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Patterns of Infertility in Romania and Implantation Deficiecy

THESIS SUMMARY

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Introduction. Topic relevance. Purpose and objectives

Infertility is a widespread worldwide couple condition that has serious medical, economic and demographic consequences. Having a multifactorial determinism, it is often difficult to define what causes difficulties in establishing and applying unitary methods of diagnosis and treatment globally. However, **the causes of infertility seem to follow certain patterns** that depend on the socio-economic development of the country.

Patients who achieve pregnancy through assisted reproductive procedures have an increased risk of obstetric complications compared to pregnant women who achieve pregnancy spontaneously. This fact is due to several factors: they are older, assisted reproduction procedures more often result in multiple pregnancies, the causes of infertility are generative of complications and, last but not least, embryogenesis, implantation and placentation can be affected.

Actively involved in the processes of implantation and placentation, signal molecules that appear already during the implantation window at the level of the endometrium and are also secreted by the blastocyst, βHCG, PAAP-A and PlGF are markers whose early observation can detect pregnancies with abnormalities genetics and those that will be complicated by abortion, preeclampsia, intrauterine growth restriction or premature birth. **Infertility affects not only the occurrence of pregnancy, but also its subsequent evolution, a fact that can be detected early through the analysis of first trimester markers.**

The profile of obstetric complications depends on the method of conception, they are more frequent in patients who have achieved pregnancy following infertility treatments. It is dependent not only on the assisted human reproduction treatment itself but also on the characteristics of the infertile population, especially on the causes of infertility. There should be a correlation between the two patterns of infertility and obstetric complications.

The aim of the thesis is to identify if of the causes of infertility can be identified in the population of infertile patients who attends to the Filantropia hospital and if pregnancies obtained through assisted reproduction techniques have a higher rate of obstetric complications that can be identified and prevented through the first trimester screening, which analyzes signal molecules that appear since the implantation period. The objectives of the thesis are:

1. identifying the pattern of infertility causes in the patient population of the Filantropia hospital

2. analysis of first trimester markers depending on the method of conception

3. comparative analysis of the evolution of tasks depending on the method of conception

1.Infertility. Patterns of Infertility

1.1. Infertility. Overview.

Infertility is a worldwide disease with serious medical psycho-social, demographic and economic consequences. Infertility is a concept that is often difficult to define because it refers to an anatomical anomaly or an impairment of the physiological processes in which the reproductive systems of both partners are involved. The major impact of infertility on the health status of the population, especially in countries under development, led the WHO to consider it a disability, the fifth worldwide [1]. Infertility affects approximately 15% of couples of childbearing age [2,3,4].

Studies have shown that the incidence and prevalence of infertility varies over time and according to sex, age and the degree of socio-economic development of the country. The rates have slightly changed compared to 1990, suggesting that the prevalence of infertility has not changed substantially over the last two decades. The highest rates were identified in Sub-Saharan Africa, the Middle East/North Africa, Central and South-Eastern Europe and Central Asia. Most of these countries are developing, with a higher rate of secondary infertility caused by increased rates of genital infections [11].

1.2. Infertility causes

Although it is considered a couple's disease, the epidemiological data on infertility mainly focus on the female side of it. The prevalence of male infertility is difficult to study, with most data coming from population surveys with interviews addressed to the couple or only to the woman.

The most prevalent cause of female infertility regardless of geographic location is ovulatory dysfunction. Female infertility causes are age dependent. Studies have identified a change of female infertility causes starting at age 35. This is the benchmark age from which an accelerated decline in oocyte quantity and quality has been observed. There is no uniform

distribution of the causes of female infertility worldwide, each population presenting its own characteristics depending on the degree of socioeconomic development.

Male infertility is characterized by alteration of sperm parameters and disorders of sexual dynamics (erectile dysfunction, premature ejaculation, retrograde ejaculation and low frequency of sexual contacts). Sperm parameters show geographic variations that appear to be related to environmental, socio-economic, nutritional factors or, in many cases, remain unknown.

1.3. Patterns of infertility causes worldwide and in Romania

Because there are a multitude of variables to consider, and definitions of infertility causes may differ from study to study, identifying a pattern of infertility causes can become problematic.

The distribution of infertility causes in couples is extremely heterogeneous worldwide, see table I.1. [5,6,7,8,9,10,11,12,13,14]

Table I.1. Infertility causes

Causes	Female Factor	Male factor	Mixt	Idiopathic
Bayagaslan 2004 Mongolia	45,8%	25,26%	18,8%	9,8%
Bablok 2011 Poland	39,28%	55,73%	18,9%	15,99%
Farhi 2011 Israel	30,6%	29,2%	18,05%	20,7%
NICE 2013 Great Britain	55%	30%	40%	25%
Anghelescu 2014 Romania	71,86%	49,85%	18,83%	23,77%
Masoumi 2015 Iran	88,6%	66%	N/A	N/A
Linsday 2015 USA	45-58%	26-30%	40%	25-28%
Sigh Kalpana 2017 India	20,48%	22,46%	8,37%	22,46%

El Hussein 2019 Sudan	42,8%	35,5%	18,4%	3,4%
Zeng 2019 China	N/A	18,8%	N/A	8,6%
van Rode New Zeeland 2015	16%	14%	5%	48%

2. Implantation and placentation deficits and their obstetric echo from the perspective of assisted reproduction technologies

2.1. Implantation and placentation deficits

Infertility also includes implantation and placentation deficits in its definition, the goal of the reproductive process being to obtain a live newborn. The process of human reproduction seems to be dominated by a multitude of inefficient processes with quite significant loss of biological material. That is why human fecundability, the percentage of pregnancies obtained/menstrual cycle is only 20% [16].

