Innovative technologies in human reproduction: challenges ahead

Chuva de Sousa Lopes, S.M.

Prof.dr. Susana M. Chuva de Sousa Lopes

Innovative technologies in human reproduction: challenges ahead

Universiteit Leiden
The Netherlands

Innovative technologies in human reproduction: challenges ahead

Inaugural lecture delivered by

Prof. dr. Susana M. Chuva de Sousa Lopes

on acceptance of the position of Professor Developmental Biology,

in particular human development

at Leiden University

on June 29, 2020

Universiteit
Leiden
The Netherlands

Esteemed Rector Magnificus, members of the Faculty board, esteemed audience,

The past and present: gaining control of human reproduction

Human reproduction is not an easy topic to discuss. The emotional or physiological desire to reproduce is deeply rooted in human beings and it is always personal. For some of us it may be truly painful, for others pure joy, a matter of hope, relief, sadness or despair. But whatever your age, sex or gender, we all have our own personal experience with aspects of human reproduction and an opinion on how far medical technology should go to support human reproduction.

Our opinion may change in time, as we ourselves grow old. Human reproduction also has different connotations for different partners in a relationship since in addition to the act of sexual intercourse, the embryo and later fetus, needs to develop inside a womb throughout pregnancy and after 9-months of gestation the baby needs to be delivered. Pregnancy and delivery are not risk free for the person carrying the pregnancy and for the baby. After birth, the first period of parental care, often includes lactation or breast feeding the new infant.

During the last 100 years, the way we (and the society around us) think about human reproduction has changed dramatically. Sexual education and fertility awareness are (to a greater or lesser extent) available and provided at schools and at home. Later in life, family planning is, again to a greater or lesser extent, available depending on the particular society and we are these days much more aware early in life that partners with ovaries have a biological clock, and their fertility starts to decline from 30 years of age until menopause completes, around 50-60 years of age.

The pill: One important technological development to control the timing of reproduction however is birth control, particularly via hormonal oral contraceptives (also known as the oral contraceptive pill). This technology started being marketed in the 1960s and although widespread in many countries, some cultures actively or passively discourage its use and for many people around the globe it is either not affordable, not available or both. However, it has empowered tremendous autonomy in reproduction with a disconnect to the sexual act from reproduction.

With family planning, a couple can now control the timing of childbirth such that it takes place when they want or are ready to reproduce. However, because of the ovarian biological clock, this time window ends with menopause. But even during the time window, starting a family may prove challenging. Environmental pollution reduces fertility, the couple may be infertile or have become infertile after an accident, disease, surgery or having had chemotherapy to treat cancer earlier in life. In addition, couples are currently older by the time they consider having a family, with consequences for overall fertility rates and the associated perceived quality of life.

MAR: Hence, another important technological development that has marked a revolution in human reproduction at an unprecedented scale is medical assisted reproduction (MAR), including in vitro fertilization (IVF). Provided mature eggs can be retrieved and sperm can be obtained so that both sex gametes are available, fertilization can take place outside the body. The embryo is then allowed to develop in culture for a couple of days and transferred (directly or after a period of cryopreservation) to the uterus where, if it manages to implant and develop, will result in live birth at term.

The first person born as a result of in vitro fertilization was Louise Brown. She was born in 1978 and is currently only 41 years old (birth date 25 July 1978). The development of this technology was so profound that the Nobel prize for Physiology or Medicine was awarded to its inventor Bob Edwards in 2010. And although the long-term consequences of this technology are still unknown, the desire to have babies is so deep that since then millions of babies (in some countries as many as 1:10) are being born from IVF or related technologies around the world.

New technologies to improve fertility preservation

In case a couple cannot provide either mature eggs or sperm (the gametes), suddenly the options to obtain a genetically own child become close to zero. This is often the case in young patients diagnosed with cancer, that need to undergo chemotherapy treatment rapidly and have high probability to become infertile afterwards. In case of a patient with ovaries, there may not be time to undergo a cycle of ovulation to retrieve mature oocytes and depending on the type of cancer undergoing a lengthy hormonal treatment may not be indicated. In this case, in the Netherlands and some other countries, it is possible to undergo a fertility preservation treatment, whereby before starting chemotherapy treatment, one ovary is surgically removed and the outer part of that ovary, containing immature eggs can be cryopreserved for later.

