MEDICALLY ASSISTED PROCREATION

SCIENTIFIC RESEARCH

using human gametes and/or embryos
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The Centrum voor Reproductieve Geneeskunde (CRG or Centre for Reproductive Medicine) of UZ Brussel specialises in treating patients with fertility problems. In many cases, such treatment includes in-vitro fertilisation which involves gametes and may result in the creation of embryos.

Of course, we primarily use these gametes and/or embryos in your treatment as (a) prospective parent(s). A number of situations may, however, arise in which they may not (no longer) be usable for your treatment or may not (no longer) be suitable for you. In that situation you are a potential donor.

As a person providing human reproductive tissue or who has embryos as a result of a treatment, you have the option of donating those gametes and/or embryos which cannot (can no longer) be used for your treatment for use in scientific research. In this brochure we will explain more about the various research projects being carried out at UZ Brussel using supernumerary gametes and/or embryos. This information should allow you to decide whether you are willing to give consent for your gametes and/or embryos that cannot be used for your treatment to be

1 Gametes is a synonym for sex cells: i.e. eggs or sperm.

2 We use the term 'supernumerary' to indicate that they no longer have any clinical usefulness for you.
used in scientific research.
The basis on which we request your co-operation is the mutual understanding that exists between scientists and patients in the area of fertility medicine. Our aim in this brochure is to enable you to make your decision in a free and fully informed way.

Since it was founded in 1983 the CRG has been propelled by two main driving forces: clinical practice, which is geared entirely towards patients, and scientific research, which takes place behind the scenes. Thanks to this powerful combination, the CRG – often working together with the medical genetics department at UZ Brussel – has made more than one breakthrough in high-technology areas of fertility medicine.

Without you, our patients who are willing to donate (a proportion of) their gametes and/or embryos, we could never have achieved this. We are therefore keen to pass on to you some of our enthusiasm for this work. We hope that after reading this brochure you will consider the option of donating the gametes and/or embryos that can no longer be used for your treatment so that they can be used in scientific research.

It is thanks to that research and your contribution to it that we have reached the place where we are today. Perhaps we can go even further tomorrow.
In Belgium – as in most other countries – there are strict laws regulating the use of human tissues in scientific research. There is also a widespread understanding in society that research is needed in order to make progress in medicine. We cannot escape the fact that proper scientific research in the area of human fertility requires human gametes and/or embryos. In other words, if you consent to the use of your reproductive tissues that can no longer be used in your treatment, this will allow us to carry out important research in the area of medically assisted reproduction and stem cell therapy.

In most cases the research that we describe here is conducted by scientific staff at the CRG laboratories. Nevertheless we also collaborate with other researchers. The genetic research work mostly takes place in the laboratories of the Centrum voor Medische Genetica (CMG or Centre for Medical Genetics) of UZ Brussel. Together, the CMG and CRG make up the pre-implantation genetic diagnosis (PGD) clinic, where we try to help couples with genetic problems to have a child free of the genetic defect.
We also work with the Human Stem Cell laboratory (hESC lab) of the Vrije Universiteit Brussel (VUB).

We have summarised below the rights and (few) obligations that you have as a donor of human reproductive tissue and/or supernumerary embryos for scientific research.

- Donation of supernumerary gametes and/or embryos takes place on a voluntary basis. It will neither increase nor reduce your chances of success and your decision will have no effect on your subsequent treatment.

- You must write down your decision as to whether or not you will donate your gametes and/or embryos for scientific research in a number of contracts.

- You are entitled to refuse to donate supernumerary gametes and/or embryos for scientific research. If you do not consent to the use of your gametes or embryos for scientific research they will be destroyed immediately.

- If you have consented to the use of your supernumerary gametes and/or embryos for scientific research, you can still withdraw your consent before the research begins. If you are a couple, this withdrawal is valid if requested by either of you. You must inform us of your decision to withdraw it in a written, signed document.

- Donating gametes and/or embryos does not result in any financial benefit, nor does it entail any additional cost.

- If you give consent to donate gametes and/or embryos for scientific research, you can be assured – this applies to both partners in the couple – that your name/names and other personal details will be kept strictly confidential. The research material is encoded to ensure your personal and clinical data are not disclosed to the researcher. In this way the research results cannot be linked to your file.

- In accordance with the Belgian law of 8 December 1992 and the Belgian Law of 22 August 2002, we respect your personal privacy. If we publish the results of the study, we also guarantee that you will be anonymous.
By consenting to the use of your supernumerary gametes and/or embryos for scientific research you are simultaneously consenting to the possible application for a patent for inventions that may result from the scientific research to which you have consented. You fully understand this and renounce all claims to any remuneration.

