WYDZIAŁ BIOLOGII
UNIWERSYTET GDAŃSKI

Joanna Liss, PhD

Are of Knowledge: ***Natural Sciences***
Field: ***Biological Sciences***
Discipline: ***Biology***

DEVELOPMENT AND APPLICATION OF MOLECULAR BIOLOGY METHODS IN GENETIC DIAGNOSTICS OF REPRODUCTIVE CELLS AND EMBRYOS

SCIENTIFIC ACHIEVEMENT

Department of Biology and Medical Genetics

Gdańsk 2019

1. **First and last name:** JOANNA LISS

2. Diplomas, scientific degrees:

Doctoral Degree in Medical Sciences in medical biology, 2000, Medical Academy in Gdańsk, Institute of Obstetrics and Women's Diseases
Title of the doctoral thesis, completed in the Independent Laboratory of Endocrinology and Laboratory Diagnostics:
„Initial assessment of the impact of HPV infection on the development of cervical cancer based on the presence of viral DNA in infected tissues"
Supervisor: Prof. dr hab. Czesław Wójcikowski

Master of Science in Biology in general biology, 1995, University of Gdańsk, Faculty of Biology, Geography and Oceanology
The title of the master's thesis, made at the Department of Biochemistry: "Attempts to express the Rz1 gene of bacteriophage λ in selected expression systems"
Supervisor: Prof. dr hab. Alina Taylor

3. Previous employment in scientific institutions:

02.2018 - present: assistant professor at the Department of Biology and Medical Genetics, Faculty of Biology, University of Gdańsk

02.2001 - present: head of the In Vitro Laboratory and the Bank of Reproductive Cells and Embryos, INVICTA

06.1999 - 01.2001: junior assistant at the Independent Public Clinical Hospital No. 2 of the Medical Academy in Gdańsk

09.1995 - 02.2001: assistant at the Independent Laboratory of Endocrinology and Laboratory Diagnostics at the Institute of Obstetrics and Women's Diseases at the Medical Academy in Gdańsk

4. Indication of the achievement based on the art. 16 sec. 2 of the Act of 14 March 2003 on academic degrees and academic title, and on degrees and title in the field of art (Journal of Laws 2017, item 1789):

a) Title of scientific achievement:

Development and application of molecular biology methods in the genetic diagnosis of reproductive cells and embryos.

b) publications included in the scientific achievement:

1. **Liss J.**, Pastuszek E., Pukszta S., Hoffmann E., Kuczynski W., Lukaszuk A., Lukaszuk K. 2018. Effect of next-generation sequencing in preimplantation genetic testing on live birth ratio. Reprod Fertil Dev. 2018 Jun 22. doi: 10.1071/RD17428.
IF: 2,105; MSHE (points according to Ministry of Science and Higher Education):30. *Percentage share on my involvement estimated at 45%.*

2. **Liss J.**, Chromik I., Szczyglińska J., Jagiełło M., Łukaszuk A., Łukaszuk K. 2016. Current methods for preimplantation genetic diagnosis. Ginekol Pol. 87(7):522-6.
IF: 0,576; MSHE : 15. *Percentage share on my involvement estimated at 50%.*

3. Łukaszuk K., Pukszta S., Wells D., Cybulska C**., Liss J**., Płóciennik Ł., Kuczyński W., Zabielska J. 2015. Routine use of next-generation sequencing for preimplantation genetic diagnosis of blastomeres obtained from embryos on day 3 in fresh in vitro fertilization cycles. FertilSteril. Apr;103(4):1031-6.
IF: 4,426; MSHE : 45. *Percentage share on my involvement estimated at 15%.*

4. **Liss J**., Kiewisz J., Zabielska J., Kulwikowska P., Lukaszuk K. 2015. Application of FISH method for preimplantation genetic diagnostics of reciprocal and Robertsonian translocations. Folia Histochem Cytobiol. 53(2):162-8.
IF: 1,06; MSHE : 15. *Percentage share on my involvement estimated at 45%.*

5. **Liss J.**, Bruszczyńska A., Łukaszuk K. 2010. Preimplantation genetic diagnosis in prevention of genetic diseases-diagnostic of spinal muscular atrophy (SMA). Ginekol Pol; 81(12):918-21.
IF: 0,367; MSHE : 9. *Percentage share on my involvement estimated at 70%.*

6. **Liss J.,** Lukaszuk K., Bruszczyńska A., Szczerkowska Z., Rebala K. 2008. Pregnancy and life after preimplantation genetic diagnosis of Smith-Lemli-Opitz syndrome. Fertil Steril. Nov;90(5):2011.e13-6.
IF: 4,167; MSHE : 24. *Percentage share on my involvement estimated at 50%.*

c) discussion of the scientific purpose of the above the work and results achieved, together with a discussion of their possible application

The scientific achievement constituting the basis for applying for the habilitation has been presented in 6 monothematic publications, including original research on the development and implementation of diagnostic methods used in preimplantation diagnosis of patients with history of genetic diseases, as well as those struggling with reproductive failures due to genetic disorders occurring in the reproductive cells . All research presented in the publications included in the scientific achievement was carried out in accordance with the protocols approved by the Bioethics Committee of the Medical University of Gdańsk. In 5 of these works, I am the first author, and the **total IF and MSHE** of these publications are **12,701** and **138**, respectively.

Preimplantation genetic testing (PGT) is a diagnostic method that can be used only in the course of infertility treatment using assisted reproduction methods. It allows genetic examination of the oocyte prior to its fertilization or embryo before it is transferred into the uterus. It was used for the first time in 1990. Thanks to this method, the first child free of the risk of the X chromosome linked adrenoleukodystrophy was born (Handyside et al. 1990).

