I. What does it consist of?

Preimplantation Genetic Diagnosis (PGD) is a technique used to complement in vitro fertilization (IVF), which aims to select those embryos free of genetic or chromosomal abnormality in each case studied.

The PGD technique is the result of combining:

1. IVF with/without sperm injection.
2. Polar body or embryonic cells biopsy.
3. Genetic Diagnosis Techniques.

II. When is it indicated?

The most common indications are:

A – Transmissible paternal/maternal genetic alterations:

- Diseases that affect a single gene or monogenic (dominant, recessive or X-linked), whose mutation is known and can be analysed.
- X chromosome-linked disorders, whose gene is not known or has some heterogeneity, but is avoided with sex selection.
- Structural chromosomal abnormalities such as Robertsonian or reciprocal translocations.

B – Screening aneuploidy:

- Repeat abortions.
- Repeated failure of implantation.
- Advanced maternal age

C – Others:

- HLA typing (histocompatibility)
- Chromosomal abnormalities in sperm

III. Procedure

In Vitro Fertilization (IVF) and Intracytoplasmic Sperm Injection (ICSI) normally begins with ovarian stimulation using drugs whose action is similar to that of certain hormones produced by the woman. Medications used include a leaflet that the patient should consult with the possibility of requesting any clarification from the medical staff of the Centre. The purpose of this treatment is to obtain the development of several follicles, inside which are the eggs. In order to prevent spontaneous ovulation other treatments with hormonal action are associated.

The process of ovarian stimulation is usually controlled by analysis of blood levels of certain ovarian hormones and/or vaginal ultrasound reporting the number and size of the developing follicles. Once proper development is achieved, other drugs are administered to achieve the final maturation of the eggs.

Many of the drugs used are injectable and presentation allows self-administration by the patient. Dosages and dosing schedules are tailored to each patient's clinical features and response to treatment may vary. Occasionally they are used in association with other types of drugs.

The eggs (oocytes) are extracted by ultrasound-guided ovarian puncture and aspiration of follicles, through the vagina. This procedure is usually performed on an outpatient basis and requires anaesthelia and subsequent observation for a variable period.

The eggs obtained are prepared and classified in the laboratory. The number of eggs that are removed in the puncture, their maturity and quality cannot be predicted with accuracy.

After obtaining the eggs, the laboratory must have the sperm from the partner or an anonymous donor, as the case may be. Semen is prepared in the laboratory in order to select...
the most suitable sperm for fertilization. To achieve the latter, when ICSI is performed, a spermatozoon is injected into each mature and morphologically normal egg recovered.

The day after IVF or ICSI the number of fertilized eggs is determined and in successive growing days the number and quality of embryos that continue their development will be assessed. The embryos will be kept in the laboratory for a period of 2-6 days after which the transfer of those not affected will be performed, after their study and selection.

The third day after the puncture the extraction is made from the polar corpuscle or one/two of the pre-embryo cells, in order to analyse them according to appropriate genetic procedure for each case, which requires 24 to 48 hours to make a diagnosis.

The embryo transfer, which with this technique will occur between the 4th and 6th day post puncture, consists of depositing the analysed disease-free embryos into the uterine cavity through the vagina. It is an outpatient procedure that requires no anaesthesia or hospital admission. In order to promote embryo implantation hormone treatment is also prescribed.

The number of embryos transferred to the uterus cannot exceed three, in a cycle. The biomedical team will provide patients with the information needed to decide the number of embryos to be transferred, in order to obtain pregnancy and avoid possible multiple gestation.

Finally, normal viable embryos for study from a PGD cycle that are not transferred will be preserved by freezing. Possible uses of cryopreserved embryos are detailed in the section on legal information on this form (Section VIII).

IV. Results

PGD knowledge of the genetic alterations present in the embryo may allow the selection of unaffected embryos for transfer, increasing to varying degrees the probability of healthy offspring. The contribution of PGD for this purpose depends on the evidence justifying its application.

The factors that determine the probability of having a sufficient number of good quality embryos are the age of the patient, the reasons for the indication of PGD and the number of good quality oocytes obtained. The possibility of pregnancy mainly depends on the age of the patient and the number and quality of embryos transferred.

