UNIVERSITY OF LATVIA

Summary of Doctoral Thesis

Violeta Fodina

REASONS FOR FAILED IMPLANTATION IN IVF CYCLES

METHODS TO IMPROVE IVF OUTCOMES

Riga 2023

UNIVERSITY OF LATVIA

FACULTY OF MEDICINE

Violeta Fodina

REASONS FOR FAILED IMPLANTATION IN IVF CYCLES
METHODS TO IMPROVE IVF OUTCOMES

SUMMARY OF DOCTORAL THESIS

Submitted for the degree of Doctoral degree (Ph.D.)
in Medicine and Health Sciences,
field of Clinical Medicine,
subfield of obstetrics and gynecology

Riga 2023

The doctoral thesis was carried out at the Department of Pathology, Faculty of Medicine and health sciences, University of Latvia, from 2014 to 2021 in iVF Riga infertility treatment clinic, Riga, Latvia.

The thesis contains the introduction, 10 chapters, reference list.

Supervisors:

Assoc. Prof. Dr. med. **Natalija Vedmedovska**,
Assoc. Prof. Dr. paed. **Margarita Pukite**

Reviewers:

1) **Dace Rezeberga**, Dr. med. Prof., Faculty of Medicine, Department of Obstetrics and Gynaecology, Rīga Stradiņš University
2) **Cristina Eguizabal Argaiz**, Dr. med. Prof., Biocruces Bizkaia Health Research Institute, Barakaldo, Spain
3) **Egle Machtejeviene**, Dr. med. Prof., Head of Fetal Medicine Centre, Department of Obstetrics and Gynaecology, Lithuanian University of Health Sciences, Lithuania, Kaunas

The thesis will be defended at the public session of the Doctoral Committee of Medicine and Health Sciences, University of Latvia, at _____: _____ on _____ _____, 2023

The thesis is available at the Library of the University of Latvia, Kalpaka blvd. 4.

This thesis is accepted for the commencement of the degree of Doctor of medicine and health sciences on _____, 2023 by the Doctoral Committee of medicine and health sciences, University of Latvia.

Chairman of the Doctoral Committee

_____/ _____/
(signature) *(name, surname)*

Secretary of the Doctoral Committee

_____/ _____/
(signature) *(name, surname)*

ISBN 978-9934-36-141-8
ISBN 978-9934-36-142-5 (PDF)

ABSTRACT

Infertility is a widespread medical and social problem affecting approximately 17.5% of couples worldwide (WHO 2021). Severe cases of infertility require *in-vitro fertilization* (IVF) treatment, and the proportion of IVF procedures performed to achieve a pregnancy is continuously growing. The main challenges facing couples and physicians during IVF treatments are recurrent implantation failure (RIF) and early pregnancy loss (EPL). There is still a lack of diagnostic and therapeutic methods to facilitate improved prognosis and to reduce the incidence of RIF and ELP.

This work was done to assess the ability of different methods and approaches to improve the outcome of IVF treatment, and to decrease RIF and EPL. Both non-invasive and invasive IVF work-up methods were assessed to reach this goal.

During the first part of the study, the impact of the non-invasive method of time-lapse microscopy (TLM) on IVF outcomes was assessed. The sample comprised 897 embryos analyzed using TLM in 146 embryo transfers. The control group included 1723 embryos cultivated and selected conventionally using the traditional static observation method which preceded 386 embryo transfers. The total EPL in the TLM group (both biochemical and clinical) was reduced statistically significantly (TML 28.4% versus control 41.8%, χ^2, $p = 0.045$). The EPL rate per embryo transfer was also significantly reduced in the TLM group (TLM 9.1% versus control 19.1%, $p = 0.043$).

During the second part of the study, the performance of two invasive methods was assessed, – preimplantation genetic testing for aneuploidies (PGT-A) of embryos and endometrial receptivity array (ERA) to improve the outcome in RIF patients. In total, 253 ICSI cycles were included in the study. They were further divided into 4 groups: Group I – frozen embryo transfers without any additional tests or procedures (FET), $n = 72$ cycles; Group II – FET with PGT-A, $n = 67$; Group III – FET with PGT-A and ERA, $n = 72$ and Group IV – FET with ERA, $n = 22$. When clinical outcomes of biochemical pregnancy, clinical pregnancy and pregnancy loss were compared to the end-point of "no pregnancy", Group II (FET+PGT-A) showed a statistically significant higher chance of achieving both biochemical pregnancy ($p = 0.01$, OR = 5.5) and clinical pregnancy ($p = 0.049$, OR = 2.3), as compared to Group I. The Group II (FET+PGT-A) and Group III (FET+PGT-A+ERA) biochemical pregnancy results showed that ERA test significantly decreases biochemical pregnancy loss. The clinical pregnancy was statistically significant in the Group III (FET+PGT-A+ERA) comparing to the Group II (FET+PGT-A).

In addition, the efficacy of PGT-A in IVF cycles was tested for a specific group of 10 patients who were carriers of balanced translocations in their karyotypes. Three of them had the most frequent type of Robertsonian translocation

and 7 had various reciprocal translocations. 180 oocytes were collected and 48 embryos were analyzed with PGT-A.

In 18 embryos where a female was a carrier of a balanced translocation, and 30 embryos where a male was a carrier of a balanced translocation, the percentages of unbalanced translocation rates in embryos were 89% and 73%, respectively. The highest euploidy rate was in the male carrier group – 27%. The unbalanced translocation rate in embryos was highest in the female carrier group – 78%. The prevalence of aneuploidy rates related to translocations was 4.5 times higher in the balanced reciprocal translocation carrier group, when compared with the balanced Robertsonian translocation carrier group.

Thus, the main finding, the application of the TLM selection model increased biochemical, clinical and ongoing pregnancy rates, and decreased EPL rates. Patients with RIF can further benefit from the embryo aneuploidy testing using the PGT-A method in combination with ERA test. The proportion of total embryo aneuploidy rates was higher in the specific group of 10 patients having translocations, when compared with the patients having normal karyotypes. Therefore, in this specific group of 10 patients, PGT-A is of utmost importance.

In conclusion, these data showed that a combination of TLM and PGT-A with the ERA test is an effective approach for improving the outcome of IVF treatment, decreasing implantation failure and early pregnancy loss in patients with normal karyotypes and appliance of the PGT-A in patients with balanced translocations provides positive results in IVF treatment.

CONTENTS

ABSTRACT 3

ACRONYMS AND ABBREVIATIONS 7

INTRODUCTION 9

Actuality of the work 9

The aim, objectives, and hypotheses of the work 10

The novelty of the work 11

Place of work performance 11

Structure and scope of work 12

Work ethic 12

1. LITERATURE REVIEW 13

1.1. Etiology of RIF 13

1.2. Uterine factors 14

1.2.1. Endometrial receptivity 14

1.2.2. Human Endometrium transcriptomics 15

1.2.3. Endometrial Receptivity Array 16

1.3. Embryonal factors 17

1.3.1. Embryo quality 17

1.3.2. Time-lapse microscopy 18

1.3.3. Embryo aneuploidy 18

2. OBJECTIVES OF THE STUDY 21

3. RESEARCH HYPOTHESES 22

4. SCIENTIFIC VALUE 23

5. MATERIALS AND METHODS 24

5.1. Patients (Sample Inclusion / Exclusion Criteria) 24

5.2. Time-lapse microscopy or imaging 27

5.3. Preimplantation genetic testing for aneuploidies (PGT-A) and endometrial receptivity array (ERA) in a group of RIF in intra-cytoplasmic sperm injection (ICSI) cycles 27

5.3.1. Whole Genome Amplification (WGA) and chromosomal analysis – preimplantation genetic testing for aneuploidies 27

5.3.2. Endometrial Receptivity Array (ERA) 28

5.4. The application of PGT-A for carriers of balanced structural chromosomal rearrangements 29
5.5. Common protocols for all the parts of the study 30
5.5.1. Ovarian stimulation 30
5.5.2. Oocyte retrieval 30
5.5.3. Intracitoplasmic sperm injection (ICSI) 30
5.5.4. Embryo cultivation 31
5.6. Outcome assessments 32
5.7. Statistical Analysis 32

6. RESULTS 33
6.1. Time lapse microscopy (TLM) or imaging study 33
6.2. Preimplantation genetic testing for aneuploidies (PGT-A) and endometrial receptivity array (ERA) in a group of RIF in ICSI cycles 40
6.3. The application of PGT-A for carriers of balanced structural chromosomal rearrangements 42

7. DISCUSSION 45

8. CONCLUSIONS 53

9. CLINICAL APPLICATIONS FOR FUTURE PRACTICE 54

10. RELATED PUBLICATIONS, LECTURES AND PRESENTATIONS 57

REFERENCES 59

ACKNOWLEDGEMENTS 68

APPENDIXES 69
Appendix I 69
Appendix II 70

ACRONYMS AND ABBREVIATIONS

2-COX 2	Cyclooxygenase
aCGH	array comparative genomic hybridization
aCLs	anticardiolipin antibodies
AFC	antral follicle count
AMH	anti-müllerian hormone
ANXA4	Annexin A4
aPLs	Antiphospholipid Antibodies
Apo A-I	Apolipoprotein A-I
APS	Antiphospholipid syndrome
ART	assisted reproductive technologies
ASRM	American Society of Reproductive medicine
aβ2GPI	anti-β2glycoprotein I antibodies
BCL6	B-cell lymphoma 6 protein
BMI	Body mass index
BRCA1	Breast cancer gene 1
Ca^{2+}	Calcium
CAM	cellular adhesion molecules
CD138	Syndecan-1, marker of plasmocytic differentiation
CD16	a molecule of the Ig superfamily known to be involved in antibody-dependent cellular cytotoxicity
CD25	α-chain of the IL-2 receptor
CD3	Glycoprotein that serves as a co-receptor for the T-cell receptor
CD4	Glycoprotein that serves as a co-receptor for the T-cell receptor
CD56	archetypal phenotypic marker of natural killer cells
CLDN4	Protein coding gene
CNV	Copy number variations
COS	controlled ovarian stimulation
cPLA2a	cytosolic phospholipase A2
CRABP2	Cellular retinoic acid-binding protein 2
CST	community state types
DET	deferred embryo transfer
DFI	DNA fragmentation index of sperm
DNA	deoxyribonucleic acid
DPP4	Dipeptidyl peptidase-4
E2	oestradiol
EDN3	gene that encodes Endothelin 3 protein
EM	Effector memory
EPL	early pregnancy loss
ERA	Endometrial Receptivity Array
ESHRE	European Society of Human Reproduction and Embryology
ET	embryo transfer
EU	European Union
FET	frozen embryo transfer
FISH	Fluorescence in situ hybridization
FOXP3	forkhead box P3, also known as scurfin
GADD45A	gene that encodes Growth arrest and DNA-damage-inducible protein
GAST	Gonadotropin-releasing Hormone Agonist Test
GLI1	Glioma-associated oncogene protein
GPX3	glutathione Peroxidase 3 protein coding gene
hCG	Human chorionic gonadotropin
hCG+7	7th day of hCG triggering or 168 hours of hCG administration
HGExERdb	Human Gene Expression Endometrial Receptivity database
HOXA-10	Homeobox A10 protein
HRT	hormone replacement therapy
ICSI	Intracytoplasmic sperm injection
IFN- γ	Interferon-γ
IGF-1	Insulin-like growth factor-binding protein
IgG	Immunoglobulin G
IL-1	Interleukin 1
IL-10	Interleukin 10
IL-11R	Interleukin 11 Receptor
IL-12	Interleukin 12
IL-13	Interleukin 13
IL-15	Interleukin 15
IL-18	Interleukin 18
IL-2	Interleukin 2
IL-21	Interleukin 21

IL-23	Interleukin 23
IL-4	Interleukin 4
IL-5	Interleukin 5
IL-6	Interleukin 6
IVF	in vitro fertilization
LA	lupus anticoagulant
LH	Luteinizing hormone
LIF	Leukaemia inhibitory factor
LPA3	lysophosphatidic acid receptor 3
MAOA	Monoamine Oxidase A protein coding gene
mIU/ml	milli-international units per millilitre
MMP7	matrix metalloproteinase-7
mRNA	messenger RNA
MUC-1	mucin 1 protein coding gene
NGS	next-generation sequencing
NK	Natural Killers
NKp46^{+}	novel triggering receptor expressed by all human NK cells that is involved in natural cytotoxicity
OLFM1	Olfactomedin 1 protein coding gene
P+3	3rd day of progesterone administration or 72 hours
P+5	5th day of progesterone administration or 120 hours
P+7	7th day of progesterone administration or 160 hours
p300	Histone acetyltransferase p300
P4	Progesterone
p53	Tumour protein P53
PAEP	Progestogen associated endometrial protein coding gene
PCR	Polymerase chain reaction
pET	personalised embryo transfer
PG	Prostaglandins
PGD	preimplantation genetic diagnostic
PGT	preimplantation genetic testing
PGT-A	preimplantation genetic testing for aneuploidies
PGT-M	preimplantation genetic testing for monogenetic diseases
pH	acidity, potential of hydrogen
POR	Poor ovarian response
qPCR	quantitative PCR
RAGs	receptivity Associated Genes
rDNA	ribosomal DNA
REM	Recurrent early miscarriage
rFSH	recombinant follicle stimulating hormone
RIF	recurrent implantation failure
RNA	ribonucleic acid
RNA-seq	RNA sequencing
ROBs	Robertsonian translocations
RPL	Recurrent pregnancy loss
RRA	Robust rank aggregation
RSA	Recurrent spontaneous abortion
SART	Society for Assisted Reproductive Technology
SCD test	Sperm chromatin dispersion test
SET	selective single embryo transfer
SFRP4	Secreted frizzled-related protein 4 coding gene
SIGs	Special Interest Groups
SIRT1	Histone deacetylase protein
SNP	single nucleotide polymorphism
sPLA2-IIA	secretory phospholipase A2
SPP1	Secreted phosphoprotein 1 protein coding gene
TGF-β	Transforming growth factor beta
TGF-β1	Transforming growth factor beta 1
Th1	Type 1 helper T cells
Th17	subset of T helper cells producing interleukin 17
Th2	Type 2 helper T cells
TLM	time-lapse microscopy
TNF-α	Tumour necrosis factor α
Treg	Regulatory T cells
TVUS	transvaginal ultrasound
UC/min	uterine contractions per minute
uNK	uterine Natural Killers
UP	uterine peristaltis
WGA	whole genome amplification
WHO	World Health Organisation
WOI	window of implantation
X3	Homeobox protein

INTRODUCTION

Actuality of the work

1978 was the year when the first IVF (*in vitro fertilization*) baby Louise Brown was born. Progress in reproductive technology grew exponentially from that time along with the expertise of reproductologist and the number of scientists working in the field. In the next years following the birth of Louise Brown, initial implantation rates were <5% per embryo (Niederberger et al. 2018). As assisted reproduction techniques (ART) progressed, many clinics replaced cleavage-stage embryos to blastocyst-stage embryos and switched from multiple embryo transfers to double- or single-embryo transfers. Those achievements led to the point reproductology has reached today.

According to European Policy Audit on Fertility, in 2017 (*Fertility Statistics – Statistics Explained*, n.d.) one of six people worldwide experience one of the forms of infertility during their reproductive years. In Europe 25 million citizens have been diagnosed as being infertile. Despite the lack of updated comparative data on Infertility and ART on wider regions, in 2021, the follow-up analysis (*Fertility Statistics – Statistics Explained*, n.d.) showed that across EU countries surveyed, nine with the highest fertility rates (France – 1.84, Czech Republic – 1.83, Iceland – 1.82, Romania – 1.81, Ireland – 1.78, Montenegro – 1.76, Denmark – 1.72, Slovenia – 1.64 and Slovakia – 1.63) remained below the replacement rate of 2.1 live births per woman. During the last 5 years the necessity for ART continued to increase.

RPL is a disorder defined by the American Society of Reproductive medicine (ASRM) as the loss of two or more consecutive clinical pregnancies prior to 20 weeks of gestation (Bashiri et al. 2018). Statistical data show that around 5% of all women experience two consecutive pregnancy losses and 75% of these are implantation failures (Comins Boo et al. 2016). There is a lack of consensus on the definition of RIF, resulting from rapidly changing ART practices and methods of infertility treatment. Today, the definition of RIF is still not clearly specified. The operational definition of RIF used in this study is: "the persisting failure of pregnancy after several attempts of IVF and embryo transfer"(Margalioth et al. 2006). This definition applied only to patients undergoing ART. The problems with definition start when we try to clarify what are exact criteria to include or correctly identify implantation failure. Through the history of ART clinics, many factors have changed, like the number of transferred embryos, the stage in which they are transferred, the percentage of collective implantation failure and others.

Lukasz T. Polanski in the year 2014 stressed that the definition of RIF given by most researchers in the last two decades has been based on one or two criteria or a combination of them, the first being the number of unsuccessful

cycles alone, and the second being the number of embryos transferred, Some definitions included the type of embryo transferred (fresh or frozen), and the quality (usually highlighting that the transferred embryo must be of good quality). There were also a few studies which mentioned the stage of embryo transfer. Polanski and his group concluded that "RIF should be defined as the absence of implantation itself defined by a negative serum hCG, 14 days after oocyte collection, following two consecutive cycles of IVF, ICSI or frozen embryo transfer, in which the cumulative number of embryos transferred was at least four cleavage-stage embryos, or at least and two blastocysts, with all embryos being of good morphological quality and in the appropriate stage of development. He also pointed out that there are many differing definitions of RIF used in these studies and no justification was given for the use of any particular one(Polanski et al. 2014).

One of the last definitions of RIF was based on publications by PGD Consortium (a specialized group of ESHRE – European Society of Human Reproduction and Embryology) and publication by Coughlan et al. 2014. This definition includes all the important factors: RIF is a failure to achieve pregnancy after ≥3 embryo transfers (ET) of high-quality embryos in women < 40 years, or transfers of ≥10 embryos in total in multiple transfers. Presence or absence of pregnancy is diagnosed by an ultrasound examination after the 5th week (Bender Atik et al. 2018; Coughlan et al. 2014; Thornhill et al. 2005).

According to ESHRE data, as of 2019, the rate of clinical pregnancies showing implantation success has increased to 34.6% and the rate of live births is up to 30.7% (Gliozheni et al. 2023), which clearly indicates that this problem is very relevant and there is still no exact answer about the reasons and tactics for patients with RIF.

As can be seen from the above literature review, however, there are two main reasons or their combination, such as embryo incompetence and endometrial incompetence, the success of implantation depends on these factors.

The aim, objectives, and hypotheses of the work

The aim of this work is to assess how additional examination of the embryo before embryo transfer (with methods such as TLM, morphological examination of the embryo and the development of embryo division), as well as the genetic stability of healthy embryos can affect embryo implantation, including specifying the quality and competence of the endometrium affects the embryo implantations and the use of both methods affects IVF results. In order to achieve the set goal, the following tasks were set:

1. To evaluate the ability of time-lapse microscopy/imaging to improve the outcome of the IVF procedures.
2. To evaluate if endometrial receptivity array and invasive embryo preimplantation genetic testing for aneuploidies used alone or in

combination can improve the outcome of IVF treatment in a group of patients with recurrent implantation failure.
3. To assess the utility of preimplantation genetic testing for aneuploidies in IVF procedures in specific group of patients – the carriers of balanced structural chromosomal rearrangements.
4. To clarify, standardize and operationally define non-invasive and invasive methods of embryo selection to decrease recurrent implantation failure rate and to promote the recommendations to other researchers and clinical professionals.

The following hypotheses were put forward:
1. Embryo selection is a vital step to increase the success of ART treatment. Selection of high implantation potency embryos using both methods – non-invasive Time-lapse microscopy/imaging and invasive preimplantation genetic testing for aneuploidies, can improve the outcome of the treatment.
2. Embryonal and endometrial factors in combination are the likely cause of failed implantation. Applying the combination of methods (Time-lapse microscopy and invasive preimplantation genetic testing together with adjusting implantation window) can reduce the number of recurrent implantation failure and early pregnancy loss.
3. Applying the preimplantation genetic testing for aneuploidy to the patients with structural imbalances in their karyotypes and determining the percentage of normal embryos have a great effect on implantation.

The novelty of the work

This study is both diverse and comprehensive. It pursues a multivariate approach, where the author in the first uses the combinations of these methods to investigate the main factors involved in recurrent implantation failure and early pregnancy loss.

The study determine which methods and factors are significant and which ones can be recommended in clinical practice for implementation in standard IVF work-up. It also identifies those methods that remain questionable. Based on the results of this study, a proposal is made to develop national guidelines for IVF treatment that will help the reproductive medical society in Latvia and abroad to improve results, of importance both for medical research and for demographical work. At the present time the standardized guidelines from ESHRE are not available.

Place of work performance

The doctoral thesis was carried out at the reproduction clinic iVF Riga from January 15, 2014, to December 9, 2021.

Structure and scope of work

The doctoral thesis is written in English, it consists of the thesis itself, written on 98 pages, 2 summaries (48 pages in Latvian and 49 pages in English) and 4 attached publications. The work contains 10 tables, 33 images and 7 appendices. The bibliography contains 245 literary sources, the oldest of which was published in 1979, and the newest in 2023.

Work ethic

All patients were informed about the purpose and methods of the study before inclusion and signed the informed consent. The study met the standards set by the Declaration of Helsinki. The Latvian Central Medical Ethics Committee has approved the study (Approval No. 01-29.1.2/1735).