Implantation and placentation are considered the key moments that ensure the success of a pregnancy. Many early and late pregnancy complications originate from implantation and placentation defects. Among the early complications, we mention recurrent abortion and abortions produced after 12 weeks of pregnancy. Intrauterine growth restriction, preeclampsia, premature birth are late complications that seem to be explained by the same defects.

2.2. The role of first trimester markers in early detection of high-risk pregnancies

The complex communication between the mother and the product of conception begins as early as the blastocyst stage by means of signal molecules. The dynamics of the secretion of some of these signal molecules is a predictive factor for the occurrence of the aforementioned complications. The best-known marker molecules are human chorionic gonadotropin, placenta-associated plasma protein (PAAP-A) and placental growth factor (PlGF).

Human chorionic gonadotropin (hCG) is a glycoprotein hormone produced by trophoblastic cells from the morula stage. The concentration in the maternal serum as well as the degree of glycosylation of this hormone varies during pregnancy and represent predictive elements for its evolution. Thus, human chorionic gonadotropin (hCG) is considered a first-trimester marker on the basis of which the quality of implantation and trophoblastic invasion

can be assessed, being included in the screening algorithm for aneuploidy, preeclampsia and growth restriction.

PAAP-A is a molecule produced by the placenta from the early stages of its appearance and development, from the stage of implantation. Because its serum level correlates with the occurrence of obstetric complications, PAAP-A analysis is part of risk calculation models for aneuploidy, abortion, intrauterine growth restriction and preeclampsia in the first trimester of pregnancy [84,85].

Placental growth factor (PlGF) is secreted by the placenta, but its presence has been detected in glandular and luminal cells as well as in stromal cells surrounding the spiral arteries as early as the implantation window [88]. PlGF is greatly decreased in pregnancies with preeclampsia, intrauterine growth restriction, trisomies 21, 13 and 18 [91,92].

2.3. First trimester screening. Comparison between spontaneous pregnancies and those achieved through assisted reproductive technologies

The first trimester screening includes the evaluation of several parameters with the establishment of risk models with a predictive role for the detection of chromosomal abnormalities such as trisomies 21, 13 and 18 as well as for the occurrence of obstetric complications such as abortions, premature birth, preeclampsia, intrauterine growth restriction.

The first trimester screening is based on signal molecules involved in the processes of implantation and placentation whose secretion is vital in establishing and continuing the pregnancy. Since infertility may be a consequence of these defects, its effects may also extend to these markers. Consequently, we expect the population of pregnant women with pregnancies achieved through assisted reproduction to have different first trimester marker values.

Genetic anomalies

The meta-analysis by Hunt et al. determined that PAAP-A has lower values in the IVF patient population. Of these, significantly reduced values were recorded in the ICSI subpopulation. The type of embryo transfer, with fresh or frozen embryos did not significantly influence this reduction [21]. The authors tried to find explanations for these particularities by issuing several hypotheses: the differences in dating between the IVF procedure and the analyzers, the super optimal level of estrogen hormones which, although causing the dilation of the arterioles at the interface between the endometrium and the placenta, causes a suboptimal placentation with a low level of PAAP-A, exposure to infertility lasting longer than 2 years. The

meta-analysis establishes that free βHCG values are not changed in IVF/ICSI patients compared to the general population [21]. Because of these abnormalities, the number of false positive cases of Down syndrome has increased, attracting by itself the increase in the number of unnecessary genetic tests, either non-invasive or invasive. In order to correct the errors that have occurred, the profile societies have established that the marker values must be adjusted according to the method of conception and related to the particularities of each population analyzed separately. Thus, in the conditions of a single pregnancy obtained by IVF/ICSI with own oocytes, PAAP-A values must be adjusted. The rest of the markers, namely β-hCG and nuchal translucency remain unchanged [22].

The **miscarriage risk** of patients with pregnancies obtained through assisted reproduction is approximately three times higher compared to the general population [23]. The risk of miscarriage can reach up to 45% around 6 weeks of age, in the first trimester it is a maximum of 7%, and after 20 weeks it drops below 2% [23,24]. The evolution of PAAP-A and free β-hCG have little predictive value for miscarriage.

Globally, pregnancies achieved through assisted reproductive procedures have a threefold higher risk of **preeclampsia** [25,26]. The incidence of preeclampsia in spontaneous pregnancies reaches 2%, while in IVF/ICSI pregnancies it can increase up to 6% [27].

