fundamentally changed the paradigms of human conception, enabling the overcoming of barriers related to reproductive disorders, genetic factors, or advanced maternal age.

Based on these general considerations and the specialized literature, the present study aims to achieve the following objectives:

- **Highlighting a relationship** between the ART method used, depending on the pathology leading to infertility, and a favorable perinatal and neonatal prognosis.
- **Determining the prevalence** of complications or various pathologies in newborns conceived through ART.
- **Establishing a causal relationship** between the ART method used and the newborns' prognosis.
- **Since ART pregnancies are closely monitored and investigated more thoroughly than spontaneous pregnancies** due to the increased compliance of this patient category, identifying and addressing various antenatal pathologies is significantly easier. This results in a favorable postnatal evolution of the newborn, ultimately aiming to optimize pre- and postnatal care.
- **Identifying factors that may lead to complications and an unfavorable prognosis** for newborns, independent of ART.

Based on the considerations described above, I will begin with a descriptive analysis of the studied cohort. Additionally, I will determine the incidence of pregnancy-induced hypertension and gestational diabetes in the studied group, followed by a comparison between the fresh versus frozen embryo groups and the donated versus own genetic material groups.

The final study aims to evaluate the neurological development of preterm infants conceived through IVF.

4. General Research Methodology

This study comprises five separate studies. The first four are retrospective observational descriptive studies conducted over a seven-year period, while the fifth study is a prospective study spanning two years, focusing on the neurological development of preterm newborns conceived through IVF.

For the retrospective studies, we selected patients who underwent an ART procedure at SCOG Panait Sîrbu Bucharest and Gynera Clinic between January 2017 and June 2024, along with their newborns admitted to the Neonatology Departments of SCOG Panait Sîrbu-Bucharest, Euromaterna-Constanța, and Sanador-Bucharest.

Data collection was performed using the electronic medical record systems of each healthcare unit, ensuring confidentiality and obtaining approval from the Ethics Committee of each institution. The analysis included patients who achieved singleton or multiple pregnancies through IVF or ICSI and their newborns.

Selected Data Variables:

- Infertility pathology (maternal, paternal, or mixed)
- Maternal age
- Type of embryo (fresh or frozen)
- Fertilization method (conventional IVF or intracytoplasmic sperm injection (ICSI))
- Timing of embryo transfer (Day 3 or Day 5)
- Occurrence of obstetric complications (preeclampsia and gestational diabetes)
- Type of cycle used for the procedure (natural or artificial)
- Type of genetic material used (own or donated)
- Gestational age
- Apgar score at 1 and 5 minutes
- Birth weight
- Need for mechanical ventilation
- Presence of congenital or genetic abnormalities in the newborn
- Length of neonatal hospitalization

Patients who underwent ovarian stimulation and those with incomplete data were excluded from the study.

The study cohort consisted of 988 newborns, including 670 from singleton pregnancies and 318 from multiple pregnancies. The data were processed and statistically analyzed using Microsoft Excel and JASP 0.19.2.0.

The fifth study aimed to compare preterm newborns conceived through ART with those conceived naturally at SCOG Panait Sîrbu between June 2022 and December 2024. A total of 378 preterm infants were examined, of whom 86 were conceived through IVF.

WHO Definitions Used in the Study:

- Preterm newborn: An infant born before 37 weeks of gestation.
- Congenital malformations: Structural or functional abnormalities that occur during intrauterine life, also referred to as birth defects, congenital anomalies, or congenital malformations. These conditions develop prenatallyand may be identified before birth, at birth, or later in life.
- Birth asphyxia: Defined as the inability to initiate breathing at birth, quantified by an Apgar score ≤7.
- Small for gestational age (SGA): Birth weight below the 10th percentile for gestational age.
- Large for gestational age (LGA): Birth weight above the 90th percentile for gestational age.

5. Descriptive Analysis of the Studied Newborn Cohort

The study includes **988 newborns** conceived through assisted reproductive technology (ART), of which **670 were from singleton pregnancies** and **318 from multiple pregnancies**. This classification was maintained across all four retrospective studies to ensure the statistical relevance of the results.

Singleton Pregnancies

- The **median gestational age** was **38 weeks**, with most births occurring at term.
- The **median Apgar score** was **9 at 1 minute** and **8 at 5 minutes**, indicating generally good neonatal health.
- **Ventilation was rarely needed**, with a **median duration of 0 days**; however, one extreme case required **56 days of ventilation**.
- The **median hospitalization duration** was **3 days**, but significant variations were observed, reaching up to **100 days**.