Thanks to the application of PGT around 1000 children were born up to year 2004 (Verlinsky et al. 2004), and by 2013 this number increased to over 10,000 (Simpson et al. 2010, Coco et al. 2014, De Rycke et al 2017). Preimplantation genetic testing is a sequence of actions that allows the selection of a genetically healthy embryo for transfer. It consists of the collection of material for examination (biopsy), the preparation of genetic material for analysis (DNA isolation and its amplification), the analysis (selection of method), and the preparation and release of the result.
Within preimplantation genetic testing, two basic types can be distinguished:
1. Preimplantation Genetic Testing for Monogenic diseases and Structural Rearrangements (PGD-M / PGD-SR) that assesses the genotype of the embryo, performed in couples affected by genetic abnormalities such as: monogenic diseases, point mutations, translocations or other genetic disorders.
2. Preimplantation Genetic Testing for Aneuploidies (PGT-A) that assesses the potential aneuploidy of all 24 chromosomes and is performed especially for patients after the age of 35, those with repeat implantation failures, recurrent pregnancy loss with normal parental karyotypes and when the cause of infertility is a significant male factor . PGT is intended to be used to select for transfer embryo or embryos with a normal karyotype (Harper et al. 2014).

I have begun the research on the use of molecular methods in the genetic diagnosis of reproductive cells and embryos in 2005 in an in vitro laboratory at

INVICTA infertility treatment clinic in Gdańsk. These studies were an innovative approach to the genetic diagnosis of reproductive cells on a national scale and in cases of some diseases also on a global scale. They allowed the introduction to routine infertility treatment of genetic diagnosis of reproductive cells or embryos in couples with a family history of genetic disease, where mutations responsible for the occurrence of a given disease entity were already diagnosed. It became, therefore, possible to prevent the occurrence of a given disease at the preimplantation stage.

Searching for the possibilities of using available molecular biology techniques, I focused, first of all, on the diagnosis of monogenic diseases. It is worth noting that such studies have not been previously conducted in Poland. The available prenatal diagnosis included only genetic tests performed during pregnancy, as part of prenatal tests, based on the analysis of maternal or fetal blood, material collected during trophoblast biopsy or amniotic fluid collected during amniocentesis. It was performed when the occurrence of genetic disease in the fetus was suspected, in order to prepare and plan therapy for the child after birth.

The first disease for which I undertook assessment and design of genetic diagnosis was the Smith, Lemli Opitz syndrome (SLOS). It is a metabolic disease, inherited in an autosomal recessive manner. It belongs to rare diseases and is characterized by multiple congenital malformations. Its incidence is estimated at 1:20,000 - 1:60,000 births. In Poland, the prevalence of this disease is higher than in other European countries and is 1:2,300 - 1:39,137, which makes this disease to be one of the most frequently occurring metabolic diseases in our country. This disease is caused by an abnormality in cholesterol biosynthesis due to mutations in the *DHCR7* gene, located on the long arm of chromosome 11, at location 11q12-13 (Nowaczyk et al. 2001). According to previous reports, there are over 120 known mutations within this gene. Most of them are one-nucleotide substitutions (90%). Deletion and insertion type mutation occur very rarely (10%) (Yu et al. 2005). This gene is responsible for the formation of DHCR7 protein - 7-dehydrocholesterol reductase, which is an enzyme involved in the conversion of 7-dehydrocholesterol to cholesterol. As a result of the mutation, the body is unable to produce enough cholesterol, which is necessary for the proper development of the embryo and fetus. Cholesterol is an element that builds, among others, cell membranes and tissues that protect nerve cells. It also serves as a basis for synthesis of many hormones. Attempts have been made to determine the correlation between the genotype and the phenotype of the disease. Mild clinical symptoms in patients (type I SLOS) are caused by missense mutations that change the codon information. The severe form of the disease (type II SLOS) is associated with mutation changing the reading frame and introducing a stop codon that may cause production of a completely dysfunctional enzyme or no enzyme at all.

The genetic pre- and postnatal diagnosis of SLOS is based on two types of tests: biochemical tests for cholesterol and 7-8- dehydrocholesterols in the blood serum and molecular tests, where the coding sequence of the *DHCR7* gene is

analyzed for mutations. So far, it has not been possible to evaluate the risk of mutations in the *DHCR7* gene at the preimplantation stage.

I have focused my research on diagnosing the nonsense mutation *W151X* G> A at position 3557 bp within the *DHCR7* gene, which was found in 2 couples during the genetic diagnosis of their children conceived naturally.

First, I designed the primer sequences that would allow the amplification of the *DHCR7* gene fragment, including the site of the above mentioned mutation. The conditions of the PCR reaction had to be selected specifically for the analyzed material, i.e. a single cell derived from an oocyte (the polar body) and the embryo (a single blastomere taken from a 6-8 cell embryo). The best method for increasing the sensitivity and specificity of amplification in this case was the nested-PCR method. While designing external and internal starters, I paid attention to the risk of primer pairing and dimer formation (primer-dimer). In addition, the melting temperatures had to differ from each other to avoid hybridization of the internal primers during the first amplification cycles. The first stage involved lysis of the cell biopsied for the analysis. In case of working with only one cell, the reaction conditions and concentration of enzymes used in this process should be noted. During this stage, I used an additional the incubation step of the collected material at -20^0C, which allowed for an effective analysis of its genome. Two-stage amplification of the *DHCR7* gene fragment allowed to obtain the sought after fragment with high sensitivity and specificity. The amplification product was designed in such a way that it also contained a restriction site for the *AluI* enzyme at the site of the mutation, which allowed for a precise analysis of the test result.