Later on, the patient can return have their own ovary pieces grafted back in the abdominal cavity, often on top of the remaining ovary, and become fertile again, at least for a period of time. However, the technology is not very efficient as the ovarian graft is often unable to make a connection to the blood supply in the body and without that it cannot survive. This technology of fertility preservation is also not suitable for patients with blood-related cancer with the risk of reintroducing malignant cells via the ovarian graft.

We are working on technology to allow the culture of the ovarian tissue containing immature eggs outside the body, and allowing the eggs to mature in vitro instead. In this case, we would avoid the necessity of an extra surgical procedure and all patients independent of the cancer type could benefit from fertility preservation technology. The ovarian pieces of the patient would be cultured, and the resulting mature eggs could be directly used in medical assisted reproduction.

Why is maturing the eggs present in the ovarian grafts so difficult in vitro? We simply know very little about human gametogenesis, both in females and males. Firstly, there are major physiological differences between humans and animals, making it difficult to extrapolate. Second, the material needed to study this process (the ovary and the testis) in very precious and difficult to obtain. Finally, although infertility can result in high levels of stress, mental illness and reduced quality of life, it does not kill you, and as such there is no priority in health programs to determine causes of infertility or for the development of suitable treatments.

As we lack suitable human-based assays to investigate gametogenesis in vitro, it remains challenging to develop disease models and apply those to personalized medicine. But that is one of our goals for the future.

The way to artificial ovaries

Of course, we need to learn how to walk before we can run. The maturation of the egg is a complex and multistep process and we need first to understand each step, making sure the egg develops exactly as it should even though the process takes place in vitro. For that, we need to know how the egg develops in vivo and so, in my group, we are first characterizing all of the phases of maturation, not only of the egg, but also of the cells surrounding it in the so-called follicle, which provide the microenvironment for the developing egg. We aim to understand the signaling networks that need to be in

place during each step to make sure that the maturation of the egg occurs successfully in culture. We need to know the 'ingredients' and have a 'recipe' in place before we can actually move forward.

In addition to understanding all of the steps required to mature the egg, we also need to have the right tools and technologies to implement the 'recipe', even when we have all the 'ingredients'. To mimic the maturation process that take place in the ovary in the laboratory, we envision that we will need to put small pieces of the ovary containing the eggs in the follicles in a gelatin-like structure to create a mini-ovary. This should provide just the right amount of stiffness and mechanical resistance so that the follicles containing the eggs can grow and mature inside the gelatin structure. To give you an idea: a follicle grows in about 100 days from a tiny cell-complex that you cannot see by eye to a structure that is about 2cm in diameter and builds sufficient pressure to trigger "ovulation", as the follicle bursts to release the egg into the oviduct. It is hard to imagine that this phenomenal growth spurt happens every menstrual cycle again and again, to produce just one mature oocyte, arguably the rarest cell in the whole human body.

My ambition is to produce mini-ovaries so that we not only can understand the process of egg maturation but may also be able to collect immature eggs from ovaries to assist reproduction in cases of involuntary infertility arising from a variety of medical conditions. To do this, we may also need to expose the small ovarian pieces seeded in the gelatin-ball to constant fluid flow to mimic the rich vasculature and blood flow in the ovary. We can do this in the laboratory using a microfluidics device, which can supply fresh nutrients and oxygen as well as certain hormones to mimic the natural hormonal cycle, while washing away unwanted secreted products. If this all works as we envision, then we can imagine a future in which we would be able to mature eggs in mini-ovaries and use those eggs in the clinic. But how can we take the step from a mature laboratory-egg to a real embryo that could be transplanted to give rise to a new "Louise Brown". How are we going to do that?

We would need to test the ability of these in vitro grown mature oocytes to be fertilized and give rise to an embryo. However, there is a major obstacle (or as we say in Dutch "een addertje onder het gras") to do this following a standard IVF protocol. This is currently not allowed in the Netherlands under the present Embryo Law, which prohibits the creation of human embryos for research. Specifically, we are allowed to investigate how to mature eggs in the laboratory, but we are not allowed to test whether those in vitro matured eggs can actually be fertilized by a sperm cell and subsequently give rise to an embryo, unless we plan to use that embryo immediately for reproductive purposes. Skipping the research part altogether is surely not acceptable without proper safety checks, which are essential for responsible implementation of new reproductive technologies. Politicians, society and legislators need to consider now if and how they would like this to move forward especially if this new technology proves feasible.