Multiple Pregnancies

- The **median gestational age** was **35 weeks**, reflecting a **higher tendency for preterm births**.
- The **Apgar scores** were lower than those in singleton pregnancies (**8 at 1 minute** and **7 at 5 minutes**), suggesting **greater neonatal adaptation challenges**.

- The **median hospitalization duration** was **9 days**, while the **mean hospitalization duration was 17.874 days**, indicating the presence of severe cases requiring prolonged care.

Correlations in Singleton Pregnancies

- **Gestational age** showed a **positive correlation** with **birth weight** ($\tau = 0.403$, $p < 0.001$) and **Apgar scores** ($\tau = 0.250$ and $\tau = 0.288$, $p < 0.001$).
- **Gestational age** was **negatively correlated** with **ventilation duration** ($\tau = -0.308$, $p < 0.001$) and **hospitalization duration** ($\tau = -0.351$, $p < 0.001$), indicating that **prematurity negatively affects neonatal outcomes**.
- **Birth weight** was strongly correlated with **Apgar scores** and negatively correlated with **the need for ventilation**and **hospitalization duration**, emphasizing the importance of **intrauterine development** for neonatal prognosis.
- **Maternal age** had weak but significant correlations with **Apgar scores**, suggesting a **minor impact on neonatal outcomes**.

Correlations in Multiple Pregnancies

- **Gestational age** had a **strong positive correlation** with **birth weight** ($\tau = 0.731$, $p < 0.001$) and **Apgar scores** ($\tau = 0.623$ and $\tau = 0.588$, $p < 0.001$), confirming **the impact of prematurity on neonatal health**.
- **Gestational age** was **negatively correlated** with **ventilation duration** ($\tau = -0.548$, $p < 0.001$) and **hospitalization duration** ($\tau = -0.701$, $p < 0.001$).
- **Apgar scores at 1 and 5 minutes** were **strongly correlated** ($\tau = 0.875$, $p < 0.001$) and showed **negative correlations** with **ventilation duration** and **hospitalization duration**, confirming that **newborns with higher Apgar scores tend to have better neonatal outcomes**

Discussions

From this first part of the doctoral study, the observed results suggest that multiple pregnancies conceived through IVF are more frequently associated with complications compared to singleton pregnancies. These findings are consistent with the specialized literature, where the topic has been widely debated due to the historical practice of transferring multiple embryos to increase pregnancy success rates.

Historically, this practice led to high rates of twin and multiple pregnancies. For example, in the mid-1990s, more than 30% of IVF cycles resulted in twin or multifetal pregnancies.(32) However, recent advancements and changes in clinical practice have significantly reduced these rates. The introduction of single embryo transfer (SET) as a standard practice, along with improved embryo culture techniques, preimplantation genetic testing, and better cryopreservation methods, has contributed to this reduction. By 2019, the twin pregnancy rate had decreased to less than 7% of IVF cycles.(32)

The cumulative probability of achieving a live birth from a multiple pregnancy after IVF varies depending on the number of cycles and maternal age. After three IVF cycles, the cumulative live birth rate for twins was 14.5%, and for triplets, 0.7%. After six cycles, these rates increased to 19.8% for twins and 1.3% for triplets.(33) The Society for Maternal-Fetal Medicine recommends careful management of pregnancies resulting from IVF, including genetic counseling, detailed obstetric ultrasound examinations, and consideration of multifetal pregnancy reduction when necessary.(34)

The risk of preterm birth is significantly higher in multiple pregnancies compared to singleton pregnancies achieved through IVF. Numerous studies have demonstrated this increased risk. For instance, a cohort study conducted in Denmark found that twins conceived through IVF/ICSI had a 10-fold higher risk of being born before 37 weeks and a 7.4-fold higher risk of being born before 32 weeks compared to singleton pregnancies.(35)

Another study comparing twin pregnancies conceived through IVF with two separate singleton pregnancies from IVF showed that preterm birth rates were dramatically higher in twins, with adjusted odds ratios ranging between 4 and 16.(36)

Additionally, a meta-analysis reported that the prevalence of preterm birth in multiple pregnancies conceived through IVF/ICSI was 51.5%, significantly higher compared to singleton pregnancies.(37)

6. Incidence of Gestational Diabetes and Pregnancy-Induced Hypertension in In Vitro Fertilization

6.1 Introduction

The specialized literature supports the hypothesis that pregnancies conceived through IVF have an increased risk of developing gestational diabetes (GDM) compared to those conceived naturally.