All research involving human embryos is strictly subject to the Law regarding research performed on in-vitro embryos of 11 May 2003, published in the Official Gazette on 28 May 2003. That law stipulates among other things that it is forbidden to use gametes and/or embryos for the following purposes:

- to insert human embryos into animals or to create chimeras;
- to implant in humans any embryos that have been used in research, except where the research was carried out with a therapeutic intent for the embryo itself or in the case of an observation method that does not harm the integrity of the embryo;
- to use embryos, gametes or embryonic stem cells for commercial purposes;
- to conduct research or develop treatments aimed at the selection or improvement of non-pathological genetic characteristics for the human race;
- to carry out research or treatments for the purpose of gender selection, except for the purpose of preventing gender-related diseases;
- to carry out cloning from human reproductive tissue;
- to carry out research on embryos after the first 14 days of development, not including the period when frozen.
Gametes and embryos that become available for scientific research may come from two sources: prospective parents and (voluntary) donors. In the context of a donation programme the latter may opt to allocate (part of) the reproductive tissue that they donate for use in scientific research.

Prospective parents are patients who are undergoing fertility treatment with a view to fulfilling their desire to have a child. During the course of their treatment they may have ‘supernumerary’ gametes or embryos, i.e. gametes or embryos that have no further clinical benefit for themselves.

We distinguish between the following situations in which gametes and embryos may be donated for scientific research. You will find the numbers set out in the brochure below at the end of the description of each type of research. You can then work out the types of tissue that are used in the various types of research.
1. GAMETES

a. eggs that cannot be used for IVF\(^4\) or ICSI\(^5\) because they have failed to reach the appropriate stage of maturation;
b. eggs that cannot be fertilised during the treatment cycle and for which cryopreservation is not an option. Eggs may be impossible to fertilise when even after persistent efforts in the IVF laboratory, the partner's ejaculate or testicular biopsy tissue\(^6\) has not yielded any sperm and where the use of donor sperm is not an option for the couple
c. eggs made available by donors;
d. eggs which have been frozen in the context of treatment and are donated for scientific research after their predefined storage period;
e. sperm left over after IVF or ICSI;
f. testicular tissue (biopsy);
g. sperm provided by donors;
d. sperm frozen in the context of treatment and donated for scientific research after the predetermined storage period.

If eggs are donated for research they can be used to create embryos using sperm from a consenting donor. This is only done if the aim of the research can only be achieved by creating embryos and subject to specific permission from the 'Federal Commission for Medical and Scientific Research on Embryos in vitro' (FCE).

\(^4\) IVF or in vitro fertilisation: the traditional method involves placing the eggs in a petri dish in the laboratory where they are brought into contact with a large (but selected) quantity of sperm. 'In vitro' literally means 'in glass'.

\(^5\) In ICSI or intracytoplasmic sperm injection, we inject a single sperm into each egg – once again this takes place in a petri dish in the laboratory.

\(^6\) In male infertility we sometimes try to collect sperm from a piece of tissue which we surgically remove from the testis or epididymis (a biopsy).
2. EMBRYOS

a. embryos originating from abnormally fertilised eggs. These therefore cannot be transferred to the uterus;

b. embryos of insufficient quality to be transferred to the uterus or cryopreserved.

c. embryos that have been genetically tested in the context of pre-implantation genetic diagnosis (PGD) and found to have a genetic defect;

d. embryos frozen as part of treatment and then donated for scientific research after the predefined storage period.

Embryos are cultured in the laboratory until no later than day 14 and are destroyed by the research technique.
The scientific research at UZ Brussel meets all the requirements of the law of 11 May 2003 on research on embryos in vitro. Every research protocol for which human gametes or embryos can be used has received a favourable recommendation before the research begins from the two authorised committees in this area: the Medical Ethics Committee of UZ Brussel (LCE) and the Federal Commission for Medical and Scientific Research on Embryos in vitro (FCE).

All studies are carried out in accordance with the guidelines of the ICH/GCP, which are included in the Declaration of Helsinki on the protection of individuals participating in clinical studies.

You will find below a brief description of the scientific projects currently taking place at our centre in which we use human gametes and/or embryos.
All the areas and associated projects are under the responsibility of the LCE. Areas C to H inclusive and associated projects are also under the authority of the FCE, while areas A & B (projects 1 and 2) are not.

We distinguish between two types of research.