The method presented above was my own contribution to the development of SLOS diagnosis at the prenatal stage. I have developed, designed and used it successfully for the first time in the world. It allowed for a healthy pregnancy and the birth of healthy children, without the risk of being affected by this disease (**Liss J. et al. 2008**). Published results of the research were recognized with a team award of the second degree by the rector of Medical University of Gdansk. This method had its application in the next two cases of diagnosed carrier of the indicated *DHCR7* gene mutations in the course of treatment with assisted reproduction methods, the treatment of which resulted in the birth of healthy children.

A positive result of the carried out research, and in particular the fact that healthy children were born without the risk of genetic disease, prompted me to continue work on designing diagnostic methods that allow the examination of individual cells in cases of other diseases. I have obtained promising results in the diagnosis of spinal muscular atrophy (SMA) in the reproductive cells of genetically burdened patients (**Liss et al. 2010**). SMA is a rare neuromuscular disorder characterized by the degeneration of the anterior spinal cord nuclei, which results in the impairment of skeletal muscles. In about 95% of cases of this disease it is caused by the mutation in the *SMN* (survival of motoneuron) gene, or more precisely telomeric copy of this gene, referred to as *SMN1*. This gene is localized in a locus on

the long arm of chromosome 5 (5q13) (Mazurczak et al. 2003, Daniels et al. 2001). The molecular defect consists of deletion of exon 7 of the telomeric copy and is responsible for about 95% of cases of SMA. In the remaining 5% of cases, substitution mutations or small deletions, located in different parts of the gene, that interfere with its normal expression are found. Deletions within the centromeric copy do not cause clinical symptoms and occur in about 5% of the healthy population. In Poland, it has not been possible to rule out the risk of having a sick child in the parents who are carriers of the defective gene. In the world pre-implantation diagnostics for SMA has been used for several years.

Also in this case, the only option for prenatal diagnosis was molecular analysis of fetal DNA originating from material collected during pregnancy (during cordocentesis, trophoblast biopsy or amniocentesis) to assess carrier status or full genetic defect within the *SMN1* gene.

Also in this case, the key element of the analysis was the preparation of individual cells for the diagnostic process. The lysis of a single cell was carried out in the PBS/PVP environment, which proved to be the most efficient in terms of the obtained amplification signal from a single cell, and the incubation step I introduced at -20^0C also additionally enhanced the efficiency of the entire process.

Considering the research results and methods used in the case of the SLOS study, I have also applied the nested-PCR design principles in this case, paying particular attention to the reaction conditions, including the melting temperatures of the primer sequences and the number of amplification cycles. Designed primers allowed for efficient amplification of the *SMN1* gene fragment including the deletion site. This fragment had in its sequence two restriction sites recognized by the *HinfI* enzyme. One of them was a site originating from the sequence of the analyzed fragment, the second one was a site designed for the analysis and was located within the internal sequence of the primer, which includes the location of the deletion. Thanks to this, in the case of the correct inheritance of the *SMN1* gene, 3 products were obtained after restriction analysis. The presence of deletions within the *SMN1* gene allowed to obtain two restriction products (only from the natural place in the sequence), due to the lack (deletion) of the nucleotide recognized by *HinfI*. Such a restrictive analysis of the amplified fragment of the *SMN1* gene allowed for an straightforward evaluation of the molecular defect in the analyzed embryos. The diagnostic tests I have developed allowed for a preimplantation test in 5 couples with a history of child births diagnosed with Werdnig-Hoffman disease (type I SMA) diagnosed and confirmed by molecular tests. In 4 of them, the treatment ended with pregnancy and the birth of healthy children. Pharmacological treatment of children with SMA is currently very expensive, childcare places special requirements on parents and requires possession of specialized rehabilitation equipment. For this reason, parents of a child with SMA often do not decide to have another one because of the risk of disease recurrence. Genetic preimplantation tests allow to avoid this risk, and the diagnostic method I have developed and implemented is a relatively

simple way to diagnose this problem and can be successfully used by other laboratories performing preimplantation diagnostics.

At this point, it is worth mentioning that the research described above focused exclusively on the indicated mutations, molecular defects responsible for the occurrence of specific diseases previously diagnosed in the family. Other genetic risks that may affect a healthy pregnancy also deserve attention.

Chromosomal structural aberrations are usually diagnosed based on the results of the patient's karyotype analysis. This type of chromosomal abnormality is balanced, with no clinical effect. The amount of genetic material is correct, only the displacement of the chromosome fragment within or between the chromosomes occurs. A parental karyotype with translocation or inversion present can contribute to genetic imbalance when the embryo is formed and develops, resulting in spontaneous miscarriage (Munne et al. 2002, Vanneste et al. 2009, Voet et al. 2011).