However, it must be remembered that not all patients starting treatment achieve the adequate follicular development to be submitted to puncture, and not all that reach this stage can receive embryo transfer, since in some cases ova fertilization fails, or a genetic study has an adverse outcome or early embryonic development fails. Therefore, the treatment performance can be expressed as a percentage of total pregnancy cycles initiated on follicular puncture cycles and transfer cycles.

The 2011 Registry of the Spanish Fertility Society reports a pregnancy rate of 18.1% per cycle, 20.8% per puncture and 37% per transfer. (https://www.registrosef.com/public/Docs/sef2011_IAFIV.pdf)

Eighty per cent of pregnancies are obtained in the first three cycles of PGD with successful embryo transfer. In case of failure it will be necessary to discuss with the health care team whether to undertake further treatment.

Furthermore, 10-20% of patients obtain embryos suitable for preserving by freezing, with only those with feasible biological characteristics considered for freezing.

Of these frozen embryos, 20-30% survive after thawing and are valid for transfer to the uterus.

V. Risks

The main risks of this therapeutic procedure are:

1) **Multiple pregnancy**: The risk of multiple pregnancy is related to the woman’s age, the number of embryos transferred to the uterus and their quality.

   The gestation of two or more foetuses poses an increase of medical risks for the mother and children, such as increasing the pregnancy pathology, prematurity, low birth weight and severe neonatal complications. The severity of this complication increases in direct proportion to the number of foetuses.

   Multiple pregnancy also implies an increase in social, economic and work difficulties for parents.

   1) **Ovarian hyperstimulation syndrome**: Sometimes ovarian response to treatment is excessive: a large number of follicles develop, ovarian size increases and the amount of

Signature of the interested parties 2
estradiol in the blood rises considerably. Furthermore, the development of this syndrome is directly related to drug administration needed for the final maturation of oocytes (HCG) and achieving pregnancy.

It is classified as mild, moderate and severe, the latter being rare (less than 2%); it is characterized by accumulation of fluid in the abdomen and even in the chest, as well as impaired renal and/or liver function. In critical cases it may be associated with respiratory failure or coagulation disorders.

It may require hospitalization and medical-surgical treatment and only rarely is pregnancy termination advisable.

3) **Ectopic pregnancy:** This involves the implantation of the embryo outside the womb, usually in the fallopian tubes. Exceptionally it can coexist with a pregnancy located in the uterus. It occurs in 3% of cases.

4) **Miscarriage:** The incidence of abortions is slightly higher than that observed in spontaneous pregnancies (15-20%), and this increase is associated with the use of the ICSI method.

5) **Advanced age, smoking and significant body weight changes** increase the risk of complications during treatment, pregnancy and for the offspring. These conditions require adaptations in the treatment necessary for ovarian stimulation and reduce success rates.

6) **Birth defects and chromosomal abnormalities of children:** Current data suggest that children born in ICSI may be at slightly increased risk for congenital and chromosomal anomalies. In PGD cycles, the risk that the offspring is a carrier of genetic or chromosomal abnormalities is reduced only for genes and/or chromosomes studied.

7) **Failures of PGD process:**
   a. Damage to the oocyte or embryo during biopsy, resulting in the arrest of development.
   b. PGD has a rate of 4-8% of diagnostic failure, due to various causes:
      - Occasionally, problems may occur in the process of biopsy, attachment or “in situ” hybridization that produce lack of results in some of the embryos under study.
      - It is possible to not get a conclusive genetic diagnosis in some embryo due to the complexity of the diagnostic techniques used.

Therefore it is recommended that additional prenatal diagnostic techniques such as ultrasound, amniocentesis or chorionic biopsy be used.

8) **Risks of anaesthesia** are detailed in the specific informed consent about this subject.

9) **Psychological risks.** Symptoms of psychological disorders such as anxiety and depressive symptoms may occur in men as well as in women. In some cases, there may be difficulties and high levels of anxiety in the waiting period between the application of the technique and the confirmation of the achievement or otherwise of the pregnancy, as well as in response to the repeated failure of the technique.