For example, a study conducted by Cai and colleagues found that IVF pregnancies had nearly twice the likelihood of developing GDM (OR = 1.83, 95% CI: 1.03–3.26) after adjusting for recognized risk factors for GDM.[1] This risk was particularly pronounced in overweight or obese women (BMI > 25 kg/m²).(38)

There are studies that have demonstrated that pregnancies conceived through IVF have an increased risk of developing hypertensive disorders of pregnancy, including gestational hypertension and preeclampsia, compared to spontaneously conceived pregnancies.

6.2 Patients and Method

This retrospective study included 779 patients who underwent IVF and developed gestational diabetes and/or pregnancy-induced hypertension during pregnancy, between January 2017 and June 2024, at SCOG Panait Sîrbu and Gynera Clinic.

Data confidentiality was maintained, and the study was conducted with the approval of the Ethics Committees of each institution. Data collection was performed using Microsoft Excel, and statistical analysis was conducted using JASP 0.19.2.0.

To ensure accuracy, the study cohort was divided into two main groups:

1. Singleton pregnancies
2. Multiple pregnancies

Each group was further divided into four subgroups:

- Singleton pregnancies with own genetic material vs. singleton pregnancies with donated genetic material
- Singleton pregnancies with fresh embryos vs. singleton pregnancies with frozen embryos
- Multiple pregnancies with own genetic material vs. multiple pregnancies with donated genetic material
- Multiple pregnancies with fresh embryos vs. multiple pregnancies with frozen embryos

Patients were excluded from the study if:

- The necessary data could not be retrieved
- They had pre-existing type 1 or type 2 diabetes
- They had a history of essential hypertension before pregnancy

6.3. Results

Incidence of Gestational Diabetes Mellitus (GDM) in Singleton Pregnancies

- ***Genetic Material*:** In the donor material group, the incidence of GDM was **27% (10/37)**, compared to **13.5% (81/602)** in the own material group. Although the Chi-Square test was significant (**p = 0.022**), the calculated odds ratio (**OR = 2.382, 95% CI: 1.111-5.106**) suggests a **weak and uncertain association**.
- ***Embryo Type*:** The GDM rate was **9.2% for fresh embryos** and **15.7% for frozen embryos**. The Chi-Square test (**p = 0.053**) and Odds Ratio analysis (**OR = 0.547, 95% CI: 0.294-1.016**) **do not confirm a statistically significant link** between embryo type and GDM incidence.

Incidence of Gestational Diabetes Mellitus (GDM) in Multiple Pregnancies

- ***Genetic Material*:** No significant difference was identified between the donor material group (**7.4%**) and the own material group (**12.4%**), with a Chi-Square test result of **p = 0.465**.

- ***Embryo Type*:** The GDM incidence was **6.7% in the fresh embryo group** and **12.7% in the frozen embryo group**. However, statistical analysis (**p = 0.355**) did not reveal a significant difference.

Incidence of Pregnancy-Induced Hypertension (PIH) in Singleton Pregnancies

- ***Genetic Material*:** The donor material group had a **significantly higher risk** of PIH (**37.8%**) compared to the own material group (**9.9%**). The Chi-Square test (**p < 0.001**) and odds ratio of **5.499 (95% CI: 2.687-11.250)** confirm a **clear association between donor material use and PIH incidence**.

- ***Embryo Type*:** No significant difference was observed between the fresh embryo group (**11.3%**) and the frozen embryo group (**11.6%**), **p = 0.922**.

Incidence of Pregnancy-Induced Hypertension (PIH) in Multiple Pregnancies

- ***Genetic Material*:** Patients with donor material had a **significantly higher** PIH incidence (**25.9%**) compared to the own material group (**9.7%**), **p = 0.024**.

- ***Embryo Type*:** No significant difference was identified between fresh and frozen embryos regarding PIH risk (**p = 0.598**).

6.4. Discussions

Although literature data and the preliminary analysis of this doctoral study indicate an increased incidence of gestational diabetes (GDM) and pregnancy-induced hypertension (PIH) in IVF pregnancies, the statistical analysis conducted on this cohort provides a new perspective on this pathology.(39)

The lack of a definitive association between GDM and the use of donated genetic material or embryo type may be explained by the mean age of the study sample, which is approximately 35 years. Studies have shown that advanced maternal age (over 35 years) is a significant risk factor for GDM in IVF pregnancies. For instance, Moaddab et al.found that women aged 40–44, 45–49, and ≥50 years had increased risks of GDM, with adjusted relative risks (aRR) of 1.23, 1.40, and 1.31, respectively, compared to women under 40 years.(40)

Additionally, Wang et al. reported that a higher proportion of mothers aged ≥40 years use assisted reproductive technologies (ART), and among them, there is a higher prevalence of GDM.(41)

Furthermore, Coussa et al. Identified advanced maternal age as a significant predictor of GDM in IVF pregnancies, with these patients exhibiting higher levels of glycated hemoglobin (HbA1C) and insulin resistance.(42)

The risk of developing GDM in IVF pregnancies in patients with increased BMI is already well known.(38) However, this information could not be obtained for the current study, representing a significant limitation of the research.