The risk of **intrauterine growth restriction (IUGR)** is higher in IVF/ICSI pregnancies. The cause of this complication is currently unknown. In the first trimester, PAAP-A is low in patients who have undergone ovarian stimulation. This decrease reflects impaired implantation [28]. First-trimester screening using an algorithm that includes maternal characteristics, uterine artery PI, PAAP-A, and PlGF has a preterm IUGR detection rate of approximately 55%, and for term IUGR only 44% [29]. Thus, some of these potential IUGR will benefit from the prophylactic role of aspirin. For the rest of the IUGR, the second trimester ultrasound performs a new stratification that identifies those patients who will be monitored frequently and will deliver preterm while the mass of patients with late IUGR will be identified by a combined screening performed in the third trimester third, at 35-36 weeks [30]. These patients were placed in a low-risk group in the second trimester, but represent approximately 90% of patients with low-birth-weight neonates.

The risk of **premature birth** is approximately twice as high in pregnant women with IVF/ICSI pregnancies [31,32,33]. There is has not yet been established an algorithm with high

predictive power for premature birth. The one based on the evaluation of first-trimester serum markers to which maternal factors are associated has a positive predictive value of only 20%, while the rate of false positive results is 10% [34]. The highest detection rate of premature birth is obtained by the combined analysis of maternal factors (age, height, race, parity) and ultrasound measurement of cervical length at 11-13 weeks. The detection rate in this case reaches 55% with a false positive rate of 10% [35].

3. Purpose

The main purpose of the doctoral thesis is to analyze the characteristics of the infertile population that addresses the Filantropia Hospital. This is epidemiologically and medically considered a primary care infertility clinic, with couples drawn from the unselected general population.

4. Objectives

The objectives of the thesis are the following:

1. Identifying the pattern of infertility causes in the population of couples who turn to Filantropia Hospital for infertility
2. Comparative analysis of pregnancies obtained through assisted reproduction techniques with those obtained spontaneously from the perspective of first trimester markers
3. Analysis of the profile of obstetric complications in pregnancies obtained through assisted reproduction procedures versus pregnancies obtained spontaneously

5. Material and method

In order to achieve these objectives, we designed three cross-sectional retrospective observational studies.

5.1. STUDY 1: *Identifying the pattern of infertility causes in the population of couples presenting to Filantropia Hospital*

5.1.1 Materials and methods

It is an observational, cross-sectional, retrospective, randomized study, in which we analyzed the data from 67 infertile couples who presented themselves to the Filantropia Clinical Hospital for infertility between 01.01.2019 and 01.03.2020.

5.1.2 Description of the analyzed variables

The socio-demographic, biometric and medical characteristics relevant to the diagnosis of infertility were analyzed such as: age, background, education level, height, weight, body mass

index, duration and causes of infertility. To these was added the study of risk factors that can reduce the chances of success of assisted reproduction treatments: smoking, alcohol consumption, coffee, drugs, chronic medication and, last but not least, stress.

5.1.3 Statistical data processing

The collected data were entered and systematized in Microsoft Excel 2016. For statistical processing, the database was converted to the IBM SPSS (The Statistical Package for the Social Sciences) Statistics version 20 software. For the descriptive analysis of the entered variables, the mean was used, standard deviation, minimum and maximum, standard error of the mean. Pearson's r (regression) bivariate correlation test was used to analyze the associations between the numerical variables, and the Chi Square test was used to analyze the associations between the qualitative variables. When the p-value was statistically significant, the strength of the association was verified by the Phi and Cramer V indicators. Categorical variables were also analyzed by the non-parametric Kruskal-Wallis test. Correlation between nominal variables and categorical variables was tested by independent sample t-test and by eta coefficient to measure effect size.

5.1.4 Results and Discussions

5.1.4.1. *Socio-demographic variables: age, background, education level*

The age of the whole group belonged to the range between 23 and 56 years (Standard Deviation (SD) = 5.599), with a mean age of 33.49 years, of which 67 participants were female and 67 participants were male representing the couple partners of the women's group.

The age of women in the study sample was between 23 and 45 years, with a mean age of 32.33 (SD – 4,765) and for men, the age range was between 25 and 56 years, with a mean age of of 34.64

5.1.4.2. *Biometric variables of the women's group: height, weight, body mass index (BMI)*

BMI (body mass index) ranged from 17.2 to 48.1 with a mean of 23.913 (SD – 5.3237)

5.1.4.3. *The variables – general risk factors identified in the study group:*

In the women group, the quantified risk factors were infection with *Chlamydia trachomatis, Ureaplasma urealyticum*, Mycoplasma hominis, associated hormonal pathologies, chronic treatments, allergies, tobacco consumption, alcohol consumption, stress level.

In the men group, the quantified risk factors were: work environment, varicocele, trauma, tobacco and alcohol consumption, allergies, stress level.

