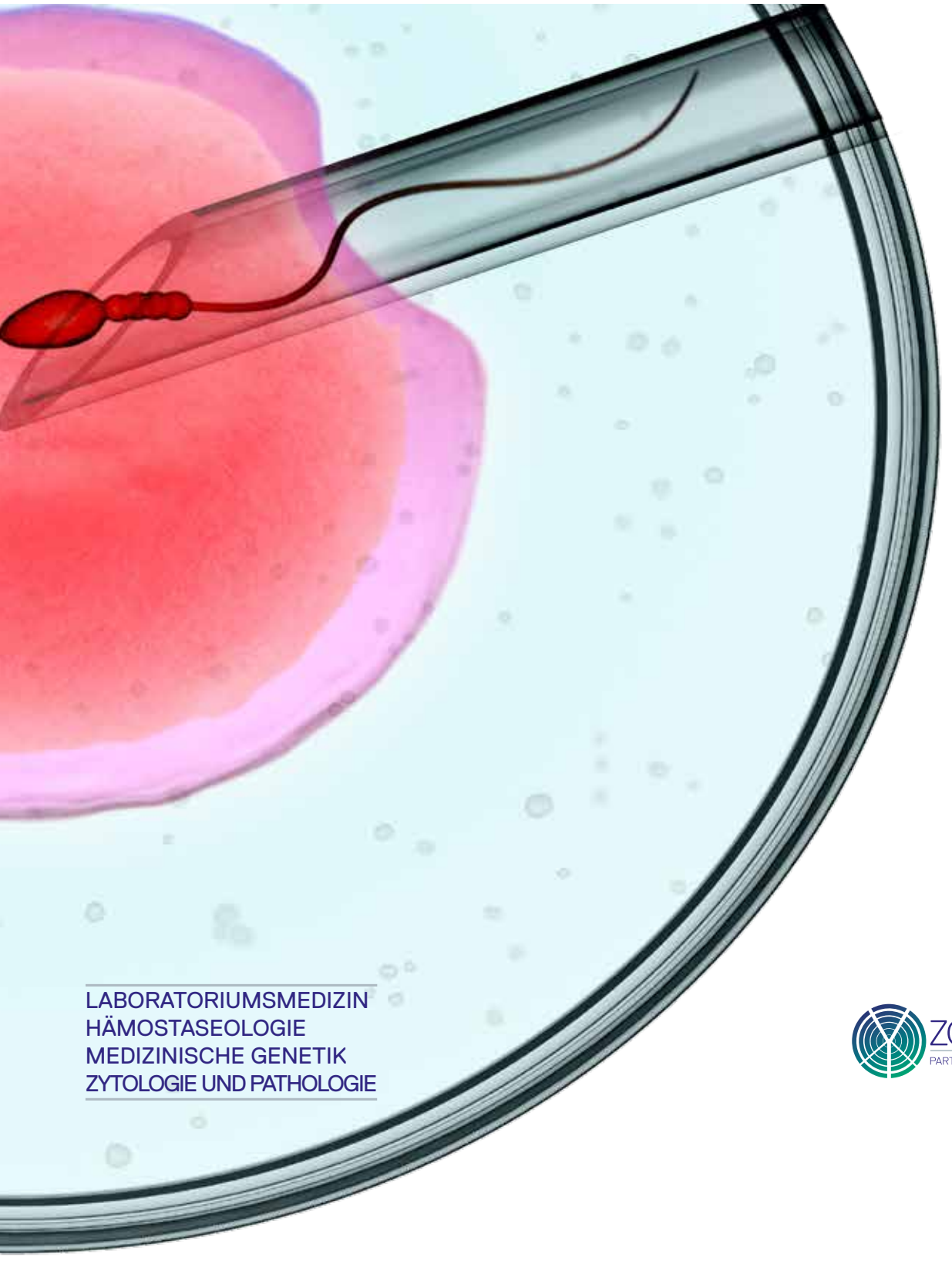
CONCEPT FOR IVF CENTRES

FROM

ZOTZ|KLIMAS - THE INTERDISCIPLINARY SUBMISSION LABORATORY



LABORATORIUMSMEDIZIN
HÄMOSTASEOLOGIE
MEDIZINISCHE GENETIK
ZYTOLOGIE UND PATHOLOGIE

ZOTZ | KLIMAS
PARTNER FÜR DIAGNOSTIK & PRÄVENTION

DEAR COLLEAGUE,

ZOTZ|KLIMAS offers a broad spectrum of diagnostics for IVF centres thanks to our expertise in genetics, pathology and laboratory diagnostics – all under one roof. These are complemented by polar body diagnostics (Polaris – Institute for Polar Body Analysis).

We have specialised in these areas for many years and employ state-of-the-art diagnostic procedures.

To improve the success rate of treatment, we can provide comprehensive diagnostics from a single source and accompany individual IVF centre strategies.

Our team has recently been strengthened by the addition of Dr Benjamin Rösing, who for many years was the head of the Department of Gynaecological Endocrinology and Reproductive Medicine at the University of Aachen.

We would be more than happy to discuss the diagnostic procedures you require for your institute and to optimise therapy recommendations. If you require, we can adjust our invoicing procedures to suit your needs.

Our aim is to support our patients by maximising their chances of conceiving a child.

We would be happy to discuss with you how we can optimise such support.