On the other hand, the clear association between PIH and IVF pregnancies using donated genetic material at younger maternal ages is a well-documented reality in the specialized literature.

Keegan et al. found that the highest PIH rate occurred in young oocyte donation recipients under 35 years (42%) compared to those over 40 years (26%).(43)

Additionally, Letur et al. demonstrated that the risk of PIH increases with the use of donated oocytes, and this risk is further influenced by maternal age.

Their study showed that PIH was more frequent in pregnancies with donated oocytes (17.8%) compared to autologous IVF pregnancies (5.3%), and the risk increased with maternal age (OR 1.08 per year).(44)

These findings were further supported by Levron et al., who showed that women who conceived using donated oocytes had a significantly higher rate of hypertensive disorders of pregnancy, including PIH, compared to those who used autologous oocytes.

Even after adjusting for maternal age, this increased risk is thought to be due to immunological factors, as the fetus represents a completely allogeneic entity for the mother, leading to defective immunological adaptation.(45)

Another important aspect to mention is the predominance of donated oocyte use in the studied cohort. Literature highlights that the use of donated sperm does not appear to significantly increase the risk of hypertensive disorders of pregnancy. Kennedy et al. found no increased risk of hypertensive disorders in pregnancies conceived with donated sperm compared to those conceived with autologous gametes (adjusted odds ratio [aOR] 0.94; 95% CI 0.73–1.21).(46)

6.5. Conclusions

Although cases of gestational diabetes (GDM) were observed in the studied cohort, no strong statistical correlationcould be established between its incidence and the type of genetic material or embryo type used.

This finding may be attributed to the mean age of the study group (approximately 35 years) or the small sample size of the subgroups. Additionally, the lack of BMI data for the included patients represents a limitation that should be considered.

Regarding pregnancy-induced hypertension (PIH), the risk of occurrence is clearly associated with IVF pregnancies using donated genetic material, in both singleton and multiple pregnancies.

These findings align with existing literature, where studies conducted on much larger cohorts have reported similar correlations.

No association could be established between embryo type (fresh vs. frozen) and the incidence of PIH.

7. Neonatal Complications Associated with the Type of Transferred Embryo

7.1. Introduction

Since the birth of Louise Brown in 1978, more than 10 million children have been born worldwide through conventional in vitro fertilization (IVF) or intracytoplasmic sperm injection (ICSI).(47-49)

For a long time, the standard IVF procedure involved the fresh transfer of embryos immediately after ovarian stimulation.

Over time, with the advancement of assisted reproductive technology (ART) techniques, fresh embryo transfers have increasingly been replaced by frozen embryo transfers, a trend that has become more prevalent in recent years.

This shift has contributed to a reduction in ovarian hyperstimulation syndrome (OHSS), a common consequence of fertility medications.(50)

7.2. Patients and Method

This retrospective study, conducted over a 7-year period (January 2017 – June 2024), included 988 newborns conceived through IVF or ICSI, of whom 670 were from singleton pregnancies and 318 from multiple pregnancies.

Data were retrieved from the electronic medical records of the following healthcare institutions: SCOG Panait Sîrbu – Bucharest, Sanador – Bucharest, Euromaterna – Constanța.

Confidentiality was maintained, and the study was conducted with the approval of the Ethics Committees of each institution. Data collection was performed using Microsoft Excel, and statistical analysis was conducted using JASP 0.19.2.0.

The objective of this study was to determine the relationship between embryo type (fresh or frozen) and various neonatal risks, including:

- Preterm birth risk
- Occurrence of congenital malformations
- Birth asphyxia
- Small for gestational age (SGA)
- Macrosomia
- Need for invasive/non-invasive ventilation

The cohort was divided into two groups:

1. Singleton pregnancies
2. Multiple pregnancies

Additionally, neonatal outcomes were compared based on the ART cycle type (natural or artificial) and embryo type (fresh or frozen).