The subject of my further research on the use of available molecular techniques to diagnose molecular defects in the reproductive cells has become a group of patients who were carriers of balanced reciprocal and Robertsonian translocations. The prenatal diagnosis in cases of translocation carriers consists of amniocyte based karyotype analysis and uses the classic cytogenetic evaluation of the tested material based on cell culture. In order to analyze a single cell derived from an embryo, it is impossible to undertake a cell culture or to multiply the cell so that it can be used for classical cytogenetic analysis. The development of a single cell analysis method, and ultimately a cell nucleus derived from a single embryo cell, became the key in the design of this type of diagnostics. Analysis of the cellular nucleus of the blastomere requires full lysis of the morphotic elements of the cell and immobilization of the nucleus on the slide to be further evaluated. Various buffers containing detergent to destabilize cell membranes may be used to lyse the cell and fix the cell nucleus. Comparing different compositions, the best effect was obtained by using cell lysis in a 0.01N HCL / 0.1% Tween20 medium, and then fixing the nucleus with a 3:1 solution of methanol and acetic acid. Additionally, it is worth noting that the method I have developed for placing and marking individual cell nuclei on a slide allowed efficient image analysis that could be quickly carried out. In order to assess chromosomal aberrations, I used commercial fluorescent-labeled molecular probes (Fluorescent In Situ Hybridization -FISH). The hybridization process has also been modified to refine the incubation times and concentrations, so that the entire analysis could take place within 36 hours, which is crucial when the decision to perform the embryo transfer needs to be made in the same IVF treatment cycle. To better assess the possible risk of aberrations, I added probes to the evaluation of the centromere and telomeres of chromosomes involved in the rearrangement. The obtained results showed a high percentage of imbalance of the genetic material in the embryos within the examined chromosomes in carriers of translocations, and the selection for transfer of only genetically balanced embryos allowed to obtain healthy pregnancies

with efficacy comparable to that obtained in patients without such risk (**Liss et al. 2015**).

The use of fluorescent probes in the preimplantation diagnosis of reproductive cells and embryos allowed to extend the scope of their use by analyzing the number of chromosomes in terms of the assessment of ploidy. However, the limitations of this method (possible analysis of up to 7 types of chromosomes in two rounds of hybridization) did not allow to study all chromosomes but only selected ones, which made it impossible to comprehensively assess the genetic status of the embryo. Only the introduction of the next generation (NGS) sequencing technique allowed for its more effective assessment of embryo ploidy.

Prenatal ploidy diagnosis is based on routine prenatal tests, where the material collected during trophoblast biopsy, amniotic fluid or peripheral blood of the mother and, depending on the diagnostic method, the fetal karyotype is analyzed. However, these studies only allow to determine the presence of an abnormal number of chromosomes and to plan further treatment after childbirth, or in specific cases contribute to the decision to terminate pregnancy.

The assessment of embryo ploidy at the preimplantation stage avoids the risk of fetal defect (Gianaroli et al. 2001, Macklon et al. 2002).

Acknowledging the technical possibilities of the NGS method, I joined the research team, which was one of the first in the world to implement this method for preimplantation genetic tests. NGS is currently the most modern diagnostic technique used in genetic analysis, giving unique possibilities in terms of the amount of information obtained and the sensitivity and reliability of the study. NGS allows sequencing of many fragments from the studied region, providing a new tool, the so-called read depth (Kobpldt et al. 2013). The analysis includes several thousand sites on all 46 chromosomes. The object of my further considerations was therefore the use of this technique in the genetic analysis of reproductive cells and embryos. In order to implement such advanced molecular method for preimplantation studies, a series of experiments was planned to prepare material for research, including material from an oocyte, an 8-cell embryo and a blastocyst, in order to verify the concordance of the obtained results. My contribution to the development of a comprehensive embryo screening method was, in this case, the development of lysis methods for the investigated cells (**Łukaszuk et al. 2015**). Every diagnostic method requires meticulous preparation of the material for analysis, and the material in the form of a single cell is very demanding. The specificity of the next generation sequencing method, due to its sensitivity, requires adjusting the environment in which the amplification is performed to the type of material being tested. Considering these requirements, I tested various types of lysis buffers, depending on the type of the analyzed cells (polar body, blastomere, blastocyst cells) and ultimately I chose the buffer where the lowest percentage of non-diagnostic results was obtained (<5%). Further analyzes confirmed the correctness of this choice and allowed it to be used in routine preimplantation testing.

The continuous development of molecular biology techniques, observed over the years, that was based on the implementation of new diagnostic methods, allowed to increase the sensitivity and specificity of preimplantation testing. Fluorescent in situ hybridization has been replaced by more reliable technologies such as microarrays (aCGH) and the next generation sequencing (NGS). Taking into account my own extensive experience in preimplantation diagnosis of embryos, I have evaluated the diagnostic possibilities of individual methods (**Liss et al. 2016**). Different genetic defects that occur during early division of reproductive cells can only be recognized thanks to specific techniques. As the NGS technique provides the greatest diagnostic possibilities, I decided to continue research using this tool.

Published literature data shows that diagnostic techniques used in preimplantation tests, including NGS, require cryopreservation of embryos after collecting the material for examination. The time of the analysis itself often does not allow to obtain a test result within one day. The effectiveness of such treatment depends on the effectiveness of freezing and thawing techniques. On the other hand it allows for a planned preparation of the patient for the embryo transfer. The subject of my further scientific considerations was therefore to estimate the effectiveness of PGT using the NGS method in cycles with frozen/thawed embryo transfers (**Liss et al., 2018**). The material for genetic testing was collected before the embryos were vitrified, on the 5th or 6th day of their culture. The preimplantation testing involved the analysis of all 46 chromosomes using the NGS technique. The obtained results allowed to unambiguously hypothesize that the assessment of embryo ploidy before its transfer to the uterus gives a higher chance of success of the treatment compared to cases lacking such information, and the next generation sequencing method can be successfully used routinely for such testing, which is also confirmed by other published literature (Fiorentino et al. 2014, Tan et al. 2014, Yang et al. 2015, Liu et al. 2016).