10) **Other risks and complications** that may occur exceptionally:
   - Intolerance to medication.
   - Peritoneal infection.
   - Bleeding from accidental puncture of blood vessels.
   - Puncture of an intestinal loop or other organs.
   - Ovarian torsion.
   - Cancellation of ovarian stimulation due to absence of or inadequate follicular development or excessive response to treatments.
   - Failure to obtain eggs in the puncture.
   - Deterioration of the embryo quality as a result of the technique.
   - Failure to carry out the transfer:
     - Egg not suitable for fertilization.
     - Absence of fertilization.
     - Normal or viable embryos not obtained.
     - Physical impossibility of transfer due to anatomic abnormalities of the uterus.
VI. Personalized risks:
Medical, social and occupational characteristics of each patient may lead to a modification of the general risks or appearance of specific risks. In this case they would be:

____________________________________________________________________________

VII. Financial information (if applicable)
The prices charged in this centre are detailed in the attached budget, indicating the impossibility of previously computing the exact total cost because treatments vary for each patient and particularly depend on the response to ovarian stimulation of each woman.

_The economic cost of maintaining the cryopreserved materials (oocytes, sperm or embryos) shall be borne by patients, whatever the decision on their disposition and during the time that they are deposited at the Centre._

VIII. Legal aspects of assisted reproduction

1.- General

_**Law 14/2006 on Assisted Human Reproductive Technologies** _primarily constitutes the legal framework governing assisted human reproduction.

Assisted reproductive technologies are aimed at solving the problems of human infertility, to facilitate procreation when other treatments have been ruled out as inadequate or ineffective.

They are also used in the prevention and treatment of diseases of genetic or hereditary origin, where possible recourse to them comes with sufficient diagnostic and therapeutic guarantees and is strictly indicated.

They can only be carried out when there is reasonable prospect of success and they do not involve a serious risk to the physical or mental health of the woman or the possible offspring; and always in older women, with full capacity to act, regardless of marital status and sexual orientation, with subjects duly informed beforehand of their chances of success, as well as the risks and conditions of such application.

The woman receiving the techniques may ask for them to be suspended at any time prior to completion of embryo transfer, and her request must be honoured.

When the woman is married, the procedure will usually require the husband's consent, unless they were legally or de facto separated and this is reliably recorded. For an unmarried couple, male consent is required both if his sperm is used in the treatment and in the use of donor sperm and such consent will determine the paternity of future offspring.

An unmarried woman, widow or a woman who is legally or de facto separated may be a recipient or user of assisted reproduction in a personal capacity, using sperm from a donor, provided she is over 18, has full capacity to act and has given her written consent in a free, conscious and explicit manner, and does not present medical contraindications for this procedure.

2.- Regarding the possibility of having a posthumous child

In case of the death of the man, parentage is only legally determined if his reproductive material was located in the uterus of the woman at the date of death, unless the husband or the man is not bound by marriage had consented in the informed consent of the techniques, in a deed, will or advance directive, that his reproductive material could be used in the twelve (12) months following his death to impregnate his wife. This consent may be revoked at any time prior to the completion of the techniques.

The law governing reproduction also provides that the consent of the deceased male is understood to have been given for the post mortem insemination of his wife (whether he is married or in a domestic partnership), when the latter has been subjected to a process of assisted reproduction already begun for the transfer of an embryo made prior to the date of the husband’s death. _From the medical point of view, it is considered starting treatment when the patient receives the first dose of medication necessary for the procedure._

3.- Disposition of surplus embryos that are cryopreserved

_**Remaining viable embryos** _from a cycle of IVF are cryopreserved in liquid nitrogen. The subsequent disposition of frozen embryos can be:

a) The use by the woman herself or, as the case may be, her female partner.
IN VITRO FERTILIZATION WITH PREIMPLANTATION GENETIC DIAGNOSIS (PGD)

b) Donation for reproductive purposes.
c) Donation for research purposes.
d) Termination of their preservation without other use.

a) **Use by the woman herself or her spouse** may be made at any time while the woman meets the **clinically appropriate requirements** to perform assisted reproductive technology (which is the maximum retention period). If the couple is separated, if she wants to use them for personal reproduction she would have to have the consent of ex-husband for the new transfer to be carried out, since the children would be both of theirs.