7.3. Results

Distribution of Cases in Singleton Pregnancies

The analysis of newborn distribution from singleton pregnancies based on embryo type (**fresh vs. frozen**) highlighted the following aspects:

- **Gestational Age:** The distribution is similar for both groups, with a slight advantage for **frozen embryos**, which tend to result in births at later gestational ages.
- **Birth Weight:** Values are comparable between groups, but **fresh embryos** show greater variability, including more extreme cases.
- **Apgar Score at 1 and 5 Minutes:** Most newborns achieve **high scores (8-10)**, with no significant differences between groups.
- **Mechanical Ventilation:** Most newborns do not require ventilation, but **a few isolated cases in the frozen embryo group** required prolonged ventilation.

- **Hospitalization Duration:** The majority of newborns have a short hospital stay, but the **frozen embryo group includes cases with prolonged hospitalizations** (over 50 days), although confidence intervals suggest a comparable distribution between groups.

Distribution of Cases in Multiple Pregnancies

Newborns from multiple pregnancies exhibited specific characteristics based on the embryo type used:

- **Birth Weight:** Most newborns weighed between **2000 and 3000 g**, with a slightly broader distribution for **frozen embryos**.
- **Gestational Age:** Multiple pregnancies are associated with **lower gestational age**. Newborns from **frozen embryos tended to be born at 36 weeks**, compared to those from fresh embryos.
- **Apgar Score:** Newborns from **frozen embryos had higher Apgar scores at 1 and 5 minutes**, suggesting **better neonatal adaptation**.
- **Mechanical Ventilation:** Most newborns did not require ventilation, but **a few isolated cases in the frozen embryo group required prolonged ventilation** (>30 days).
- **Hospitalization Duration:** The distribution is similar between groups, with **no significant differences in average hospitalization duration**.

Neonatal Complications in Singleton Pregnancies

Preterm Birth Incidence

- The **preterm birth rate** was **22% in the fresh embryo group** and **19% in the frozen embryo group**.
- The **Chi-Square test did not indicate significant statistical differences (p = 0.341)**, but the **Odds Ratio (OR = 1.647, 95% CI: 0.985-2.754)** suggests a **higher likelihood of preterm birth in fresh embryos**.

Congenital Malformations Incidence

- Malformations were **more frequent in the fresh embryo group (16%)** than in the frozen embryo group (10%).
- The **Chi-Square test showed marginal significance (p = 0.055)**, and the **Odds Ratio (1.645)** suggests an **increased risk**, although without clear confirmation of an association.

Birth Asphyxia Incidence

- The **asphyxia rate** was **10% for fresh embryos** and **8.4% for frozen embryos**.
- No statistically significant differences were observed between groups (**p = 0.522**).

Small for Gestational Age (SGA) Incidence

• The **SGA rate** was **13% for fresh embryos** and **11.5% for frozen embryos**.

• The **Chi-Square test did not indicate a significant association (p = 0.573)**.

Large for Gestational Age (LGA) Incidence

• **Macrosomia** was more frequent in **frozen embryos (6.2%)** compared to **fresh embryos (1.8%)**.

• Although the **Chi-Square test suggested a difference (p = 0.030)**, the **Odds Ratio analysis and Fisher's exact test did not confirm a clear association**.

Mechanical Ventilation in Singleton Pregnancies

• **Mechanical ventilation** was required for **6 newborns from the fresh embryo group** and **27 newborns from the frozen embryo group**.

• **No significant statistical differences were recorded between groups (p = 0.442)**.

Neonatal Complications in Multiple Pregnancies

Preterm Birth Incidence

• The **preterm birth rate** was **70% for fresh embryos** and **72% for frozen embryos**.

• **No statistically significant differences were identified between groups (p = 0.764)**.

Congenital Malformations Incidence

• **Congenital malformations were significantly more frequent in fresh embryos (28%) compared to frozen embryos (14%)**.

• The **Chi-Square test (p = 0.004)** and **Odds Ratio (OR = 2.398)** confirm a **higher risk for fresh embryos**.

Birth Asphyxia Incidence

• The **asphyxia rate** was **42% for fresh embryos** and **37% for frozen embryos**.

• **No statistically significant differences were observed between groups (p = 0.427)**.

Small for Gestational Age (SGA) Incidence

• The **SGA rate** was **67% in the fresh embryo group** and **60% in the frozen embryo group**.

• **No statistically significant difference was identified (p = 0.267)**.

Large for Gestational Age (LGA) Incidence

• **No cases of macrosomia** were reported in the fresh embryo group, while **1.7% of newborns from frozen embryos had macrosomia**.

• **No statistically significant association was found between embryo type and macrosomia (p = 0.235)**.

Mechanical Ventilation in Multiple Pregnancies

• **Mechanical ventilation** was required for **25 newborns from fresh embryos** and **52 newborns from frozen embryos**.