Dr Rainer B. Zotz

Dr Dietmar Klimas

Dr Benjamin Rösing

OUR RANGE OF SERVICES AT A GLANCE

LABORATORY DIAGNOSTICS

- We offer a broad spectrum of human genetic diagnostics with comprehensive specialist panels to assess female and male infertility
- KIR typing – analysis of the precision of embryo-maternal “cross-talk”
- Carrier screening to discover recessive disorder carrier status
- Single gene analysis through to complete exome analysis – also, with the aim of improving the quality of sperm banks
- Polar body diagnostics in the cryopreserved cycle and at a very attractive price
  - within 36 hours for the fresh cycle – see price list
    - for €220.00 per egg in the cryopreserved cycle
- Endometrial diagnostics to assess endometrial receptivity
- Microbiome diagnostics with NGS (next generation sequencing) and therapy recommendations in partnership with dus.ana-Düsseldorf.Analytik
- Reproductive endocrinology with therapy recommendations
- Diagnosis of haemostasis disorders for implantation failure, miscarriage and pre-eclampsia

OUR EXPERTS

HUMAN GENETICS

Dr Diana Mitter
Specialist in human genetics
Medical Director of Medical Genetics

Dr Robert Maiwald
Specialist in human genetics
ABMG-certified specialist in clinical molecular genetics | clinical cytogenetics

Claudia Behrend
Specialist in gynaecology and obstetrics – medical genetics
Medical Director of Cytogenetics

Dr Bärbel Überlacker
Graduate biologist
Head of Molecular Genetics

REPRODUCTIVE ENDOCRINOLOGY

Dr Benjamin Rösing
Specialist in gynaecology and obstetrics
Specialisation in gynaecological endocrinology and reproductive medicine

COAGULATION DIAGNOSTICS

Dr Rainer B. Zotz
Specialist in laboratory medicine,
transfusion medicine and
haemostaseology

Dr Dagmar Lammerting
Specialist in laboratory medicine

Dr Birgit Fleiter
Specialist in transfusion medicine

ENDOMETRIAL DIAGNOSTICS

Sibylle Spieth
Specialist in pathology | MIAC

MICROBIOME DIAGNOSTICS

Dr Patrick Finzer
Specialist in laboratory medicine | microbiology
dus.ana - Düsseldorf.Analytik

COUNSELLING AND DIAGNOSTICS FOR INFERTILITY

- **DÜSSELDORF**
  IMMERMANNSTR. 65 A | 40210 DÜSSELDORF | GERMANY

- **COLOGNE**
  BONNER STRASSE 178 | 50968 COLOGNE | GERMANY

- **AACHEN**
  THEATERPLATZ 6–12 | 52062 AACHEN | GERMANY

AFFILIATE LABORATORIES

- **DUISBURG**
  KÖNIGSTR. 53 | 47051 DUISBURG | GERMANY

- **ESSEN**
  RÜTTENSCHEIDER STR. 14 | 45128 ESSEN | GERMANY

- **KREFELD**
  VIOLSTR. 92 | 47800 KREFELD | GERMANY

- **MÖNCHENGLADBACH**
  at THE ELISABETH HOSPITAL
  HUBERTUSSTR. 100 | 41239 MÖNCHENGLADBACH | GERMANY

CONTACT

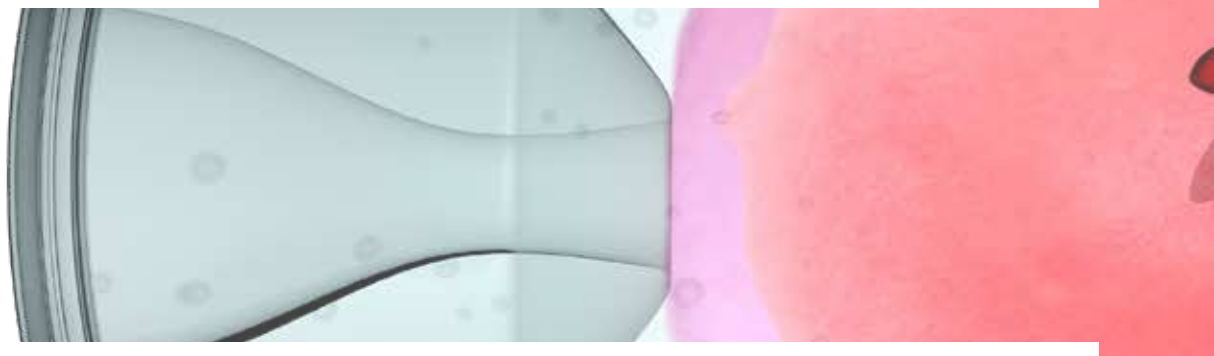
Tel.: +49 (0)211 27 101 117
Email: kinderwunsch@zotzklimas.de

Your contact person: Mr Jan Vogt | +49 (0)174 685 47 94

PRICING STRUCTURE FOR OUR SERVICES

Genetic counselling	Statutory and private health insurance coverage
Genetic laboratory diagnostics	Statutory and private health insurance coverage
Laboratory diagnostics | haemostaseology	Statutory and private health insurance coverage
Polar body diagnostics	Generally, only covered by private health insurance, see price list*
KIR typing	Generally, only covered by private health insurance, see price list*
Endometrial diagnostics	Generally, only covered by private health insurance, see price list*
Microbiome diagnostics	Generally, only covered by private health insurance, see price list*
Carrier screening	Generally, only covered by private health insurance, see price list*

** For private patients, cost estimates can be prepared for health insurance providers if required.*



PRICE LIST

ENDOMETRIAL DIAGNOSTICS	
ENDOMETRIAL RECEPTIVITY ANALYSIS OF ENDOMETRIAL THICKNESS HE, PAS, OESTROGEN, PROGESTERONE, KI-67	€ 168.46
MONITORING | TWO ANALYSES	€ 298.00
PLASMA CELL MARKERS	€ 40.00
KIR TYPING	€ 167.67
MICROBIOME (ENDOMETRIAL/VAGINAL)	€ 198.17
CARRIER SCREENING	
DEPENDENT ON THE NUMBER OF GENES EXOME ON REQUEST	€300.00 – 1,400.00

POLAR BODY DIAGNOSTICS

ARRAY CGH OR NGS FOR COMPREHENSIVE RESULTS				
Fresh transfer				Cryoconservation
Number of eggs	Price	Number of eggs	Price	Price
1–2	€ 1,000	8	€ 2,250	€ 220/egg*
3	€ 1,400	9	€ 2,420	
4	€ 1,570	10	€ 2,590	
5	€ 1,740	11	€ 2,760	
6	€ 1,910	12	€ 2,930	
7	€ 2,080			

