Genome and epigenome approaches in human assisted reproduction

Impact Statement

Infertility is an important global health issue that one in six people will experience at some point in their lives indiscriminately of their income-status or geographic location[1]. Nonetheless, our understanding of the underlying pathophysiology remains primitive and for 30% of couples presenting with infertility no cause is identified[2]. Even when a cause is identified, very few evidence-based disease-specific treatments are available[3] and therefore assisted reproductive technology (ART) procedures, such as *in vitro* fertilisation (IVF) and intracytoplasmic sperm injection (ICSI), persist as the mainstay of fertility treatment. Access to ART varies widely from country to country and often poses a significant financial burden for couples who are obligated to shoulder treatment cost themselves[4,5]. Additionally, the limited availability of ART treatments can, in part, be attributed to their relatively low success rate, which has plateaued at approximately 30% per embryo transferred[6]. In turn, many couples face multiple rounds of treatment that make ART both a time consuming and resource intensive endeavour. As such, innovations that improve the success rates or reduce the complexity or cost of ART procedures are urgently needed.

The first part of this thesis focused on the *in vitro* embryo culture environment (ART culture media), not only as an avenue to improve ART success rates, but also as a potential strategy to ameliorate the increased perinatal and childhood cardiometabolic morbidity seen in children conceived through ART[7]. We focused specifically on the methylome of neonates and children born after embryo culture in different culture media as a potential mechanism underlying their phenotypic differences because periconception environmental factors are thought to modulate the epigenome with potential implications for future disease development[8]. Reassuringly, we did not find large methylation differences between children conceived after embryo culture in the media that we studied. This suggests that the culture media either did not differentially module the methylome, or if they did that the differences did not persist until the studied time points and as such, we would not expect differences in the occurrence of DNA methylation-mediated disease in these individuals. Importantly, we also excluded DNA methylation at imprinting regions. This is of relevance, as a marginally increased rate of imprinting disorders is observed in ART offspring compared to their naturally conceived counterparts[9]. Our studies imply that the culture media we investigated would not differentially affect the incidence of imprinting disorders.

Next, we focused on innovations in preimplantation genetic testing (PGT). Although PGT was initially developed to allow couples with heritable genetic disorders to prevent these from being passed to their offspring, it has since attracted substantial interest as a tool for embryo ranking and selection with the aim of improving ART success rates[6].

Firstly, we described the development and validation of barcoded oligonucleotides for sample tracking as part of an embryo tracking system (ETS). Implementation of the ETS positively impacted the laboratory workflow by eliminating the need for the four-eyes principle, previously employed during error-prone steps, thereby directly reducing the time requirement of highly skilled laboratory technicians, and in turn the associated labour cost. Furthermore, the ETS could identify switched or mixed samples and therefore prevent associated misdiagnoses that could have devastating consequences for patients undergoing PGT with the aim of preventing disease in their offspring. Secondly, we described a whole-genome sequencing (WGS)-based approach for PGT. Compared to our prior reduced-representation genotyping by sequencing protocol, WGS library preparation could be carried out in less than half of the time and yielded vastly more relevant data. With this approach a greater number of embryos surpassed the minimum diagnostic threshold for PGT for monogenic disorders (PGT-M), especially in cases with challenging indications, such as consanguineous couples, couples with indications in difficult to sequence genomic locations and couples with multiple indications. The improved diagnostic capacity of this approach will increase the number of unaffected embryos available to couples and therefore reduce the number of ovarian stimulation and ovum pick-up procedures that are needed. Not only does this save time and resources, but it also reduces the number of times patients are exposed to procedures that carry a risk of complications. Furthermore, we demonstrated that the WGS-PGT approach could be applied (simultaneously) for all forms of PGT, including PGT for (multiple) monogenic disorders, structural re-arrangements, (meiotic) aneuploidies (PGT-A) and mitochondrial disorders. Developing PGT protocols with robust but simplified laboratory and analytical protocols will allow more centres to adopt these approaches and move away from commonly used time-consuming patient specific PGT protocols. Ultimately, universally applicable PGT methods will reduce the processing time per couple, therefore reducing waiting lists and increasing the availability of PGT without the need for more highly specialised clinical and laboratory staff.

In this thesis we have also looked at the genomic landscape of (recurrent) pregnancy loss. Furthering our understanding of the genetic factors underlying pregnancy loss is useful to guide which patients would likely benefit from IVF/ICSI and whether PGT, for aneuploidies or other genetic indications, should be conducted as part of their treatment. Additionally, gaining insights into the genome dynamics of early *in vivo* human development is beneficial to interpret the significance of PGT-A results and may contribute to more accurate embryo ranking/selection practises in the future.

Finally, we have explored the potential of liquid biopsy in reproductive medicine. We highlighted the potential of liquid biopsy for PGT where a non-invasive test analysing cell-free DNA in spent embryo culture medium could supersede embryo biopsies.

Replacing invasive embryo biopsies, that are potentially detrimental to the ongoing development of an embryo, could increase the number of well-developed embryos that are available to couples for transfer. Furthermore, collection of spent culture medium can be achieved without specialised equipment and with minimal training so it could be easily implemented in many IVF clinics. We also consider how non-invasive prenatal testing (NIPT), which is currently used to identify common chromosomal abnormalities, could be expanded to detect other genetic abnormalities thereby eliminating the need for invasive pre-natal diagnostic testing and the associated risk of miscarriage. Additionally, NIPT samples could be analysed for biomarkers of pregnancy-related conditions such as pre-eclampsia that benefit greatly from timely diagnosis, surveillance, and management and could therefore be implemented to reduce the morbidity and mortality associated with such conditions.

References

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