• **No statistically significant difference was identified between groups (p = 0.124)**.

7.4. Discussions

Regarding the risk of preterm birth in the singleton pregnancy group, the analysis indicates a slightly higher rate of preterm births in fresh embryo transfers (22%) compared to frozen embryo transfers (19%).

Although the Chi-square test did not show a statistically significant difference (p=0.341), the Odds Ratio (OR) analysis revealed a 64.7% higher risk associated with fresh embryo transfers (OR=1.647; 95% CI: 0.985–2.754).

The marginal significance identified through Fisher's exact test (p=0.041) suggests the need for further investigation.

These results may indicate a weak association between fresh embryo transfers and an increased risk of prematurity, but conclusions should be interpreted cautiously due to the small sample size. However, several large-scale studieshave reported similar findings:

• Westvik-Johari et al. found that infants conceived through fresh embryo transfer had a higher likelihood of preterm birth (OR 1.27, 95% CI: 1.17–1.37) compared to their naturally conceived siblings, whereas the risk was lower for frozen embryo transfers (OR 1.18, 95% CI: 1.00–1.41).(51)

• Maheshwari et al. reported that singleton pregnancies from fresh embryo transfers had a relatively higher risk of preterm birth (RR 0.90, 95% CI: 0.84–0.97) compared to those from frozen embryo transfers.(52)

• Pelkonen et al. observed that the risk of preterm birth was lower in the frozen embryo transfer (FET) group compared to the fresh embryo transfer (ET) group (adjusted OR 0.83, 95% CI: 0.71–0.97).(53)

• Raja et al. found that frozen embryo transfer was associated with a lower risk of preterm birth (adjusted RR 0.93, 95% CI: 0.88–0.97) compared to fresh embryo transfer.(54)

7.5. Conclusions

Conclusion for Singleton Pregnancies

The results indicate marginally significant differences in preterm birth and congenital malformation rates for fresh embryo transfers. However, the evidence is not strong enough to establish definitive causal relationships. Further studies are needed to explore the underlying biological mechanisms.

Conclusion for Multiple Pregnancies

The higher rate of congenital malformations associated with fresh embryos supports the use of frozen embryo transfer protocols to reduce risks. However, other clinical variables, such as prematurity or birth weight, do not appear to be significantly influenced by the type of embryo used.

8. Neonatal Complications Associated with the Type of Genetic Material Used

8.1 Introduction

The first pregnancy achieved using donated oocytes was reported in 1983, marking a significant milestone in assisted reproductive technology (ART).(55,56) This innovation allowed women who were unable to conceive with their own oocytes, due to conditions such as premature ovarian insufficiency, advanced reproductive age, or genetic disorders, to achieve pregnancy.

Oocyte donation involves retrieving oocytes from a donor, which are then fertilized in vitro with sperm. The resulting embryos are transferred to the recipient's uterus, which has been prepared with hormonal treatments to ensure endometrial receptivity.(57)

Over the years, this process has been refined through advancements in ovarian stimulation protocols, oocyte cryopreservation, and preimplantation genetic testing (PGT).(56)

The use of donated oocytes has increased significantly, with over 50,000 births recorded in the United States by 2018, accounting for more than 10% of all IVF cycles performed annually.(55)

This practice has also provided valuable insights into reproductive biology, including the ability to differentiate the effects of oocytes from those of the uterine environment on pregnancy outcomes.(55,57)

In vitro fertilization (IVF) using donated sperm began in the mid-1980s. The first successful IVF pregnancy with donated sperm was reported in 1986. Over the following five years, 303 treatment cycles were performed for 185 couples, with 33% achieving a successful pregnancy, including those who underwent subsequent frozen embryo transfers.(58)

8.2 Patients and Method

This retrospective study, conducted over a 7-year period (January 2017 – June 2024), included 988 newborns conceived through IVF or ICSI, of whom 670 were from singleton pregnancies and 318 from multiple pregnancies.

Data were retrieved from the electronic medical records of the following healthcare institutions: SCOG Panait Sîrbu – Bucharest, Sanador – Bucharest, Euromaterna – Constanța.

Confidentiality was maintained, and the study was conducted with the approval of the Ethics Committees of each institution. Data collection was performed using Microsoft Excel, and statistical analysis was conducted using JASP 0.19.2.0.