1. LITERATURE REVIEW

1.1. Etiology of RIF

Successful embryo implantation is an interactive process between the blastocyst and the uterus. Synchronized development of embryos with uterine differentiation to a receptive state is necessary to facilitate pregnancy. Implantation failure may occur even at the early stages during embryo attachment or migration. As a result, there will be no objective evidence of a pregnancy, i.e., negative urine or blood pregnancy tests hCG. Alternatively, the embryo can migrate through the luminal surface of the endometrium and start to produce hCG, which may be detected in the blood or urine. But even at this stage, the process could be disrupted before the formation of an intrauterine gestational sac. Clinically this condition is called biochemical pregnancy.

The International Committee Monitoring Assisted Reproductive Technologies (ICMART) and the World Health Organization (WHO) defined biochemical pregnancy as the detection of hCG in blood or urine without subsequent clinical signs of pregnancy(Maesawa et al. 2015).

From the clinical point of view as defined by the ASRM, implantation is considered successful when there is ultrasonographic evidence of an intrauterine gestational sac or by histopathological examination (Bashiri, Halper, and Orvieto 2018). Despite the acceptance of the general implantation definition, many authors still unsure about the optimal hCG level and in many publications, numbers vary between > 5 to > 25 mIU/ml (Zeadna et al. 2015; R. Yang et al. 2015; Bates and Ginsburg 2002).

With hundreds of potential causes to consider, etiology of implantation failure is still a complex challenge for reproductologists. To reduce the complexity, some researchers attempted to summarize all the putative variables and factors involved in RIF which are helpful, because they facilitate the proposal of new, beneficial solutions for the treatment and management of patients. For example, T. Timeva et al. (2014) has divided RIF causes into three main groups:

1. The first group is "Multifactorial RIF" having the subgroups of maternal (endocrine diseases such as thyroid, diabetes mellitus, thrombophilia), male, hormonal or metabolic disorders, infections and thrombophilias.
2. The second group is "Endometrial RIF", which results from thin (≤ 6 mm) endometrium, with or without variations in vascularity.
3. The third group is "Idiopathic RIF", which is unexplained failure to achieve pregnancy after ET of good quality embryos, without any anatomical and histological changes in uterine cavity and endometrium, and without any other disturbances in the patient, the patient-partner and the embryos (Timeva, Shterev, and Kyurkchiev 2014).

Other authors have divided etiologic groups into decreased endometrial receptivity, defective embryonic development, and also multifactorial causes – which include endometriosis, hydrosalpinxes and suboptimal ovarian stimulation (Margalioth et al. 2006).

In all these cases can be noticed that the two main causes of implantation failure are always present. They are Uterine and Embryonal factors. Embryonal factors usually include embryo quality and embryo aneuploidy. Regarding the uterus, a lot of biological factors can alter the process of successful implantation, for example, uterine cavity abnormalities – Mullerian anomalies, fibroids, polyps and also tubal disorders. At the micro level, there are altered expressions of adhesive molecules, and specific immunological factors (Bashiri, Halper, and Orvieto 2018).

1.2. Uterine factors

1.2.1. Endometrial receptivity

The endometrial environment plays a crucial role in embryo implantation and early placental development. There is a specific period of endometrial maturation during which the trophectoderm of the blastocyst can attach to the endometrial epithelial cells and subsequently proceed to invade the endometrial stroma and the vasculature. This is called endometrial receptivity (Lessey and Young 2019). This complex process facilitiates the embryo to attach normally, implant and continue to develop.

In the menstrual cycle, there is a short period of time during which endometrial receptivity is optimal and embryo implantation is possible. This period is called the "window of implantation" (WOI). Studies with donor embryos in humans have shown that this receptive period starts at day 6 post-ovulation and continues 4–5 days (or 3–6 days) within the secretory phase being days 20–24 of the cycle) in most healthy women. In certain pathologic conditions, this window is narrowed or shifted, which disrupts normal implantation, leading to infertility or pregnancy loss (Lessey and Young 2019; Bergh and Navot 1992). Endometrium is unique in its ability to block embryos from implanting, except during this narrow window of receptivity, where the endometrium undergoes morphological, cytoskeletal, biochemical, and genetic changes (Mahajan 2015). As shown in the mice models, the "implantation window", is regulated by ovarian steroid hormones. In the receptive endometrium, crucial hormones are progesterone (P4) and 17β-estradiol (E2) (Tan et al. 1999; Paria, Huet-Hudson, and Dey 1993). In the mice models, researchers observed, that on the first day of pregnancy E2, which is preovulatory secreted, a proliferation of uterine epithelial cells was induced. From the formed corpus luteum, P4 levels start to increase, and on day 3, stromal cell proliferation is initiated. Due to rising levels of P4 and E2 secretion, the pre-receptive uterus becomes receptive on the fourth day (Huet-Hudson YM, Andrews GK 1989; Matsumoto 2017; Talbi et al.

2006). Devroey et al., and Papanikolaou EG et al., also discussed the importance of these two hormones in humans and stated that there is increasing evidence that endometrial function in the stimulated cycle is adversely affected by levels of estrogen and premature secretion of progesterone (Papanikolaou et al. 2010; Devroey et al. 2004).

In one of the studies of 189 pregnancies, the risk of early pregnancy loss increased with later implantation ($P < 0.001$). Among the 102 blastocysts that implanted by the ninth day, 13% ended with early loss. This proportion rose to 26% with implantation on day 10, and to 52% on day 11, then to 82% following day 11. The conclusion of this is that most successful human pregnancies and blastocyst implantations occur 8 to 10 days after ovulation. The risk of early pregnancy loss increases with later implantation (Wilcox, Baird, and Weinberg 1999).

1.2.2. Human Endometrium transcriptomics

The transcriptome reflects the genes that are actively expressed at any given time within a specific cell population or tissue (Garrido-Gómez et al. 2013).

Human endometrial receptivity transcriptomes pose a rather complex issue and the quantity of crucial genes that play a main role in receptivity is still being debated.

A publication done by Riesewijk et al. in 2003, compared pre-receptive endometrium 2 days after the LH peak with receptive endometrium 7 days after the LH peak, by sequencing whole-genome expression profiles. Bhagwat et al. studied 179 genes that can be called Receptivity Associated Genes (RAGs). These Genes were compiled from a Human Gene Expression Endometrial Receptivity database (HGExERdb) (Riesewijk et al. 2003; Bhagwat et al. 2013).

The HGExERdb database, created by Bhagwat et al. should be reported separately, as it contains information about 19,285 genes, in 312 datasets retrieved from 51 publications of human studies. It also provides information on the relative expression level of individual genes, when compared under different physiological conditions.

One of the most valuable meta-analyses of transcriptomic biomarkers for receptivity was done in 2016 and published by Altmäe in "Nature". In this study 14 publications were selected for qualitative analysis from 57 eligible publications. In this meta-analysis, a robust rank aggregation (RRA) method was used (Kolde et al. 2012), using enrichment analysis to identify the meta-signature of highly presumed biomarkers of endometrial receptivity (Altmäe et al. 2017). A statistically significant meta-signature of 52 up-regulated and five down-regulated genes was identified (D. Zhang et al. 2012; Horcajadas, Pellicer, and Simón 2007; Tseng et al. 2010; Tapia et al. 2011; Bhagwat et al. 2013).

Interestingly, commercial ERA by Igenomix (Ruiz-Alonso et al. 2013; Díaz-Gimeno et al. 2011) shares 47 genes in common with the identified 57 putative receptivity biomarkers.

1.2.3. Endometrial Receptivity Array

Traditional histologic endometrial dating criteria defined by Noyes (Noyes, Hertig, and Rock 1975), have been a standard procedure over the last 60 years. Despite the historical importance, because of the questioned accuracy and functional relevance of this procedure, a scientific group from the Igenomix company has attempted to make an analyses to clinically improve detection of embryonic–endometrial desynchrony due to accelerated or delayed endometrial luteal-phase differentiation. In 2009, after more than 10 years of research for transcriptomic signatures of endometrial receptivity, an "Endometrial receptivity array" was designed, developed and patented (Díaz-Gimeno et al. 2011). Garrido Gomez from Igenomix developed a clinical algorithm with a computational predictor which tests results, based on the expression analysis of 248 genes. Expression profiling was accomplished by assaying mRNA levels with microarrays or next-generation sequencing technologies (RNA-seq), that allowed identification of the transcriptomic signature of the window of implantation (WOI) (Hung and Weng 2017).

The reproducibility of ERA results depends on two points. First, the test must be performed repeating the exact same conditions that match embryo transfer cycle of a particular patient. It should always be hormone replacement therapy cycles (HRT) or natural cycles. Controlled ovarian stimulation cycles are not allowed.

The second point is the exact time in which endometrial biopsy must be done. In HRT cycle first endometrial biopsy should be performed after 5 full days of progesterone administration (or 120 hours of progesteron administration). In the natural cycle biopsy should be done at 7^{th} day of hCG triggering (or after 168 hours of hCG administration). Since the ERA checks the endometrium at the moment of implantation, in the case of day-3 embryo transfers, instructions for biopsy are the same (P+5 or hCG+7). After sequencing the mRNA from an endometrial biopsy, the endometrium can be receptive or non-receptive (pre-receptive, post-receptive, or proliferative).

If the endometrium is receptive, the blastocyst should be transferred at P+5, or at P+3 in the case of day-3 embryo. In 10% of cases the second biopsy is needed in the specific day designated by the first ERA test. Detecting specific point in time of endometrial cycle in which the WOI starts, allows doctors to perform personalized embryo transfer (pET), after which each patient receives personal recommendations that are based on her specific endometrial profile. Moreover, the term personalized embryo transfer and the procedure itself started to exist because of ERA (Garrido-Gómez et al. 2013).

The accuracy and consistency of the ERA test had been demonstrated in multiple trials, that showed that the ERA test is a reliable and reproducible method for determination of the exact time of the WOI that can be used with better results in comparison to histological dating of endometrial receptivity (Díaz-Gimeno et al. 2013).

1.3. Embryonal factors

In cases of RIF, the investigation of embryo quality becomes crucial for patients undergoing IVF treatment. According to the Istanbul Consensus recommendations, evaluating the morphokinetic properties of embryos using the Embryo grade system is essential for selecting high-quality embryos. Additionally, testing for embryo ploidy status is necessary (Balaban et al. 2011).

1.3.1. Embryo quality

Blastocysts (day-5 embryos) are graded according to expansion and quality of the inner cell mass and trophectoderm. Other criteria include blastomeres being of equal size and regular in distribution, distribution of cytoplasm without granularity and less than 10% fragmentation (Cutting et al. 2008). A good-quality embryo needs to have the correct number of cells corresponding to the day of its development. The present stage of embryo evaluation, including the description of all its related pathological elements and the development of time-lapse imaging took over 4 decades of research.

The grading system involves the assessment of three main quality parameters: the degree of blastomere (embryonic cell) expansion, Inner Cell Mass (ICM) and Trophectoderm (TE).

Based on Paynes work, Mio and Maeda (Mio and Maeda 2008) extended the analysis period to blastocyst stage and obtained 286 images of human oocytes and embryos. In the same year, Lemmen and colleagues (Lemmen, Agerholm, and Ziebe 2008) analyzed the events that occur during the first day of the development after fertilization of 102 oocytes using a microscope with an enclosed camera system. They were the first group that studied embryo kinetic properties, by finding a link between the early disappearance of pronuclei after fertilization, early first cleavage, and many blastomeres on day 2 of the development.

At first the main focus was on the timing of the first embryonic division or early cell division at which point an embryo became a 2-cell organism. The correlation between pregnancy rate and time of early cell division was first studied by Edwards group (Edwards et al. 1984). They had concluded that that the transfer of embryos that cleaved 24–26 h after insemination resulted in higher implantation and pregnancy rates than embryos transferred which had delayed division. Later many other publications showed that too fast or too slow cleavage has a negative impact on the embryo (M. Meseguer et al. 2011; Cruz et al. 2012; Natalia Basile and Meseguer 2012; Chamayou et al. 2013; Tsai et al. 2002).

A more accurate approach is to compare the times of early cleavage with embryo quality. Several studies have shown that early cleavage embryos have significantly higher numbers of cells than late cleaving embryos (Sakkas et al. 1998; Lundin, Bergh, and Hardarson 2001; Fenwick et al. 2002).

It is likely that divisions that occurred on time reflect the good quality of the cytoplasmic component of the embryo, an optimal level of energy provided

by mitochondria. They also reflect properly reacted components of activation, such as Ca^{2+} oscillations (Milewski and Ajduk 2017). It had been proven in several mouse studies that Ca^{2+} oscillations impact on embryo growth many days after fertilization. More specifically, they increase ATP levels in mitochondria. They could be related to culture conditions, as well as to intrinsic factors of sperm, age, genetic competence and metabolic conditions (Lundin, Bergh, and Hardarson 2001).

1.3.2. Time-lapse microscopy

Time-lapse microscopy (TLM) is a noninvasive embryo selection method for evaluation of embryonic development. This method provides 24-h monitoring of the embryo morphology and timing of cleavages without disrupting the constant culturing environment. The TLM method is based on embryo selection according to cell division timing, excluding morphologically normal embryos with discordant cleavage timing.

Modern time-lapse observation systems are developed for more optimized and accurate selection of viable embryos that include morphological grading with the possibility of registering kinetic parameters (M. Meseguer et al. 2011). Time-lapse imaging has its own benefits, like low light exposure in relation to traditional morphology observation methods (Ottosen, Hindkjær, and Ingerslev 2007) and the possibility to observe the embryo inside the incubator without moving it. This provides stable and uninterrupted conditions, beneficial to the final result (Marcos Meseguer et al. 2012).

Many predictive models that are based on different statistical methods of analysis were created and can predict blastocyst stage development and quality. Algorithms can be very simple, with only one parameter that needs to be calculated, or they could have a complex hierarchical structure, to give more predictive value than simple algorithms and be more popular in reproductive medicine (Milewski and Ajduk 2017; Cruz et al. 2012; Motato et al. 2016; S. T. Yang et al. 2015).

ESHRE also described the positive effect of TLM, but acknowledged that it is difficult to compare the outcomes of different studies since the methodologies used are not consistent (Apter et al. 2020).

1.3.3. Embryo aneuploidy

As people age, body defense systems are not always able to protect all the cells properly. This results in errors in chromosome segregation during meiosis I and II. Some reports claim that segregation errors in meiosis II occurs more frequently that in meiosis I (Alan H. Handyside et al. 2012). Because each woman from birth has limited reserve of oocytes, all errors lead to increase of embryos having abnormal chromosome numbers. The reasons for these chromosome segregation errors involve many factors, like incorrect formation of bivalents,

deterioration of cohesins, sister kinetohores separation by large distances and incorrect attachment of spindle microtubules to kinetochores during meiosis (Webster and Schuh 2017). Despite the decreasing quality of oocytes that correlates with age, many women in current times, especially in high income countries, more frequently delay having their first child until later (Webster and Schuh 2017).

Detection and management of embryo anomalies that occur due to age, poor quality oocytes or sperm abnormalities, may be accomplished by performing preimplantation genetic testing (PGT) that allows selection and transfer of blastocysts having normal genetic constitution.

The history of PGT goes back to 1989 when A. Handyside performed the first preimplantation genetic diagnostic (PGD) study detecting a Y chromosome-specific region with PCR in a case having X-linked adrenoleukodystrophy and X-linked mental retardation (A. H. Handyside et al. 1990). Now, defining embryo gender is known as sexing and can complement genetic testing of monogenic disorders linked to the sex chromosomes (Coonen et al. 2020).

With time, PGT underwent significant metalogical and approach changes, starting from polar body testing and blastomere analysis to adapting trophectoderm biopsies with subsequent blastocyst freezing (Renwick et al. 2006). In early days, the blastomeres were analyzed using FISH method for chromosomes X, Y, 18, 13, and 21. At that time it was shown that women of advanced age, after PGT, had significantly lower live birth rates as compared with those undergoing IVF without PGT (Maxwell and Grifo 2018). The analysis of more than just single cells led to comprehensive downstream molecular investigations, which resulted in development of the blastocyst stage biopsy strategy (Cimadomo et al. 2016). Molecular genetic testing started as analysis of single loci by the PCR method and grew to become sophisticated single cell whole genome amplifications (Fiorentino 2012). Instead of using PGT, many groups have tried to develop algorithms to detect ploidy based on morphokinetic properties. There have been several attempts to create such algorithms using time-lapse monitoring. The idea was based on the assumption that embryos display different cleavage dynamics depending on their genetic material, but research has not yet fully documented this (Chavez et al. 2012; Vera-Rodriguez et al. 2015).

PGT-A (Preimplantation Genetic Testing for Aneuploidy) is a procedure that allows one to determine the chromosomal status of IVF embryos by screening all 23 pairs of human chromosomes including sex chromosomes. Many different methods are used, which include the array of comparative genomic hybridization (aCGH), quantitative PCR (qPCR), single nucleotide polymorphism array (SNP array) and next-generation sequencing (high and low resolution) (NGS). The difference between those methods is in the quantity of genomic amplification, the ability to detect balanced or unbalanced translocations, partial aneuploidies, polyploidy, and mosaicism. For example, Array CGH, SNP array, and high resolution NGS use whole genome amplification (WGA) of genomic

DNA but at the same time, can introduce an artefact. Quantitative PCR and low resolution NGS are not able to amplify the whole genome and due to their low genomic coverage, small deletions or duplications could not be detected (Maxwell and Grifo 2018).

After the PGT-A embryos can be diagnosed three ways – as euploids with the normal number of chromosomes; as aneuploid with abnormal numbers of chromosomes, and as mosaics – wheree 2 different cell lines are present in the same embryo (often one euploid cell line and one aneuploid cell line). It has been shown that mosaicism rates decrease with extended embryo cultures. This could happen due to the embryos ability to self-correct, or because euploid cell lines predominate at later developmental stages (Santos et al. 2010).

PGT-M is a Pre-implantation genetic testing of embryos for monogenic (or single gene) diseases.

Balanced rearrangements represent one of the most common forms of genetic abnormality affecting approximately 1 in every 500 (0.2%) individuals. Difficulties processing the abnormal chromosomes during meiosis lead to an elevated risk of chromosomally abnormal gametes, resulting in high rates of miscarriage and/or children with congenital abnormalities (Alfarawati et al. 2022). This rearrangement in embryos may occur as a de novo event or can be the product of an abnormal karyotype in one of the parents. Carriers of balanced translocations are most often identified by karyotyping or by genetic analysis of products of conception after an embryonic demise which necessitated the subsequent karyotyping of parents (Sundheimer et al. 2018). Two types of balanced structural chromosomal rearrangements are known- Robertsonian translocations and reciprocal translocations. Robertsonian translocations (ROBs) are chromosomal rearrangements that result from the fusion of the entire long arms of two acrocentric chromosomes. Robertsonian translocations are among the most frequent reorganizations in human genes. Most of the cases analyzed correspond to rearrangements with chromosomes from the D-group (chromosomes 13, 14 and 15), whereas some rare Robertsonian translocations are seldom found in the literature, except those with both chromosomes from the G-group (chromosomes 21 and 22) and those involving chromosomes from both groups (D; G translocations) (Anton, Blanco, and Vidal 2010; Zhao et al. 2015; Alfarawati et al. 2022). Reciprocal translocation is a type of chromosome rearrangement that is involved in the exchange of chromosome segments between two chromosomes that do not belong to the same pair of chromosomes (H. G. Zhang et al. 2016).

ESHRE guidelines indicates the necessity of the genetic screening of the embryos before embryo transfer with different methods (PGT-A, PGT-SR, PGT-M), the scientific review still continues for different group of patients (recurrent implantation failure, recurrent pregnancy loss, for patients with the structural aberrations in their karyotype or rare inherited gene diseases) (Coonen et al. 2020).

2. OBJECTIVES OF THE STUDY

This study focused on patients having recurrent early pregnancy loss and recurrent implantation failure. The **aim** of the work is to assess the ability of different methods and approaches to improve the outcome of the IVF treatment, and to decrease recurrent implantation failure and early pregnancy loss.

The following objectives have been set for achieving this aim:

1. To evaluate the ability of time-lapse microscopy/imaging to improve the outcome of the IVF procedures.
2. To evaluate if endometrial receptivity array and invasive embryo preimplantation genetic testing for aneuploidies used alone or in combination can improve the outcome of IVF treatment in a group of patients with recurrent implantation failure.
3. To assess the utility of preimplantation genetic testing for aneuploidies in IVF procedures in specific group of patients – the carriers of balanced structural chromosomal rearrangements.
4. To clarify, standardize and operationally define non-invasive and invasive methods of embryo selection to decrease recurrent implantation failure rate and to promote the recommendations to other researchers and clinical professionals.

3. RESEARCH HYPOTHESES

1. Embryo selection is a vital step to increase the success of ART treatment. Selection of high implantation potency embryos using both methods-non-invasive Time-lapse microscopy/imaging and invasive preimplantation genetic testing for aneuploidies, can improve the outcome of the treatment.
2. Embryonal and endometrial factors in combination are the likely cause of failed implantation. Applying the combination of methods (Time-lapse microscopy and invasive preimplantation genetic testing together with adjusting implantation window) can reduce the number of recurrent implantation failure and early pregnancy loss.
3. Applying the preimplantation genetic testing for aneuploidy to the patients with structural imbalances in their karyotypes and determining the percentage of normal embryos have a great effect on implantation.

4. SCIENTIFIC VALUE

Novelty of this study: existing studies and publications regarding the different factors that affect outcomes of IVF procedures are controversial and present conflicting evidence. Some studies suggest different methods assessing embryonal or uterine factors to improve the outcome of ART and to decrease implantation failure and early pregnancy loss. Some of the studies do not recommend these methods and question the credibility of this procedure. The purpose of this study is to investigate and evaluate this important issue. Large samples of patients were studied, and different embryonal (morphological, genetical) and uterine methods were used, which are widely known and commonly used in research and clinical practice.