In vitro gametogenesis

So far, I have discussed maturation of eggs using the ovary containing a pool of immature eggs as starting material. But what if a patient also does not have this initial pool of immature eggs? Wouldn't it be an option if we could make eggs from stem cells? Pluripotent stem cells can form any cell of the body, so we assume also the gametes. So what if we used not just any type of stem cells, but stem cells isolated from the patient and known as induced pluripotent stem cells (iPSCs). In mice, this has already been accomplished, but we know a lot more about mouse than human gametogenesis.

To make patient-specific pluripotent stem cells, we need to collect cells from tissue of the patient (somatic cells), for

example skin, blood or simply kidney cells that are shed into the urine. These cells then receive 4 transcription factors (Oct4, Sox2, Klf4 and Myc) and over a period of weeks undergo a process called reprogramming during which they transit from a somatic- to a pluripotent cell state. This technology has changed the way we understand developmental potency and its discoverers Shinya Yamanaka and John Gurdon were awarded the Nobel prize for Physiology or Medicine in 2012 for this game-changing technology in cell biology. Moreover, it is accelerating the development of human-based, or even patient-based, disease models and is helping create novel platforms for drug testing and discovery.

Once somatic cells acquire pluripotency, we can keep them in culture for a long period of time, but we can also direct them to become different cell types, such as neurons, liver cells or muscle cells using different types of culture media supplements. So, in theory, if we understand the steps necessary to make a mature egg in vivo, we should be able to generate functionally mature eggs from patient-specific stem cells. Importantly, in contrast to other potential clinical applications of stem cells in regenerative medicine, for example to treat diabetes, liver disease or heart disease, where many millions of cells may be necessary to treat one patient, treating infertility would only need one egg (or one sperm cell) at a time.

The first step to make gametes, starting from human pluripotent stem cells, is already well understood. And although perhaps somewhat disappointing, this is as far as we are able to get for the time being using human cells. One of the next important steps involves the process of meiosis and this has proved really challenging to recapitulate in vitro. But this is a critical event: without it, gametes will not develop properly. It involves the pairing of chromosomes and the exchange of pieces of genetic material between them, generating chromosomes with a unique combination of genes from you and your partner in each germ cell, so that children are all different even with the same parents. As you may appreciate, this exchange of genetic material (during meiosis) needs to occur very precisely to avoid genetic defects.

Moreover, as mentioned earlier, the egg also needs the microenvironment of the follicle to provide support for the egg to mature. The problem is we do not know what the follicle cells provide to the egg or how to mimic that in vitro. Even in the mouse, after decades of trial-and-error, we still do not know how to induce meiosis and follicle formation from stem cells in vitro. And so, each time we want to differentiate mouse pluripotent stem cells into gametes, we need to use ovaries of mouse fetuses, so that those cells can provide the signals and environment to the stem cells directly, instructing them through meiosis and follicle formation. Even in the mouse, we are still dependent on the presence of these fetal cells to make eggs from stem cells and have to date not discovered what it is that they provide to the eggs.

Limitations and opportunities associated with in vitro gametogenesis

Culturing human patient-specific stem cells with cells from human fetal gonads from abortion material to induce the formation of gametes, as in mice, may be scientifically interesting to test as proof of principle but, it is not going to be feasible or desirable to develop as procedure for clinical applications. Not least because there will be objections from society to this "instrumental use" of aborted fetuses, aside from the feasibility of the supply. This is why it is important that we invest in understanding the role that cells of the fetal ovary play in inducing the formation of gametes from stem cells, so that we can eventually mimic these chemical signals or physical properties in vitro. Secondly, we need to invest in developing protocols to differentiate not only the early germ cells in vitro, but in parallel also follicular cells so that these could subsequently be brought together and in a best case

scenario, self-organize into a mini-ovary in which the stem cell-derived follicular cells could induce the maturation of stem cell-derived gametes. Only after we circumvent the use of fetal tissue to make stem-cell derived gametes, will we be able to move on to develop standardized procedures that could be potentially used in the clinic or industry.