b) **Donation for reproductive purposes** can be carried out if the woman was not older than 35 when the freezing occurred and the embryos can be donated to infertile couples or women who need them. The donation is **voluntary, free, anonymous and altruistic** and requires **prior specific written consent and updated serology**. Recipients and the children born are entitled to obtain general information about the donors, which does not include their identity. In extraordinary circumstances that entail a danger to life or health of the child, or where appropriate in accordance with the criminal procedural law, the identity of the donor may be revealed, in restriction and without ever modifying the previously established parentage.

c) In **donation for research purposes** the embryos are transferred altruistically for biomedical research projects in specifically authorized health care facilities and for specific projects that are also authorized. The effective exercise of this option will lead to the signing of an additional and specific consent that explains the research goals to be pursued and their implications, and will make specific reference also to the use of the technique or specific techniques to be applied (Law 14/2007 on Biomedical Research).

d) The **termination of their preservation without other use**, shall only apply **once the maximum storage period has ended** without having opted for other possible destinations. The doctors responsible for treatment shall set the maximum retention period with the favourable opinion of independent specialists from outside the centre when they consider that the recipient woman does not meet the clinically appropriate requirements for the practice of assisted reproduction.

*The economic cost of maintaining the cryopreserved materials (oocytes, sperm or embryos) shall be borne by patients, as long as these are deposited in the Medical Centre.*

4.- **Obligation renewal of consent regarding cryopreserved embryos**

At least every **two years** the parenting woman or partner will be asked for a **renewal or amendment of consent**. If the parenting woman or partner stops signing the periodic renewal of consent, after two consecutive requests from the facility by reliable means (burofax with return receipt, registered letter with return receipt, telegram with acknowledgment of receipt, notarized letter, etc.) embryos **will be at the disposal of this centre**, which may use them for any of the purposes mentioned in paragraph 3, maintaining the requirements of confidentiality and anonymity as well as the absence of payment and profit.

IX. **Particular legal aspects of preimplantation genetic diagnosis (PGD)**

Law 14/2006 on assisted reproductive technologies considers PGD a technique supplementary to “in vitro” fertilization and ICSI, which is intended to meet one of its basic objectives such as the prevention and treatment of diseases of genetic origin, provided that there are sufficient diagnostic and therapeutic guarantees and they are properly authorized.

In this rule, the legal regime to carry out the PGD provides two different situations, depending on the system of authorization established (art. 12):

1).- Without the need for prior administrative authorization. The law provides for direct authorization of the technique in two cases, in which the centre simply communicates its implementation to the relevant health authority (that of the Autonomous Community), which will in turn report to the National Commission on Assisted Human Reproduction: a) the detection of serious hereditary diseases, of early onset and not subject to curative postnatal treatment in accordance with current scientific knowledge in order to carry out embryo selection from the embryos not affected for their transfer; b) the detection of other abnormalities that may compromise pre-embryo viability (mainly chromosomal).
2). With requirement for express administrative authorization. -- For all other situations that may arise in the selection of embryos by PGD, the law requires for its realization the express approval, case by case, of the relevant health authority, following a mandatory favourable report from the National Commission evaluating the clinical, therapeutic and social characteristics of each case.

This second track of approval also includes the authorization of extensive or therapeutic PGD for third parties, providing that the application of PGD techniques, combined with the determination of histocompatibility antigens of “in vitro” embryos for therapeutic purposes for third parties, will also require the express authorization, case by case, of the relevant health authority, following a mandatory favourable report of the Commission referred.

X. Alternatives if the technique fails

If one or more attempts at sperm microinjection with PGD do not achieve pregnancy, you might want to adopt, after due reflection, one of the following alternatives:

- Begin treatment again.
- Perform supplementary studies.
- Apply modifications to the technique used.
- Conduct a pre-implantation genetic diagnosis (PGD).
- Perform new treatments with donated gametes (eggs and/or sperm).
- Use donated embryos.
- Give up the assisted reproduction treatments.

The contents of this document reflect the current state of knowledge, and therefore are subject to change if new findings or scientific progress so warrant.

In __________________ on the ______ day of __________________year ___________

Signed Physician (License no.)  Patient signature  Partner signature