This study is both diverse and comprehensive. It pursues a multivariate approach, where the author in the first uses the combinations of these methods to investigate the main factors involved in recurrent implantation failure and early pregnancy loss.

The study determine which methods and factors are significant and which ones can be recommended in clinical practice for implementation in standard IVF work-up. It also identifies those methods that remain questionable. Based on the results of this study, a proposal is made to develop national guidelines for IVF treatment that will help the reproductive medical society in Latvia and abroad to improve results, of importance both for medical research and for demographical work. At the present time the standardized guidelines from ESHRE are not available.

5. MATERIALS AND METHODS

This is prospective cohort study. It was conducted in the reproductive clinic iVF Riga from January 15, 2014 until December 9, 2021.

Different approaches were used to support the hypotheses of the effect of embryonic and endometrial factors on implantation failure. In the first study (Paper I), the effect of time-lapse imaging on embryo morphokinetics with further influence on implantation outcome was evaluated. In the second study (Paper II), the impact of selecting embryos with the PGT-A method, as well as the impact of endometrial receptivity as a single factor, and in combination with embryonic factors on implantation outcomes was evaluated. The third study (Paper III) compared the PGT-A results of patients with structural imbalances in their karyotypes – to determine the percentage of normal embryos and their effect on implantation.

All patients were informed of the goal and methods of the study before enrolment and signed the informed Consent form before being included in the study. The study conformed to the standards set by the Declaration of Helsinki. The Latvian Central Medical Ethical Committee has approved the study (approval no. 01-29.1.2/1735, available in the Appendix I).

5.1. Patients (Sample Inclusion / Exclusion Criteria)

For the study (The study No. 1) that evaluated the effect of time-lapse imaging of 367 patients. In this part of the study recruited couples received autologous or oocyte donation ICSI cycles. Study group included 897 embryos analyzed using TLM for 146 embryo transfers. Control group included 1723 embryos cultivated/ selected conventionally using traditional static observations method prior to 386 embryo transfers (ETs) (Figure 5.1.1.).

- Inclusion criteria:
  - Patient aged 35–39 years;
  - Patients who had failed to achieve a clinical pregnancy after transfers of at least three good-quality embryos in different single fresh or frozen embryo transfers;
  - IVF cycles only with ICSI procedure;
  - Only single embryo transfers and double embryo transfer;
  - Only good quality embryos were transferred, *i.e.* those that had the correct number of cells corresponding to the day of development – the day-5 embryos (blastocysts).
- Exclusion criteria:
  - Man with obstructive azoospermia and oligospermia;

- Patients with abnormal karyotypes (translocations, inversions), or severe endocrine disorders, or atrophic endometrium;
- Embryo transfers with three embryos.

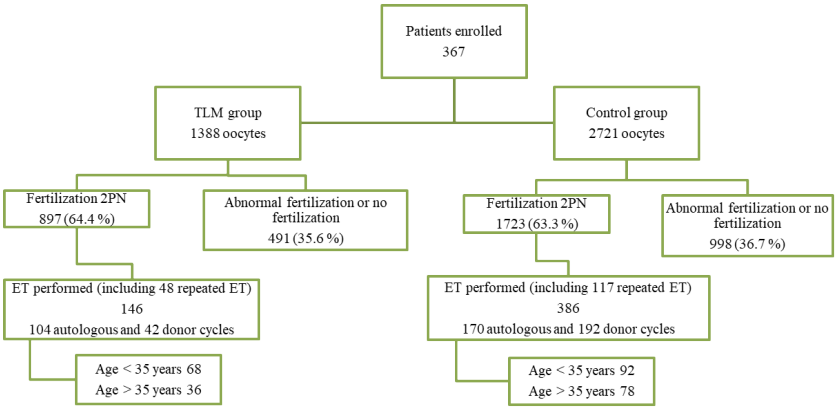


Figure 5.1.1. **Patients enrolled for the Time-lapse microscopy or imaging study (The study No. 1)**

For the study (The study No. 2) that evaluated the effect of selecting embryos with the PGT-A and endometrial receptivity in total 253 cycles of the assisted reproduction by means of ICSI were included, and further randomly divided into 4 groups: Group I – frozen embryo transfers without any additional tests or procedures (FET), $n = 72$ cycles; Group II – FET with PGT-A, $n = 87$; Group III – FET with PGT-A and ERA, $n = 72$; Group IV – FET with ERA, $n = 22$ (Figure 5.1.2.).

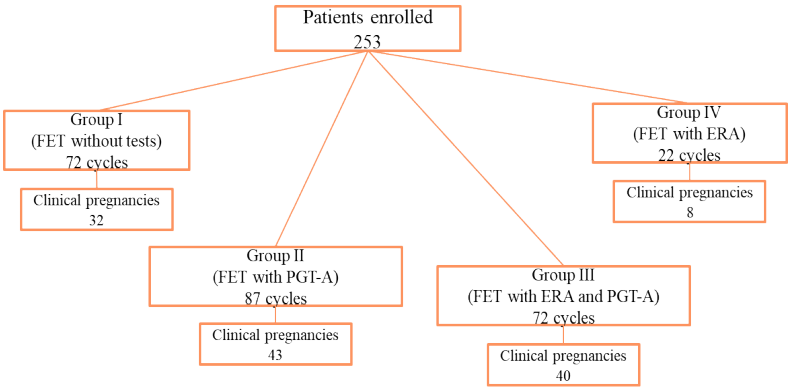


Figure 5.1.2. **Patients enrolled for the preimplantation genetic testing for aneuploidies and endometrial receptivity array in a group of recurrent implantation failure in the intra-cytoplasmic sperm injection cycles (The study No. 2)**

- Inclusion criteria: Patients aged 34–36 with at least one previous implantation failure in their medical history.
- Exclusion criteria:
  - Patients with poor ovarian reserve (less than 5 oocytes);
  - Abnormalities in the endometrium such as inflammation, Asherman's syndrome, organic changes, polyps, and submucosal myomas;
  - Patients with aberrations in the karyotype.

For the study (The study No. 3) about the patients with structural imbalances, from the 36 patients with changes in karyotypes that were found in iVF Riga clinic from period 2014–2018, 10 patients undergoing IVF with PGT-A were eligible for the study (Figure 5.1.3.). Three patients were with the most frequent type of Robertsonian translocation from the D group (between 13 and 14 chromosomes), and 7 patients with various reciprocal translocations. As noted above: 180 oocytes were collected. 140 fertilized normally. 90 of these 140 embryos were frozen. Of these 90, 69 were biopsied, and of these 69, 48 were analyzed with PGT-A.

- Inclusion criteria: patients with structural imbalances.

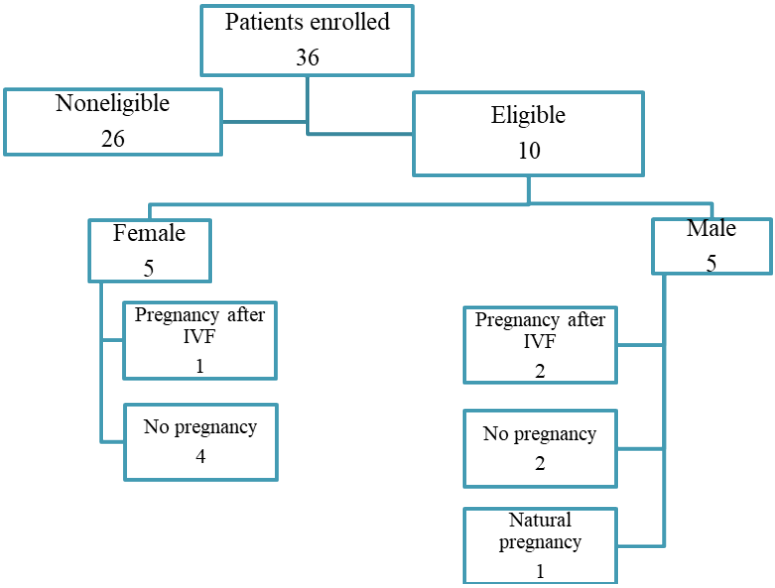


Figure 5.1.3. **Enrolled patients for the "The application of preimplantation genetic testing for aneuploidies for carriers of balanced structural chromosomal rearrangements" (The study No. 3)**

5.2. Time-lapse microscopy or imaging

In this part of the study, 367 couples participated, receiving treatment involving autologous or oocyte donation ICSI cycles. Study group included 897 embryos analyzed using TLM (EmbryoScope, Vitrolife) for 146 embryo transfers.

Control group included 1723 embryos cultivated/selected conventionally using traditional static observations method before 386 embryo transfers (ETs).

Embryos were classified in four groups: complete trophectoderm and high cell number compact inner cell mass – group A; incomplete trophectoderm and several grouped cells – group B; few cells in trophectoderm or inner cell mass – group C, sparse distribution of large or flat or degenerate cells in trophectoderm and no cells or degenerate or necrotic cells in ICM – group D. Top embryos were selected for day 3 ET – control group only. Human blastocysts with trophectoderm cells and inner cell mass in A or B groups were selected for the embryo transfer or vitrification on day 5. Embryo evaluation and selection with morphokinetic method was performed by analysis of time-lapse images of each embryo on an external computer with software developed for time-lapse image analysis (EmbryoViewer workstation; Unisense Fertilitech A/S, Aarhus, Denmark). The Meseguer classification was used (Marcos Meseguer et al. 2012) to describe morphokinetic classification depending on embryo cell division timing: time of cleavage to two-blastomere embryo (t2), time of cleavage to three-blastomere embryo (t3), time of cleavage to four-blastomere embryo (t4), and time of cleavage to five-blastomere embryo (t5). Additionally, the duration of the second cell cycle (cc2) – that is, the duration of the two-blastomere embryo phase (t3–t2) – and synchrony (s2) in divisions from a two-blastomere embryo to a four-blastomere embryo (t4–t3) were calculated.

5.3. Preimplantation genetic testing for aneuploidies (PGT-A) and endometrial receptivity array (ERA) in a group of RIF in intra-cytoplasmic sperm injection (ICSI) cycles

In this study, the ICSI results were evaluated from couples with RIF. The patients who failed to achieve a clinical pregnancy after transfers of at least three good-quality embryos in different single fresh or frozen embryo transfers were considered RIF. Patients with an abnormal karyotype (translocations, inversions), with severe endocrine disorders, with atrophic endometrium were not included in the study.

5.3.1. Whole Genome Amplification (WGA) and chromosomal analysis – preimplantation genetic testing for aneuploidies

PGT-A was performed as described elsewhere (C. Rubio et al. 2013). Sureplex reagent kit (Illumina) was used for WGA of trophectodermal cells.

Electrophoresis and DNA quantification with Qubit was followed to evaluate the success of amplification. PGT-A was done either by aCGH (array Comparative Genomic Hybridization) with Illumina 24Sure arrays from 2015 to 2017 or Illumina VeriSeq PGS library preparation kit from 2018 to 2019 due to methodology change (Figure 5.3.1.). Both methods allow to detect imbalanced aberrations of autosomes 1–22 and X, Y sex chromosomes in preimplantation embryos, but due to technical differences patients were divided in groups after methods used – aCGH or NGS. Low level mosaicism, uniparental disomy, 69XXX variant and aberrations under 20 Mb cannot be determined. Copy number variations (CNV) analysis in both cases was performed with Bluefuse software. At least two molecular geneticists and at least one clinical geneticist interpreted the results. Chromosomal aberrations were classified according to guidelines: euploid, low level mosaic (20–50%), high level mosaic (50–80%), aneuploid. Decision regarding transfer was made according to chromosomes involved and type of aberration (whole chromosome versus partial). Embryos with aneuploidy were not transferred.

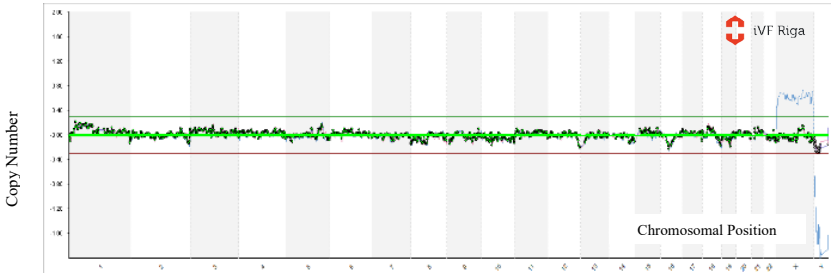


Figure 5.3.1. **Figure 5.3.1. Preimplantation genetic testing for aneuploidies with NGS.** Image from iVF Riga archive

5.3.2. Endometrial Receptivity Array (ERA)

Endometrial biopsies were collected from the uterine fundus following the manufacturer's protocol (Díaz-Gimeno et al. 2011), and samples were sent for the analysis by iGenomix. Endometrium were classified by expression profile as receptive, pre- or post-receptive based on transcriptome signature (Díaz-Gimeno et al. 2011).

5.4. The application of PGT-A for carriers of balanced structural chromosomal rearrangements

The most frequent type of Robertsonian translocation is presented in figure 5.4.1.

Whole Genome Amplification (WGA) and chromosomal analysis – pre-implantation genetic testing for aneuploidies was performed as described above. Parental karyotyping was performed using classical G band cytogenetic approach. Peripheral blood cells (lymphocytes) were cultivated for 72 h in the PB-MAX™ Karyotyping Medium. The colcemide solution was added and put to a thermostat to stop the cell division. Fixative, consisting of methanol and acetic acid, was added to each sample of cells. Processed cells in their metaphase state were fixated on the glass slide with chromosome staining using Giemsa stain. At least 15 metaphases analyzed for each patient with a microscope using Lucia Kario software program. Karyotype was analyzed based on the International System for Human Cytogenomic Nomenclature (ISCN 2016) criteria.

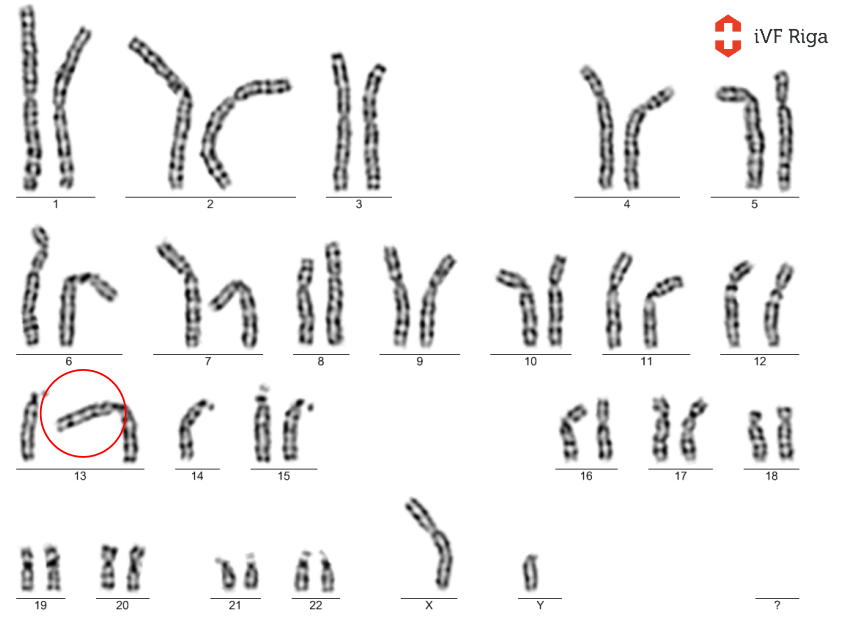


Figure 5.4.1. **Karyotype with Robertsonian translocation.**
Image from iVF Riga archive

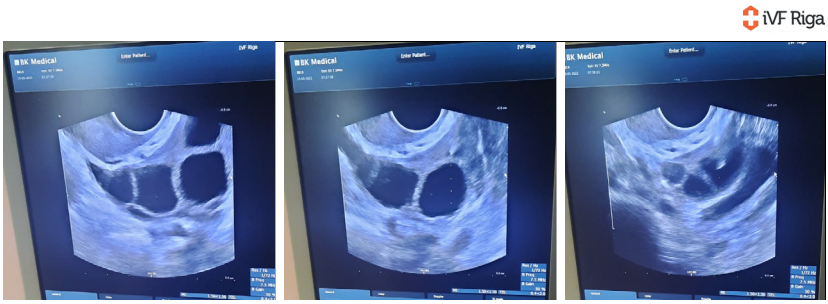
5.5. Common protocols for all the parts of the study

5.5.1. Ovarian stimulation

The long or short controlled ovarian stimulation protocols were used for both autologous and donor cycles with recombinant follicle stimulating hormone (rFSH). Controlled ovarian stimulation was performed using recombinant follicle-stimulating hormone (Follitropin Alfa) and gonadotropin-releasing hormone antagonist (Ganirelix acetate injection or Cetrorelix acetate). All the dosages were used considering ovarian reserve and anti-mullerian hormone values in patients' medical histories. When the lead follicle reached its mean diameter 18–20 mm, 6500 IU human chorionic gonadotropin (hCG) agonist were injected subcutaneously for ovulation induction.

5.5.2. Oocyte retrieval

Oocyte retrieval was performed 35–36 h after hCG injection. Follicles were aspirated (Figure 5.5.1.), and oocytes were washed in mHTF washing medium (Life Global, USA) with human serum albumin supplement. After washing, oocytes were cultured in fertilization medium with human serum albumin supplement (Life Global, USA) at 5.5% CO_2 and 37 °C (Planer benchtop BT-37 incubator) for 2 h before oocyte denudation. Oocyte denudation was performed by mechanical pipetting 40 IU/ml hyaluronidase in the same medium and incubated at 5.5% CO_2 and 37 °C (Planer benchtop BT-37 incubator) for 2 h before intracitoplasmic sperm injection.



Ovary with follicles before the aspiration | Aspiration of the first follicle | Aspiration of the second follicle

Figure 5.5.1. **Oocyte retrieval manipulation.** Images from iVF Riga archive

5.5.3. Intracitoplasmic sperm injection (ICSI)

ICSI was performed in a medium with human serum albumin supplement (Life Global, USA) at 200 x magnification using Nicon Ts-i microscope.

Immediately after ICSI, the injected oocytes for time-lapse microscopy cycles were placed individually in pre-equilibrated culture dishes (EmbryoSlide;

Unisense Fertilitech A/S) with 25 µl medium supplemented with human serum albumin (Life Global, USA) under oil (Lite Oil, Life Global) at 37 °C and 5% O_2, 6% CO_2 in a time-lapse incubator (EmbryoScope). Zygotes for conventional incubator (Planer benchtop BT-37) cycles were placed in normal Petri dishes (Nunc, Roskilde, Denmark) containing culture media (Life Global, USA) with human serum albumin supplement under oil (Lite Oil, Life Global) at 37 °C and 5% O_2, 6% CO_2.

5.5.4. Embryo cultivation

All embryos were incubated at 37 °C and 5% O_2, 6% CO_2 and were cultured individually (Figure 5.5.2.) until embryo transfer or embryo biopsy and freezing.

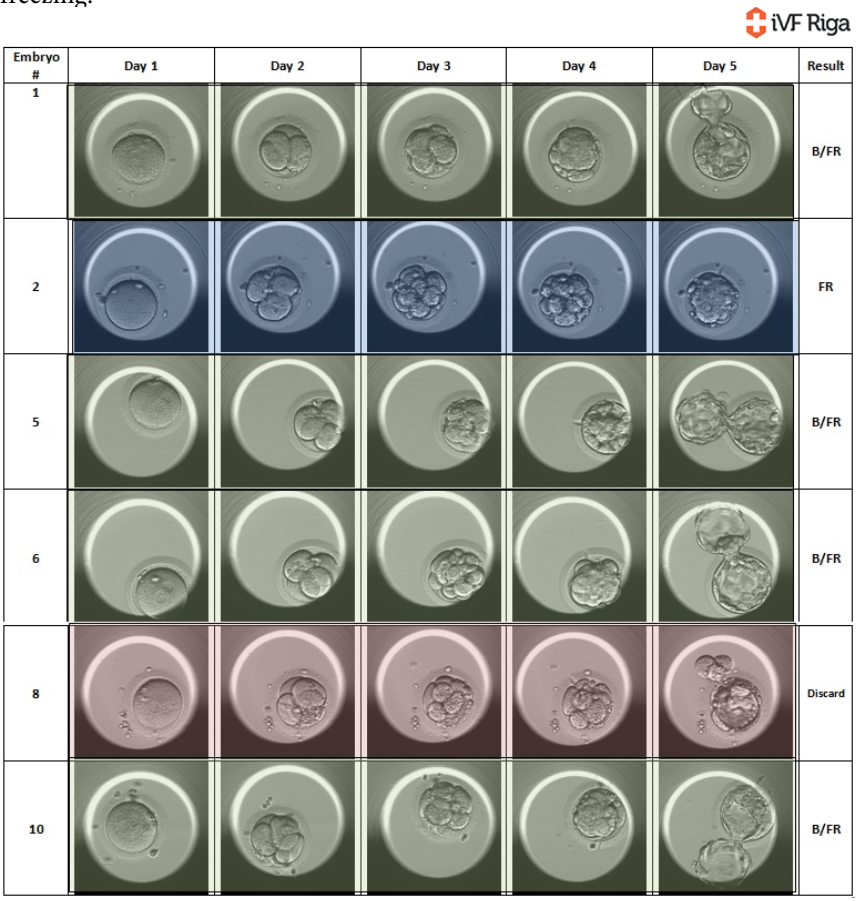


Figure 5.5.2. **Time-lapse view of embryos development by days.**
Images from iVF Riga archive

5.6. Outcome assessments

The main end-points were divided into:

A) no pregnancy – no symptoms or signs of pregnancy ("How To Be Reasonably Certain That a Woman Is Not Pregnant" 2016);
B) biochemical pregnancy (defined as the occurrence of a low peak in β-hCG (< 100 mIU/mL), a rapid decline in urinary or serum β-hCG concentration, and the absence of a significant delay in the onset of the next menstrual period (Annan et al. 2013) (not proceeding further to the clinical pregnancy);
C) clinical pregnancy (diagnosed by an increasing level of hCG and by ultrasonography of at least one fetus with the presence of heart beat (Zegers-Hochschild et al. 2009));
D) pregnancy loss (Spontaneous pregnancy demise before 10 weeks of gestational age or before 8th developmental week (Kirk et al. 2020).