Considering the overall results and research described in the above publication cycle, the use of molecular biology methods in preimplantation diagnostics brings a very tangible benefit in the treatment of infertile couples. It is possible to exclude the risk of having a sick child in families with a history of genetic disease. Another positive aspect is also the possibility of avoiding termination of pregnancy, especially in cases where the risk of genetic defects may be conditioned on parental carrier status, e.g. translocations, genetic diseases inherited in an autosomal dominant or recessive manner. Summing up my achievements in this field, it is worth noting that the research methods developed by me allowed to diagnose genetic problems in reproductive cells and embryos at the preimplantation stage, which measurably translates into the better effect of treatment in couples using assisted reproduction methods. Many of the methods can be successfully used by other laboratories performing preimplantation diagnostics.

Literature *(includes items not listed in point 4b)*

1. Coco R. Reprogenetics: Preimplantational genetic diagnosis. Genetic and Molecular Biology. 2014, 37:271-284.
2. Daniels G, Pettigrew R, Thornhill A et al. Six unaffected livebirths following preimplantation diagnosis for spinal muscular atrophy. 2001. Mol Hum Reprod. 7:995-1000.
3. De Rycke M, Goossens V, Kokkali G, Meijer-Hoogeveen M, Coonen E, Moutou C. ESHRE PGD Consortium data collection XIV-XV: cycles from January 2011 to December 2012 with pregnancy follow-up to October 2013. 2017. Hum Reprod. 32:1974-1994.
4. Fiorentino, F., Biricik, A., Bono, S., Spizzichino, L., Cotroneo, E., Cottone, G., Kokocinski, F., and Michel, C.-E. 2014. Development and validation of a next-generation sequencing-based protocol for 24-chromosome aneuploidy screening of embryos. Fertil. Steril. 101:1375□1382.e2.
5. Gianaroli L, Magli MC, Ferraretti AP The in vivo and in vitro efficiency and efficacy of PGD for aneuploidy. 2001. Mol Cell Endocrinol. 22;183 Suppl 1:S13-8.
6. Handyside AH, Kontogianni EH, HardyK,WinstonRM. Pregnancies from biopsied human preimplantation embryos sexed by Y-specific DNA amplification. 1990. Nature;344 (6268):768□770.
7. Harper JC, Geraedts J., Borry P. Current issues in medically assisted reproduction and genetics in Europe: research, clinical practice, ethics, legal issues and policy. 2014. Human Reproduction, 29,8:1603□1609.
8. Kobpldt DC, Steinberg KM, Larson DE, Wilson RK, Mardis ER. The next-generation sequencing revolution and its impact on genomics. 2013. Cell;155:27-38.
9. Liu, M., Su, Y., andWang, W.-H. 2016. Assessment of clinical application of preimplantation genetic screening on cryopreserved human blastocysts. Reprod. Biol. Endocrinol. 14,16.
10. Macklon NS, Geraedts JP, Fauser BC Conception to ongoing pregnancy: the 'black box' of early pregnancy loss. 2002. Hum Reprod Update;Jul-Aug;8(4):333-43.
11. Mazurczak T, Hausmanowa-Petrusewicz I, Zaremba J i wsp. 2003. Zastosowanie technik biologii molekularnej w diagnostyce rdzeniowego zaniku mięśni (SMA). Ekspertyza naukowa wykonana na zlecenie Ministerstwa Zdrowia, Warszawa.
12. Munne S. Preimplantation genetic diagnosis of numerical and structural chromosome abnormalities. 2002. Reprod Biomed Online;4:183□196.
13. Nowaczyk MJM, Waye JS. The Smith Lemli-Opitz syndrome: a novel way of understanding developmental biology, embryogenesis and dysmorphology. 2001. Clin Genet;59:375□86.
14. Simpson JL. 2010. Preimplantation genetic diagnosis at 20 years. Prenat Diagn. 30: 682-695.
15. Tan, Y., Yin, X., Zhang, S., Jiang, H., Tan, K., Li, J., Xiong, B., Gong, F., Zhang, C., Pan, X., Chen, F., Chen, S., Gong, C., Lu, C., Luo, K., Gu, Y., Zhang, X., Wang, W., Xu, X., Vajta, G., Bolund, L., Yang, H., Lu, G., Du, Y., and Lin, G. 2014.

Clinical outcome of preimplantation genetic diagnosis and screening using next generation sequencing. Gigascience 3, 30.
16. Vanneste E, Voet T, Le Caignec C, Ampe M, Konings P, Melotte C, Debrock S, Amyere M, Vikkula M, Schuit F, Fryns JP, Verbeke G, D'Hooghe T, Moreau Y,Vermeesch JR. 2009. Chromosome instability is common in human cleavage-stage embryos. Nat Med. May;15(5):577-83.
17. Verlinsky Y, Cohen J, Munné S. 2004. Over a decade of experience with preimplantation genetic diagnosis: a multicenter report. Fertility and Sterility. 82: 292□294.
18. Voet T, Vanneste E, Van der Aa N, Melotte C, Jackmaert S, Vandendael T, Declercq M, Debrock S, Fryns JP, Moreau Y, D'Hooghe T, Vermeesch JR. 2011. Breakage-fusion-bridge cycles leading to inv dup del occur in human cleavage stage embryos. Hum Mutat. Jul;32(7):783-93.
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5. Discussion of other scientific and research achievements.

5.1. Scientific publications

In addition to the publications that make up the academic achievement presented above, my work consists **of 29 publications** in the Journal Citation Reports database, which I co-authored and **5 publication** in scientific journals without IF scores. The total IF for these works is **62,435** (**MSHE: 678).**

Below is a brief overview of these works with the indication of publications related to a given research topic.

