**Blok 3: Toegepast onderzoek Onderzoek/wetenschap**

**Name: SM Chuva de Sousa Lopes, LUMC**

-   Wat zijn nu al de toepassingsmogelijkheden bij het laten ontstaan van embryo’s voor wetenschappelijk onderzoek en welk onderzoek kan nu nog niet gedaan worden?

 The research on embryos generated specifically for scientific research, includes:

* research on the preimplantation period, to better understand fertilization, resumption of meiosis, paternal DNA decondensation, second polar body segregation, pro-nuclear fusion, embryonic genome activation, compaction, epigenetic reprogramming of the paternal and maternal genome, lineage restriction, blastocyst formation). This is a species-specific, so using mice is not an alternative.
* research on gene editing (safety and efficacy)
* research on innovative methods for artificial reproductive technologies, such as improving fertility preservation (see example here bellow)

I work with ovarian material from oncological patients, often (pre)puberal oncological female patients. Before oncological patients undergo chemo/radiotherapy and depending on their age and type of cancer, the patients can be offered fertility preservation (ie oocyte maturation/preservation; or the ovary is surgically removed and the cortical part where the immature eggs are located is isolated and cryopreserved in liquid nitrogen). Later in life and in case the patient underwent ovary cryopreservation, the patient can opt for ovarian tissue transplantation to restore fertility. This procedure is rather inefficient and requires an additional surgery. It would be a welcome alternative if we would be able to culture the cryopreserved ovarian tissue and mature the patient’s eggs before undergoing an IVF procedure. This would also allow patients with blood-related cancers to preserve their fertility.

Currently, we are still working on protocols on how to mature the (oncological) patient’s eggs starting from cryopreserved ovarian tissue. However, once we are able to mature the patient’s eggs, it would be beneficial to test the quality of the eggs by allowing fertilization and testing the quality of the resulting embryo. If indeed the resulting embryo develops properly and passes all quality controls, this protocol could be offered in the clinic to oncological patients.

I also work on the generation of stem-cell based gametes (still not that developed currently) and haploidization (introduce a somatic cell in G1 phase in an enucleated egg, and stimulate it to undergo mito-meiosis, as it segregates half of the chromosomes). Using this method, would circumvent the generation of gametes using patient derived pluripotent stem cells, but would go directly from a somatic cell into a gamete (egg), that could be fertilized. In contrast to a natural egg, the genetic material of the resulting ‘egg’ does not undergo DNA recombination in each chromosome, but undergoes reduction of the number of chromosomes by half, allowing it to be fertilized. The epigenetic of this newly generated egg, still needs to be investigated. This procedure has given viable pups in mice.

-   Welke mogelijkheden zijn er om de (fertiliteits)zorg te verbeteren met onderliggend voorstel?

 Examples of research that is performed in Belgium with embryos generated for scientific research to improve fertility treatments:

* Improve options for fertility preservation: develop maturation protocols for semi-immature eggs: fertilize the eggs afterwards

(those still immature eggs are now simply discarded)

* Understand why eggs from transmasculine people are unable to mature properly: they have performed nuclear transfer to cisgender mature oocytes and fertilized those: these cis eggs with trnas nucleaus were able to develop into high quality embryos (the problem seem to be in the cytoplasm of the mature transmasculine egg)
* Doing proteomics on human embryos (at the single cell embryos) during pre-implantation
* Performing CRISPR-CAS for several genes of interest and investigate their functionality: for example, maternally inherited PADI6 mutation gives rise to defects in early development. They are investigating the function of PADI6 by inducing a PADI6 mutation (using CRISPR-CAS) and investigating its effect.
* Investigating haploidization: can they induce mito-meiosis using for example granulosa cells introduced in enucleated egg and understanding meiotic resumption, fertilization and subsequent embryo development

-   Waarom is het wel/niet belangrijk dat dit onderzoek in Nederland plaats kan vinden? Tot welke relatie staat dit onderzoek en de (on)mogelijkheden tot landen om ons heen?

1. In the Netherlands, we have access to suitable material to investigate and optimize egg maturation. For example, we have available:
* ovarian cortex from transmasculine donors (to investigate oocyte maturation) and hence perform the necessary tests, on material that is actually to be discarded.
* fetal human material (from elective abortions) (to investigate how to generate gametes)

2) In the Netherlands, we have strong and international competitive research on:

- single cell and single embryo omics

- human pluripotent stem cells (such as iPSCs) and differentiation protocols

- organoid and organs on chip and other innovative culture platforms

- genomics and patient genetic information (analysis and storage)

that could give us a clear advantage to other countries.

1. In the Netherlands, we have excellent national service of fertility care associated with UMCs, that would allow efficient collaboration between clinicians and researchers, ensuring concentration of available material and resources and safe storage of data.

-   Hoe kijken de sprekers aan tegen het doen ontstaan van embryoachtige structuren of pluripotente stamcellen ten behoeve van vruchtbaarheidsonderzoek?

 Current stem cell embryo models do not recapitulate the period from zygote to morula.

 Only using stem cell-derived gametes followed by fertilization would generate an ‘embryo model’ that could mimic the whole spectrum of events that occur during the period of early human development.

Current stem cell embryo models can be used to investigate aspects of blastocyst development and the implantation period (equivalent from day 5-12 post fertilization, blastoid model), aspects of gastrulation (equivalent from day 10-15 post fertilization, ETX model), body axes (equivalent from day 10-15 post fertilization, certain gastruloid models), organogenesis of certain organs/structures (equivalent from day 15-25 post fertilization, axioloid models, somitoid models).

The blastoid model may have value to investigate the implantation period, but as the blastoids develops notorious aneuploidies during formation, the applicability of the model as such remains to be proven. Pluripotent stem cells are notorious unstable in the naïve state, necessary to generate blastoids.

Except the blastoid model, the other embryo models have reduced applicability regarding fertility, they are more valuable to acquire fundamental knowledge on specific lineage restriction and pattern of specific organs.