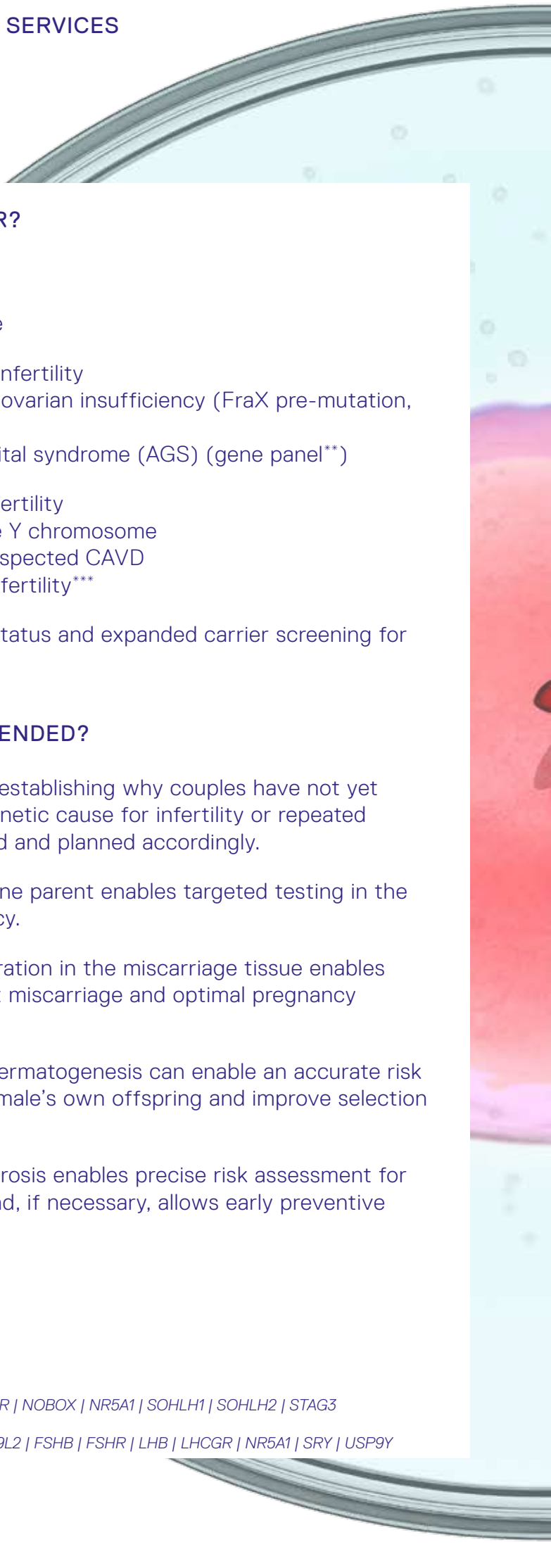
* The above prices represent prices per sample. In other words, they apply to the pooled polar bodies method (first and second polar body of an egg in one reaction step). If requested, each polar body can be examined individually. The price will be adapted accordingly. If no array CGH analysis is possible with the submitted material (for instance in the case of fragmented polar bodies), we only charge a flat fee of € 175.00.

FISH METHOD TO DIFFERENTIATE COMPLEX ISSUES RELATING TO TRANSLOCATION	
Number of eggs	Price
After prior arrangement	After prior arrangement

FISH METHOD Aneuploidy diagnostics for six chromosomes: 13, 16, 18, 21, 22 and X.	
Number of eggs	Price
1–8	€390
9-16	€540

We are happy to provide you with information on transport costs in advance, which are calculated based on expenses incurred.

GENETICS



WHICH GENETIC ANALYSES DO WE OFFER?

- Chromosome analysis for both partners
- Chromosome analysis on miscarriage tissue
- Specific genetic analyses to clarify female infertility
  - In cases of suspected premature ovarian insufficiency (FraX pre-mutation, gene panel*)
  - In cases of suspected adrenogenital syndrome (AGS) (gene panel**)
- Specific genetic analyses to clarify male infertility
  - Analysis of the AZF region on the Y chromosome
  - Analysis of the CFTR gene for suspected CAVD
  - Gene panel in the case of male infertility***
- Analysis to discover cystic fibrosis carrier status and expanded carrier screening for severe paediatric genetic diseases

WHY IS GENETIC DIAGNOSTICS RECOMMENDED?

The success of fertility treatment depends on establishing why couples have not yet been able to conceive. If we can establish a genetic cause for infertility or repeated miscarriages, further treatment can be adapted and planned accordingly.