5.1.1. Thematic scope: the importance of ovarian reserve in the treatment of infertility.

1. Kunicki M, Łukaszuk K, **Liss J**, Jakiel G, Skowrońska P (2018). Demographic characteristics and AMH levels in rural and urban women participating in an IVF programme. Ann Agric Environ Med. 2018 Mar 14;25(1):120-123.
IF: 1,116; MSHE : 20

2. **Liss J**, Kunicki M, Czyzyk A, Pastuszek E, Zabielska J, Meczekalski B, Lukaszuk K. (2017). Clinical utility of different anti-Müllerian hormone - AMH assays for the purpose of pregnancy prediction. Gynecol Endocrinol. Apr 27:1-6.
IF=1,453; MSHE : 15

3. Nelson SM, Pastuszek E, Kloss G, Malinowska I, **Liss J**, Lukaszuk A, Plociennik L, Lukaszuk K. (2015). Two new automated, compared with two enzyme-linked immunosorbent, antimüllerian hormone assays. Fertil Steril. Oct;104(4):1016-1021
IF=4,426; MSHE : 45

4. Lukaszuk K, Kuczynski W, Kunicki M, Ludwikowska B, **Liss J**, Malinowska I, Lukaszuk A, Bednarowska A, Kuczynska A, Kuc P, Pastuszek E. (2014). Comparison of the second-generation Beckman Coulter IVD and first-generation AnshLabs ELISA assays for anti-Müllerian hormone in patients undergoing IVF treatment. Ginekol Pol.;85(10):778-83.
IF=0,601; MSHE : 15

5. Lukaszuk K, **Liss J**, Kunicki M, Jakiel G, Wasniewski T, Woclawek-Potocka I, Pastuszek E. (2014). Anti-Müllerian hormone (AMH) is a strong predictor of live birth in women undergoing assisted reproductive technology. Reprod Biol.;14(3):176-81. *IF=1,524; 15; MSHE : 15*

6. Lukaszuk K, Ludwikowska B, **Liss J**, Kunicki M, Sawczak M, Lukaszuk A, Plociennik L, Jakiel G, Wasniewski T, Woclawek-Potocka I, Bialobrzeska D. (2014). Decreasing quality of the new generations of anti-Müllerian hormone assays. Biomed Res Int; 165352. doi: 10.1155/2014/165352.
IF=1,579; MSHE : 20

7. Lukaszuk K, Kunicki M, **Liss J**, Bednarowska A, Jakiel G. (2014). Probability of live birth in women with extremely low anti-Müllerian hormone concentrations. Reprod Biomed Online;28(1):64-9
IF=3,015; MSHE : 35

8. Lukaszuk K, Kunicki M, **Liss J**, Lukaszuk M, Jakiel G. (2013). Use of ovarian reserve parameters for predicting live births in women undergoing in vitro fertilization. Eur J Obstet Gynecol Reprod Biol. Jun;168(2):173-7.
IF=1,627; MSHE : 25

For many years, the assessment of ovarian reserve has been an important element in the diagnosis and treatment of infertility. In the early childbearing period, it allows to diagnose the fertility problem even before its occurrence, allowing the patient to be aware of the risks and able to take preventive measures. Also awareness

of the chances of success during the treatment is an important element of the therapy. The primary indicator in the diagnosis of infertility is currently the level of Anti-Müllerian hormone (AMH). The AMH level allows to determine the woman's ovarian reserve, and thus to determine her reproductive potential. AMH is a very good marker of the patient's ability to obtain their own oocytes and allows to determine the chances of having her own offspring. Its level decreases with age, and therefore it may also be a marker of fertility decline, including premature ovarian failure. Due to the wide diagnostic use of AMH, the reliability and reproducibility of the test results is of fundamental importance. AMH is a relatively new marker of ovarian reserve, which has replaced the FSH level commonly used to assess fertility. The widespread use of AMH, however, faces many difficulties. Rapid commercialization of scientific research causes a lack of standardization of products, in this case hormone assay kits. In addition, the push to spread the use of this test by the network laboratory companies further reduced the quality of data. The randomness of the blood draws and pre-laboratory error can significantly reduce the reliability of results.

An important achievement of the author is active participation in team research on the standardization of AMH testing results and the development of prediction models currently used in the treatment of infertility (**items 1-8**).

5.1.2. Thematic scope: use of current available research methods in the treatment of partner infertility

1. Pastuszek E, Kiewisz J, Skowronska P, **Liss J**, Lukaszuk M, Bruszczynska A, Jakiel G, Lukaszuk K. (2017). An investigation of the potential effect of sperm nuclear vacuoles in human spermatozoa on DNA fragmentation using a neutral and alkaline Comet assay. Andrology. Mar;5(2):392-398.
IF= 2,734; MSHE : 35

One of the important parameters to be evaluated during the diagnosis and treatment of infertile couples is the assessment of partner's sperm. The sperm quality cannot be currently determined based on the basic sperm analysis according to WHO standards, especially that its importance in assisted reproduction is increasingly being questioned. Currently the most important sperm parameter is the quality of its genetic material, which is best represented by the measurement of fragmentation of sperm DNA. In addition, the presence of characteristic morphological forms in the sperm (vacuole) allows to classify the sperm in more detail and on this basis estimate their potential for the development of a healthy embryo. The level of damage in the sperm DNA, and more specifically the chromatin of the sperm, has a large impact on

fertility. Considering the entire process of spermatogenesis and the use of a portion of the sperm during assisted reproduction treatment, the examination of DNA fragmentation is limited only to the prognostic value. It is not currently possible to investigate the level of DNA fragmentation in a specific sperm used for fertilization of the oocyte during the actual process of in vitro fertilization. The available methods allow only for optical image analysis under high magnification, that is for detailed evaluation of the sperm morphology. Therefore, it was important to determine the correlation between the sperm DNA quality and its morphology, so that this parameter could be used in clinical practice (**position No. 1**).