I. PROJECTS FOR WHICH EMBRYOS ARE NOT CREATED

Area A – Project 1
Refining IVF techniques
IVF, ICSI, embryo biopsy\(^7\), embryo culture\(^8\), embryo cryopreservation\(^9\) and in vitro maturation (IVM)\(^{10}\) are routine procedures at the CRG. In order to guarantee the quality of these procedures, it is important for us to train our laboratory staff so that they can learn to carry out the technical procedures as perfectly as possible. To do this we need ‘real’ gametes and/or embryos. We are also constantly seeking to improve existing procedures and to develop and validate new procedures.

Research material: 1a, 1e, 1g.

Area B – Project 2
Refining PGD techniques
In pre-implantation genetic diagnosis (PGD) the CMG of UZ Brussel carries out a genetic test on one or two cells taken from embryos three days old that have developed in vitro. Before an embryo is transferred to the uterus of the prospective parent, the laboratory diagnoses which embryos are carrying the genetic disorder that is affecting one or both of the parents. Once the results are known, embryo transfer can take place on day five after in-vitro fertilisation. Consequently only those embryos that do not carry the disease being tested for are eligible.

Since the embryos are tested before they are transferred (and therefore before a pregnancy has occurred) PGD is an alternative to prenatal diagnosis. It is an option for couples who are at high risk of having children with a genetic disease and who want to avoid having a termination of pregnancy.
In PGD, the genetic tests must be highly efficient and accurate. They must also be adapted to be carried out on a single cell. In order to train laboratory workers in the technical and practical aspects of these single-cell tests and to develop and validate new tests, we sometimes use cells from embryos.
Research material: 2a, 2b.

Area C – Project 19
The (epi)genome of human embryos
Scientific research tells us that many new changes (mutations) can occur in the DNA of embryos which we were unable to detect before because the technology was not available. In addition to this there are two important characteristics of the embryo’s genome we want to research:
• the chemical or structural changes of the DNA, referred to as epigenetics. These epigenetic changes are important because they control the creation of proteins which the embryo needs to grow; and
• the DNA of the mitochondria. Mitochondria are the organelles in our cells responsible for the production of energy.
The latest genetic technologies we want to use in our research will allow us to fully analyse the embryo’s genome. Strongly developed IT processes will make it possible to integrate all these different elements (epigenetics, mitochondria, new mutations) and to obtain an overall picture of the embryo’s genome status. Ultimately this will result in much higher prediction scores as to which embryos will develop into healthy babies, and which ones won’t.

Project 19 – Study of mitochondrial DNA mutations in human pluripotent stem cells by massive parallel sequencing
AdV058 - LCE (BUN 143201526468, permit 9/12/2015);
FCE (permit 25/01/2016, completion date 25/01/2021).
Research material: 2b, 2c, 2d.
Area D

Derivation and culture of embryonic stem cell lines

Human embryonic stem cells are derived from human embryos about six days old. In this process the embryos themselves are destroyed.

In a dish in the laboratory we can cultivate stem cells for a very long time in the primitive form in which they were present in the embryo so that they continue to be a source of new cells. Given the right stimuli they can also grow into any type of tissue in the human body.

That is why stem cells (and stem cell research) are of such great importance to medicine. Stem cells could, for example, be used in the treatment of diabetes or Parkinson’s disease. The pharmaceutical industry could use them to develop and test new medicines, which would considerably reduce the number of laboratory animals needed for pharmaceutical research.

In this research we are initially trying to improve the techniques that are used to derive stem cells from human embryos and to develop new techniques.

What is more, and before stem cells can be used for transplantation into patients, we need to investigate the safety of these cells when used as a treatment. One such safety aspect is the genetic stability of embryonic stem cells. We know that if we culture embryonic stem cells for a long time in the laboratory, they begin to display chromosome errors. These errors could have negative consequences if we were ever to use them for transplantation in patients. We are therefore carrying out in-depth research into this aspect using specific (molecular biological) measurement techniques.

Another area in which human embryonic stem cells are very important is in the study of genetic disorders. Since we can derive stem cells from embryos that have one of these diseases, we can study them under laboratory conditions.

In embryos with a genetic mutation we look for confirmation of all findings produced by the research on stem cells.
At UZ Brussel we are mainly interested in diseases\textsuperscript{10} which are caused by so-called dynamic mutations, e.g. fragile X syndrome, myotonic dystrophy (also known as Steinert’s disease) and Huntington’s disease. These diseases have one characteristic in common: an unstable gene that changes over time, also resulting in changes in the patient’s clinical condition. We are also interested in stem cells with cystic fibrosis, SMA and Duchenne muscular dystrophy.

Finally, we also want to cultivate stem cells with other genetic diseases that can be used by other researchers both in Belgium and abroad to study those diseases. Researching stem cells with a genetic mutation may help us to understand these diseases better and might even help us to develop targeted treatments such as specific medicines.