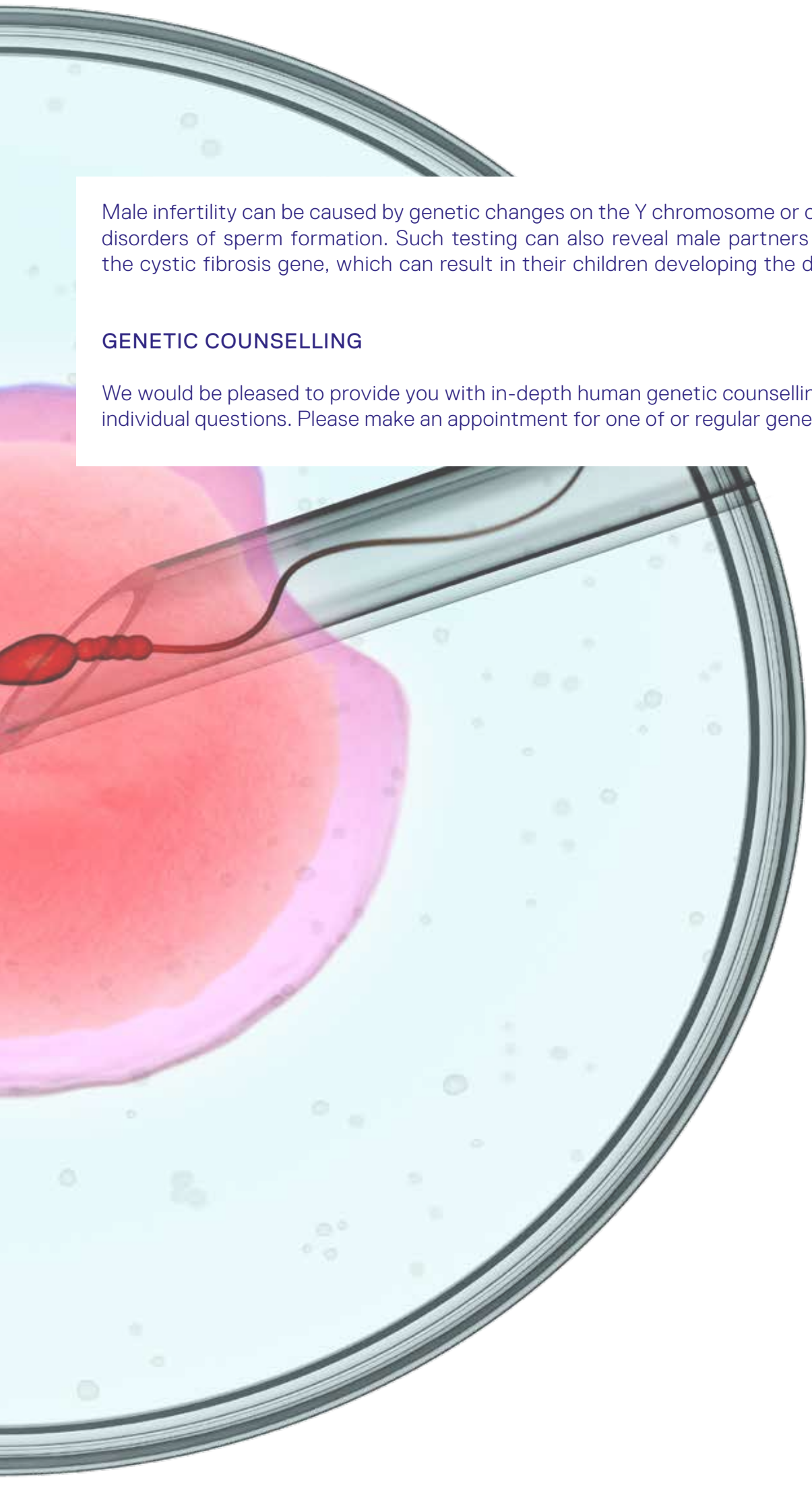
- Identification of a familial translocation in one parent enables targeted testing in the course of artificial insemination or pregnancy.
- Identification of a novel chromosomal aberration in the miscarriage tissue enables accurate assessment of the risk of a repeat miscarriage and optimal pregnancy planning.
- Identification of a hereditary disorder of spermatogenesis can enable an accurate risk assessment of a repeat miscarriage of the male's own offspring and improve selection of artificial insemination methods.
- Identification of carrier status for cystic fibrosis enables precise risk assessment for the disease in offspring of both partners and, if necessary, allows early preventive measures to be taken.

* *BMP15 | DIAPH1 | ESR1 | FIGLA | FOXL2 | FSHR | GDF9 | INHA | LHCGR | NOBOX | NR5A1 | SOHLH1 | SOHLH2 | STAG3*
** *CYP21A2 | CYP11B1 | CYP17A1 | HSD3B2*
*** *AR | AURKC | CATSPER1 | CATSPER2 | CFTR | DAZL | DDX25 | DPY19L2 | FSHB | FSHR | LHB | LHCGR | NR5A1 | SRY | USP9Y*

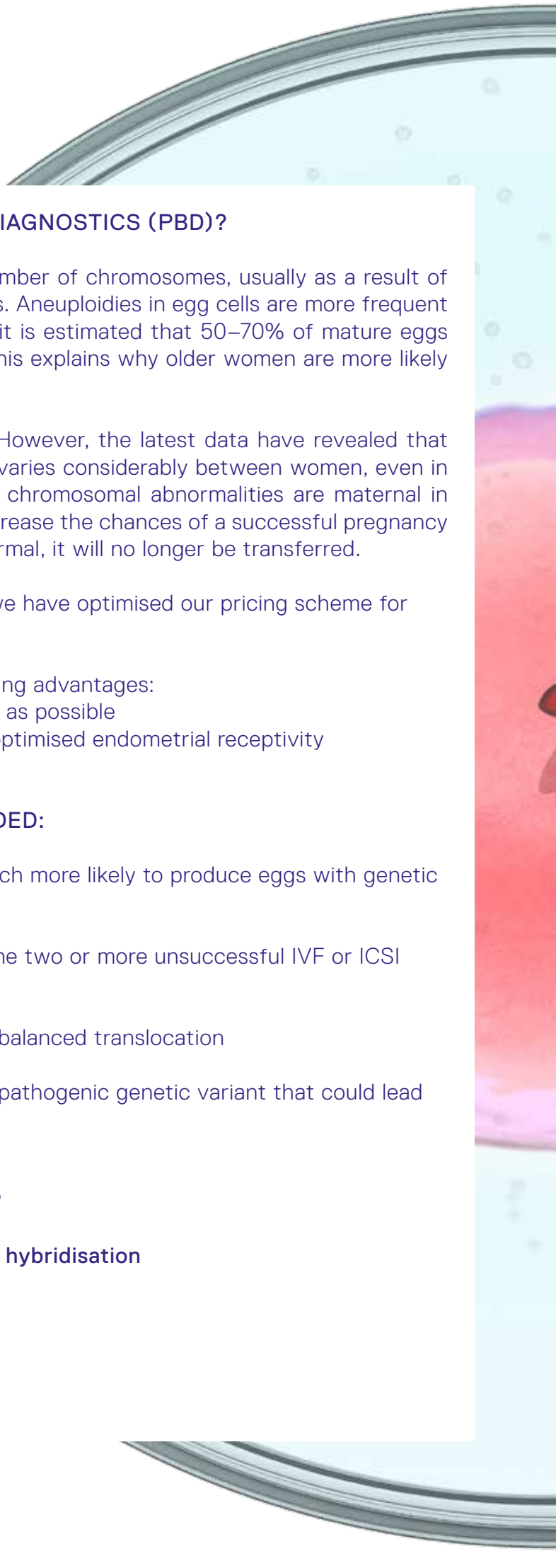
Male infertility can be caused by genetic changes on the Y chromosome or other rare genetic disorders of sperm formation. Such testing can also reveal male partners to be carriers of the cystic fibrosis gene, which can result in their children developing the disease.