5.1.4.4. *Quantitative clinical variables for the sample of women.*

They are represented by the duration of infertility, ovarian dysfunction, the presence of tubal factor, congenital anomalies, cervical factor, the presence of leiomyomatosis, endometriosis.

In the studied group, the average time until the presentation of women for the specialist evaluation of infertility is 2.596 years, with a minimum value of 6 months and a maximum value of 11 years (SD – 2.1469)

In Table V.2. are presented the prevalence of the causes of female infertility:

Table V.2. Causes of infertility in women identified in the study group

	Number	Percentage %	Total
Anovulatory infertility	32	49.2%	65
Ovarian insufficiency	11	16.4%	67
Tubal factor	18	34%	53
Mullerian anomalies	5	7.5%	67
Uterine fibroids	9	13.4%	67
Cervical factor	4	6.7%	60
Endometriosis	17	26.15%	12.7

The associations and the size of the effect between stress risk factors and female causes of infertility were studied, with the following results: the cervical factor was statistically significantly associated, p = 0.044, with the level of stress, with a moderate effect size (Eta – 0.338), no statistically significant results were obtained for the rest of the parameters.

In the women's group, anovulatory infertility and ovarian dysfunction were statistically significantly correlated with chronic medication (p=0.308 respectively p=0.010). Tubal factor was associated with Ureaplasma urealyticum infection, p= 0.000. Also, duration, causes of female infertility and risk factors were not associated with background and level of education.

In the men's group, the association between age, duration of infertility in spermogram years and the presence of sexual dynamics disorder was tested without statistically significant results, except for the association between sexual dynamics abnormalities and age, variables with significant association, p = 0.017. The duration of infertility was not associated with the environment of origin (p = 0.499) nor with the level of education (p = 0.223). Associations between sperm parameters and identified risk factors were tested with the following results:

number abnormalities are associated with stress, p = 0.029, motility abnormalities are associated with genital trauma, p = 0.036, morphological abnormalities are associated with chronic medication, p = 0.040 and with varicocele, p=0. 036, relationship; combinations of anomalies are associated with trauma, p = 0.012, anomalies of sexual dynamics are associated with medication, p = 0.025.

The study revealed the following pattern of the causes of infertility represented in Fig. 5.1.

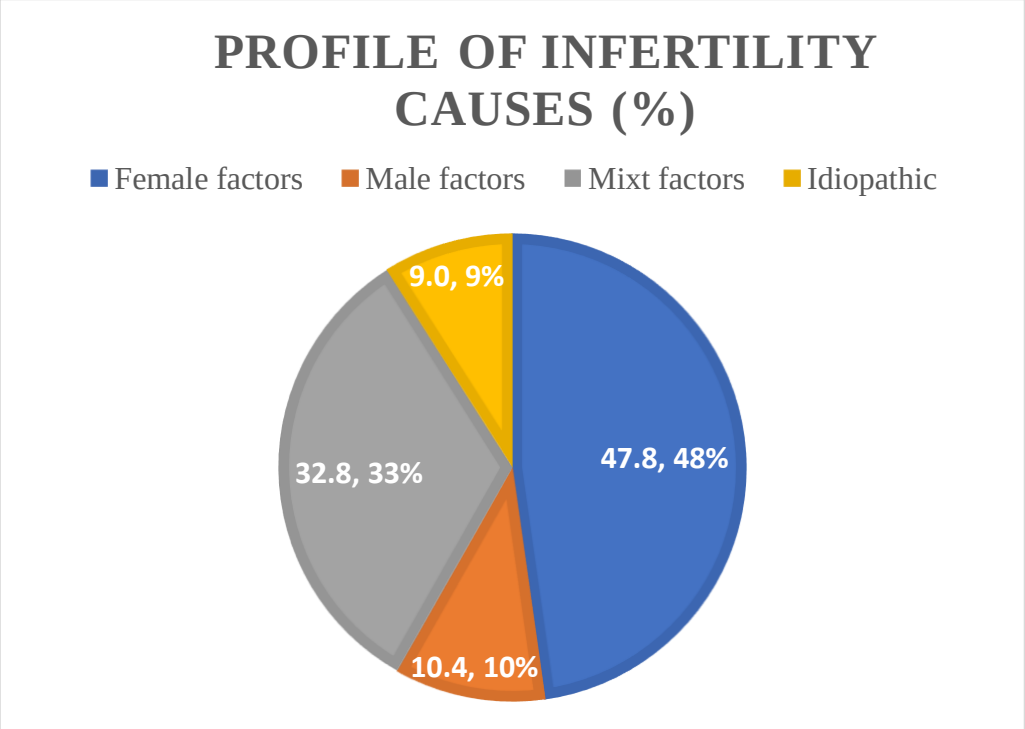

Fig. 5.1. The profile of the causes of infertility in the population of infertile couples who addressed the Filantropia hospital, primary care center for infertility

5.2. STUDY 2. ***Comparative analysis of pregnancies achieved through assisted reproductive techniques and those achieved spontaneously from the perspective of first trimester markers***

5.2.1. Material and method

The second descriptive, transversal, retrospective study is based on a sample consisting of a total number of 5566 patients (evaluated in the Filantropia clinical hospital between 01.01.2014 and 31.12.2018 with the approval of the hospital's Ethics Commission and compliance the confidentiality rules that preceded accessing the data archive). The sample includes a group of patients who got pregnant spontaneously (5454 in number), also representing the control group. The second group consists of a number of 31 patients who

obtained pregnancy through ovulation induction, and the third group consists of a number of 81 patients who obtained pregnancy through IVF.