5.1.3. *Thematic scope: the role of hormone levels, including DHEA, in developing strategies for the treatment of infertility*

1. Kunicki M, Łukaszuk K, **Liss J**, Skowrońska P, Szczyptańska J. (2017). Granulocyte colony stimulating factor treatment of resistant thin endometrium in women with frozen-thawed blastocyst transfer. Syst Biol Reprod Med. Feb;63(1):49-57.
IF=1,582; MSHE : 20

2. Lukaszuk K, **Liss J**, Kunicki M, Kuczynski W, Pastuszek E, Jakiel G, Plociennik L, Zielinski K, Zabielska J. (2015). Estradiol Valerate Pretreatment in Short Protocol GnRH-Agonist Cycles versus Combined Pretreatment with Oral Contraceptive Pills in Long Protocol GnRH-Agonist Cycles: A Randomised Controlled Trial. Biomed Res Int. 2015:628056
IF=2,134; MSHE : 20

3. Łukaszuk K, Kunicki M, Kulwikowska P, **Liss J**, Pastuszek E, Jaszczołt M, Męczekalski B, Skowroński K. (2015). The impact of the presence of antithyroid antibodies on pregnancy outcome following intracytoplasmatic sperm injection-ICSI and embryo transfer in women with normal thyreotropine levels. J Endocrinol Invest. Dec;38(12):1335-43
IF=1,994; MSHE : 15

4. Kunicki M, Łukaszuk K, Jakiel G, **Liss J**. (2015). Serum Dehydroepiandrosterone Sulphate Concentration Is Not a Predictive Factor in IVF Outcomes before the First Cycle of GnRH Agonist Administration in Women with Normal Ovarian Reserve. PLoS One. 4;10(3)
IF=3,057; MSHE : 40

5. Kunicki M, Łukaszuk K, Woclawek-Potocka I, **Liss J**, Kulwikowska P, Szczyptańska J. (2014). Evaluation of granulocyte colony-stimulating factor effects

on treatment-resistant thin endometrium in women undergoing in vitro fertilization. Biomed Res Int. 2014
IF=1,579; MSHE : 20

6. Łukaszuk K., **Liss J**. , Łukaszuk M., Maj B. (2005). Optimization of estradiol supplementation during the luteal phase improves the pregnancy rate in women undergoing in vitro fertilization-embryo transfer cycles. Fertil. Steril.; vol. 83, nr 5, 1372-1376.
IF=3,114; MSHE : 24

Treatment of infertility with assisted reproduction methods requires the analysis of many factors before commencement of therapy. It is important to interpret and implement proper hormonal treatment for abnormal levels of hormones, including DHEA, which has been recently shown to be an important factor in predicting the quality of responses to hormonal stimulation, including oocyte quality. The application of an individualized stimulation protocol based patient's needs turns out to be crucial in obtaining the intended treatment effect (**items 1-4**).

The quality of the endometrium is also important. The success of embryo implantation in the course of treatment with assisted reproduction methods depends on many factors, including the quality of the embryo and the receptivity of the endometrium, i.e. the timing of the so-called implantation window. It is estimated that 50-75% loss of chances for pregnancy in the course of treatment is due to the lack of adequate endometrial receptivity (**item No. 5**). Implantation is a highly complicated process involving both the endocrine and immune systems and requires further complex treatment in the form of luteal phase supplementation after the treatment with assisted reproduction methods (**item No. 6**).

5.1.4. Thematic scope: the role of HPV in the pathogenesis of cervical cancer.

1. **Liss J**., Łukaszuk K., Gulczyński J., Zwaliński M., Woźniak I., Emerich J., Wójcikowski Cz. (2002). Występowanie DNA wirusa HPV u pacjentek z rakiem szyjki macicy w regionie gdańskim. Gin. Pol.; 73(9), 740-745.
MSHE =5

2. Łukaszuk K., **Liss J**. (2002). Wartość kliniczna stosowanych testów diagnostycznych na obecność infekcji HPV w profilaktyce i leczeniu raka szyjki macicy. Gin. Pol.,;73(8):719-726.
MSHE =5

3. Łukaszuk K., **Liss J**., Wójcikowski Cz. (2002). Recombinant DNA templates as a competitive internal standard in qualitative PCR of human papillomavirus detection in cervical cytology. Pol. J. Gynaecol. Invest.; 5, 253-260.
MSHE =4

4. Łukaszuk K., **Liss J**., Woźniak I., Emerich J., Wójcikowski Cz. (2003). Human Papillomavirus Type 16 Status in Cervical Carcinoma Cells DNA Given by Multiplex PCR. J Clin. Microbiol.; 41, 608-612.
IF=3,489; MSHE : 14

5. Łukaszuk K., **Liss J**., Emerich J., Wójcikowski Cz. (2003). Zastosowanie ilościowej oceny kopii genów E2/E6 HPV16 jako markera diagnostycznego raka szyjki macicy. Ginekol. Pol.; t. 74, nr 9, 793-798
MSHE =5