5.7. Statistical Analysis

Descriptive statistics are presented using medians, interquartile ranges and percentages. Differences between groups were assessed using Chi-square tests and Fisher's exact test. Multivariate regression analysis was used to assess the results for the covariates. Potential covariates tested included the age of the female, the number of acquired oocytes after the pick-up procedure, and the number of acquired blastocysts.

In the multivariate regression analysis, the results of Groups II–IV were compared with the "reference" Group I (FET without any additional genetic tests). To compare the results of the ICSI cycles, the end-points b), c) and d) were compared with an end-point a) – no pregnancy: in order to assess the ability of each approach to achieve better clinical outcomes as compared to the reference group FET approach, and as compared with the negative outcome (no pregnancy).

Two-tailed, χ^2 tests were used, $p < 0.05$ was the significance level. Statistical analyses were performed using SPSS 20.0 (SPSS Inc., Chicago, IL, USA).

6. RESULTS

6.1. Time lapse microscopy (TLM) or imaging study

In the study of time-lapse based morphokinetic (TLM) evaluation of the embryonic development, selection of high implantation potency embryos was found to improve treatment outcomes, and to decrease EPL and multiple pregnancy rate when implementing selective single embryo transfers (SET).

Three hundred and sixty-seven female patients participated in the study. The TLM group comprised 98 patients. The control group included 269 patients (Figure 6.1.1.).

The median age of control group patients was 39. It was 35 years for the TML group, which is significantly lower ($p < 0.0001$).

Data on sperm, oocyte and embryo characteristics per ET are summarized in Table 6.1.1.

Table 6.1.1. **Sperm, oocyte, and embryo characteristics per ET**

Parameters	TLM		Control		*p* value
	%	*n*	%	*n*	
ET from autologous oocytes	71.2	104	44.3	170	< 0.0001
Autologous oocytes		10.7		7.4	< 0.0001
Donor oocytes		13.6		11.8	0.07
Fertilization rate (2PN)	64.4	897/1388	63.3	1723/2721	0.41
2 PN		7.4		6.2	0.004
ET day		4.7±0.7		4.4±0.9	
ET day 3	13		22.3		0.002
ET day 5	81.5		71.5		0.054
Embryo per ET		1.7±0.5		1.7±0.5	
SET	33.6		34.7		0.8
DET	66.4		65.3		
Sperm quality					
Concentration > 18	84.9	124	86	332	0.75
Concentration < 18					
18–10	68.2	15	33.3	18	0.005
10–5	13.6	3	35.2	19	0.06
> 5	13.6	3	29.6	16	0.15
DNS fragmentation		33		13	
35–50	33.3	11	53.8	7	0.21
> 50	15.2	5	0	0	0.14
ET difficult	8.3	12	10.9	42	0.38

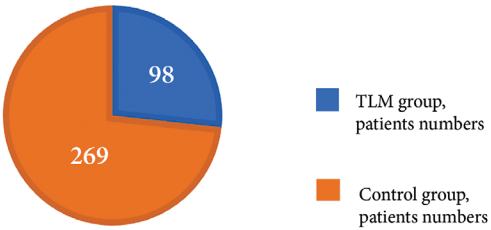


Figure 6.1.1. **Patient distribution in the Time lapse microscopy (TLM) study**

ETs resulting from autologous oocyte were more common in the TLM group than in the control group (71% versus 44.3%, $p < 0.0001$). In the TLM group more oocytes were retrieved per transfer (10.7 versus 7.4, $p < 0.0001$). There was no significant difference in retrieved donor oocytes (13.6 versus 11.8, $p = 0.07$) (Figure 6.1.2.).

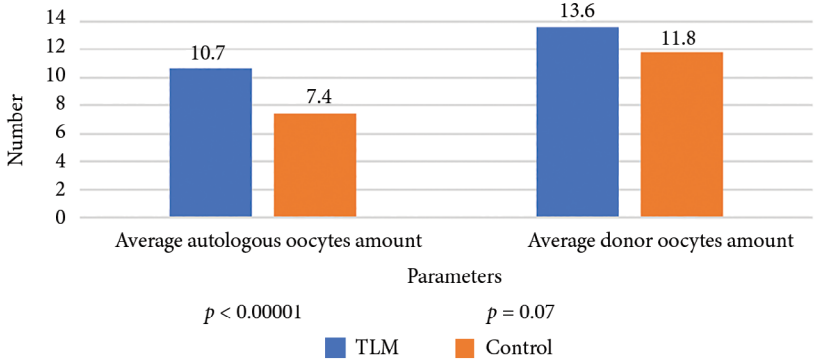


Figure 6.1.2. **TLM group and control group oocytes characteristics**

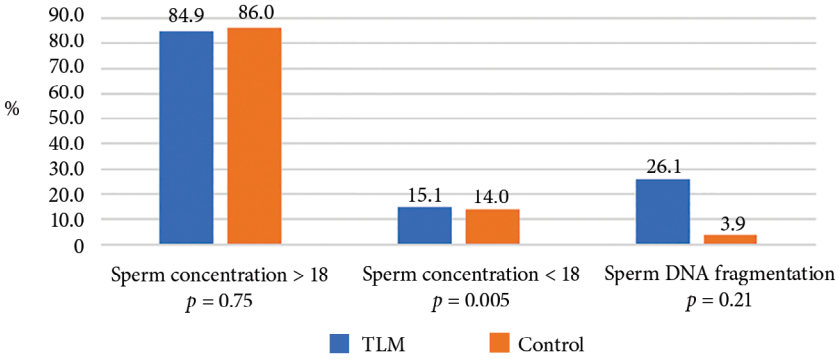


Figure 6.1.3. **Characteristics of the sperm quality in TLM group and control group**

Sperm used for fertilisation was good quality. There were no statistically significant differences in the sperm concentration (Figure 6.1.3.).

There was no significant difference in the fertilization rates (64.4% versus 63.3%, $p = 0.41$). In the TLM group however, there were 7.4 fertilized oocytes (with 2 pronucleus: 2 PN) per transfer compared to 6.2 in control group ($p = 0.004$) (Figure 6.1.4.).

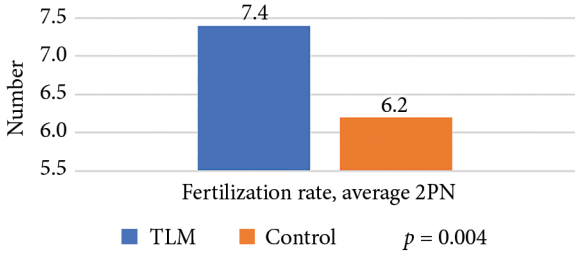


Figure 6.1.4. **TLM group and control group oocytes fertilization rates**

In both groups blastocysts were transferred more often than day-3 embryos. DET (double ET) were performed on 66.4% of the TLM group and 65.3% of the control group, but SET (single ET), found in 33.6% and 34.7%, is not statistically significant (Figure 6.1.5. and in the Table 6.1.1.). The biochemical pregnancy rate per ET in the TLM group was 50.7% versus 50.3% for the control group ($p = 0.93$, OR = 1.02, 95% CI = 0.7–1.49).

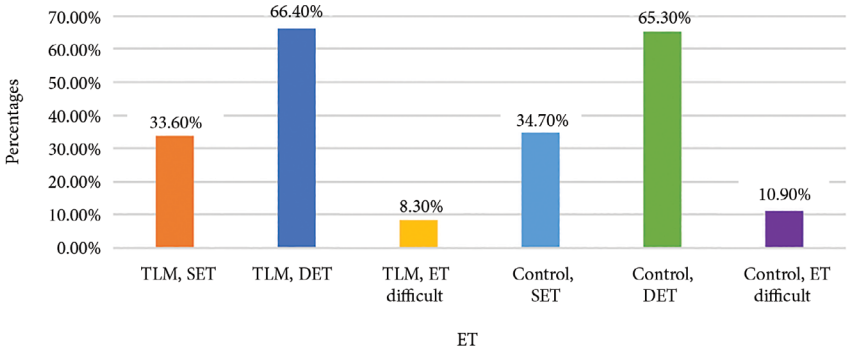


Figure 6.1.5. **Transfer characteristics in TLM group and control group**

The clinical pregnancy rate per ET were both increased in the TLM group (Table 6.1.2).

Table 6.1.2. **Outcome measures**

Parameters	TLM		Control		*p* value	Odds ratio	95% CI
	%	*n*	%	*N*			
ET		146		386			
Biochemical pregnancy per ET	50.7	74	50.3	194	0.93	1.02	0.70–1.49
Clinical pregnancy per ET	41.1	60	36.6	141	0.33	1.21	0.82–1.79
Ongoing pregnancy per ET	36.3	53	29.8	114	0.13	1.36	0.91–2.03
Early clinical pregnancy loss (of all clinical pregnancies)	12.1	7	20.7	27	0.23	0.58	0.24–1.42
Biochemical loss (of all biochemical pregnancy)	18.9	14	27.5	54	0.14	0.61	0.31–1.17
Early pregnancy loss (biochemical and clinical loss)	28.4	21	41.8	81	0.045	0.55	0.31–0.99
All transferred embryos		243		638			
Implantation rate (of all transferred embryos)	31.7	77	28.1	179	0.31	1.19	0.86–1.64
Early pregnancy loss (of all implanted embryos)	9.1	7	19.1	35	0.043	0.41	0.17–0.97
Autologous cycles younger than 35 years							
ET		68		92			
Biochemical pregnancy per ET	54.4	37	47.8	44	0.41	1.3	0.69–2.44
Clinical pregnancy per ET	39.7	27	38	35	0.83	1.07	0.56–2.04
Ongoing pregnancy per ET	39.7	27	31.5	29	0.28	1.43	0.74–2.76
Early clinical pregnancy loss (of all clinical pregnancies)	0	0	17.1	6	0.03	0.53	0.41–0.68
Biochemical loss (of all biochemical pregnancy)	27	10	20.5	9	0.49	1.44	0.51–4.04
Early pregnancy loss (biochemical and clinical loss)	27	10	34.1	15	0.5	0.72	0.28–1.86
All transferred embryos		110		161			
Implantation rate (of all transferred embryos)	35.5	39	29.8	48	0.33	1.29	0.77–2.17
Early pregnancy loss (of all implanted embryos)	0	0	25	12	0.001	0.48	0.38–0.61

Table 6.1.2. **Outcome measures. Continued**

Parameters	TLM		Control		*p* value	Odds ratio	95% CI
	%	*n*	%	*N*			
Over 35 years, only with autologous oocytes							
ET		36		78			
Biochemical pregnancy per ET	33.3	12	30.8	24	0.79	1.13	0.48–2.62
Clinical pregnancy per ET	27.8	10	21.8	17	0.49	1.38	0.56–3.42
Ongoing pregnancy per ET	25	9	15.4	12	0.22	1.83	0.69–4.85
Early clinical pregnancy loss (of all clinical pregnancies)	10	1	29.4	5	0.24	0.27	0.03–2.70
Biochemical loss (of all biochemical pregnancy)	16.7	2	29.2	7	0.41	0.49	0.08–2.81
Early pregnancy loss (biochemical and clinical loss)	25	3	50	12	0.15	0.33	0.07–1.54
All transferred embryos		63		121			
Implantation rate (of all transferred embryos)	15.8	10	14	17	0.75	1.15	0.49–2.70
Early pregnancy loss (of all implanted embryos)	10	1	29.4	5	0.24	0.27	0.03–2.70

The clinical pregnancy rate per ET was 41.1% for the TLM group versus control group 36.6% (OR = 1.21, 95% CI = 0.82–1.79, $p = 0.33$), not significantly different. The ongoing pregnancy rate per ET in TLM group was 36.3% versus the control group 29.8% (OR = 1.36, 95% CI = 0.91–2.03, $p = 0.13$). The biochemical (TLM group 18.9% versus control group 27.5% OR = 0.61, 95% CI = 0.31–1.17, $p = 0.14$) and the clinical pregnancy loss rates (TLM group 12.1% versus control group 20.7% OR = 0.58, 95% CI = 0.24–1.42, $p = 0.045$) rates were significantly reduced in the TLM group.

The total EPL rate in the TLM group was significantly reduced (TLM 28.4% versus control 41.8% OR = 0.55, 95% CI = 0.31–0.99, $p = 0.043$) (Figure 6.1.6.).

The implantation rates per ET in both groups were not significantly different (TLM group 31.7% versus control group 28.1% OR = 1.19, 95% CI = 0.86–1.64, $p = 0.31$), but EPL rate per ET was significantly reduced in the TLM group (TLM 9.1% versus control 19.1% OR = 0.41, 95% CI = 0.17–0.97, $p = 0.043$).

To reduce the maternal age influence on EPL, patients in both the groups were divided into two sub-groups: younger than 35 years and 35 years and older (autologous cycles only).

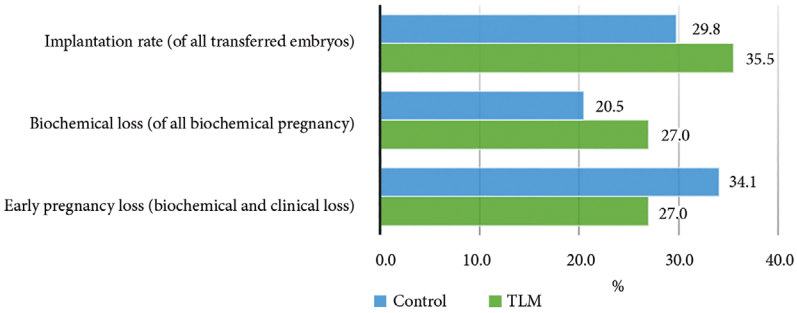


Figure 6.1.6. **Implantation and EPL rates in TLM and control group**

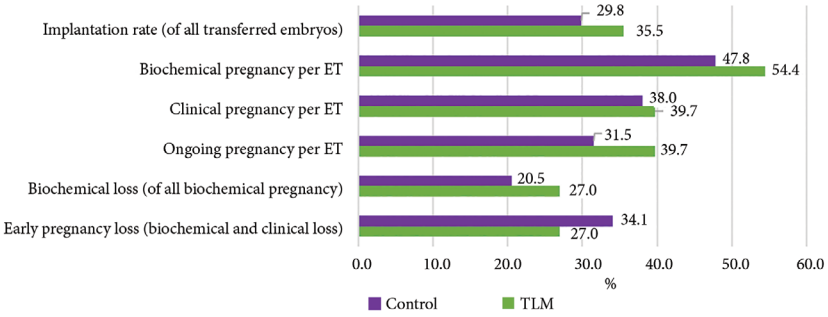


Figure 6.1.7. **Autologous cycles patients younger than 35 years implantation, biochemical and clinical pregnancy rates and EPL rate in the TLM group and control group**

In autologous cycles the patients younger than 35 years became pregnant biochemically and clinically with no statistically significant difference, but the ongoing pregnancy rate was increased in the TLM group (TLM 39.7% versus control 31.5% OR = 1.43, 95% CI = 0.74–2.76, p = 0.28) (Figure 6.1.7.). It should be noted that only biochemical pregnancy loss was documented in the TLM group (TLM 27% versus control 20.5% OR = 1.44, 95% CI = 0.51–4.04, p = 0.49), but clinical pregnancy loss was not observed in the TLM group (TLM 0% versus control 17.1% OR = 0.53, 95% CI = 0.41–0.68, p = 0.03).

The implantation rate per ET was 35.5% for the TLM group and 39.8% for the control group (OR = 1.29, 95% CI = 0.77–2.17, p = 0.33), which is not statistically significant, but EPL per ET was greatly decreased in the TLM group (TLM 0% versus control 25% OR = 0.48, 95% CI = 0.38–0.61, p = 0.001).

Autologous cycle patients older than 35 years had decreased outcome measures:

- biochemical pregnancy rate (TLM group 33.3% versus control group 30.8% OR = 1.13, 95% CI = 0.48–2.62, p = 0.79);
- clinical pregnancy rate (TLM group 27.8% versus control group 21.8% OR = 1.38, 95% CI = 0.56–3.42, p = 0.49);
- ongoing pregnancy rate (TLM group 25% versus control group 15.4% OR = 1.83, 95% CI = 0.69–4.85, p = 0.22). Biochemical, clinical pregnancy, implantation and EPL rates per ET were not significantly different. Main outcome measures are summarized in Figure 6.1.8.

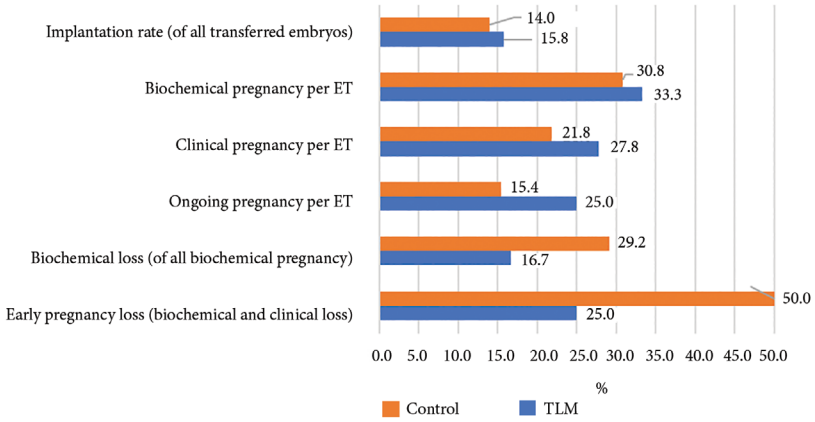


Figure 6.1.8. **Autologous cycles patients older than 35 years implantation, biochemical and clinical pregnancy rates and EPL rate in the TLM group and control group**

Table 6.1.3. **Logistic regression analysis adjusted by age and autologous oocytes**

Parameters	***p* value**	**Odds ratio**	**95% CI**
Per ET			
Biochemical pregnancy	0.6	1.12	0.74–1.68
Clinical pregnancy	0.18	1.33	0.88–2.01
Ongoing pregnancy	0.11	1.42	0.93–2.17
Per cycle			
Early clinical pregnancy loss	0.39	0.67	0.27–1.68
Biochemical pregnancy loss	0.11	0.57	0.29–1.14
Early pregnancy loss (biochemical and clinical pregnancy)	0.07	0.57	0.31–1.04
Per ET			
Implantation rate	0.31	1.19	0.85–1.66
Early pregnancy loss	0.044	0.41	0.17–0.98

Logistic regression analysis was done for the TLM group and control group, and, adjusted for age and autologous oocyte rates. There were no significant differences in biochemical, clinical, and ongoing pregnancy rates per ET. However, the EPL rate per ET was significantly reduced: OR = 0.41, 95% CI = 0.17–0.98, $p = 0.044$ (Table 6.1.3).

6.2. Preimplantation genetic testing for aneuploidies (PGT-A) and endometrial receptivity array (ERA) in a group of RIF in ICSI cycles

The present research suggests that RIF depends on several factors. Successful embryo implantation is an interactive process between the blastocyst and the uterus. Synchronized development of an embryo with uterine differentiation to a receptive state is a prerequisite for attaining pregnancy. The main reasons for implantation failure are summarized in this study.

Table 6.2.1. **Descriptive statistics for the study groups. (Group I – cycles with FET; Group II – cycles with FET+PGT-A; Group III – cycles with FET+PGT-A+ERA test; Group IV – cycles with FET+ERA test)**

Variable	Group I	Group II	Group III	Group IV	*p*
Age (median, IQR)	34.0 (37.0–32.0)	36.0 (38.0–34.0)	35.0 (37.0–33.0)	34.0 (38.0–32.5)	0.21
Acquired oocytes (median, IQR)	13.0 (16.0–10.0)	13.0 (17.0–9.0)	11.5 (18.0–8.0)	11.5 (17.5–6.8)	0.83
Acquired blastocysts (median, IQR)	5.0 (7.0–3.0)	7.0 (9.0–3.0)	5.0 (7.0–3.0)	4.0 (5.5–3.0)	**0.02**

The median age of the entire study group for the females was 35 years (Figure 6.2.1.). Female age and acquired oocytes count showed no differences between the all Groups I–IV. Table 6.2.1 shows the all statistics for this study cohort.

The counts of acquired blastocysts were higher for Group II, compared both to the Group I ($p = 0.03$) and Group IV ($p = 0.004$) (Table 6.2.2.). All these factors were used as covariates in multivariate regression analysis.

Table 6.2.3. shows the clinical outcomes (no pregnancy, biochemical pregnancy, clinical pregnancy, and pregnancy loss) in Groups I–IV. Group I – cycles with FET; Group II – cycles with FET+PGT-A; Group III – cycles with FET+PGT-A+ERA test; Group IV – cycles with FET+ERA test.