The study aims to verify whether the markers used in first trimester screening are influenced by the mode of conception.

5.2.2. Description of variables

The analyzed variables were: the mother's biometric data (age, race, height, weight), serum biomarkers (ß-hCG, PAAP-A, PlGF), ultrasound and clinical parameters: nuchal translucency, craniocaudal length (LCC) as well as mean blood pressure. The units of measure for some of these parameters are expressed in multiples of the median (MoM).

5.2.3. Statistical data processing

The statistical analysis was performed in the IBM SPSS Statistics version 20 software. Descriptive statistics and significance tests were performed. The confidence interval was set at 95%. The Shapiro-Wilk test was performed to test for normality. Pearson correlation tests, T-test for independent variables were used to verify intervariable associations and the non-parametric Mann Whitney U test was used when the condition of normality of data distribution was not validated. The Kruskal-Wallis test was used to compare groups according to the type of conception.

5.2.4. Results and discussions

5.2.4. *Statistical results for the comparison of the 3 samples* (Table V.3.)

Table V.3 Correlations between study groups by conception type.

	Spontaneous Pregnancy	Ovulation Induction	IVF	Coefficient p (df)	Chi-Square
Age -average (DS)	30.10 (4.454)	30.31 (3.681)	34.8 (3.950)	$p < 0.001$ (2)	80.725
Smoking	24.1%	22.6%	12.3%	$p = 0.048$ (2)	6.067
Parity					29.680
-annulment	60.3%	67.7%	88.9%	$p = 0.02$	
-multiparous	39.7%	32.3%	11.1%	(16)	

BMI	/	24.948 (4.1611)	23.294 (3.2729)	p = 0.134 (1)	2.247
Weight (average, DS)	63.462, (12.1952)	/			
Height (average, DS)	164.81, (6.089)	/			
LCC (average, DS)	69.077, (7.7484)	67.619, (6.9847)	67.398 (8.0295)	p = 0.121 (2)	4.217
TN (average, DS)	1.895, (0.4310)	1.1815, (0.2863)	1.8451 (0.38997)	p = 0.485 (2)	1.449
ß-hCG free MoM (average, DS)	1.2253, (0.818624)	1.1683, (0.73494)	1.12105 (0.58432)	p = 0.090 (2)	2.871
PAPP-A MoM (average, DS)	1.22979, (0.876596)	1.019, (0.424449)	1.38134 (0.58432)	p = 0.022 (2)	7.643
PlGF MoM (average, DS)	1.13031, (0.47336)	/	0.7617 (0.2321)	p = 0.090 (2)	2.871
TAM (average, DS)	87.295, (9.0404)	90.483, (8.2901)	90.421 (9.5219)	p = 0.112 (2)	4.377
TAM MoM (average, DS)	1.0355, (0.10093)				
Equivalent		1.0558, (0.08017)	1.0738 (0.1024)	p = 0.556 (2)	0.347

5.3. STUDY 3. *Analysis of the profile of obstetric complications in pregnancies obtained through assisted reproduction procedures versus spontaneous pregnancies*

5.3.1. Material and method

The study presented below is an observational, retrospective, transversal study conducted on a number of 10,190 patients who presented and gave birth in the Filantropia hospital between 01.01.2016 and 31.12.2018 with the approval of the hospital's Ethics Commission and compliance with the norms of confidentiality that preceded accessing the data archive. The sample includes a group of patients who got pregnant spontaneously, numbering 10190, also

representing the control group. The second group consists of 94 patients who got pregnant through IVF.

The purpose of the study was to analyze if there are differences between the profile of complications during pregnancy and at birth between patients who got pregnant spontaneously and those who resorted to assisted human reproduction techniques.

5.3.2. Description of variables

The analyzed variables were: mother's age, parity, gestational age at birth, Apgar score at 1 minute and at 5 minutes, birth weight and the presence of hypertension.

5.3.3. Statistical data processing

Statistical analysis was performed with the same computational coordinates used in study 2.

5.3.4. Results and discussions

Descriptive analysis of the data according to the method of conception

In the studied sample, the maternal age differs statistically significantly according to the type of conception, thus the age is older in the IVF conception group. The median age for spontaneous pregnancies is 29 years, compared to 34 years for IVF pregnancies. Infertile couples in Romania turn to reproductive medicine specialists late. Delays occur for various reasons: postponement of conception, infertility as a stigma, refusal to accept specific treatment and last but not least financial reasons.

Gestational age at birth also differs by conception, with IVF pregnancies being more prone to premature births. The risk of premature birth is 3 times higher in pregnancies obtained through IVF. (Table V.4.)