As the formation of functional gametes depends on the cellular niche that they are exposed to: what would happen if we would co-culture male stem cells with female follicular cells? Would those male stem cells be able to develop into mature eggs, instead of sperm? The answer is no. And the other way around, would female stem cells cultured together with testis cells be able to turn into sperm cells, instead of eggs? The answer is also no. Why is it that the stem cells cannot turn into gametes of the opposite sex even if they receive the correct signals to do so?

The explanation lies in the nature of the sex chromosomes: female cells have two XX chromosomes and male cells have one X and one Y chromosome. Using gene editing, my group is also trying to understand not only gametogenesis, but also how we can influence their choice of gamete (egg or sperm). In other words, which genes of the Y chromosome would we have to modify so that male stem cells could actually develop into an egg instead of sperm cell? And what additional parts of the X chromosome would we need to duplicate? And on the other side of the spectrum, what would be the strategy to turn female cells into sperm: we may need to first silence the whole second X chromosome, before we can test what Y genes would be essential to add to allow sperm formation. This is a fundamental question that almost strikes the heart of nature itself: why do we need an egg and a sperm? Will it always be like that? We have yet to find out.

We may of course find out that developing gametes of the opposite sex is simply impossible, but in the process, we will learn a lot about human female and male gametogenesis.

Future technologies

Looking into the future, I would like to briefly mention 3 controversial technologies that make newspaper headlines and are the topic of many science-fiction movies, some of which have unexpectedly become reality. One of those is the use of CRISPR-CAS9 technology in gene editing for reproductive purposes. The world was shocked when a Chinese scientist announced in a YouTube video (on the 25 Nov 2018) that he had used CRISPR-CAS for gene-editing in IVF embryos and that resulted in the birth of two gene-edited baby girls with a third one on the way. The gene of choice was CCR5 and his idea was to provide the babies with a mutation, that occurs naturally in that gene, that would confer resistance to HIV.

Obviously, all work involving human reproduction is strictly evaluated by national ethical committees, put in place to ensure national legal regulations are followed and are ethically sound. These ethical committee scrutinize not only the experiments to be done, but the documentation that is provided for the couples as well as the text for informed consent. In this particular case, the ethical review permit seemed to have been forged.

Several countries currently allow research on the efficacy and safety of CRISPR-CAS technology in human embryos, provided that those edited-embryos are not used for reproductive purposes. But in the future, our society may have to decide whether to use gene editing technology for human reproductive purposes. And if we decide to allow it, what do we want to use it for? Should we allow only the repair of genes for monogenetic diseases or shall we consider enhancing some traits as well? Will we say "yes" or "no" to designer babies?

Other controversial technology that I would like to mention is the development of the so-called artificial embryos. Imagine you can use your own stem cells not only to make gametes of specific cell types of your body, but that you could actually

make a whole embryo, your clone, clumps of cells that could develop into a complete twin if transferred to a uterus. In mice, these clumps of cells look like an embryo, although they lack the potential to develop into a mouse pup. But if artificial embryos turn out to be able to develop into a viable clone or twin, what ethical and legal status should these artificial embryos have?

And finally, I would also like to mention controversial technology that would lead to the generation of an "artificial womb" or "exowomb". This means the development of a device where an embryo or fetus could develop outside the body. This device would mimic the uterine environment, allowing the embryo to develop its own placenta and grow as if in a womb. To date, a human embryo is only allowed to grow outside the body until day 14, hence the exowomb to allow a baby to grow for 9-months may never become a reality.

However, devices could also be developed not to mimic the uterus, but to mimic the placental function in an amniotic tank. These devices could be used to provide very premature babies, that are too premature for the neonatal intensive-care incubator system, with a physiological environment to complete the gestational period. These would function like an extra-uterine life support machine ("biobag"), functioning like a dialysis machine, providing the fetus with nutrients and oxygen and removing waste products, while the fetus would still develop in an aquatic environment that would support the development of lungs and brain as if it would still be inside the maternal womb. This technology has only been used in lambs so far and although the lambs developed well it may never be ready to be tested in humans.

All these technologies raise important bioethical and legal considerations, with important consequences for our reproductive rights. Even though the law provides guidance and we all work within the law, it is a very personal choice where one's own ethical border is. Ongoing technological advances may have the potential to radically change the way we reproduce, we should reflect not only on how far we can go, but also on how far we want to go and on how far we should go.

I have come to the end of my inaugural lecture and I would like to thank some people:

Firstly, I would like to thank the Executive Board, the Faculty Board as well as all those who contributed to the realization of my appointment.