6. Łukaszuk K., **Liss J**., Woźniak I., Śliwiński W., Emerich J., Wójcikowski Cz. (2004). HPV and histological status of pelvic lymph node metastases in cervival cancer: a prospective study. J. Clin. Pathol.; 57:472-476
IF=2,619; MSHE : 12

7. Łukaszuk K., **Liss J**., Woźniak I., Emerich J. (2006). Ocena korelacji obecności DNA HPV w węzłach chłonnych chorych na raka szyjki macicy z parametrami histopatologicznymi w zmianie pierwotnej. Przeg. Lek.:63, 3, 113-116.
MSHE =5

8. Łukaszuk K., **Liss J**., Gulczyński J., Nowaczyk M., Nakonieczny M., Piątkowski M., Śliwiński W., Baay M., Woźniak I., Maj B., Łukaszuk M.. (2007). Predictive value of HPV DNA in lymph nodes in surgically treated cervical carcinoma patients - a prospective study. Gynecol Oncol. Mar;104(3):721-6
IF=2,614; MSHE : 24

9. Łukaszuk K., **Liss J**., Nowaczyk M., Śliwiński W., Maj B., Woźniak I., Nakonieczny M., Barwińska D. (2007). Survival of 231 cervical cancer patients, treated by radical hysterectomy, according to clinical and histopathological features. Eur J Gynaecol Oncol.;28(1):23-7.
IF=0,587; MSHE : 10

10. Baay MF, Nakonieczny M, Wozniak I, Deschoolmeester V, **Liss J**, Lukaszuk K, Sotlar K, Emerich J, Vermorken JB. (2009). Microsatellite instability and HPV genotype in Polish women with cervical cancer. Eur J Gynaecol Oncol.;30(2):162-6.
IF=0,614; MSHE : 10

Cervical cancer is the most frequently occurring genital cancer in Poland and the fourth most common in terms of the incidence of cancer in women. Based on retrospective and prospective epidemiological studies conducted in many centers around the world, HPV infection was considered the main etiological factor of cervical cancer. The virus, after cell penetration, in the early phase of infection, is contained in it in an episomal form (not related to the genome of the infected cell). It is found both in the cytoplasm and in the nucleus. Probably, one of the factors increasing the risk of developing cancer is the inclusion of HPV DNA into the epithelial cell genome. At the beginning of the 21st century, there were no epidemiological studies in Poland that would determine the incidence of HPV infection in a population of clinically healthy women.

In the years 2001-2002, a commonly used commercial test was Hybrid Capture, a hybridization test that uses RNA probes that detect DNA of specific HPV types. The probes used in this test were based on DNA sequences of genes for HPV capsid proteins, which resulted in the detection of HPV infections in cases of a mild infection. In cases where HPV DNA is integrated into cellular DNA, i.e. in a large percentage of cervical carcinomas and in cases of progressive dysplasia in the absence of episomal forms of the virus, it is not possible to detect HPV infection by the above-mentioned method. The European Scientific Organization of Genital Infections and Cancer - EUROGIN at the international cervical cancer conference in 1997 recommended using the PCR method to detect HPV infection only by specialized diagnostic laboratories. Tests based on hybridization methods were recommended for laboratories without highly specialized diagnostic facilities. Therefore, an attempt was made to develop diagnostic tests based on molecular biology methods in order to reliably evaluate both forms of virus in the evaluated material (**items 1-2**). An important issue was also the development of diagnostic tests with a wide control panel, not only negative and positive control, but also an internal positive control of a given PCR reaction and their implementation in clinical practice (**item No. 3**).

Detailed studies on the neoplastic transformation of epithelial cells of the cervix have additionally assessed the contribution of HPV oncoproteins (E6 and E7) and confirmed their importance in the entire carcinogenesis process. For this purpose, detailed molecular methods for detection of specific HPV genes in one reaction mixture were developed, which allowed to assess the quantitative ratios of the products of the amplified virus genes (**entries 4-5**). This method can be used to assess the severity of the viral infection and to determine additional prognostic criteria in patients with HPV-positive cervical cancer (**items 6-10**).

5.2. Other manifestations of scientific activity

In summary, I am the author or co-author of **40 publications** with the total **IF: 75,136 and MSHE: 816**. The number of **citations** of my works listed on the Web of Science is **344**. **Hirsch index** is **10**.

I have prepared and delivered **21 lectures** at international and national conferences and scientific meetings.

I am a board member of the Polish Society for Reproductive Medicine and Embryology and **a member of 5 other scientific societies**.

I was the scientific supervisor of **4 Master's theses** in cooperation with the Faculty of Biology of the University of Gdańsk. Currently, from October 2018 I have been the **supervisor of one master's thesis**, and since February 2019 I have been **supervising** theses of **two undergraduate students**.

I delivered postgraduate training sessions for doctors during specialist courses in gynecological endocrinology and reproduction in the field of infertility and assisted reproduction techniques.

I participated in **9 professional courses** concerning assisted reproduction techniques and preimplantation diagnostics.

I took an active part in **1 international** and **5 national research projects**.

In 2004, I received a **first-degree team award from the Rector of the Medical Academy in Gdańsk** for assessing the presence and integration of HPV virus with the cell genome in patients with cervical cancer. In 2009 I received from **the Rector of the Medical Academy in Gdansk a first-degree team award** for research on the prognostic value of selected molecular parameters in patients with cervical cancer and colorectal cancer and a also **the second-degree team award** for research on the use of preimplantation genetic diagnosis.

A detailed description of my scientific, didactic and organizational achievements can be found in a separate appendix (Appendix No 4.).

Liss Joanna