The objective of this study was to determine the relationship between genetic material type (own or donated) and various neonatal risks, including:

- Preterm birth risk
- Occurrence of congenital malformations
- Birth asphyxia
- Small for gestational age (SGA)
- Macrosomia
- Need for invasive/non-invasive ventilation

The cohort was divided into two groups:

1. Singleton pregnancies
2. Multiple pregnancies

8.3 Results

Distribution of Newborn Cases Based on Neonatal Complications and Type of Genetic Material Used

Singleton Pregnancies

- Gestational Age: Newborns from own embryos have a clear peak between 38-40 weeks, while those from donated embryos show a more dispersed distribution.
- Birth Weight: Newborns from own embryos have weights concentrated between 2500-3500 g, while those from donated embryos show greater variability.

- Apgar Score: Scores at 1 and 5 minutes are high in both groups, but newborns from own embryos have more concentrated scores between 9-10.
- Mechanical Ventilation: Most newborns do not require ventilation, but in the case of donated embryos, the average duration is higher.
- Hospitalization Duration: Newborns from own embryos show greater variation in hospitalization duration, with more prolonged cases compared to donated embryos.

Multiple Pregnancies

- Birth Weight: Newborns from own embryos tend to weigh more (2000-3500 g), while those from donated embryos are more frequently in the 1500-2500 g range.
- Gestational Age: Own embryos tend to be delivered around 36-38 weeks, while donated embryos between 32-35 weeks.
- Apgar Score: Newborns from own embryos have higher Apgar scores compared to those from donated embryos.
- Mechanical Ventilation: Prolonged ventilation cases are more frequent among newborns from donated embryos.
- Hospitalization Duration: Newborns from donated embryos require, on average, more days of hospitalization compared to those from own embryos.

Results Regarding Neonatal Complications

Preterm Birth Incidence in Singleton Pregnancies

- Preterm birth rate: 32% for donated embryos vs. 18% for own embryos.
- Chi-Square test suggests marginal statistical significance ($p = 0.039$), and Odds Ratio indicates a 2.095 times higher risk of preterm birth in the donated embryo group.

Congenital Malformations Incidence in Singleton Pregnancies

- Congenital malformations are more frequent in the donated embryo group (16%) than in the own embryo group (11%), but without clear statistical significance ($p = 0.235$).

Birth Asphyxia Incidence in Singleton Pregnancies

- Birth asphyxia rate: 16% in the donated embryo group vs. 8.4% in the own embryo group.
- No statistically significant difference identified ($p = 0.102$).

Small for Gestational Age (SGA) Incidence in Singleton Pregnancies

- SGA rate: Higher in the donated embryo group (21%) vs. 11.4% in the own embryo group, but without clear statistical significance ($p = 0.062$).

Large for Gestational Age (LGA) Incidence in Singleton Pregnancies

- Macrosomia was more frequent in the own embryo group (5.3%) compared to the donated embryo group (2.7%), but without statistically significant difference ($p = 0.478$).

Mechanical Ventilation in Singleton Pregnancies

- Mechanical ventilation was required for 16.2% of newborns from donated embryos and 4.2% from own embryos.
- A statistically significant association was found between donated embryos and the need for mechanical ventilation ($p = 0.001$).

Results Regarding Neonatal Complications in Multiple Pregnancies

Preterm Birth Incidence in Multiple Pregnancies

- Preterm birth was significantly more frequent in the donated embryo group (91%) compared to the own embryo group (68%).
- Chi-Square test ($p < 0.001$) and Odds Ratio (4.693) confirm a more than fourfold increased risk of preterm birth for donated embryos.

Congenital Malformations Incidence in Multiple Pregnancies

- Malformations were more frequent in the donated embryo group (27.3%) compared to the own embryo group (15.6%).
- The difference is statistically significant ($p = 0.039$), and Odds Ratio indicates a 2.03 times higher risk for donated embryos.

Birth Asphyxia Incidence in Multiple Pregnancies

- Birth asphyxia rate: 63.6% in newborns from donated embryos vs. 33.8% in those from own embryos.
- The difference is statistically significant ($p < 0.001$), with an Odds Ratio of 3.421.

Small for Gestational Age (SGA) Incidence in Multiple Pregnancies

- SGA is more frequent in newborns from donated embryos (85.5%) compared to own embryos (57%).
- The difference is statistically significant ($p < 0.001$), with an Odds Ratio of 4.426.

Large for Gestational Age (LGA) Incidence in Multiple Pregnancies

- No cases of macrosomia were reported in the donated embryo group, compared to 1.5% in the own embryo group.
- No statistically significant difference identified ($p = 0.357$).

Mechanical Ventilation in Multiple Pregnancies

- Mechanical ventilation was required for 23.6% of newborns from donated embryos and 24.3% from own embryos.

• No statistical difference was found between groups (p = 0.912).