\textbf{Domain I – Projects 15, 16 and 21}

\textbf{Interaction between the embryo and the endometrium}

Implantation is a crucial stage in reproduction in which the embryo and the endometrium must interact perfectly. In humans this happens on day 7 after ovulation. The implantation process – for which both the embryo and the endometrium have to be optimally prepared – consists of three steps:

- apposition, i.e. the initial loose attachment between the embryo and the endometrium;
- adhesion, or the stronger attachment of the embryo to the endometrium; and
- invasion, when the embryo penetrates the endometrium.

In humans, the implantation mechanisms are not well known, which means that in an IVF programme implantation failure is the main limiting factor. In order to gain a better understanding of implantation, and implantation failure in particular, we will be setting up an in-vitro model for implantation in humans. Human embryos will be grown in the presence of human endometrial cell lines or biopsies. Using molecular biological techniques and a microscope, we will examine the role played by adhesion molecules, growth

\textsuperscript{10} A word of explanation about the genetic disorders mentioned here:

Fragile X syndrome forms the basis for a mental handicap.

SMA (spinal muscular atrophy), Steinert’s disease and Duchenne muscular dystrophy are muscle diseases.

Huntington’s disease is a neurological disorder.
factures, hormones and the immune system during the three phases of implantation. By fostering or preventing implantation in vitro, we hope to discern the factors that play a crucial role. More specifically, we hope to understand the causes of implantation failure and recurrent miscarriage.

**Project 15 – Research into regulators of implantation in the human embryo**
AdV045 – LCE (BUN143201316309, permit 08/02/2013); FCE (permit 30/04/2013, completion date 30/04/2017 - prolonged).
Research material: 2a, 2b, 2c, 2d.

**Project 16 – Investigation of trophectoderm regulators playing a role in human embryo implantation**
AdV049 – LCE (BUN143201419672, permit 11/02/2014); FCE (permit 24/03/2014, completion date 24/03/2018 - prolonged).
Research material: 2a, 2b, 2c, 2d.

**Projet 21 – Impact of maternal decidualisation on human blastocyst development**
AdV066 – LCE (BUN 143201629028, permit 10/08/2016); FCE (permit 19/09/2016, completion date 18/09/2021).
Research material: 1a, 1b, 1c, 1d, 1g, 2a, 2b, 2c, 2d.
II. PROJECTS FOR WHICH EMBRYOS ARE CREATED

Area E – Project 20
Fertilisation and development of the embryo
We have a very limited knowledge of human pre-implantation development. This is the period between the fertilisation of the egg and implantation of the early embryo in the uterus. During this period, the fertilised egg develops into a multicellular embryo and then into a blastocyst. The blastocyst consists of an inner cell mass – the future fetus – and the trophectoderm, which is part of the placenta. The trophectoderm is necessary for implantation of the embryo in the uterus.
After it is transferred to the uterus, implantation takes place on around day six or day seven of embryonic development.
To study early development, we ascertain which cells in the early embryo have the capacity to develop into a complete embryo in the laboratory. Using specific molecular biological measurement techniques and microscopic analysis we determine the presence or absence of specific substances (proteins and RNA) in embryonic cells as they divide.

Information on the timing and mechanism of differentiation of cells in the preimplantation embryo, will contribute to our knowledge of human embryology. More specifically this will help us to find out how the first differentiation – into placental tissue – and the second differentiation – into the yolk sac – take place in humans. It will also allow us to find out what happens to an embryo after it loses cells. This cell loss may be caused by fragmentation during in-vitro development, it may be the result of a biopsy for PGD or caused by damage during freezing.
Finally, we will culture stem cells from some embryos which will in turn be used for further research. This research will contribute towards our knowledge of stem cell biology, more specifically the origins of embryonic stem cells in the embryo.
Project 20 – Signalling pathways controlling trophectoderm lineage differentiation in early human embryos
AdV057 - LCE (BUN 143201526417, permit 9/12/2015);
FCE (permit 24/02/2016, completion date 24/02/2021)
Research material: 1a, 1b, 1c, 1d, 1g, 2a, 2b, 2c, 2d.

Area F
Safety of IVF techniques
The results of numerous follow-up studies in children born thanks to IVF/ICSI indicate that the techniques used are safe. This is true of results at the time of birth and also in relation to later child development.
Further research is still needed, however, since we have not yet investigated all the risks. For example, studies in sheep and cattle have shown that media used for in-vitro culture can cause epigenetic disruption in the embryos. This epigenetic disruption in the investigated sheep and cattle caused 'Large Offspring Syndrome' (LOS), one characteristic of which is overweight. In mice, however, a lower birth rate has been observed.