GENETIC COUNSELLING

We would be pleased to provide you with in-depth human genetic counselling to answer your individual questions. Please make an appointment for one of or regular genetic consultations.



POLAR BODY DIAGNOSTICS



WHO CAN BENEFIT FROM POLAR BODY DIAGNOSTICS (PBD)?

Aneuploidy is the presence of an abnormal number of chromosomes, usually as a result of misdistribution of chromosomes during meiosis. Aneuploidies in egg cells are more frequent after the age of 35. In a 40-year-old woman, it is estimated that 50–70% of mature eggs are affected by a chromosomal abnormality. This explains why older women are more likely to suffer miscarriages.

In general, all women can benefit from PBD. However, the latest data have revealed that the number of chromosomally abnormal eggs varies considerably between women, even in young women. More than 90% of embryonic chromosomal abnormalities are maternal in origin. Polar body diagnostics can therefore increase the chances of a successful pregnancy in all women. If an embryo is found to be abnormal, it will no longer be transferred.

To enable more women to benefit from PBD, we have optimised our pricing scheme for cryopreserved cycles.

Cryopreserved embryo transfer has the following advantages:
- Collection and fertilisation of as many eggs as possible
- Cryotransfer in one treatment cycle, with optimised endometrial receptivity

WOMEN FOR WHOM PBD IS RECOMMENDED:

- Women over the age of 35, as they are much more likely to produce eggs with genetic defects
- Women who have already already undergone two or more unsuccessful IVF or ICSI attempts
- Women who are known to be carriers of a balanced translocation
- Women who are known to be carriers of a pathogenic genetic variant that could lead to severe disease in the offspring

WHICH PBD TECHNIQUES DO WE OFFER?

- **ARRAY CGH: array comparative genomic hybridisation**
- **NGS: next generation sequencing**
- **FISH: fluorescence** in situ **hybridisation**

ARRAY CGH AND NGS

Using several methods (array CGH, NGS), all chromosomes (chromosomes 1–22 and X) of the polar body DNA are analysed and examined in parallel for aneuploidies. Even in women who are known carriers of a balanced translocation, these methods are **rapid and yield comprehensive results.**

FISH

This method makes the chromosomes "visible" by means of a specific technique, after which they are examined under the microscope. The six chromosomes (13, 16, 18, 21, 22 and X) that are most frequently associated with defects are examined. Down's syndrome (trisomy 21), for example, is one such defect.

FISH can only analyse a limited number of chromosomes. It cannot detect potential aneuploidies involving any other chromosomes. The technique can also be used on eggs from women who are known to carry a translocation.

GENETIC COUNSELLING

The decision to undergo a polar body analysis is deeply personal and can be discussed with the doctors at IVF clinics.
If patients have any further questions, please feel free to contact us and schedule an in-depth personal genetic counselling session.

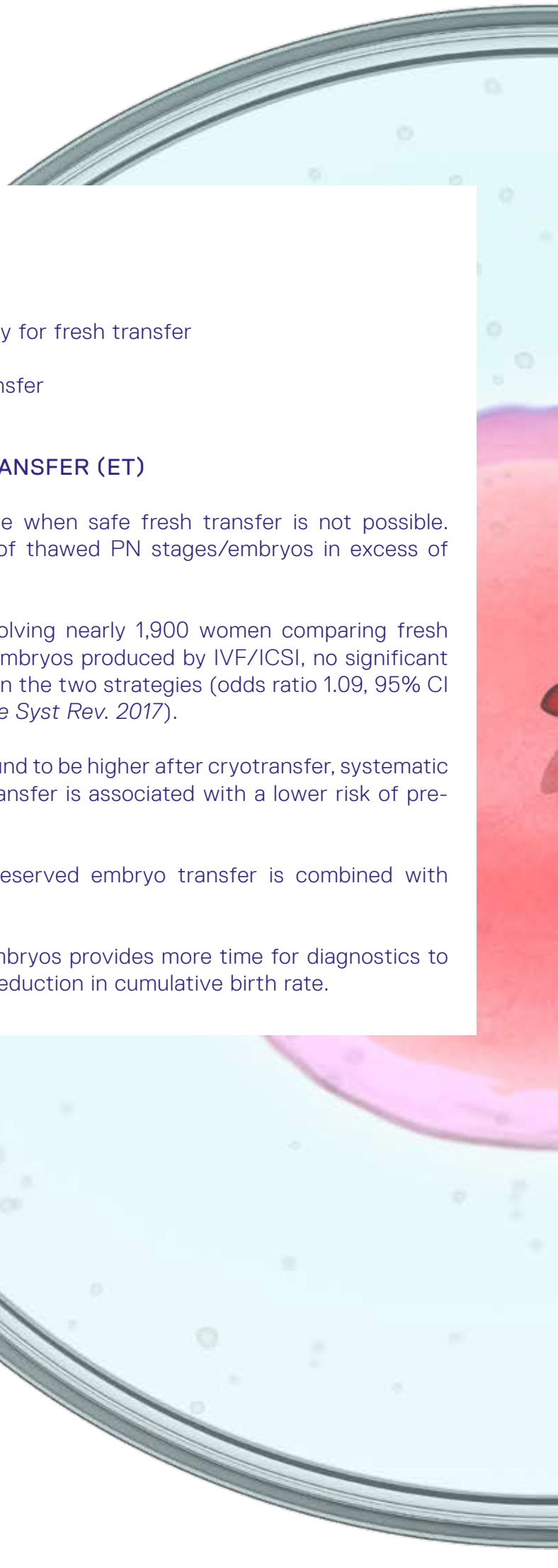
SUMMARY

PBD can be used to indirectly analyse eggs for

- numerical chromosomal defects (aneuploidies)
- structural chromosomal defects (translocations)
- the presence of a known pathogenic sequence variant leading to a severe monogenic disease (especially in X-linked recessive diseases such as haemophilia A/B)

Aims of PBD

- Increasing the chances of implantation
- Reducing the risk of miscarriage
- Increasing the rate of live births



HOW MUCH DOES PBD COST?