Table V.4.Descriptive data analysis according to the mode of conception

		Conception					
		Spontaneous			IVF		
		Pregnancy type			Pregnancy type		
		Singleton	Twin		Singleton	Twin	
			DCDA	MCDA		DCDA	MCDA
		Nr (%)	Nr (%)	Nr (%)	Nr (%)	Nr (%)	Nr (%)
VG	< 28 weeks	58 (0.6%)	6 (4.8%)	2 (7.1%)	0	0	0
	29-34 weeks	258 (2.6%)	30 (24%)	13 (46.4%)	3 (4.8%)	5 (16.1%)	0
	35-37 weeks	879 (8.8%)	57 (45.6%)	11 (39.3%)	8 (12.9%)	16 (51.6%)	1 (100%)
	> 38 weeks	8841 (88.1%)	32 (25.6%)	2 (7.1%)	51 (82.3%)	10 (32.3%)	0

Legend: MC-Monochorionics; DA- Diamniotics; DC- dichorionic; VG-gestational age

Twin pregnancies are significantly more common in the IVF conception group. Apparently, there is no tendency to adhere to the single transfer policy, but if we look carefully at the age of the patients in the IVF group, we notice that the average age is 35 years. From this age, the safe transfer of 2 embryos is allowed to increase the chances of success. (Table V.5.)

Table V.5. Descriptive analysis of the data according to the method of conception

	Conception		
	Spontaneous	IVF	p
Age (median,IQR)	29 (7)	35 (6)	< 0.001
VG (median,IQR)	39 (2)	38 (3)	< 0.001
Risk categories VG	extreme = 0.6% severe = 3% easy = 9.3% on time = 87.1%	extreme = 0 severe = 8.5% easy = 26.6% on time = 64.9%	< 0.001
Parity (average,IQR)	1.52 (1)	1.18 (0)	< 0.001
Pregnancy type	singleton = 98.5% DCDA = 1.2%	singleton = 66% DCDA = 33%	< 0.001

	MCDA = 0.3%	MCDA = 1.1%	
Twin pregnancy	yes = 1.5% no = 98.5%	yes = 34% no = 66%	< 0.001
APGAR 1′ fetus 1(average,IQR)	8.52 (1)	8.51 (1)	0.407
APGAR 5′ fetus 1(average,IQR)	9.08 (1)	9.02 (0)	0.173
APGAR 1′ fetus 2(average,IQR)	7.46 (2)	8.13 (1)	0.073
APGAR 5′ fetus 2(average,IQR)	7.84 (1)	8.56 (1)	0.026
Fetus sex1	male = 51.8% female = 48.2%	male = 48.9% female = 51.1%	0.605
Fetus sex 2	male = 47.1% female = 52.9%	male = 31.2% female = 68.8%	0.120
Fetus weight 1 (median,IQR)	3300 (650)	3100 (613)	< 0.001
Fetus weight 2 (median,IQR)	2300 (850)	2450 (713)	0.156
Birth mode	Caesarean= 46.8% SP/OP = 49.6% Instrumental birth = 3.7%	Caesarean = 85.1% SP/OP = 9.6% Instrumental birth = 5.3%	< 0.001

Birth weight differs according to the type of conception and the degree of pregnancy, p = 0.003, thus, twin pregnancies tend to have a higher weight if achieved by IVF, and singleton pregnancies have similar weight IVF versus spontaneous.

Gestational age at birth differed by type of conception and degree of pregnancy, p = 0.001. In IVF pregnancies, the gestational age decreases for singleton pregnancies, but increases for twin pregnancies.

The APGAR score at 1′ differs according to the degree of pregnancy and the mode of conception. Twin pregnancies have a lower score both in the group of spontaneous conceptions

and in the group of IVF conceptions. The APGAR score at 5′ differs according to the degree of pregnancy and mode of conception. Twin pregnancies have a lower score both in the group of spontaneous conceptions and in the group of IVF conceptions.

The incidence of hypertensive conditions is influenced by the mode of conception. They occur with a 3 times higher frequency in IVF pregnancies compared to spontaneous pregnancies, see Table V.6.

Table V.6. The incidence of hypertensive disorders according to the mode of conception

Hypertensive disorders	TOTAL	384	Population	%
	IVF	12	n=94	12.76596
	spontaneous	372	n=10190	3.650638

The method of delivery is influenced by the method of conception, pregnancies obtained through IVF have almost double the chance of ending by caesarean section (79% versus 46%). Only 8% of IVF pregnancies end in vaginal birth, while 50% of spontaneous pregnancies are vaginal. The prevalence of instrumental births is higher in pregnancies obtained through IVF versus spontaneous births (8.1% versus 3.7%), justified by the greater degree of anxiety that burdens the supervision of a pregnancy obtained through IVF .

In twin pregnancies, cesarean delivery becomes the rule, only 23% of spontaneous DC/DA pregnancies, respectively 3.2% of IVF pregnancies will be born vaginally.