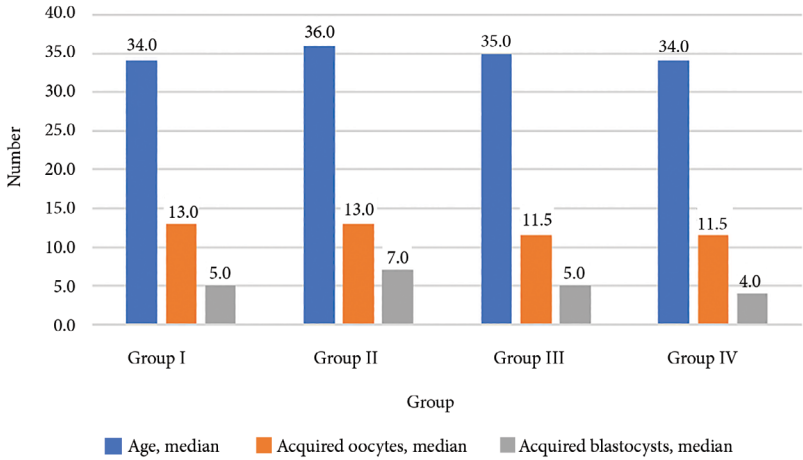


Figure 6.2.1. **Descriptive statistics for the study groups (Group I – cycles with FET; Group II – cycles with FET+PGT-A; Group III – cycles with FET+PGT-A+ERA test; Group IV – cycles with FET+ERA test)**

Table 6.2.2. **Comparative analysis (p values) for counts of acquired blastocysts between the Groups I–IV**

	Group I	Group II	Group III	Group IV
Group I		**0.03**[1]	0.52	0.13
Group II			0.11	**0.004**[1]
Group III				0.07
Group IV				

Table 6.2.3. **Clinical outcomes in Groups I–IV**

Parameters Group I		Group				Total
		Group II	Group III	Group IV		
No pregnancy	Number	35	19	30	11	95
	%	48.6	28.4	41.7	50.0	40.8
Biochemical pregnancy	Number	4	12	1	1	18
	%	5.6	17.9	1.4	4.5	7.7
Clinical pregnancy	Number	32	33	40	8	113
	%	44.4	49.3	55.6	36.4	48.5
Pregnancy loss	Number	1	3	1	2	7
	%	1.4	4.5	1.4	9.1	3.0
Total	Number	72	67	72	22	253

[1] Statistically significant.

Analysis with chi-square test of the clinical outcomes of biochemical pregnancy, clinical pregnancy and pregnancy loss to compare with the end-point of "no pregnancy", Group II (FET+PGT-A) showed a statistically significant higher chance of achieving both biochemical (p = 0.01, OR = 5.5) and clinical pregnancy (p = 0.049, OR = 2.3), as compared with the Group I (FET with no additional tests).

The more detailed analysis of the results by the Fisher's exact test was done to compare the Group II (FET+PGT-A) and the Group III (FET+PGT-A+ERA) biochemical pregnancy results, which showed that ERA test statistically significantly decreases biochemical pregnancy loss (Table 6.2.4.).

Table 6.2.4. **Statistical analysis of the biochemical pregnancy results in Groups I–IV**

	Group I	Group II	Group III	Group IV
Group I		**0.005**[1]	0.37	0.99
Group II			**0.001**[1]	0.07
Group III				0.49
Group IV				

The clinical pregnancy was statistically significant in the Group III (FET + PGT-A + ERA) comparing to the Group II (FET + PGT-A) (Table 6.2.5.).

Table 6.2.5. **Statistical analysis of the clinical pregnancy results in Groups I–IV**

	Group I	Group II	Group III	Group IV
Group I		**0.08**[2]	0.18	0.99
Group II			**0.002**[1]	0.43
Group III				0.33
Group IV				

6.3. The application of PGT-A for carriers of balanced structural chromosomal rearrangements

Balanced rearrangements represent one of the most common forms of genetic abnormality. This study examined 10 couples who had abnormalities in their karyotypes and previous histories of reproductive results. One of the main factors of recurrent pregnancy loss and implantation failure is aneuploid embryos. The study shows that expectancy of total embryo aneuploidy rates is higher in those carriers, than in people with normal karyotypes (Table 6.3.1.).

A total of 180 oocytes were collected. 140 (77.8%) fertilized normally, resulting in 90 (64%) of the embryos reaching the maturation stage on day 5.

[2] Statistically significant.

Table 6.3.1. **All the patient's pregnancy history with current results**

Pair No.	Karyotype	Number of euploid embryos	Number of aneuploid embryos	Results
1	46,XX,t(1;6) (p22;p25) 46,XY	1	3	Pregnancy did not occur
2	46,XX 46, XY,t(4;12) (q31;q13)	2	2	Natural pregnancy
3	46,XX 45,XY,der(13;14) (q10;q10)	4	2	Pregnant after IVF
4	46,XX,t(9;17) (p10;p10) 46,XY	1	2	1 child after IVF
5	46,XX,t(6;10) (p21.3;q22.3) 46,XY	0	1	Needs new stimulation
6	46,XX 45,XY,der(13;14) (q10;q10)	0	2	Needs new stimulation
7	46,XX 46,XY,t(1;3) (q12;q29) [4]/46,XY[15]	2	2	Pregnant after IVF
8	46,XX,t(13;20) (q34q13) 46,XY	0	6	Needs new stimulation
9	46,XX,t(8;11) (p23;p15) 46, XY	0	4	Needs new stimulation
10	46,XX 45,XY,der(13;14) (q10;q10)	0	4	Needs new stimulation

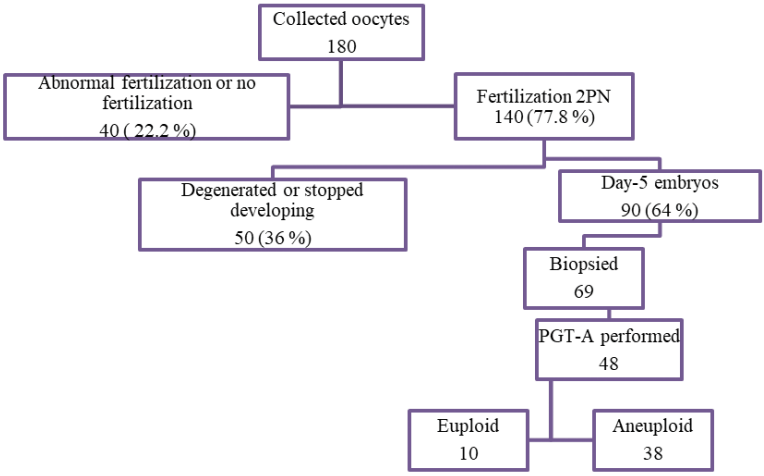


Figure 6.3.1. **Diagram of collected oocytes and embryo biopsy with current results**

A biopsy was performed on 69 blastocysts and PGT-A was done for biopsies from 48 embryos. The total abnormality rate from the 48 embryos was 79.2% (including 10 – euploid, 38 – having chromosomal structural aberrations or aneuploidies) (Figure 6.3.1.).

Twenty-two embryos were analyzed from the carriers of Robertsonian translocations.

Of these 22, the rate of unbalanced translocations was 81.8%, whereas from the reciprocal translocation carrier group, the unbalanced translocation rate of embryos was 76.9%.

Unbalanced translocation rates in embryos resulting from woman were higher than those from men, 88.9% versus 73.3%. This study involved 18 embryos from 5 couples where the female was a carrier of balanced translocation and 30 embryos from 5 couples where the male was carrier.

The women ranged from 31–36 years of age with a median age of 34 and the men ranged 28–46 years with a median age of 39.

The highest euploidy rate was in the male carrier group – 26.7%, and the lowest was in the Robertsonian translocation carrier group – 18.2%. Sporadic aneuploidy of 68.2% was also highest in the Robertsonian translocation carrier group, and lowest in the female carrier group – 11.1%.

The unbalanced translocation rate in embryos was highest in the female carrier group – 77.8%, and lowest in Robertsonian group – 13.6%.

One female patient with a reciprocal translocation between 13 and 20 chromosomes had unbalanced chromosomal translocations inherited from her mother in all six embryos tested. One male patient with type D Robertsonian translocation between 13 and 14 chromosomes had aneuploidy in 12 embryos from 16 that were tested.

After PGT-A results were obtained, 5 of 10 female patients needed new stimulation as PGT-A showed 0 euploid embryos. In 1 female patient with reciprocal translocation, pregnancy did not occur after FET. One patient, whose husband was a reciprocal translocation carrier, conceived naturally. Two patients having husbands being Robertsonian and reciprocal translocation carriers, got pregnant after iVF treatment. One child was born from a woman carrier of reciprocal translocation (Detailed information is available in the Table 6.2.3. in the Appendix II).

7. DISCUSSION

Infertility is a widespread problem that affects many couples worldwide. It has been estimated that up to 15% of couples experience infertility. It can be caused by many different factors, including problems with ovulation, sperm quality, or the reproductive system itself. One of the most common causes of infertility is a recurrent implantation failure, which occurs when embryos fail to implant in the uterus after transfer. Recurrent implantation failure is an important challenge in assisted reproduction, as it significantly reduces the chances of a successful pregnancy. In this thesis, strategies and techniques are discussed that can be used to decrease the incidence of early pregnancy loss and increase the chances of successful pregnancy in patients with infertility. The research focused on three studies that investigated the use of different methods and combination of them to reach the result in the fastest way. The idea of investigation of different levels (embryonic and endometrial) of the IVF processes may provide the answers to the questions which factors affect the implantation most and what we need to avoid or identify in the group of patients with recurrent implantation failure. The studies that are included into the present thesis investigated the following embryonic factors such as morphology, kinetics and genetics, which represent the life perspective and potential of the embryo. One of the uterine factors, such as endometrium receptivity also reflects the women's health condition and have been studied by the author of the thesis.

The main goal of assisted reproductive technologies is to improve quality and effectiveness of infertility treatment in IVF cycles, and to reduce RIF and complications during procedures. For this purpose several methods of embryo selection were tested – one of them is time-lapse system, another – PGT-A and clarification of the endometrium functionality. As complies with the research of the both authors (Cozzolino et al. 2020; Lessey and Young 2019). Altogether they elucidate the individual capabilities of patients to produce high-quality gametes, the ability of these gametes to fertilize and further divide, as well as the individual characteristics of a woman regarding implantation of the fertilized gametes or – an embryo. It is established that the quality of the gametes and the quality of the embryos strongly correlates (Campbell et al. 2013). The "gold standard" for preparing and conducting an IVF procedure is the evaluation of three fundamental factors – the embryonic, uterine and woman's health factors (immune factor, coagulation factors), upon which the outcome of the IVF directly depends.

Study I – during this study the main focus was to understand whether the TLM (time-lapse system) can affect the fertilization of the oocytes and developing of the embryos in comparison with the control group, in which the embryos were cultivated in the standard incubator, and also how the incubation in the closed system (TLM) can affect pregnancy outcomes and pregnancy loss until

seventh week of pregnancy. Also, utilizing a TLM system can offer additional advantages, such as improved and flexible management of laboratory workload. The selection of embryos based on visual indicators of presumed quality has mostly relied on subjective application of a decision tree. The incorporation of multiple visual parameters has resulted in enhanced outcomes, and the widespread adoption of the widely known 'Gardner criteria' serves as a good illustrative example (Apter et al. 2020). Kirkegaard group and later on Milewski group also showed that TLM system positively affected fertilization and cultivation of embryo till blastocyst stage in comparison with the standard incubation (Kirkegaard et al. 2013; Milewski and Ajduk 2017). Considering that the embryonic factor is one of the main factors influencing implantation (Balaban et al. 2011), one of the main tasks is to ensure the quality of gametes (oocytes and spermatozoa).

Thus, the specific selection of embryos is fundamental to the process of obtaining positive results in the IVF cycle. To accomplish successful outcome, precise standardized methods need to be employed, including the selection of embryos according to three parameters. The first being morphology, the second – kinetic properties of the embryo, and the third – genetic evaluation of the blastocyst.

The study found that the time-lapse imaging and monitoring system is a safe non-invasive method to improve reproductive outcomes. Stable and controlled incubation conditions and embryo monitoring *in-situ* provide a consistent milieu for the developing embryo. Changes in temperature, air and pH which the embryo is exposed to in conventional incubators can be harmful (De Mouzon et al. 2010). The study demonstrated increased biochemical, clinical, and ongoing pregnancy rates, and a statistically significant decrease in early pregnancy loss rates.

The median age of patients involved in IVF treatment (37±2 years) (Eurostat, "Fertility Statistics – Statistics Explained," n.d.) has increased to 38±6 years in recent years, which is a risk factor for obtaining an insufficient number of good quality oocytes. To improve this aspect various modified, personalized protocols are used to acquire a larger quantity and usually better quality of cells. In the Study I the median age of the patients which were involved in to the study was 35.9 years for the TLM group and 39.2 years for the control group, that is statistically different ($P < 0.0001$). That why in this study patients were divided in two subgroups – until and over 35 years. The quality and the age of the oocytes are one of the most important factors for the future embryo quality, its ability to cleavage and evolve, and the possibility of implantation later on. As indicated by many authors, maternal age have been associated with a strong decline in the production of healthy and high-quality oocytes which results in reduced fertility in women that are older than 35 years (Mikwar, MacFarlane, and Marchetti 2020).

Also, the quality of the sperm can affect the prospects of the embryo development and quality. In the Study I only two characteristics of sperm from male partners was tested – the sperm concentration and DNA integrity, and in the both groups (TLM and control group), the parameters of the sperm were the same ($p = 0.21$ and $p = 0.14$, respectively). Since the preparation of the sperm includes swim-up method that selects the most motile sperm in the upper fraction and leaves low or no motility sperm in the lower fraction, the concentration of the sperm does not play a crucial role in the fertilization. It is known that sperm DNA integrity plays more important role in fertilization and proper division of embryos than the sperm concentration (Bungum et al. 2007), as it affects the fragmentation of the blastocysts and their quantity and directly their ability for implantation. Therefore, the obtained results from the TLM and control groups are comparable.

The fertilization of the oocytes till 2 PN stage in both subgroups (younger 35 and over 35 years of age) in the TLM group shows statistically significant better results ($p = 0.004$) comparing to the controls. This confirms that the comfortable environment, which is provided by TLM system influences the fertilization rate and embryo developing. Interestingly, higher cleavage rate till the 3rd day and the 5th day was statistically higher in the TLM group in comparison with control group. It is in line with the study of the Milewski group (Milewski and Ajduk 2017). The results of the study support the use of the TLM system as successful tool for improving the fertilization of oocytes to the 2PN stage, as well as for the cultivation of embryos up to 3–5 days in all study groups (donor cells, patients younger and older than 35 years) compared with the controls.

The TLM selection model has been validated in several other studies (I. Rubio et al. 2014; Pribenszky et al. 2010; Wong et al. 2010). It also specifies the conditions for excluding impaired embryos from the transfer, in order to prevent early pregnacy loss. TLM system is not only a high-quality incubator, which provides correct media for better embryo development, but also the specific artificial intelligence, which contributes the algorithm impeding correct choice of the embryo for the transfer. As described in the Basile research the current methods of embryo evaluation relies mostly on static observations of the morphology. These observations are critically restricted to specific times and, as the development of the embryo is a dynamic process, many critical stages may go unnoticed. This imaging system in the embryology and the possible use of it is a novel selection tool (Natalia Basile and Meseguer 2012). This approach is also supported by ESHRE guidelines (Apter et al. 2020).

ESHRE guidelines provide the long list of recommended condition for embryologist regarding education of the staff, finding the appropriate system settings. During Study I the effectiveness, utility and accessibility of the technology was validated and proved, as well as counsel patients alongside offering described method.

The use of TLM and the selection of high-quality of embryos directly affect the further onset of pregnancy and its outcome. In the Study I it has been shown that in spite of similar implantation rate in all transfer groups, the early pregnancy loss significantly decreases in the TLM group in comparison with the controls ($p = 0.043$). The same outcome have been demonstrated by Meseguer, as clinical pregnancy rate for treatments using TLM, comparing to standard incubator, significantly increased (Marcos Meseguer et al. 2012).

Therefore, TLM can be recommended for the implementation in the clinical routine of IVF clinics. Especially important is TLM ability to decrease early pregnancy loss rates, which is a devastating clinical problem for both the couple and the physician. Implementing this non-invasive method would be the first step to improve the outcomes in IVF treatment, and to decrease EPL rates.

Genetic testing of embryos for chromosome composition is one of the fundamental criteria for the quality of embryos, as well as factors affecting implantation and reducing the number of unsuccessful cycles. It is worth noting that both methods – non-invasive TLM and invasive PGT-A – are interconnected and give the best result only in combination. Further, an invasive method for examining embryos at the blastocyst stage requires TLM due to the impossibility to coordinate the time of the trophectoderm biopsy.

Chromosomal aberrations in embryos occur for several reasons, the most common of which are an insufficient maturity of the oocytes or age-related changes in the oocytes associated with a weakening of the spindle apparatus, as well as various hormonal and immune factors. The male factor plays an important role in the formation and further development of the embryo. Abnormal DNA integrity can cause aneuploidy in embryos. It is well known that the percentage of euploid and aneuploid embryos depends on the woman's age: in the age group up to 35 years, every 5th oocyte leads to the formation of an euploid embryo; in the age group 35–39 years – every 9th oocyte, and after 39 years – every 16th oocyte (Esteves et al. 2018). The POSEIDON criteria were developed to provide a more detailed picture of patients with poor ovarian response and to help physicians in the management of these patients (Roque et al. 2021). The new standards are challenging the current criteria of patients with poor ovarian response in favor of the newly defined concept of "low prognosis" (Esteves et al. 2018). Some of the authors implies that the selection of the embryos until the blastocysts stage is completely enough to achieve the clinical pregnancy (Cruz et al. 2011). Thus, the use of the PGT-A method makes it possible to reduce the number of obviously negative embryo transfers (or transfers with aneuploid embryos) in genetically healthy couples. It also makes it possible to find embryos free from chromosomal defects in patients having an altered karyotype, thereby reducing the percentage of implantation failure.

For the purpose to improve the results of IVF treatment and to decrease early pregnancy loss, in the Study II two invasive methods were tested: preimplantation genetic testing for aneuploidies and endometrial receptivity array – which

both focus on patients having recurrent implantation failure. It is well established that maternal age is associated with a rapid decline in the production of healthy and high-quality oocytes and euploid embryos which results in reduced fertility in women older than 35 years (Mikwar, MacFarlane, and Marchetti 2020). In the last ESHRE guidelines edition (ESHRE PGT Consortium good practice recommendations for the detection of structural and numerical chromosomal aberrations) it is recommended to use PGT-A method as one of the treatment factors for patients with recurrent implantation failure and recurrent pregnancy loss (Coonen et al. 2020).

In this study the women of of all groups were the similar age ($p = 0.21$) with recurrent implantation failure in the medical history were analyzed. The embryonic outcomes in four groups showed statistical differences ($p = 0.02$), due to variations in the quantity of acquired blastocysts. However, as the single embryo transfer (SET) for patient in each group was performed, the results are comparable. Furthermore, in the study groups, additional examination was provided for both the embryo and the endometrium. In the control group – Group I, SET was performed without any additional examination of the embryo (PGT-A) or the endometrium (ERA test).

In the Group II PGT-A method was provided to determine the chromosomal status of an embryo prior to transfer. An euploid embryo refers to an embryo that possesses 23 haploid chromosome pairs and 2 sex chromosomes. On the other hand, aneuploid embryos are those that exhibit alterations in the number or structure of chromosomes.

In this study for Groups II and III the euploid embryos have been used, but for Groups I and IV PGT-A test was not provided. Therefore, the chromosomal status of embryo in the latest groups was not clear. From another studies (Cozzolino et al. 2020) it is known that the euploid embryos have highest possibility of implantation, less percent of early pregnancy loss, higher percent of live birth and the similar results have been shown in this thesis. The possibility of the implantation considerably reduces in a case of embryos aneuploidy. For example, the changes in the 1st, 2nd, and 3rd chromosomes stops the development of the embryo in the first 8 days. The changes in the 16th, 19^{th,} and 22nd chromosomes predict the obturation of the pregnancy around week 7–8th of pregnancy (C. Rubio et al. 2013). The missing one of the pair of the chromosomes blocks the development of the embryo in the early stages (Thornhill et al. 2005). The results of the studies presented in this thesis confirmed these data as well. Therefore PGT-A is considered essential for women over 35 to improve IVF outcomes and to decrease RIF rates.

In Group III the PGT-A and ERA test were provided in combination. ERA tests determination of the implantation windows in the endometrium is one of the possible factors to reduce implantation failure rates. The implantation windows are forming in the endometrial structure in day 7 after ovulation. The different factors such as inflammation process in the uterus, Asherman

syndrome, as well as organic changes as polyps or submucosal myomas, can affect implantation process negatively (Bender Atik et al. 2022). Therefore, patients with these confounding factors were excluded from the present study. Based on that, the ERA test, which provides genetic profiling of the endometrium and is not directly linked to the factors mentioned above. The ERA test is more precise and more important method to determine the day and hour for the implantation, as the result patients with recurrent pregnancy loss, have higher rate for clinical pregnancy and significantly decrease biochemical pregnancy loss, if this method combined with PGT-A. Ruiz-Alonso with the group also shows decrease in the early pregnancy loss with the ERA tests (Ruiz-Alonso et al. 2013). In the current ESHRE guidelines the effect of the ERA test for the recurrent pregnancy loss patient group was not described. General focus in ESHRE Recurrent pregnancy loss guidelines was made on the morphological and immunological condition of the endometrium, but the genetic status of the endometrium was not reviewed. (Bender Atik et al. 2022). However, data from Study II confirm that combination of several factors play a crucial role in recurrent implantation failure, including embryo quality (both morphological and genetic) and also endometrial receptivity (window of implantation). Two groups that showed the best results of the achieving clinical pregnancy were the Groups when combined treatment methods were used (FET+PGT-A 49.3% and FET+PGT-A+ERA 55.6%, respectively). The clinical pregnancy rate is the main criterion for determining treatment effectiveness. These results demonstrate that the combination of genetic testing of the embryo (PGT-A) prior embryo transfer and genetic assessment of the endometrium (ERA test), which establish the exact time of the embryo transfer are the best treatment model, as they decrease biochemical loss and increase the clinical pregnancy rate. Combined implementation of the PGT-A and ERA test improves biochemical and clinical pregnancies rates either in patients with recurrent implantation failure, compared to cycles where embryo transfers were carried out without additional tests or if the methods were provided one at a time.