The costs depend on the diagnostic context:

- Diagnostics that are conducted immediately for fresh transfer
- Diagnostics for planned cryopreserved transfer

FRESH VS CRYOPRESERVED EMBRYO TRANSFER (ET)

Embryos can be cryopreserved for future use when safe fresh transfer is not possible. Vitrification methods result in survival rates of thawed PN stages/embryos in excess of 90%.

In a recent meta-analysis of four studies involving nearly 1,900 women comparing fresh versus cryopreserved transfer strategies for embryos produced by IVF/ICSI, no significant difference in live birth rates was found between the two strategies (odds ratio 1.09, 95% CI 0.91–1.31) (*Wong KM* et al. *Cochrane Database Syst Rev. 2017*).

Although the risk of pre-eclampsia has been found to be higher after cryotransfer, systematic reviews of observational studies reveal cryotransfer is associated with a lower risk of pre-term deliveries and low birth weights.

One possible reason for this is that cryopreserved embryo transfer is combined with methods to optimise endometrial receptivity.

At any rate, cryopreservation of pronuclear embryos provides more time for diagnostics to be performed on the egg or embryo, with no reduction in cumulative birth rate.

CARRIER SCREENING BY MEANS OF INDIVIDUAL PANELS OR THE HORIZON™ TEST

horizon™ carrier screening tests the carrier status of up to 274 autosomal recessive or X-linked serious genetic disorders.
Such genetic changes can result in severe disease in the child if the partner is also a carrier, even if both parents themselves are healthy.

WHEN SHOULD HORIZON® CARRIER SCREENING BE PERFORMED?

horizon™ can be performed at any time before or during pregnancy. Some people prefer to find out about their carrier status before conception in order to make informed family planning decisions.

WHAT CAN HORIZON™ RESULTS TELL ME AND WHEN?

Your doctor will receive the results in about two to three weeks.
If the results are abnormal, this means that a genetic alteration associated with a disease has been detected. If this happens, it is important to discover the carrier status of the partner as well, to determine the likelihood of a hereditary disease in any joint children. If the results are normal, this means that no genetic abnormalities causing the disease have been detected. Even if a normal result suggests a much reduced likelihood of being a carrier, carrier screening cannot detect all disease-causing variants.

HOW MUCH DOES CARRIER SCREENING COST?

horizon™ carrier screening is not covered by health insurers. The costs depend on the the extent of the analysis selected. We recommend privately-insured patients obtain a cost estimate.
See price list.

KIR (KILLER-CELL IMMUNOGLOBULIN-LIKE RECEPTOR) TYPING

WHAT IS KIR TYPING AND IN WHAT CIRCUMSTANCES IS IT USEFUL?

KIRs are expressed on the surface of natural killer (NK) cells. They bind specific HLA molecules, and their function is to identify cells that lack or have reduced HLA expression.

To date, 16 KIR genes have been characterised, whose products are specific for those of the HLA genes. Certain KIR types have been found to be associated with implantation failure, pre-eclampsia and repeated miscarriage. Such findings did not identify specific genes for multiple activating KIRs in women who have suffered multiple miscarriages.

For example, the activating receptors KIR-2DS1, -2DS5 and -3DS1 are found significantly less frequently in women with fertility disorders than in women without fertility disorders. KIR-dependent immunological processes can, thus, be assumed to represent a potential reason for implantation disorders and other complications of pregnancy.
Findings are obtained on the basis of indication.

WHAT NEEDS TO BE SUBMITTED?
2 ml EDTA, application for assumption of costs and declaration of consent as per the German Genetic Diagnostics Act

HOW LONG DOES KIR TYPING TAKE?
1–2 weeks

HOW MUCH DOES KIR TYPING COST?
See price list

ENDOMETRIAL RECEPTIVITY ANALYSIS

WHAT IS ENDOMETRIAL RECEPTIVITY ANALYSIS?

The endometrium is only receptive for implantation of an embryo within a comparatively short time frame of approx. 72 hours in each cycle. This period is known as the “window of implantation” (WOI) and is characterised by morphological, immunological, microbial, molecular and genetic factors involved in tissue remodelling. Identifying the WOI allows doctors to precisely time fertility treatment.

We perform WOI diagnostics, adopting an interdisciplinary approach.
In addition to communicating differentiated findings, we make suggestions on specific therapies, such as hormonal therapy, coordinated antibiotic therapy, dietary optimisation and recommendations on metabolic modulation. The aim of treatment is to optimise the implantation environment and the course of pregnancy.

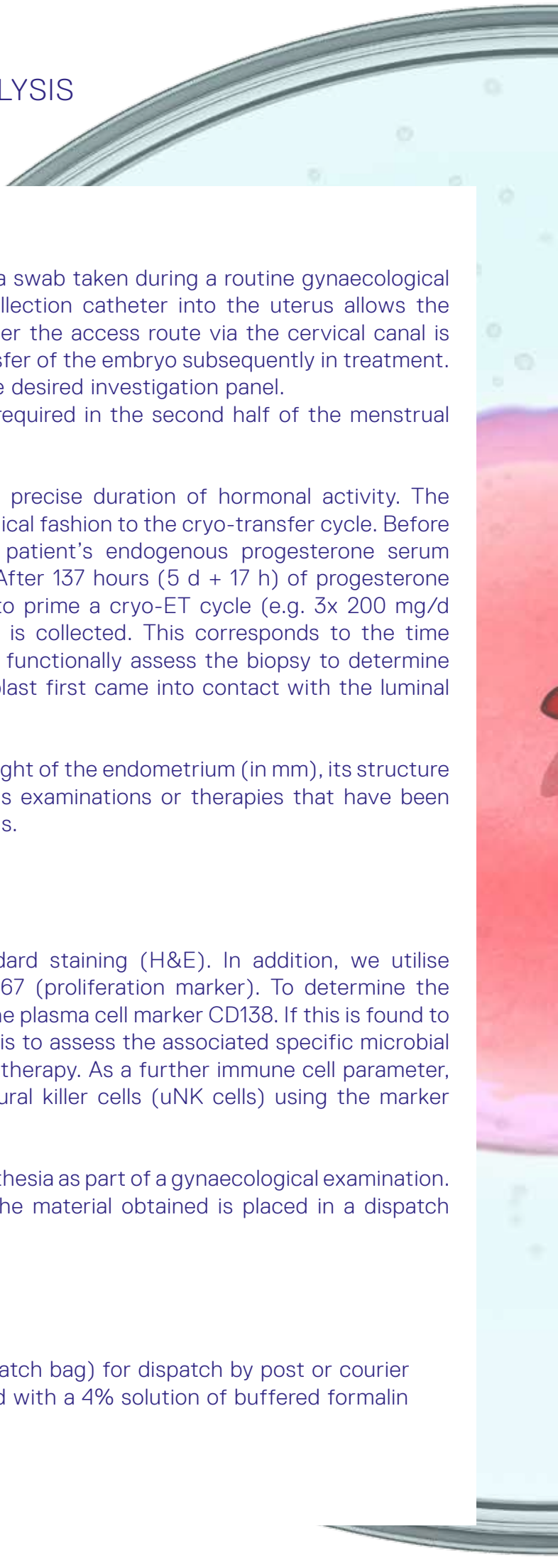
Two diagnostic scenarios exist for endometrial pipelle sampling.