Hospitalization Duration

• Newborns from donated embryos required an average of 21.4 days of hospitalization, compared to 17.1 days for those from own embryos.

• The difference is statistically significant (p < 0.001), indicating a higher risk of complications in newborns from donated embryos.

8.4. Discussions

The analysis of singleton pregnancies using donated genetic material revealed a significantly increased risk of preterm birth (OR = 2.095, p = 0.039). Additionally, this category of newborns required mechanical ventilation more frequently compared to the singleton pregnancy group using own genetic material (OR = 4.344, p = 0.001).

The Mann-Whitney U test showed that newborns in the donor group had a significantly higher average number of ventilation and hospitalization days, highlighting the need for closer monitoring of this patient category.

For multiple pregnancies, the use of donated genetic material was associated with a significantly higher likelihood of preterm birth (OR = 4.67, p < 0.001). However, unlike singleton pregnancies, no statistically significant differencewas found regarding the need for mechanical ventilation. This may be explained by differences in pulmonary maturation between multiple and singleton pregnancies, due to fetal stress.

8.5. Conclusions

• The use of donated genetic material doubles the likelihood of preterm birth compared to own genetic material in singleton pregnancies and quadruples the need for mechanical ventilation.

• Consequently, this category of patients requires a longer duration of ventilation and hospitalization.

• In singleton pregnancies, no significant differences were found between the two groups regarding congenital malformations or intrauterine growth restriction (IUGR).

• In multiple pregnancies, the risk of preterm birth is approximately five times higher in the donor groupcompared to the own genetic material group.

• Additionally, the risk of birth asphyxia was approximately three times higher in the donor group.

• The incidence of low birth weight was four times higher in newborns conceived with donated genetic material.

• Unlike singleton pregnancies, in multiple pregnancies, the risk of congenital malformations was twice as highin the donor group.

9. Neurological Follow-up of Preterm Newborns Conceived Through IVF

A total of 86 infants conceived through IVF were evaluated.

At the 3-month assessment, 9 out of 86 infants showed motor development disorders with distal spasticity, which improved with physical therapy programs.

During the follow-up period, 8 infants were diagnosed with global motor development disorders, with some cases also presenting epileptic spasms (West Syndrome). The etiology was primarily genetic or structural, while the remaining 78 children showed normal age-related development up to the present.

At this stage, a definitive correlation between potential neurodevelopmental delays and conception through IVF cannot be established, as the sample size is too small.

10. Final Conclusions and Personal Contributions

10.1. Final Conclusions

This doctoral study allows for the following final conclusions:

• Pregnancy-induced hypertension (PIH) is a complication associated with IVF using donated genetic material, in both singleton and multiple pregnancies.

• No statistical correlation was found between the risk of developing gestational diabetes (GDM) and the type of embryo or genetic material used in IVF.

• Preterm birth is associated with fresh embryo transfer in singleton pregnancies and with donated genetic material in both singleton and multiple pregnancies.

• The need for invasive or non-invasive respiratory support is statistically associated with the use of donated genetic material in singleton pregnancies.

• Birth asphyxia is three times more frequent in newborns conceived with donated genetic material compared to own genetic material in multiple pregnancies.

• Intrauterine growth restriction (IUGR) is more frequently associated with the use of donated genetic material in multiple pregnancies.

• Regarding congenital malformations, the statistically significant risk is higher in multiple pregnancies, regardless of whether fresh embryo transfer or donated genetic material was used. In singleton pregnancies, only a marginal association was observed with fresh embryo transfer.

10.2. Personal Contributions

- Conducted a literature review focusing on fresh vs. frozen embryo transfer, resulting in a meta-analysis that led to a published article. Later, the review was expanded to cover all aspects of IVF, which were included in the general section of the thesis.
- Designed and developed the study models.
- Collected patient and newborn data from electronic medical records, compiled them into Microsoft Excel, and built the database of IVF patients and newborns.
- Performed statistical analysis using JASP 0.19.2.0 and interpreted the results.
- Determined the incidence of pregnancy-induced hypertension (PIH) and gestational diabetes (GDM), with preliminary results published in an article.
- Evaluated neonatal adverse outcomes, including preterm birth, congenital malformations, and the need for ventilatory support, based on embryo type and genetic material, with results published in two articles.
- Compared and aligned the obtained data with existing literature.
- Conducted neurological follow-up of former preterm infants and formulated conclusions regarding those conceived through IVF vs. natural conception.
- Synthesized the final conclusions of the doctoral research in accordance with the initial objectives, providing a valuable reference point for current medical practice.

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