The results in the Group IV possibly can be explained by the small number of patients for whom only the ERA test was applied. Therefore, the use of the ERA test alone is still debatable. However its use in combination with PGT-A enhances the results of implantation, as PGT-A gives complete enlightenment about the chromosomal structure of the transferable embryo, its potential for implantation and development. Clarification of the day and time of transfer is determined by the ERA test.

As mentioned before, other putative factors can have an impact on fertility treatment, which were not investigated in this study, including uterine microbiome, different immunological factors such as increase of pro-inflammation interleukins, overproduction of the TNF-a, abnormal function of natural killers (NK) and specifically uterine NK cells along with non-immunological factors. Also, as mentioned earlier, the male factor or sperm DNA integrity is thought to play a

role in RIF (Zini et al. 2008). Other studies, however, failed to confirm this (Carol Coughlan et al. 2015). Assessing of their relative importance is crucial. Such studies would help clinicians reduce recurrent implantation failure and assist couples to achieve successful pregnancies. Perspective studies should be continued.

Study III also demonstrated that PGT-A becomes important not only for a group of patients with recurrent implantation failure having normal karyotypes, but more importantly, for a group of patients having balanced translocations. The results showed that rates of aneuploid embryos in these patients are higher when compared to patients with normal karyotypes. Therefore, they are at significantly higher risk of developing RIF – especially if a female is the carrier of a translocation. This is considered as good practice and has been strongly recommended by the ESHRE PGT Consortium (Coonen et al. 2020).

Comparing male and female carriers, the total aneuploidy rates higher in the maternal group, and the percentage of embryos related to translocation is 4.7 times higher in the maternal group than in the paternal one, even taking maternal age into account. This explains the phenomenon, that female translocations carriers have higher percentage of miscarriages and fetal abnormalities than partners of male carriers. This difference in the rate of embryos with unbalanced translocations between male and female translocation carriers presented in this study is statistically significant (12% vs. 24%, $p < 0.05$), which is in concordance to other authors (Idowu et al. 2015).

There are only a few studies investigating patients with balanced translocations and comparing them for all possible aspects of IVF outcomes (Idowu et al. 2015). Usually, the number of the patients is small as this is a rare disease. The present study provides additional data to this rare condition, as well support the evidence of mandatory implementation of PGT-A in the infertile treatment patients with Robinson translocation. More effective consultations and patient evaluations are needed regarding prognostic accuracy of aneuploid embryos. More data on balanced chromosomal changes are required.

In summary, the reasons for implantation failure are multifactorial. The most common reasons are chromosomal aberrations of an embryo, endometrium abnormality, different kinds of coagulopathies, endocrine disorders, and combinations and interplay between these factors. The present work conducted during the last years analyzed and observed the patients facing these problems. In these 3 studies the most important factors of implantation – as embryonic and endometrial – were investigated and analyzed. Taking into consideration review of the literature and the results of described studies, the algorithm of fertility treatment is outlined in chapter 9. This unique algorithm provides detailed practical clinical steps for IVF procedures regarding fertility treatment of patients having a recurrent implantation failure in the medical history. To date, the ESHRE Recurrent Implantation Failure Guidelines, have not delineated specific recommendation for the patients having implantation failure, however, the ESHRE group is currently updating these guidelines.

For patients having 2 failed pregnancies during the IVF cycles, the fundamental recommendation is a detailed examination of both partners including karyotyping them to exclude different kinds of aberrations in their own chromosomes. It is also important to make detailed examination of the embryos, including embryo cultivation until the day 5 (blastocyst stage) through daily or on-line monitoring using time-lapse microscopy. This step is expected to improve the process of excluding embryos with increased fragmentation and of morphologically poor quality. The use of this standardized algorithm and performing the appropriate selection (kinetic and morphological) will increase ET implantation potential. This method provides information about kinetics of embryo development (or embryo meiosis rate) using different validated algorithms (M. Meseguer et al. 2011; I. Rubio et al. 2014; N. Basile et al. 2015). It also provides a good image of embryo development following its transfer to the uterus cavity.

PGT-A is the most promising method in the evaluation of embryo quality and prognosis for implantation. It is worth noting that more precise prognostic results are obtained analyzing material from the embryo trophectoderm and not from the blastomere (3rd day embryo). Examination of specifically those cells gives reliable testing results. This method improves pregnancy outcomes in patients having implantation failure. It also reduces most of the serious diseases that might occur in their future offspring. Accordingly, the complete examination of the embryo is the first step for patients having 2 failed pregnancies during IVF cycles.

One of the aspects for patients with recurrent implantation failure is the detailed examination of the endometrium, which includes evaluation of endometrial thickness in the first and second phase of the menstrual cycle, to exclude the presence of organic damage like polyps, hyperplasia, adhesions as well as myomas, adenomyosis and congenital anomalies. Observation of patients with implantation failure confirms that better prognostic potential requires examination of the endometrium regarding the implantation window. Different factors, like abortion, inflammatory processes of endometrium, severe or chronic hormonal imbalance, can lead to a significant shift of the window of implantation. The examination of window of implantation can be recommended in cases of implantation failure as an additional examination.

It is important to stress that the recommendations mentioned do not guarantee absolute or ultimate success following embryo transfer. More studies are necessary including endometrial T regulatory lymphocytes, plasma cell (CD138+) infiltration, and the state of immunity of the endometrium.

8. CONCLUSIONS

Based on the results of this thesis, the following conclusions are reached:

1. The TLM selection model helps to exclude impaired embryos for transfer and prevents early pregnancy loss due to embryo morphological and kinetic developmental changes. Implementation of TLM increases biochemical, clinical pregnancy and decreases early pregnancy loss rates. It might serve as the step one in the treatment algorithm for the patients with recurrent implantation failure.
2. Use of TLM system is essential as it provides the next step of embryo examination – pre-implantation selection of the embryos of aneuploidy or PGT-A.
3. Excluding transfer of aneuploid embryo, the PGT-A procedure positively affects infertility care and reduces recurrent implantation failure, even if implemented as a single treatment method.
4. The combination of morphological, kinetical and genetical embryo assesment and clarification of the implantation window by ERA test decreases extremely the implantation failure rate and increases the rate of clinical pregnancy.
5. For patients with the balanced translocations in their karyotype, pre-implantation selection of the embryos of aneuploidy is a "Gold standard" in the case of recurrent implantation failure, as it increases the pregnancy rate and decreases biochemical and clinical pregnancy loss.
6. Based on results obtained, the recommendations for the clinical practice have been developed and are described in Chapter 9.

9. CLINICAL APPLICATIONS FOR FUTURE PRACTICE

The reasons for implantation failure are multifactorial. The most common causes are chromosomal aberrations of embryos, endometrium failure, different kinds of coagulopathies, endocrine disorders, and the combination or interaction between mentioned determinants. This research conducted in the iVF Riga clinic during the last 10 years, including clinical analyses and observation of patients has resulted in the development of a new algorithm for the examination and management of IVF patients (see Figure 9.1.).

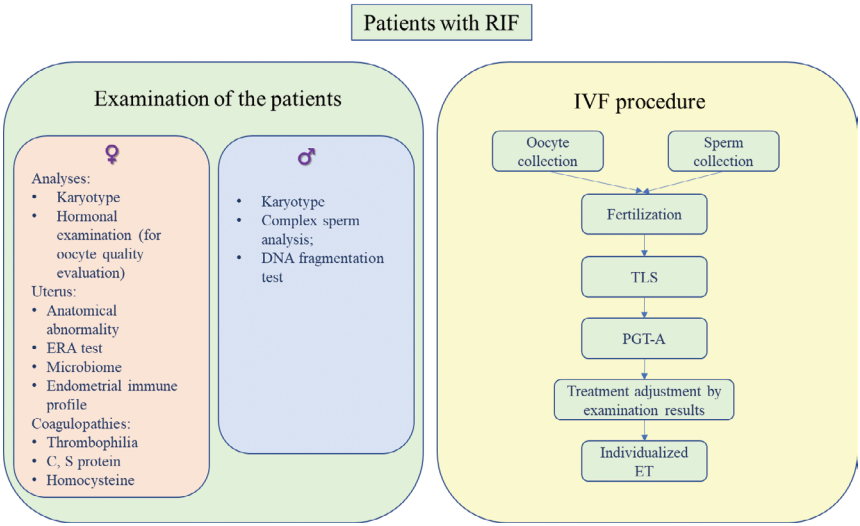


Figure 9.1. **Algorithm for examination of patients having an implantation failure**

Patients with 2 or more implantation failures in IVF cycles must be examined before undergoing new IVF cycles.

1. At the first stage of examination, karyotyping of both partners is required to assess the risk of developing abnormal embryos.
2. Detailed examination of the man includes sperm analysis using the DNA fragmentation test. Both factors work to avoid defects during fertilization and embryo development, and increase good morphological embryos.
3. Other factors in examination of the woman includes hormonal analyses and coagulation criteria, which affect the maturation quality of the oocyte and also help professional to choose the best strategy for the stimulation.

4. In IVF cycles it is recommended to use TLM for the embryo selection by morphological, kinetic factors and also to specify the day and time of the embryo biopsy.
5. The PGT-A method is recommended for patients who have normal karyotypes but have a complicated medical history. This method is essential for patients with balanced translocations in their own karyotype.
6. A combination of TLM and PGT-A with the ERA test is an effective approach for improving the outcome of IVF treatment, decreasing implantation failure and early pregnancy loss in patients with normal karyotypes and appliance of the PGT-A in patients with balanced translocations provides positive results in IVF treatment.

This algorithm design has been used for infertility treatments of patients who have a medical history of recurrent implantation failure. The clinical and biochemical pregnancy rate, achieved at iVF Riga in 2022 is shown in Figure 9.2. For comparison, the data provided by the ESHRE registry are presented in Figure 9.3. (ESHRE 2022).

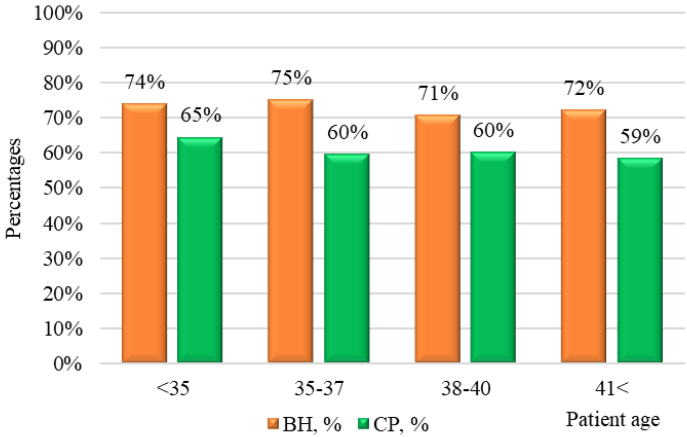


Figure 9.2. **Statistical data of iVF Riga biochemical and clinical pregnancy outcomes, 2022**

This data involved FET results present higher clinical pregnancy success rates than European mean pregnancy rates in the Figure 9.3. below.

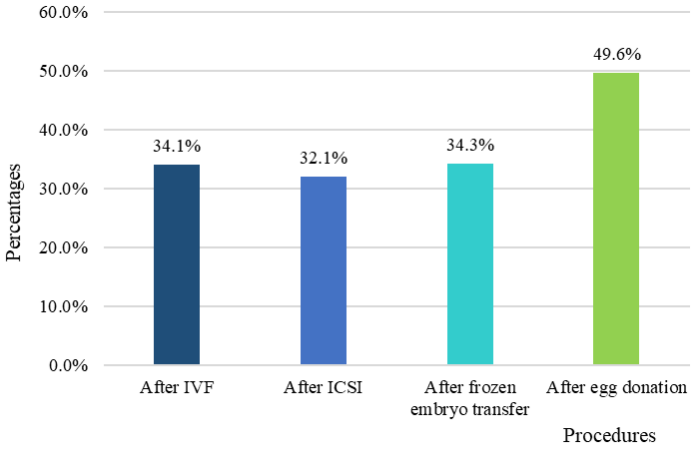


Figure 9.3. **Statistical data of ESHRE clinical pregnancy outcomes, 2018**

Continuous studies are recommended to identify other reasons for recurrent implantation failure, including uterine microbiome, immunological and male factors.

10. RELATED PUBLICATIONS, LECTURES AND PRESENTATIONS

This thesis is based on the following publications:

Fodina, V., Andzane, D., Pimane, E., Miskova, A. (2015). How to decrease EPL rate treating infertility? *Gynecol Endocrinol*, 31(S1): pp. 51–54.

Fodina, V., Dudorova, A., Alksere, B., Dzalbs, A., Vedmedovska, N., Andersone, S., Conka, U., Erenpreiss, J., Berzina, D. (2019). The application of PGT-A for carriers of balanced structural chromosomal rearrangements, *Gynecol Endocrinol*, 35:sup1, pp. 18–23.

Fodina, V., Dudorova, A., Erenpreiss, J. (2021). Evaluation of embryo aneuploidy (PGT-A) and endometrial receptivity (ERA) testing in patients with recurrent implantation failure in ICSI cycles, *Gynecol Endocrinol*, 37:sup1, pp. 17–20.

Fodina, V., Dudorova, A., Erenpreiss, J. (2021). Reasons and Mechanisms of Recurrent Failed Implantation in IVF. In: Wei-Hua Wand, ed. "Infertility and Assisted Reproduction," ISBN 978-1-83962-825-2. IntechOpen, doi: 10.5772/intechopen.98301. http://mts.intechopen.com/articles/show/title/reasons-and-mechanisms-of-recurrent-failed-implantation-in-ivf

Results of the this thesis were presented in the following conferences and lectures:

Fodina, **V.,** Miskova, A., Voložonoka, L. Embryo selection impact on early pregnancy loss. ESHRE annual meeting, July 15–19, 2015, Lisbon, Portugal.

Fodina, V. Reduction of reproductive loss in the treatment of infertility, The health of reproduction is the national security guarantee, IV International Congress of obstetricians and gynecologists, October 16–17, 2015, Yekaterinburg, Russia.

Fodina, V. Understanding IVF failures in patients with complicated reproductive history, 3rd Annual Baltic Fertility Society Meeting, May 20–21, 2016, Riga, Latvia.

Fodina, V. Endometrium receptivity as one of the improving factors of ART, StemcellBio-2016, November 10, 2016, Saint Petersburg, Russia.

Fodina, V. How to deal with patients who have had a miscarriage, iVF Riga clinic organized lecture cycle on "Innovations in Latvian medicine", February 28, March 28, April 4, 18, May 2, 16, June 6, 2017, Riga, Latvia.

Fodina, V. Genetics and infertility: how it can help in practice, iVF Riga clinic offsite seminar, May 30, 2017, Ventspils, Latvia and June 13, 2017, Daugavpils, Latvia.

Fodina, V. How to help couples who are unable to conceive on their own, or families with sick children. How to avoid? MFD conference, March 29, 2018, Riga, Latvia.

Fodina, V. "Neveiksmīgas implantācijas iemesli IVF ciklos. Diagnostikas taktika. Problēmu risināšana". 8. Latvijas Dzemdību speciālistu un Ginekologu kongress, 2018. 20.–21. aprīlī, Rīgā. Mutiskā prezentācija un tēzes.

Fodina, V. Diagnostic options and treatment tactics in complicated IVF cases, January 26, 2019, Stockholm, Sweden.

Fodina, V. *et al.* Prior miscarriage and preterm delivery in assisted reproductive technology pregnancies, RĪGA STRADIŅŠ UNIVERSITY INTERNATIONAL CONFERENCE on Medical and Health Care Sciences "Knowledge for Use in Practice", April 1–3, 2019, Riga, Latvia.

Fodina, V. Female infertility diagnosis and new treatment options for various pathologies. Action plan in cases when the development of the embryo stops within the IVF cycle. Novel Avenues in the treatment of female and male infertility, Scientific Practical Conference, June 6–7, 2019, Vilnius, Lithuania.

Fodina, V. Diagnostic options and treatment tactics in complex IVF cases, E-visit lecture, February 2, 2020, Riga, Latvia.

REFERENCES

1. Alfarawati, S., Fragouli, E., Colls, P., & Wells, D. (2022). Erratum: Embryos of Robertsonian Translocation Carriers Exhibit a Mitotic Interchromosomal Effect That Enhances Genetic Instability during Early Development(PLoS Genet (2012) 8:10 (e1003025) DOI: 10.1371/journal.pgen.1003025). *PLoS Genetics*, *18*(8), 23133396. https://doi.org/10.1371/journal.pgen.1010377
2. Altmäe, S., Koel, M., Võsa, U., Adler, P., Suhorutšenko, M., Laisk-Podar, T., Kukushkina, V., Saare, M., Velthut-Meikas, A., Krjutškov, K., Aghajanova, L., Lalitkumar, P. G., Gemzell-Danielsson, K., Giudice, L., Simón, C., & Salumets, A. (2017). Meta-signature of human endometrial receptivity: A meta-analysis and validation study of transcriptomic biomarkers. *Scientific Reports*, *7*(1), 1–16. https://doi.org/10.1038/s41598-017-10098-3
3. Annan, J. J. K., Gudi, A., Bhide, P., Shah, A., & Homburg, R. (2013). Biochemical Pregnancy During Assisted Conception: A Little Bit Pregnant. *Journal of Clinical Medicine Research*, *5*(4), 269–274. https://doi.org/10.4021/jocmr1008w
4. Anton, E., Blanco, J., & Vidal, F. (2010). Meiotic behavior of three D;G Robertsonian translocations: Segregation and interchromosomal effect. *Journal of Human Genetics*, *55*(8), 541–545. https://doi.org/10.1038/jhg.2010.67
5. Apter, S., Ebner, T., Freour, T., Guns, Y., Kovacic, B., Le Clef, N., Marques, M., Meseguer, M., Montjean, D., Sfontouris, I., Sturmey, R., & Coticchio, G. (2020). Good practice recommendations for the use of time-lapse technology†. *Human Reproduction Open*, *2020*(2), 1–26. https://doi.org/10.1093/hropen/hoaa008
6. Balaban, B., Brison, D., Calderon, G., Catt, J., Conaghan, J., Cowan, L., Ebner, T., Gardner, D., Hardarson, T., Lundin, K., Cristina Magli, M., Mortimer, D., Mortimer, S., Munne, S., Royere, D., Scott, L., Smitz, J., Thornhill, A., van Blerkom, J., & Van den Abbeel, E. (2011a). The Istanbul consensus workshop on embryo assessment: proceedings of an expert meeting. *Human Reproduction*, *26*(6), 1270–1283. https://doi.org/10.1093/humrep/der037
7. Balaban, B., Brison, D., Calderon, G., Catt, J., Conaghan, J., Cowan, L., Ebner, T., Gardner, D., Hardarson, T., Lundin, K., Cristina Magli, M., Mortimer, D., Mortimer, S., Munne, S., Royere, D., Scott, L., Smitz, J., Thornhill, A., van Blerkom, J., & Van den Abbeel, E. (2011b). The Istanbul consensus workshop on embryo assessment: proceedings of an expert meeting. *Human Reproduction*, *26*(6), 1270–1283. https://doi.org/10.1093/humrep/der037
8. Bashiri, A., Halper, K. I., & Orvieto, R. (2018). Recurrent Implantation Failure-update overview on etiology, diagnosis, treatment and future directions 11 Medical and Health Sciences 1114 Paediatrics and Reproductive Medicine. *Reproductive Biology and Endocrinology*, *16*(1), 4–7. https://doi.org/10.1186/s12958-018-0414-2
9. Basile, N., & Meseguer, M. (2012a). Time-lapse technology: Evaluation of embryo quality and new markers for embryo selection. *Expert Review of Obstetrics and Gynecology*, *7*(2), 175–190. https://doi.org/10.1586/eog.12.8