The biopsy can be performed 1) during a spontaneous cycle or 2) after targeted hormonal priming of the endometrium.

1) In spontaneous cycle endometrial biopsies, the main emphasis is to assess the homogeneity of the development of the individual cell compartments (specifically with regards to luminal and glandular epithelia, secretion patterns, stromal cells, decidualisation, vascularisation and immune cells). It is also possible to check for signs of endometritis.
Chronic endometritis occurs in about 10–20% of patients with unexplained repeated miscarriages and repeated implantation failures.

2) Specific WOI diagnostics are conducted after targeted hormonal priming of the endometrium. In addition to the factors mentioned above, the process can assess how closely the temporal pattern of tissue remodelling coincides with the implantation window. This assessment helps in synchronising cryotransfer of the embryo and endometrial development.

ENDOMETRIAL RECEPTIVITY ANALYSIS



COURSE OF THE ANALYSIS

The sample is taken using a pipelle, much like a swab taken during a routine gynaecological examination. The process of inserting the collection catheter into the uterus allows the gynaecologist to simultaneously assess whether the access route via the cervical canal is restricted. The route needs to be clear for transfer of the embryo subsequently in treatment. The timing of the investigation depends on the desired investigation panel.
In the spontaneous cycle, a biopsy is usually required in the second half of the menstrual cycle but, in principle, is possible at any time.

WOI diagnostics require consideration of the precise duration of hormonal activity. The patient is administered progesterone in an identical fashion to the cryo-transfer cycle. Before progesterone administration is initiated, the patient's endogenous progesterone serum concentration must be analysed < 1.5 ng/ml. After 137 hours (5 d + 17 h) of progesterone substitution as per the customary algorithm to prime a cryo-ET cycle (e.g. 3x 200 mg/d of progesterone vaginally), the pipelle biopsy is collected. This corresponds to the time of blastocyst hatching in a transfer cycle. We functionally assess the biopsy to determine the time period when the cells of the trophoblast first came into contact with the luminal epithelium of the endometrium.

Clinical information such as the sonographic height of the endometrium (in mm), its structure (trilaminar pattern) and indications of previous examinations or therapies that have been carried out all support the histological diagnosis.

TECHNICAL INFORMATION

Staining and markers used

The endometrial tissue is subjected to standard staining (H&E). In addition, we utilise oestrogen and progesterone markers and Ki-67 (proliferation marker). To determine the presence of chronic endometritis, we employ the plasma cell marker CD138. If this is found to be positive, we can perform a follow-up analysis to assess the associated specific microbial profile to improve the success of an antibiotic therapy. As a further immune cell parameter, we conduct numerical analysis of uterine natural killer cells (uNK cells) using the marker CD56.

Endometrial biopsy is performed without anaesthesia as part of a gynaecological examination. In general, this is well tolerated by women. The material obtained is placed in a dispatch vessel and sent for analysis.

On request, we can provide you with:

- Pipelles
- Dispatch materials (order form and dispatch bag) for dispatch by post or courier
- Dispatch vessels (10–20 ml in size) filled with a 4% solution of buffered formalin

MICROBIOME AND IVF SUCCESS (REPRODUCTIVITY OUTCOME)

THE FEMALE GENITAL MICROBIOME STRONGLY INFLUENCES FERTILITY AND THE SUCCESS OF IVF

- Bacterial vaginosis (BV) pathogens in the vagina are associated with decreased pregnancy rates.
- A high vaginal microbiome diversity on the day of embryo transfer (IVF) is negatively correlated with “resultant live births”.
- Pregnancy rates are particularly high when the vaginal microbiome is dominated by ‘ lacotobacilli.
- Lactobacillus levels in the endometrium are a determining factor for pregnancy rates.
- Treatment of a microbial imbalance (“dysbiosis”) in infertile women significantly increases pregnancy rates.

THE SUCCESS OF IVF CAN BE IMPROVED BY

- vaginal/endometrial microbiome diagnostics
- control of microbial IVF conditions
- specialist microbiological and laboratory medical findings
- a high level of expertise in molecular/innovative diagnostics
- targeted antibiotic and probiotic therapy counselling

Sample collection material: Swabs (e.g. eNAT)

Time of sampling: At any time during the menstrual cycle
Record the day of the menstrual cycle on the order form.