The question is whether the in-vitro culture system used in IVF techniques in humans may give rise to this type of epigenetic disruption. In this study we use specific molecular biological measurement techniques and microscopic analysis to study the presence or absence of certain epigenetic proteins and certain epigenetic properties, such as DNA methylation, in dividing embryonic cells.

Area G
Suitability of eggs and embryos for freezing
Not all eggs and embryos survive the freezing and thawing process. When frozen human embryos are thawed, they often have both intact and damaged blastomeres. These embryos have a smaller chance of implantation after being transferred to the uterus than intact embryos. It is clearly possible to optimise the procedure used in freezing eggs and embryos.

11 By ‘epigenetic’ we mean genetic characteristics or disorders that may also be caused by external circumstances and not by genetic transmission alone.
When freezing eggs we use vitrification, which is a new freezing technique. Vitrification involves completely transforming a solution into amorphous ice by cooling it rapidly, in this case to −196°C. One of the tasks now facing us is the development of a vitrification protocol, i.e. a calibrated way of working that can be followed by everyone. This should, among other things, allow comparisons with techniques involving slow freezing and thawing. There are four aims of this research:

- to gain better insights into the causes of damage after freezing and thawing human eggs and embryos;
- to gain more knowledge about the consequences of cell loss due to the developmental capacity of thawed embryos;
- to develop an optimised vitrification technique for cryopreservation of human eggs and embryos; and
- to test the safety of vitrification using molecular techniques that guarantee the normal function of vitrified eggs and embryos.

Area H

Characteristics of embryonic stem cells

When creating human embryonic stem cells, part of the embryo is used, as a result of which it is also destroyed. We can, however, also create human embryonic stem cells from a single embryonic cell. This is done at an early stage when the embryos are only two or three days old and contain only four to eight cells. By removing one cell to create stem cells the donor embryo is not destroyed and it can then develop further.

Through this research we are initially aiming to improve the techniques used to derive stem cell lines from a single cell. In addition we want to find out whether embryonic stem cells from a single cell have the same characteristics as stem cells derived in the conventional way. We will use specific molecular biological measurement techniques and microscopic analysis for this.
Area J – Project 22
Chromosomes in embryos

Through many years of research into embryos resulting from in-vitro fertilisation, we know that many of them – up to half – have abnormal chromosomes. Most embryos with chromosome abnormalities fail to survive after being transferred to the uterus. Even after years of research we still don’t know the causes of these abnormalities.

Thanks to the recent development of powerful new methods to look into our genome and because embryos remain in culture in the laboratory longer, we have found out a number of things. We now know that on day 5 less embryos have chromosomal abnormalities than on day 3. It is as if after day 3 embryos are able to correct the errors in their chromosomes.

We now also know that some embryos with normal chromosomes are unable to implant in the uterus, and some embryos with chromosomal abnormalities do result in a healthy pregnancy and baby. But we don’t know why this is. We expect the answers lie in the embryos’ genomes.

The latest genetic technologies we want to use in our research will allow us to fully analyse the embryo’s genome, both in terms of the chromosomes and the proteins that are expressed. Strongly developed IT processes will make it possible to integrate all these different elements (chromosomes, proteins) and to obtain an overall picture of the embryo’s genome status. Ultimately this will result in much higher prediction scores as to which embryos will develop into healthy babies, and which ones won’t.

Project 22 – The search for the origin of chromosomal abnormalities in human preimplantation embryos
ADV069 - LCE (BUN 143201628722, permit 15/06/2016); FCE (permit 24/10/2016, completion date 23/10/2021)
Research material: 1b, 1c, 1d, 1e, 1g, 1h, 2c, 2d
DISCLAIMER
This brochure contains all the information that you need if you are considering taking part in scientific research conducted by UZ Brussels in the area of reproductive medicine. You should therefore read it carefully. The agreements that you sign in relation to your fertility treatment and in which you have to decide on the fate of your supernumerary gametes and/or your supernumerary embryos refer to this brochure is referred to as the SR* brochure.
By signing this contract you indicate that you have read this brochure and understood the information in it.

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Magelaan cvba
Gent – België

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Centrum voor Reproductieve Geneeskunde
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Publication date: October, 2018
Sixth print – unrevised
Advances in fertility medicine owe a great deal to the scientific research that is constantly taking place in this area. This would not be possible, however, without the help of patients who are willing to donate their tissues. This brochure explains more about the various research projects at UZ Brussel and your rights as a (participating) patient.