6. Conclusions. Personal contribution

6.1. Conclusions. The novelty of the research. Limits of research. New directions

The research set out and succeeded in identifying whether there is a pattern to the causes of infertility in the general population of the country. It started from the premise that the Filantropia hospital is the first filter of this type of patients, and a pattern/profile associated with this population can be extrapolated to the general population.

The pattern of infertility causes takes into account their prevalence in the studied group as well as their association with different risk factors. Following the analysis of these parameters, a complete, integrated diagnosis can be established followed by optimal treatment.

The study identifies the following **prevalences of female infertility causes**: 49.2% had anovulatory infertility; 16.4% had ovarian insufficiency; In 34% the tubal factor was present; 7.5% presented congenital uterine anomalies; 13.4% presented leiomyomatosis; In 6.7% the cervical factor was present; 26.6% had endometriosis.

Infertility is a multifactorial disease in which small defects and risk factors combine to prevent pregnancy. Therefore, during the diagnostic process, each factor must be analyzed and integrated, an overall picture must be made and the best treatment option must be established. The analyzed risk factors are: age, duration of infertility, body mass index, smoking, coffee consumption, alcohol, drugs and stress.

Age is the main risk factor associated with the diagnosis of infertility. This parameter correlates with most of the risk factors that characterize the infertile couple. The average age of women who call for specialized medical services is 32 years, while the average age of partners is 34 years.

The duration of infertility is an important parameter that guides the diagnosis and treatment of infertility. In the studied group, the average time until the presentation of women for the specialist evaluation of infertility is 2.596 years, with a minimum value of 6 months and a maximum value of 11 years.

In the study group, both women's age and men's age were statistically significantly correlated with the duration of infertility. The older the age, the longer we expect the duration of infertility, the more severe the underlying cause and the more aggressive the treatment. The study detects a statistically significant association between anovulatory infertility and ovarian insufficiency and age.

Among the other risk factors, we mention that. Tubal factor was associated with Ureaplasma infection. Leiomiomatosis was associated with hipothyroidism.

In the group of men, the starting point in the diagnosis of infertility was the analysis of the **spermogram**, thus 9.8% presented number anomalies, 33.3% motility anomalies, 37.3% morphological anomalies and 21.6% had combinations of anomalies. 10.4% of partners had a positive sperm culture.

Spermogram abnormalities and sexual dynamics dysfunctions were correlated with the identified risk factors. There is a significant association between number abnormalities and stress.

Genital traumas are associated with sperm motility morphology and combination abnormalities. Chronic medication affects morphology, and can produce disturbances of sexual dynamics. Morphological abnormalities are influenced by varicocele and trauma.

The profile of the causes of infertility at the level of the couple is as follows: 48% exclusively female causes, 10% strictly male causes, 33% mixed causes and 9% idiopathic. This pattern corresponds to a primary care infertility clinic serving the general, unselected infertile population. In contrast to this profile, that of a specialized assisted human reproduction clinic tends to have a higher percentage of male infertility.

The limits of the research are represented by the relatively small number of couples analyzed and the limited duration of the study. Such a study is difficult to carry out, infertility being a plurifactorial disease that requires integrated diagnosis, often expensive and time-consuming for couples. Keeping them compliant can sometimes become a challenge both ways. It is not possible to judge whether these patterns are not dependent on the time factor, large population studies over consistent time periods are needed to track their evolution.

The research continues with the second objective, that of analyzing the outcome of assisted reproduction treatments, pregnancy, from the perspective of first trimester markers. These markers appear from the period of implantation and placentation and can guide possible therapeutic prophylactic interventions.

The trend of increasing age within the study groups was statistically validated ($p < 0.001$). The first study of the thesis discovered that the average age at which infertile patients turn to specialized institutions in Romania is 32 years old with an average duration of pregnancy of approximately 3 years, the average age of patients with pregnancies obtained through IVF being 35 years.

Smoking is considered a risk factor for infertility and pregnancy. Following a process of awareness of the negative effects of smoking, most of the patients who obtained pregnancies through assisted human reproduction techniques are non-smokers. This fact can be seen by the progressive decrease from 24.1% to 22.6% to 12.3% of the rate of smoking patients depending on the type of conception ($p = 0.048$).

Parity naturally correlates with conception type. We expected that IVF patients would be predominantly nulliparous (60.3% versus 88.9%). This finding may be due to the small number of IVF performed in Romania to date.

No statistically significant differences were found between cranio-caudal length, nuchal translucency and ß-hCG value depending on the method of conception.

PAPP-A MoM is significantly higher in patients who conceived through IVF, $p = 0.022$, but this fact is explained by a higher number of multiple pregnancies in the group of pregnancies obtained through IVF. This correlation was no longer valid after we have adjusted the data by grade.

In conclusion, from the perspective of first-trimester markers, pregnancies obtained through assisted reproduction techniques present a similar profile to those obtained spontaneously.