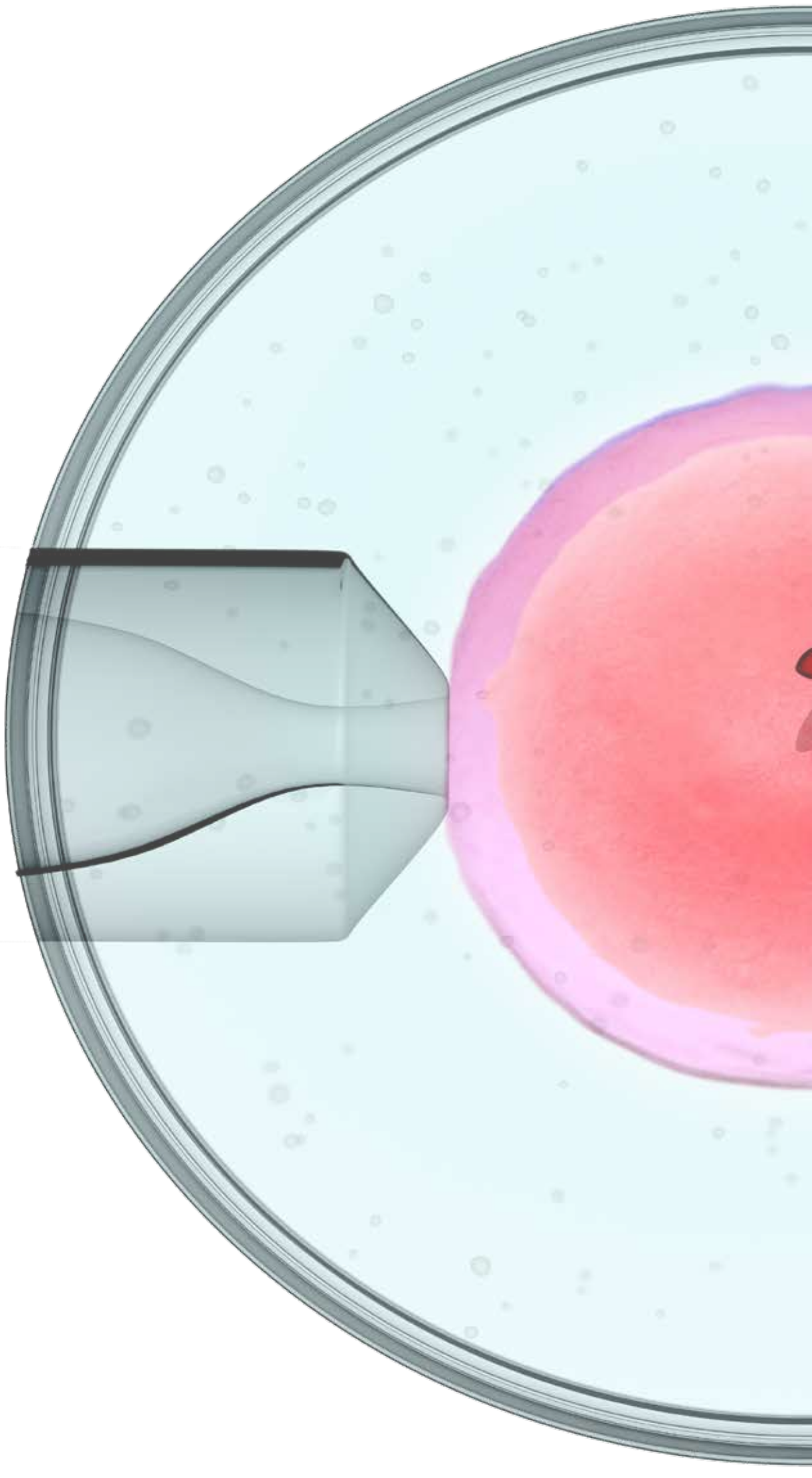
Methodology: NGS/microbiome

NEW: GENITAL MICROBIOME AND CHRONIC ENDOMETRITIS

Together with our pathology department, we perform immunological testing of the endometrium (in addition to hormone receptors and the proliferation marker) to detect NK cells, plasma cells and regulatory T cells (Tregs) to establish a diagnosis of chronic endometritis.

Health insurers cover the costs of this service.

If requested, we can issue an additional report formulated jointly by the pathologist and the microbiologist to cover immunological and microbiome analyses of the endometrium.



ASSOCIATION OF HAEMOSTASIS DISORDERS IN IMPLANTATION FAILURE, MISCARRIAGE AND PRE-ECLAMPSIA

A propensity to miscarry may be due to a haemorrhagic or thrombophilic blood clotting disorder (tendency to bleed or for thrombosis).

The main causes of a tendency to bleed are fibrinogen and factor XIII deficiency. It has long been known that an association exists between an elevated susceptibility to thrombosis (thrombophilia) and miscarriage, pre-eclampsia and implantation failure. The thrombophilic risk of such patients is therefore identified by means of laboratory diagnostics to assess their potential increased risk of thrombosis.
In addition to the increased risk of implantation failure, miscarriage and pre-eclampsia, there is also a significant risk that severely raised risk factors will result in thromboembolic complications during pregnancy. Thromboembolism is one of the most important causes of death in pregnant women, which is why it should be diagnosed before pregnancy, in particular if there is a familial history of thrombosis.

A number of studies have found that women with risk factors for thrombophilia who received heparin are significantly less likely to miscarry or suffer from pre-eclampsia. However, other studies were unable to confirm this effect. We can provide you with specific recommendations on therapy options, taking current guidelines into full consideration.

There is an international consensus that heparin and ASA should be administered in any future pregnancy if antiphospholipid antibodies are detected. This measure has increased the rate of successful pregnancies from approximately 20% to 50–60%. An analogous treatment is administered in the event of implantation failure.

If the patient suffers from a haemorrhagic disorder of blood coagulation such as fibrinogen or factor XIII deficiency, administration of factor concentrates throughout pregnancy may be necessary to sustain the pregnancy. We can perform such factor administration in our practice if desired.

COUNSELLING AND CARE OF PATIENTS

Patients who suffer from implantation failure, miscarriage and pre-eclampsia are offered individual counselling sessions. If indicated, our clinical specialists can prescribe heparin, ASA or factor concentrates (e.g. fibrinogen) for high-risk patients during future pregnancies.

ZOTZ|KLIMAS
MVZ Düsseldorf-Centrum
Immermannstraße 65 A
40210 Düsseldorf
Germany

Tel.: +49 (0)211 2710 1117
Email: kinderwunsch@zotzklimas.de
www.zotzklimas.de