10. Basile, N., & Meseguer, M. (2012b). Time-lapse technology: Evaluation of embryo quality and new markers for embryo selection. *Expert Review of Obstetrics and Gynecology*, *7*(2), 175–190. https://doi.org/10.1586/eog.12.8
11. Basile, N., Vime, P., Florensa, M., Aparicio Ruiz, B., García Velasco, J. A., Remohí, J., & Meseguer, M. (2015). The use of morphokinetics as a predictor of implantation: A multicentric study to define and validate an algorithmfor embryo selection. *Human Reproduction*, *30*(2), 276–283. https://doi.org/10.1093/humrep/deu331
12. Bates, G. W., & Ginsburg, E. S. (2002). Early pregnancy loss in in vitro fertilization (IVF) is a positive predictor of subsequent IVF success. *Fertility and Sterility*, *77*(2), 337–341. https://doi.org/10.1016/S0015-0282(01)02988-0
13. Bender Atik, R., Christiansen, O. B., Elson, J., Kolte, A. M., Lewis, S., Middeldorp, S., Mcheik, S., Peramo, B., Quenby, S., Nielsen, H. S., van der Hoorn, M.-L., Vermeulen, N., & Goddijn, M. (2022). ESHRE guideline: recurrent pregnancy loss: an update in 2022. *Human Reproduction Open*, *2023*(1), 1–7. https://doi.org/10.1093/hropen/hoad002
14. Bender Atik, R., Christiansen, O. B., Elson, J., Kolte, A. M., Lewis, S., Middeldorp, S., Nelen, W., Peramo, B., Quenby, S., Vermeulen, N., & Goddijn, M. (2018). ESHRE guideline: recurrent pregnancy loss. *Human Reproduction Open*, *2018*(2). https://doi.org/10.1093/hropen/hoy004
15. Bergh, P. A., & Navot, D. (1992). The impact of embryonic development and endometrial maturity on the timing of implantation. *Fertility and Sterility*, *58*(3), 537–542. https://doi.org/10.1016/s0015-0282(16)55259-5
16. Bhagwat, S. R., Chandrashekar, D. S., Kakar, R., Davuluri, S., Bajpai, A. K., Nayak, S., Bhutada, S., Acharya, K., & Sachdeva, G. (2013). Endometrial Receptivity: A Revisit to Functional Genomics Studies on Human Endometrium and Creation of HGEx-ERdb. *PLoS ONE*, *8*(3). https://doi.org/10.1371/journal.pone.0058419
17. Bungum, M., Humaidan, P., Axmon, A., Spano, M., Bungum, L., Erenpreiss, J., & Giwercman, A. (2007). Sperm DNA integrity assessment in prediction of assisted reproduction technology outcome. *Human Reproduction*, *22*(1), 174–179. https://doi.org/10.1093/humrep/del326
18. Campbell, A., Fishel, S., Bowman, N., Duffy, S., Sedler, M., & Hickman, C. F. L. (2013). Modelling a risk classification of aneuploidy in human embryos using non-invasive morphokinetics. *Reproductive BioMedicine Online*, *26*(5), 477–485. https://doi.org/10.1016/j.rbmo.2013.02.006
19. Chamayou, S., Patrizio, P., Storaci, G., Tomaselli, V., Alecci, C., Ragolia, C., Crescenzo, C., & Guglielmino, A. (2013). The use of morphokinetic parameters to select all embryos with full capacity to implant. *Journal of Assisted Reproduction and Genetics*, *30*(5). https://doi.org/10.1007/s10815-013-9992-2
20. Chavez, S. L., Loewke, K. E., Han, J., Moussavi, F., Colls, P., Munne, S., Behr, B., & Reijo Pera, R. A. (2012). Dynamic blastomere behaviour reflects human embryo ploidy by the four-cell stage. *Nature Communications*, *3*(1), 1251. https://doi.org/10.1038/ncomms2249
21. Cimadomo, D., Capalbo, A., Ubaldi, F. M., Scarica, C., Palagiano, A., Canipari, R., & Rienzi, L. (2016). The Impact of Biopsy on Human Embryo Developmental Potential during Preimplantation Genetic Diagnosis. In *BioMed Research International* (Vol. 2016). https://doi.org/10.1155/2016/7193075

22. Comins Boo, A., Segovia, A. G., del Prado, N. N., Fuente, L. de la, Alonso, J., & Ramon, S. S. (2016). Evidence-based Update: Immunological Evaluation of Recurrent Implantation Failure. *Reproductive Immunology: Open Access*, *01*(04). https://doi.org/10.21767/2476-1974.100024
23. Coonen, E., Rubio, C., Christopikou, D., Dimitriadou, E., Gontar, J., Goossens, V., Maurer, M., Spinella, F., Vermeulen, N., & De Rycke, M. (2020). ESHRE PGT Consortium good practice recommendations for the detection of structural and numerical chromosomal aberrations†. *Human Reproduction Open*, *2020*(3), 1–20. https://doi.org/10.1093/hropen/hoaa017
24. Coughlan, C., Clarke, H., Cutting, R., Saxton, J., Waite, S., Ledger, W., Li, T., & Pacey, A. A. (2015). Sperm DNA fragmentation, recurrent implantation failure and recurrent miscarriage. *Asian Journal of Andrology*, *17*(4), 681–685. https://doi.org/10.4103/1008-682X.144946
25. Coughlan, C., Ledger, W., Wang, Q., Liu, F., Demirol, A., Gurgan, T., Cutting, R., Ong, K., Sallam, H., & Li, T. C. (2014). Recurrent implantation failure: Definition and management. *Reproductive BioMedicine Online*, *28*(1), 14–38. https://doi.org/10.1016/j.rbmo.2013.08.011
26. Cozzolino, M., Diaz-Gimeno, P., Pellicer, A., & Garrido, N. (2020). Evaluation of the endometrial receptivity assay and the preimplantation genetic test for aneuploidy in overcoming recurrent implantation failure. *Journal of Assisted Reproduction and Genetics*, *37*(12), 2989–2997. https://doi.org/10.1007/s10815-020-01948-7
27. Cruz, M., Gadea, B., Garrido, N., Pedersen, K. S., Martínez, M., Pérez-Cano, I., Muñoz, M., & Meseguer, M. (2011). Embryo quality, blastocyst and ongoing pregnancy rates in oocyte donation patients whose embryos were monitored by time-lapse imaging. *Journal of Assisted Reproduction and Genetics*, *28*(7), 569–573. https://doi.org/10.1007/s10815-011-9549-1
28. Cruz, M., Garrido, N., Herrero, J., Pérez-Cano, I., Muñoz, M., & Meseguer, M. (2012). Timing of cell division in human cleavage-stage embryos is linked with blastocyst formation and quality. *Reproductive BioMedicine Online*, *25*(4). https://doi.org/10.1016/j.rbmo.2012.06.017
29. Cutting, R., Morroll, D., Roberts, S. A., Pickering, S., Rutherford, A., & ACE, on behalf of the B. F. S. and. (2008). Elective Single Embryo Transfer: Guidelines for Practice British Fertility Society and Association of Clinical Embryologists. *Human Fertility*, *11*(3), 131–146. https://doi.org/10.1080/14647270802302629
30. De Mouzon, J., Goossens, V., Bhattacharya, S., Castilla, J. A., Ferraretti, A. P., Korsak, V., Kupka, M., Nygren, K. G., & Nyboe Andersen, A. (2010). Assisted reproductive technology in Europe, 2006: Results generated from European registers by ESHRE. *Human Reproduction*, *25*(8), 1851–1862. https://doi.org/10.1093/humrep/deq124
31. Devroey, P., Bourgain, C., Macklon, N. S., & Fauser, B. C. J. M. (2004). Reproductive biology and IVF: ovarian stimulation and endometrial receptivity. *Trends in Endocrinology & Metabolism*, *15*(2), 84–90. https://doi.org/10.1016/j.tem.2004.01.009
32. Díaz-Gimeno, P., Horcajadas, J. A., Martínez-Conejero, J. A., Esteban, F. J., Alamá, P., Pellicer, A., & Simón, C. (2011a). A genomic diagnostic tool for human endometrial

receptivity based on the transcriptomic signature. *Fertility and Sterility*, *95*(1). https://doi.org/10.1016/j.fertnstert.2010.04.063

33. Díaz-Gimeno, P., Horcajadas, J. A., Martínez-Conejero, J. A., Esteban, F. J., Alamá, P., Pellicer, A., & Simón, C. (2011b). A genomic diagnostic tool for human endometrial receptivity based on the transcriptomic signature. *Fertility and Sterility*, *95*(1). https://doi.org/10.1016/j.fertnstert.2010.04.063
34. Díaz-Gimeno, P., Ruiz-Alonso, M., Blesa, D., Bosch, N., Martínez-Conejero, J. A., Alamá, P., Garrido, N., Pellicer, A., & Simón, C. (2013). The accuracy and reproducibility of the endometrial receptivity array is superior to histology as a diagnostic method for endometrial receptivity. *Fertility and Sterility*, *99*(2), 508–517. https://doi.org/10.1016/j.fertnstert.2012.09.046
35. Edwards, R. G., Fishel, S. B., Cohen, J., Fehilly, C. B., Purdy, J. M., Slater, J. M., Steptoe, P. C., & Webster, J. M. (1984). Factors influencing the success of in vitro fertilization for alleviating human infertility. *Journal of In Vitro Fertilization and Embryo Transfer*, *1*(1). https://doi.org/10.1007/BF01129615
36. ESHRE. (2022). *ART fact sheet January 2022* (Issue january).
37. Esteves, S. C., Roque, M., Bedoschi, G. M., Conforti, A., Humaidan, P., & Alviggi, C. (2018). Defining low prognosis patients undergoing assisted reproductive technology: POSEIDON criteria-the why. *Frontiers in Endocrinology*, *9*(AUG), 1–10. https://doi.org/10.3389/fendo.2018.00461
38. Fenwick, J., Platteau, P., Murdoch, A. P., & Herbert, M. (2002). Time from insemination to first cleavage predicts developmental competence of human preimplantation embryos in vitro. *Human Reproduction*, *17*(2), 407–412. https://doi.org/10.1093/humrep/17.2.407
39. *Fertility statistics – Statistics Explained*. (n.d.). https://www.eshre.eu/-/media/sitecore-files/Publications/PolicyAuditonFertilityAnalysis9EUCountriesFINAL16032017.pdf?la=en&hash=A573990078239BEC146FB44B192284FD4E29FE0A
40. Fiorentino, F. (2012). Molecular genetic analysis of single cells. *Seminars in Reproductive Medicine*, *30*(4). https://doi.org/10.1055/s-0032-1313906
41. Garrido-Gómez, T., Ruiz-Alonso, M., Blesa, D., Diaz-Gimeno, P., Vilella, F., & Simón, C. (2013). Profiling the gene signature of endometrial receptivity: Clinical results. *Fertility and Sterility*, *99*(4), 1078–1085. https://doi.org/10.1016/j.fertnstert.2012.12.005
42. Handyside, A. H., Kontogianni, E. H., Hardy, K., & Winston, R. M. L. (1990). Pregnancies from biopsied human preimplantation embryos sexed by Y-specific DNA amplification. *Nature*, *344*(6268), 768–770. https://doi.org/10.1038/344768a0
43. Handyside, A. H., Montag, M., Magli, M. C., Repping, S., Harper, J., Schmutzler, A., Vesela, K., Gianaroli, L., & Geraedts, J. (2012). Multiple meiotic errors caused by predivision of chromatids in women of advanced maternal age undergoing in vitro fertilisation. *European Journal of Human Genetics*, *20*(7). https://doi.org/10.1038/ejhg.2011.272
44. Horcajadas, J. A., Pellicer, A., & Simón, C. (2007). Wide genomic analysis of human endometrial receptivity: new times, new opportunities. *Human Reproduction Update*, *13*(1), 77–86. https://doi.org/10.1093/humupd/dml046
45. *How To Be Reasonably Certain that a Woman Is Not Pregnant*. (2016). Division of Reproductive Health, National Center for Chronic Disease Prevention and Health

Promotion. https://www.cdc.gov/reproductivehealth/contraception/mmwr/spr/not-pregnant.html

46. Huet-Hudson YM, Andrews GK, D. S. (1989). Cell Type-Specific Localization of c-Myc Protein in the Mouse Uterus: Modulation by Steroid Hormones and Analysis of the Periimplantation Period*. *Endocrinology*, *125*(3), 1683–1690. https://doi.org/10.1210/endo-125-3-1683
47. Hung, J.-H., & Weng, Z. (2017). Analysis of Microarray and RNA-seq Expression Profiling Data. *Cold Spring Harbor Protocols*, *2017*(3), pdb.top093104. https://doi.org/10.1101/pdb.top093104
48. Idowu, D., Merrion, K., Wemmer, N., Mash, J. G., Pettersen, B., Kijacic, D., & Lathi, R. B. (2015). Pregnancy outcomes following 24-chromosome preimplantation genetic diagnosis in couples with balanced reciprocal or Robertsonian translocations. *Fertility and Sterility*, *103*(4), 1037–1042. https://doi.org/10.1016/j.fertnstert.2014.12.118
49. Kirk, E., Ankum, P., Jakab, A., Le Clef, N., Ludwin, A., Small, R., Tellum, T., Töyli, M., Van den Bosch, T., & Jurkovic, D. (2020). Terminology for describing normally sited and ectopic pregnancies on ultrasound: ESHRE recommendations for good practice. *Human Reproduction Open*, *2020*(4), 1–21. https://doi.org/10.1093/hropen/hoaa055
50. Kirkegaard, K., Kesmodel, U. S., Hindkjær, J. J., & Ingerslev, H. J. (2013). Time-lapse parameters as predictors of blastocyst development and pregnancy outcome in embryos from good prognosis patients: a prospective cohort study. *Human Reproduction*, *28*(10), 2643–2651. https://doi.org/10.1093/humrep/det300
51. Kolde, R., Laur, S., Adler, P., & Vilo, J. (2012). Robust rank aggregation for gene list integration and meta-analysis. *Bioinformatics*, *28*(4), 573–580. https://doi.org/10.1093/bioinformatics/btr709
52. Lemmen, J. G., Agerholm, I., & Ziebe, S. (2008). Kinetic markers of human embryo quality using time-lapse recordings of IVF/ICSI-fertilized oocytes. *Reproductive BioMedicine Online*, *17*(3). https://doi.org/10.1016/S1472-6483(10)60222-2
53. Lessey, B. A., & Young, S. L. (2019). What exactly is endometrial receptivity? *Fertility and Sterility*, *111*(4), 611–617. https://doi.org/10.1016/j.fertnstert.2019.02.009
54. Lundin, K., Bergh, C., & Hardarson, T. (2001). Early embryo cleavage is a strong indicator of embryo quality in human IVF. *Human Reproduction*, *16*(12). https://doi.org/10.1093/humrep/16.12.2652
55. Maesawa, Y., Yamada, H., Deguchi, M., & Ebina, Y. (2015). History of biochemical pregnancy was associated with the subsequent reproductive failure among women with recurrent spontaneous abortion. *Gynecological Endocrinology*, *31*(4), 306–308. https://doi.org/10.3109/09513590.2014.994601
56. Mahajan, N. (2015). Endometrial receptivity array: Clinical application. *Journal of Human Reproductive Sciences*, *8*(3), 121–129. https://doi.org/10.4103/0974-1208.165153
57. Margalioth, E. J., Ben-Chetrit, A., Gal, M., & Eldar-Geva, T. (2006). Investigation and treatment of repeated implantation failure following IVF-ET. *Human Reproduction*, *21*(12), 3036–3043. https://doi.org/10.1093/humrep/del305
58. Matsumoto, H. (2017). Molecular and cellular events during blastocyst implantation in the receptive uterus: clues from mouse models. *Journal of Reproduction and Development*, *63*(5), 445–454. https://doi.org/10.1262/jrd.2017-047

59. Maxwell, S. M., & Grifo, J. A. (2018). Should every embryo undergo preimplantation genetic testing for aneuploidy? A review of the modern approach to in vitro fertilization. In *Best Practice and Research: Clinical Obstetrics and Gynaecology* (Vol. 53). https://doi.org/10.1016/j.bpobgyn.2018.07.005

60. Meseguer, M., Herrero, J., Tejera, A., Hilligsoe, K. M., Ramsing, N. B., & Remohi, J. (2011a). The use of morphokinetics as a predictor of embryo implantation. *Human Reproduction*, *26*(10), 2658–2671. https://doi.org/10.1093/humrep/der256

61. Meseguer, M., Herrero, J., Tejera, A., Hilligsoe, K. M., Ramsing, N. B., & Remohi, J. (2011b). The use of morphokinetics as a predictor of embryo implantation. *Human Reproduction*, *26*(10), 2658–2671. https://doi.org/10.1093/humrep/der256

62. Meseguer, M., Rubio, I., Cruz, M., Basile, N., Marcos, J., & Requena, A. (2012). Embryo incubation and selection in a time-lapse monitoring system improves pregnancy outcome compared with a standard incubator: A retrospective cohort study. *Fertility and Sterility*, *98*(6). https://doi.org/10.1016/j.fertnstert.2012.08.016

63. Mikwar, M., MacFarlane, A. J., & Marchetti, F. (2020). Mechanisms of oocyte aneuploidy associated with advanced maternal age. *Mutation Research - Reviews in Mutation Research*, *785*, 108320. https://doi.org/10.1016/j.mrrev.2020.108320

64. Milewski, R., & Ajduk, A. (2017a). Time-lapse imaging of cleavage divisions in embryo quality assessment. In *Reproduction* (Vol. 154, Issue 2, pp. R37–R53). https://doi.org/10.1530/REP-17-0004

65. Milewski, R., & Ajduk, A. (2017b). Time-lapse imaging of cleavage divisions in embryo quality assessment. In *Reproduction* (Vol. 154, Issue 2, pp. R37–R53). https://doi.org/10.1530/REP-17-0004

66. Mio, Y., & Maeda, K. (2008). Time-lapse cinematography of dynamic changes occurring during in vitro development of human embryos. *American Journal of Obstetrics and Gynecology*, *199*(6), 660.e1-660.e5. https://doi.org/10.1016/j.ajog.2008.07.023

67. Motato, Y., de los Santos, M. J., Escriba, M. J., Ruiz, B. A., Remohí, J., & Meseguer, M. (2016). Morphokinetic analysis and embryonic prediction for blastocyst formation through an integrated time-lapse system. *Fertility and Sterility*, *105*(2), 376-384.e9. https://doi.org/10.1016/j.fertnstert.2015.11.001

68. Niederberger, C., Pellicer, A., Cohen, J., Gardner, D. K., Palermo, G. D., O'Neill, C. L., Chow, S., Rosenwaks, Z., Cobo, A., Swain, J. E., Schoolcraft, W. B., Frydman, R., Bishop, L. A., Aharon, D., Gordon, C., New, E., Decherney, A., Tan, S. L., Paulson, R. J., … LaBarbera, A. R. (2018). Forty years of IVF. *Fertility and Sterility*, *110*(2), 185-324.e5. https://doi.org/10.1016/j.fertnstert.2018.06.005

69. Noyes, R. W., Hertig, A. T., & Rock, J. (1975). Dating the endometrial biopsy. *American Journal of Obstetrics and Gynecology*, *122*(2), 262–263.