The third study also validates that the maternal age is a parameter that varies statistically significantly with the mode of conception. The average age of patients who got pregnant through IVF is significantly higher compared to that of patients who got pregnant spontaneously, 35 years versus 29 years. Romanian couples hardly accept the diagnosis of infertility, they turn to specialized medical services late, they even have difficulties in accepting the treatment itself, reasons that additionally delay getting pregnant by at least 2-3 years. The reasons why they end up in this situation are social and economic: postponing conception until building a career, infertility is still considered not a disease but a stigma and, last but not least, assisted reproduction treatments are financially expensive and underfunded by healthisurance.

The study identified a higher incidence of preterm birth in IVF patients. The risk of premature birth is 3 times higher in IVF pregnancies compared to spontaneous pregnancies. Also, in the case of IVF pregnancies, the gestational age at birth is lower compared to spontaneous ones. This fact is mainly due to the increased prevalence of caesarean section in this group of patients. The same cannot be said about IVF twin pregnancies, in their case the gestational age at birth is earlier compared to spontaneous ones.

The incidence of hypertensive conditions is affected by the mode of conception. In the groups analyzed, 3.6% of spontaneously obtained pregnancies were complicated by hypertensive conditions. A 4 times higher percentage was registered in the IVF group, 12.76%.

Parity is influenced by mode of conception. Most of the patients who got pregnant through IVF are primiparous, a fact that justifies the efforts made to get pregnant.

The percentage of twin pregnancies is obviously higher in IVF pregnancies. Since the average age in this group of patients is 34 years, we expected it to include a larger subset of patients over 35 years of age, from which age 2 embryos can be transferred at a time to increase the success rate.

By comparing the samples, it was found that birth weight varies depending on the mode of conception and the degree of pregnancy. In the case of singleton pregnancies, the birth weight is similar regardless of the method of conception, IVF versus spontaneous. Girls from IVF twin pregnancies tend to be heavier than those conceived spontaneously

The condition of the newborn immediately postpartum, reflected by the Apgar score at 1 min and 5 min, varied according to the method of conception and the degree of pregnancy. The Apgar score at 1 min and at 5 min are similar in singleton pregnancies regardless of the mode of conception, while in twin pregnancies the Apgar score at 1 min and at 5 min tend to be lower in IVF pregnancies versus those obtained spontaneously.

The study concludes that mode of delivery is influenced by mode of conception, with IVF pregnancies almost twice as likely to end by caesarean section. The incidence of vaginal delivery of patients with pregnancies obtained through IVF was 8%. In contrast, 50% of the patients with spontaneous pregnancy gave birth vaginally. Instrumental births, when they occur, are more frequent in the group with IVF pregnancies.

The grade of pregnacy is statistically significant associated with the mode of delivery, cesarean delivery becoming the rule, regardless of the mode of conception.

In terms of the third objective, the research validates the finding that IVF pregnancies have a higher risk of obstetric complications, risk of preterm birth and hypertensive conditions.

The research was unable to identify the risk of intrauterine growth restriction due to heterogeneous data and non-uniform definitions.

The greatest difficulty encountered was the inability to create a unitary database to follow the prospective evolution of patients. In Romania, they can choose the clinic where they will perform the assisted reproduction treatment as well as the hospital where they will be monitored and give birth. In most cases all three institutions are different.

To choose any research direction, it is necessary to create such a database that allows comparative analysis and the identification of a multitude of pathologies that have their starting point from the implantation phase. Among the directions, the research suggests the creation of studies that follow the evolution of pregnancies according to the cause of infertility and the type of embryo transfer, fresh or frozen or according to the type of preparation of the endometrium for embryo transfer, natural cycle or artificial cycle, with or without the presence of a corpus luteum.

6.2. Personal contributions

I carried out the research presented in the thesis over 4 years, in between 2018 to 2020. The data used in the research came from the medical documents of the patients of the Filantropia hospital.

I was directly involved in the process of designing the studies, collecting data from the medical files and observation notes as well as in their statistical processing.

In the case of infertile couples, we took a complete anamnesis, we designed an investigation plan based on which we were able to develop an integrated diagnosis of the causes of infertility. I thus created a database with all the characteristics of this population, which allowed me to create a profile/pattern that can be used to select the optimal treatment that shortens the time to pregnancy. For the pregnant population, we analyzed hospital databases and created specific databases for the objectives pursued. I checked the medical files and the presentation notes to fill in the various missing results from the already existing databases.

We conducted an extensive literature review to identify patterns of infertility causes and their effects on pregnancies. I wrote the thesis myself.

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SCIENTIFIC PAPERS:

1. **Mirona Furtună**, Anca Panaitescu, Ana Huszti, Corina Gică, Nicolae Gică, Gheorghe Peltecu Patterns of Infertility-a Primary Care Clinic Study Obstetrica și Ginecologia. Revista Societății Române de Obstetrică și Ginecologie. Vol LXVIII. Nr. 2, aprilie-iunie 2020. **DOI**: 10.26416/OBSGIN.68.2.3859. Revista este indexată în baza de date Index Copernicus International, impact factor 2016: 5.656, SJR 2016: 2.899BDI (Capitol 5)

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