I would like to thank my teachers, scientific supervisors and mentors throughout the years.

In particular, my PhD supervisor Prof. Christine Mummery, still the single most important person in my scientific career. She is a role model in leadership, esteemed boss, dear friend. I would also like to mention two other ladies who had a huge impact on my life and career. One is Dr. Kirstie Lawson, now in her 80s, and still exemplary for her scientific rigor, hard-work and passion for science, in particular developmental biology. The other is Dr. Anne McLaren, who sadly passed away in 2007, for her extraordinary multidisciplinary engagement in the field of reproductive biology.

I would like to thank not only my colleagues in the Dept ANA (and welcome the new head of the Dept Prof. Niels Geijsen and his group), but I would like to expend my gratitude to everybody that works at the LUMC, for making it such an inspiring and pleasant place to work.

I would like to thank all the current and past members of my group at the LUMC.

Spending time together and working on scientific problems every day is an absolute joy and a privilege. Thanks to you

I have the impression that I have never worked a day in my life. I would like to thank my former technician Liesbeth van Iperen, for staying with me until her retirement for her kindness and positivity managing the group and being my right hand for so many years. I would like to take a moment to remember another technician who played an important role in my Hubrecht Institute life: Alie Feijen, who unexpectedly passed away recently.

I would like to thank the members of the Ghent-Fertility and Stem cell Team at the Ghent University Hospital, Belgium where I am Visiting Professor, and in particular Prof Petra de Sutter and Prof Bjorn Heindryckx, for the opportunity to interact with your team and students. I really value our long-term interaction and participating in the research you do.

I would like to thank my many current and past collaborators.

In particular, the fruitful local collaborations with members of the LU and LUMC, highlighting the Dept GYN via Dr. Lucette van der Westerlaken, head of the IVF-lab and the Dept MOLEPI via Dr. Hailiang (Leon) Mei, manager of the Sequencing Analysis Support Core. I hope our circle of expertise will increase and keep us excelling for a long time to come. I would also like to acknowledge my longstanding collaboration with several abortion clinics in the Netherlands (previously with CASA and now with Vrelinghuis and Gynaikon) that are vital to my research.

Finally, I would like to bring a very special 'thank you' message for my friends and family in Portugal, the Netherlands and everywhere else, for their unconditional love and support. Last, but not least, I would like to thank my husband Bernard Roelen, for sharing his life with me during the last 2 decades and accepting me as I am.

I have spoken

Prof.dr. Susana M. Chuva de Sousa Lopes

Susana M. Chuva de Sousa Lopes is Full Professor in the Dept. of Anatomy and Embryology at the Leiden University Medical Center, Leiden the Netherlands and appointed Guest Professor at the Dept. Reproductive Medicine, University Ghent, Belgium from 2013. She studied Fundamental Biomedical Sciences at the University of Utrecht followed by a PhD in the group of Prof. Christine Mummery at the Hubrecht Institute, where she became interested in gametogenesis. This was followed by two postdocs in the group of Prof. Azim Surani and of Dame Anne McLaren at the Gurdon Institute, University of Cambridge, UK supported by a NWO TALENT grant and a Isaac Newton grant. She was awarded a VENI fellowship in 2006 and returned to the Hubrecht Institute to continue research on stem cells and germ cell development and was awarded the de De Snoo-van't Hoogerhuijs Foundation prize in that year. Her group focuses on investigating the developmental trajectories of human organs and tissues, with a particular interest for the germline and urogenital organs. Based on the transcriptional profiles of over a hundred different human (fetal) organs, together with her collaborators, her group has developed a mathematical algorithm called KeyGenes to facilitate the comparison of transcriptional data from differentiated human pluripotent stem cells or organoids to their in vivo counterparts. Her group currently investigates the composition dynamics of the human fetal and adult ovary at the single-cell level and is developing strategies to mature oocytes in vitro in artificial follicles. Chuva de Sousa Lopes is currently funded by an ERC Consolidator and a prestigious Dutch-grant VICI. She has been member of the Committee of National Representatives (Netherlands) from 2014-2020 and is since 2019 coordinator of the Special Interest Group "Stem Cells" of the European Society of Human Reproduction and Embryology (ESHRE).

Universiteit
Leiden
The Netherlands