70. Ottosen, L. D. M., Hindkjær, J., & Ingerslev, J. (2007). Light exposure of the ovum and preimplantation embryo during ART procedures. *Journal of Assisted Reproduction and Genetics*, *24*(2–3). https://doi.org/10.1007/s10815-006-9081-x

71. Papanikolaou, E. G., Bourgain, C., Fatemi, H., Verpoest, W., Polyzos, N. P., De Brabanter, A., Kolibianakis, E., Tarlatzis, B., Devroey, P., & Tournaye, H. (2010). Endometrial advancement after triggering with recombinant or urinary HCG: a randomized controlled pilot study. *Reproductive BioMedicine Online*, *21*(1), 50–55. https://doi.org/10.1016/j.rbmo.2010.03.020

72. Paria, B. C., Huet-Hudson, Y. M., & Dey, S. K. (1993). Blastocyst's state of activity determines the "window" of implantation in the receptive mouse uterus. *Proceedings of the National Academy of Sciences of the United States of America*, *90*(21), 10159–10162. https://doi.org/10.1073/pnas.90.21.10159

73. Polanski, L. T., Baumgarten, M. N., Quenby, S., Brosens, J., Campbell, B. K., & Raine-Fenning, N. J. (2014). What exactly do we mean by "recurrent implantation failure"? A systematic review and opinion. *Reproductive BioMedicine Online*, *28*(4), 409–423. https://doi.org/10.1016/j.rbmo.2013.12.006

74. Pribenszky, C., Mátyás, S., Kovács, P., Losonczi, E., Zádori, J., & Vajta, G. (2010). Pregnancy achieved by transfer of a single blastocyst selected by time-lapse monitoring. *Reproductive BioMedicine Online*, *21*(4), 533–536. https://doi.org/10.1016/j.rbmo.2010.04.015

75. Renwick, P. J., Trussler, J., Ostad-Saffari, E., Fassihi, H., Black, C., Braude, P., Ogilvie, C. M., & Abbs, S. (2006). Proof of principle and first cases using preimplantation genetic haplotyping - A paradigm shift for embryo diagnosis. *Reproductive BioMedicine Online*, *13*(1). https://doi.org/10.1016/S1472-6483(10)62024-X

76. Riesewijk, A., Martín, J., van Os, R., Horcajadas, J. A., Polman, J., Pellicer, A., Mosselman, S., & Simón, C. (2003). Gene expression profiling of human endometrial receptivity on days LH+2 versus LH+7 by microarray technology. *Molecular Human Reproduction*, *9*(5), 253–264. https://doi.org/10.1093/molehr/gag037

77. Roque, M., Haahr, T., Esteves, S. C., & Humaidan, P. (2021). The poseidon stratification-moving from poor ovarian response to low prognosis. *Jornal Brasileiro de Reproducao Assistida*, *25*(2), 282–292. https://doi.org/10.5935/1518-0557.20200100

78. Rubio, C., Bellver, J., Rodrigo, L., Bosch, E., Mercader, A., Vidal, C., De Los Santos, M. J., Giles, J., Labarta, E., Domingo, J., Crespo, J., Remohí, J., Pellicer, A., & Simón, C. (2013). Preimplantation genetic screening using fluorescence in situ hybridization in patients with repetitive implantation failure and advanced maternal age: Two randomized trials. *Fertility and Sterility*, *99*(5). https://doi.org/10.1016/j.fertnstert.2012.11.041

79. Rubio, I., Galán, A., Larreategui, Z., Ayerdi, F., Bellver, J., Herrero, J., & Meseguer, M. (2014). Clinical validation of embryo culture and selection by morphokinetic analysis: A randomized, controlled trial of the EmbryoScope. *Fertility and Sterility*, *102*(5), 1287-1294.e5. https://doi.org/10.1016/j.fertnstert.2014.07.738

80. Ruiz-Alonso, M., Blesa, D., Díaz-Gimeno, P., Gómez, E., Fernández-Sánchez, M., Carranza, F., Carrera, J., Vilella, F., Pellicer, A., & Simón, C. (2013). The endometrial receptivity array for diagnosis and personalized embryo transfer as a treatment for patients with repeated implantation failure. *Fertility and Sterility*, *100*(3), 818–824. https://doi.org/10.1016/J.FERTNSTERT.2013.05.004

81. Sakkas, D., Shoukir, Y., Chardonnens, D., Bianchi, P. G., & Campana, A. (1998). Early cleavage of human embryos to the two-cell stage after intracytoplasmic sperm injection as an indicator of embryo viability. *Human Reproduction*, *13*(1), 182–187. https://doi.org/10.1093/humrep/13.1.182

82. Santos, M. A., Teklenburg, G., MacKlon, N. S., Van Opstal, D., Schuring-Blom, G. H., Krijtenburg, P. J., De Vreeden-Elbertse, J., Fauser, B. C., & Baart, E. B. (2010). The fate of the mosaic embryo: Chromosomal constitution and development of Day

4, 5 and 8 human embryos. *Human Reproduction*, *25*(8). https://doi.org/10.1093/humrep/deq139

83. Sundheimer, L. W., Liu, L., Buyalos, R. P., Hubert, G., Al-Safi, Z., & Shamonki, M. (2018). Diagnosis of parental balanced reciprocal translocations by trophectoderm biopsy and comprehensive chromosomal screening. *Journal of Assisted Reproduction and Genetics*, *35*(1), 165–169. https://doi.org/10.1007/s10815-017-1042-z
84. Talbi, S., Hamilton, A. E., Vo, K. C., Tulac, S., Overgaard, M. T., Dosiou, C., Le Shay, N., Nezhat, C. N., Kempson, R., Lessey, B. A., Nayak, N. R., & Giudice, L. C. (2006). Molecular Phenotyping of Human Endometrium Distinguishes Menstrual Cycle Phases and Underlying Biological Processes in Normo-Ovulatory Women. *Endocrinology*, *147*(3), 1097–1121. https://doi.org/10.1210/en.2005-1076
85. Tan, J., Paria, B. C., Dey, S. K., & Das, S. K. (1999). Differential uterine expression of estrogen and progesterone receptors correlates with uterine preparation for implantation and decidualization in the mouse. *Endocrinology*, *140*(11), 5310–5321. https://doi.org/10.1210/endo.140.11.7148
86. Tapia, A., Vilos, C., Marín, J. C., Croxatto, H. B., & Devoto, L. (2011). Bioinformatic detection of E47, E2F1 and SREBP1 transcription factors as potential regulators of genes associated to acquisition of endometrial receptivity. *Reproductive Biology and Endocrinology*, *9*(1), 14. https://doi.org/10.1186/1477-7827-9-14
87. Thornhill, A. R., deDie-Smulders, C. E., Geraedts, J. P., Harper, J. C., Harton, G. L., Lavery, S. A., Moutou, C., Robinson, M. D., Schmutzler, A. G., Scriven, P. N., Sermon, K. D., & Wilton, L. (2005). ESHRE PGD Consortium "Best practice guidelines for clinical preimplantation genetic diagnosis (PGD) and preimplantation genetic screening (PGS)." *Human Reproduction*, *20*(1), 35–48. https://doi.org/10.1093/humrep/deh579
88. Timeva, T., Shterev, A., & Kyurkchiev, S. (2014). Recurrent implantation failure: The role of the endometrium. In *Journal of Reproduction and Infertility* (Vol. 15, Issue 4).
89. Tsai, Y. C., Chung, M. T., Sung, Y. H., Tsai, T. F., Tsai, Y. T., & Lin, L. Y. (2002). Clinical value of early cleavage embryo. *International Journal of Gynecology and Obstetrics*, *76*(3). https://doi.org/10.1016/S0020-7292(01)00591-4
90. Tseng, L. H., Chen, I., Chen, M. Y., Yan, H., Wang, C. N., & Lee, C. L. (2010). Genome-based expression profiling as a single standardized microarray platform for the diagnosis of endometrial disorder: an array of 126-gene model. *Fertility and Sterility*, *94*(1), 114–119. https://doi.org/10.1016/j.fertnstert.2009.01.130
91. Vera-Rodriguez, M., Chavez, S. L., Rubio, C., Pera, R. A. R., & Simon, C. (2015). Prediction model for aneuploidy in early human embryo development revealed by single-cell analysis. *Nature Communications*, *6*(1), 7601. https://doi.org/10.1038/ncomms8601
92. Webster, A., & Schuh, M. (2017). Mechanisms of Aneuploidy in Human Eggs. In *Trends in Cell Biology* (Vol. 27, Issue 1). https://doi.org/10.1016/j.tcb.2016.09.002
93. WHO. (2021). *Infertility prevalence estimates, 1990–2021.*
94. Wilcox, A. J., Baird, D. D., & Weinberg, C. R. (1999). Time of Implantation of the Conceptus and Loss of Pregnancy. *New England Journal of Medicine*, *340*(23), 1796–1799. https://doi.org/10.1056/NEJM199906103402304

95. Wong, C. C., Loewke, K. E., Bossert, N. L., Behr, B., De Jonge, C. J., Baer, T. M., & Pera, R. A. R. (2010). Non-invasive imaging of human embryos before embryonic genome activation predicts development to the blastocyst stage. *Nature Biotechnology*, *28*(10), 1115–1121. https://doi.org/10.1038/nbt.1686

96. Yang, R., Yang, S., Li, R., Chen, X., Wang, H., Ma, C., Liu, P., & Qiao, J. (2015). Biochemical pregnancy and spontaneous abortion in first IVF cycles are negative predictors for subsequent cycles: an over 10,000 cases cohort study. *Archives of Gynecology and Obstetrics*, *292*(2), 453–458. https://doi.org/10.1007/s00404-015-3639-8

97. Yang, S. T., Shi, J. X., Gong, F., Zhang, S. P., Lu, C. F., Tan, K., Leng, L. Z., Hao, M., He, H., Gu, Y. F., Lu, G. X., & Lin, G. (2015). Cleavage pattern predicts developmental potential of day 3 human embryos produced by IVF. *Reproductive BioMedicine Online*, *30*(6). https://doi.org/10.1016/j.rbmo.2015.02.008

98. Zeadna, A., Son, W. Y., Moon, J. H., & Dahan, M. H. (2015). A comparison of biochemical pregnancy rates between women who underwent IVF and fertile controls who conceived spontaneously. *Human Reproduction*, *30*(4), 783–788. https://doi.org/10.1093/humrep/dev024

99. Zegers-Hochschild, F., Adamson, G. D., de Mouzon, J., Ishihara, O., Mansour, R., Nygren, K., Sullivan, E., & Vanderpoel, S. (2009). International Committee for Monitoring Assisted Reproductive Technology (ICMART) and the World Health Organization (WHO) revised glossary of ART terminology, 2009*. *Fertility and Sterility*, *92*(5), 1520–1524. https://doi.org/10.1016/j.fertnstert.2009.09.009

100. Zhang, D., Sun, C., Ma, C., Dai, H., & Zhang, W. (2012). Data mining of spatial-temporal expression of genes in the human endometrium during the window of implantation. *Reproductive Sciences*, *19*(10). https://doi.org/10.1177/1933719112442248

101. Zhang, H. G., Zhang, X. Y., Zhang, H. Y., Tian, T., Xu, S. B., & Liu, R. Z. (2016). Balanced reciprocal translocation at amniocentesis: Cytogenetic detection and implications for genetic counseling. *Genetics and Molecular Research*, *15*(3), 1–8. https://doi.org/10.4238/gmr.15038556

102. Zhao, W. W., Wu, M., Chen, F., Jiang, S., Su, H., Liang, J., Deng, C., Hu, C., & Yu, S. (2015). Robertsonian translocations: An overview of 872 Robertsonian translocations identified in a diagnostic laboratory in China. *PLoS ONE*, *10*(5), 1–15. https://doi.org/10.1371/journal.pone.0122647

103. Zini, A., Boman, J. M., Belzile, E., & Ciampi, A. (2008). Sperm DNA damage is associated with an increased risk of pregnancy loss after IVF and ICSI: Systematic review and meta-analysis. *Human Reproduction*, *23*(12), 2663–2668. https://doi.org/10.1093/humrep/den321

ACKNOWLEDGEMENTS

I would like to express my gratitude to all my colleagues at the clinic without whom this work would be difficult to fulfill. Especially I would like to thank following persons:

- MSc. biol. Evija Pīmane, MSc. biol. Arita Blumberga from the Embryology laboratory of iVF Riga for their endless caring about the embryos;
- MSc. biol. Dace Enkure, MSc. biol. Una Čonka, MSc. biol. Baiba Alkšere, Dr. Liene Korņejeva, Dr. Aigars Dzalbs and MSc. biol. Santa Andersone from the Genetic Centre, iVF Riga, for their help in genetic and cytogenetic analysis, and interpretation of results in this study;
- Dr. med. Juris Erenpreiss for his help in drafting the manuscripts;
- MSc. biomed., MSc. chem. Anna Dorondo and Dr. biol. Marija Mihailova for helping with organizational issues;
- My supervisors Asoc. Prof. Dr. med. Natalija Vedmedovska and Asoc. Prof., Dr. paed. Margarita Pukite for kind advice and support during this time; I am very greatly indebted to them for Their instructive and stimulating scientific discussions, support, and encouragement during this period.
- I am grateful to my friend Gregory MacDonald, PhD, the Royal College of Surgeons in Ireland, who skillfully revised English language of this thesis.
- My family for understanding and endless support during this period.

APPENDIXES

Appendix I

Centrālā medicīnas ētikas komiteja

Brīvības iela 72, Rīga, LV-1011 • Tālr. 67876182 • Fakss 67876071 • E-pasts: vm@vm.gov.lv
Rīgā

Datums skatāms laika zīmogā Nr. 01-29.1.2/1735
Uz Nr.

Rīgas Stradiņa Universitātes
Molekulārās ģenētikas
zinātniskajai laboratorijai
jerenpreiss@gmail.com
baiba.alksere@rsu.lv
violeta.fodina@ivfriga.eu

Par atļaujas saņemšanu pētījumam "GENĒTISKO VARIĀCIJU IDENTIFIKĀCIJA PACIENTIEM AR REPRODUKTĪVIEM TRAUCĒJUMIEM MĒRĶTIECĪGAS ĀRSTĒŠANAS STRATĒĢIJAS IZVĒLĒ"

Centrālā medicīnas ētikas komiteja izskatīja Rīgas Stradiņa Universitātes Molekulārās ģenētikas zinātniskās laboratorijas vadošā pētnieka, Dr. Med. Jura Ērenpreiss pieteikums par pētījumu "*GENĒTISKO VARIĀCIJU IDENTIFIKĀCIJA PACIENTIEM AR REPRODUKTĪVIEM TRAUCĒJUMIEM MĒRĶTIECĪGAS ĀRSTĒŠANAS STRATĒĢIJAS IZVĒLĒ*" (reģistrēts Veselības ministrijā 2021.gada 3.martā Nr.3605).

Atbilstoši Centrālā medicīnas ētikas komitejas 2021.gada 18.marta sēdes protokola Nr.2021-10 II.daļas "*PRECIZĒTIE IESNIEGUMI*" 4.punktam tiek sniegts atzinums, ka pētījums nav pretrunā bioētikas normām.

Centrālās medicīnas ētikas
komitejas priekšsēdētājs (paraksts*) V. Sīlis

Andrejs Rubins 67876062
andrejs.rubins@vm.gov.lv

*Dokuments ir parakstīts ar drošu elektronisko parakstu un satur laika zīmogu

Table 6.3.1. **All the patient's pregnancy history with data of ovarian stimulation, insemination, embryo biopsy with current results**

Pair No.	Karyotype	Pregnancy	Childbirth	Children	Pregnancy with fetal abnormality	Life-born child with pathology	
1	46,XX,t(1;6) (p22;p25)	0	0	0	0	0	
	46,XY						
2	46,XX	1	0	0	0	0	
	46, XY,t(4;12) (q31;q13)						
3	46,XX	0	0	0	0	0	
	45,XY,der(13;14) (q10;q10)						
4	46,XX,t(9;17) (p10;p10)	0	0	0	0	0	
	46,XY						
5	46,XX,t(6;10) (p21.3;q22.3)	1	1	1	0	0	
	46,XY			0			
6	46,XX	1	1	1	0	0	
	45,XY,der(13;14) (q10;q10)			0			
7	46,XX	0	0	0	0	0	
	46,XY,t(1;3) (q12;q29) [4]/46,XY[15]						
8	46,XX,t(13;20) (q34q13)	2	0	0	1	0	
	46,XY						
9	46,XX,t(8;11) (p23;p15)	3–5	1	1	0	0	
	46, XY			1			
10	46,XX	2	0	0	0	0	
	45,XY,der(13;14) (q10;q10)						

Obtained oocytes	Fertilized oocytes	Obtained blastocysts	Biopsied blastocysts	Blastocysts analyzed	Euploidy	Aneuploidy	Results
18	9	8	5	4	1	3	Pregnancy did not occur
8	8	5	4	4	2	2	Natural pregnancy
1 cycle – 13 2 cycle – 21	1 cycle – 11 2 cycle – 18	1 cycle – 4 2 cycle – 13	1 cycle – 4 2 cycle – 12	1 cycle – 4 2 cycle – 12	4	2	Pregnant after IVF
18	14	8	4	3	1	2	1 child after IVF
13	5	5	4	1	0	1	Needs new stimulation
1 cycle – 11 2 cycle – 4	1 cycle – 9 2 cycle – 3	1 cycle – 9 2 cycle – 0	1 cycle – 2 2 cycle – 0	1 cycle – 2 2 cycle – 0	0	2	Needs new stimulation
24	20	16	15	4	2	2	Pregnant after IVF
8	8	8	6	6	0	6	Needs new stimulation
25	18	5	4	4	0	4	Needs new stimulation
17	17	9	9	4	0	4	Needs new stimulation

Table 6.3.2. **Patients' data of karyotype, number and grade of obtained blastocysts with PGT-A results**

No.	Gender	Age	Karyotype	Blastocysts analysed	Blastocysts grade	
1	Female	35	46,XX,t(1;6) (p22;p25)	4	1) BC3BB 2) BC3BB 3) BC4AA 4) BC3AB	
2	Male	46	46, XY,t(4;12) (q31;q13)	4	1) BC3BB 2) BC3BA 3) BC2BB 4) BC3AB	
3	Female	34	46,XX,t(9;17) (p10;p10)	3	1) BC2BB/3/D 2) BC2BB/2/A 3) BC2BB/5/A	
4	Female	32	46,XX,t(6;10) (p21.3;q22.3)	1	1) BC3BB	
5	Male	28	46,XY,t(1;3) (q12;q29)[4]/ 46,XY[15]	4	1) BC2BB/4/A 2) BC3BB/4/A	
6	Female	31	46,XX,t(13;20) (q34q13)	6	1) BC2BC/5/A 2) BC2BB/5/A 3) BC2AB/5/A 4) BC2AB/4/A 5) BC3BB/5/BG 6) BC2BB/5/A	

PGT-A results
1) arr1p36.331p22.1(102664–92589017)x1, 6p25.3p24.3(103351–8874411)x3; 2) arr1p36.331p22.1(102664–92589017)x3,6p25.3p24.3(103351–8874411)x1; 3) arr(1–22)x2,(XY)x1; 4) arr1p36.331p22.1(102664–92589017)x1,6p25.3p24.3(103351–8874411)x3
1) arr4q32.3q35.2(169102980–190815481)x1,12q21.1–q24.33(72408753–133435933)x3 2) arr16p13.3p12.2(137326–23760229)x3 3) arr(1–22,X)x2 4) arr(1–22,X)x2
1) arr9p24.3q13(104476–66534426)x1, 17p13.3p11.2(556474–20230594)x1 2) arr(9)x1 3) arr(1–22)x2, (XY)x1
1) arr(2)x1~2,6p25.3p21.32(672168–3233433100)x1,10p23.1q26.3(82574455–135336967)x3,(12–22,X)cx
1) seq[GRCh37]dup(4)(p16.3q31.21)chr4:g. 602,531_144,926,382 del(4)(q31.21q35.2) chr4:g.146,137,523_190,874,077 2) seq[GRCh37](1–22)x2, (XY)x1
1) seq[GRCh37](1–22)cx dup(13)(q12.11q33.3) chr13:g.19,812,720_108, 833, 167 del(13) (q34q34) chr13:g. 110,994,782_114, 494, 829 del(20) (p13q13.2) chr20:g. 621, 362_52, 103, 980 dup(20) (q13.2q13.33) chr20:g. 53,221,087_62,715,666 2) seq[GRCh37] (1–22,X)cx dup(13) (q12.11q 33.3) chr13:g. 19,812,720_108,833,167 del(13) (q34q34) chr13:g. 110,994,782_114,494, 829 del(20) (q13.2q13.33) chr20:g. 53,221,087_62,715,666 3) seq[GRCh37]dup(13) (q12.11q33.3) chr13:g. 19,812, 720_108, 833, 167 del(13) (q34q34) chr13:g. 110,994, 782_114,494,829 del(20)(p13q13.2) chr20:g. 621, 362_52, 103,980 dup(20)(q13.2q13.33) chr20:g.53,221,087_62,715,666 4) seq[GRCh37](1)x1del(13) (q34q34) chr13:g. 110,994,782_114,494,829(17)x1 dup(20) (q13.2q13.33) chr20:g. 53,221,087_62,715,666 del(20) (q13.2q13.33) chr20:g. 53,221,087_62,715,666 5) seq[GRCh37]dup(13)(q12.11q33.3) chr13:g. 19,812,720_108, 833,167 del(13) (q34q34) chr13:g.110,994,782_114,494,829 6) seq[GRCh37]del(13)(q34q34) chr13:g. 110,994,782_114,494,829 dup(20) (q13.2q13.33) chr20:g.53,221,087_62,715,666

Table 6.3.2. **Patients' data of karyotype, number and grade of obtained blastocysts with PGT-A results. Continued**

No.	Gender	Age	Karyotype	Blastocysts analysed	Blastocysts grade	
7	Female	36	46,XX,t(8;11) (p23;p15)	4	1) BC2AB/5/A 2) BC2BC/CC/5/A 3) BC5BC/CC/1/D 4) BC3AB/2/A	
8	Male	46	45,XY,der(13;14) (q10;q10)	1st cycle – 4 2nd cycle – 12	1) BC2BB/5/D 2) BC2BB/5/A 3) BC2BB/5/A 4) BC2BB/4/A 1) BC2BB/5/A 2) BC2BB/2/A 3) BC3BB/4/A 4) BC3AB/5/A 5) BC3AA/5/A 6) BC3BC/3/D 7) BC3AB/1/C 8) BC3AA/1/C 9) BC3BB/5/A 10) BC3BB/5/A 11) BC2BB+cells/2/A 12) BC3BB/2/A	
9	Male	40	45,XY,der(13;14) (q10;q10)	1st cycle – 2 2nd cycle – 0	1) BC2AB 2) BC3BB	
10	Male	35	45,XY,der(13;14) (q10;q10)	4	1) BC2BB 2) BC3AB 3) BC3BB 4) BC3BB	

	PGT-A results
	1) seq[GRCh37]del(8)(p23.3p23.1) chr8:g.663,894_9,478,357, dup(11)(p15.5p15.4) chr11:g.721,142_6,682,406,(13)x1, (14)x1 2) seq[GRCh37]dup(X)(p22.33p22.12)chrX:g.3,354,528_21,343,697 3) seq[GRCh37] dup(8)(p23.3p23.1) chr8:g.663,894_9,478,357, del(11) (p15.5p15.4) chr11:g.663,894_6, 682,406 4) seq[GRCh37]del(6) (q26q27)chr(6):g.161, 462,989_170,976,006,cxmos
	1) arrmos(2)x1, 11q23.2q25(114304322–134564974)x1 2) arr(15)x1,(16)x3 3) arr(16)x1 4) arr(13)x1,(16)x1 1) arr(16)x1 2) arr18q21.2q23(49776191–77856022)x1_2 3) arr2q12.2q37.3(10656227–242058943)x1_2,6q23.3q27(138010907–170931603)x1_2; 4) arr(1–22),(XY)x1 5) arr(1–22),(XY)x1 6) arr(22)x1 7) arr(1–22,X)x2 8) arr(16)x3 9) arr(4)x3,(16)x3 10) arr(1–22)x2,(XY)x1 11) arr(4)x1, (19)x3 12) arr(16)x3
	1) seq[GRCh37]6q13q27 (75,871,171–170,976,006)x1 2) seq[GRCh37](1–22)cx
	1) seq[GRCh37](1–22,X)cx(13)x1,(21)x3 2) seq[GRCh37](1–22)cx (21)x2~3 3) seq[GRCh37]del(12)(q12q24.33) chr12:g. 44, 629,644_133,380,179 4) seq[GRCh37](1–22)cx(13)x1~2,(16)x1