Liver disease accounts for approximately 2 million deaths per year worldwide. Non-alcoholic fatty liver disease (NAFLD) is the most common chronic liver disease, which affects 30% of the population, and it is strongly associated with increased mortality due to insulin resistance, type 2 diabetes and cardiovascular disease. The increasing prevalence of these disorders places a substantial burden on public health resources. The aetiology of liver diseases is complex, however it is believed that a suboptimal environment or exposures during critical times of embryo development (e.g. undernutrition, overnutrition) can lead to permanent placental and foetal adaptations that can predispose the individual to disease later in life. Assisted Reproductive Technologies (ART- methods to treat infertility), that involve hormonal treatment, gamete and embryo manipulation, has been considered as environmental insults that can affect embryo development and cause adverse perinatal and postnatal outcomes. Previous studies have reported that ART procedures result in adverse metabolic phenotypes, such as impaired glucose tolerance, increased insulin resistance, higher risk of cardiovascular disease, and increased fat deposition in the liver. These factors represent the main alterations of several metabolic diseases, therefore this underlines the need for more focused research to identify risk of disease, such as chronic liver disease. It is believed that epigenetic modifications are responsible for the adverse outcomes caused by ART. These epigenetic modifications are defined as changes of gene expression that do not involve alteration of the genetic code, but can affect the phenotype of the organism. In the last decades the field of epigenetics has gained relevance, especially since it has been shown that these alterations in the epigenome can be transmitted over generations, thus providing a mechanism of transgenerational inheritance of diseases.

Based on this, I formulated the hypothesis that *ART can induce risk of chronic liver disease that is transmitted across generations by epigenetic mechanisms.* To test my hypothesis, a first generation of mouse offspring will be conceived through transfer of embryos developed after Intra Cytoplasmatic Sperm Injection (ICSI), a commonly used ART technique nowadays; and by natural mating for the control group. General metabolic health will be determined by biochemical analysis. The incidence of NAFLD will be assessed by evaluation of epigenetic modifications (DNA methylation and miRNAs), gene expression, markers for oxidative stress and morphology of the liver, and general metabolic health. Next, a second and third generation of mouse offspring will be obtained by natural mating, the same markers analysed in the first generation will be performed in offspring of the next generations to assess the incidence of transmission of liver and metabolic alterations.

This project will provide answers and contribute to the ongoing debate about the risks of current reproductive trends, identify transgenerational inheritance of chronic liver disease caused by ART and identify epigenetic markers that can be used as early diagnostic tools for chronic liver disease. This will improve the health care system by educating and counselling ART patients on the potentially detrimental effects of these procedures. Also, it will help prevent the onset of more severe disease in later life by proper planning and counseling of therapeutic interventions and lifestyle modifications.