The general objective of this project is to evaluate the risk of arousal of autistic traits in offspring from aged parents as well as in offspring conceived by Assisted Reproductive Technologies (ART), collectively called Contemporary Conception Trends (CCT). Almost half of European men and a quarter of women now choose to postpone parenthood beyond the age of 35 years. More than 5 million ART babies have been born worldwide. Advanced parental age (APA) and the use of ART have been associated with higher risk of autism spectrum disorder (ASD) in offspring.

We recently reported that the offspring conceived by aged fathers displayed distinctive symptoms of ASD that were partially transmitted to the second generation of mice. This suggests that the risk of ASD may develop over generations, consistent with heritable mutations or epigenetic alterations associated with CCT. An increased risk of epigenetic errors, such as abnormal DNA methylation has been observed in APA and ART offspring. To test our working hypothesis that epigenetic modifications caused by CCT in humans are associated with an increased risk of ASD we will perform comprehensive behavioural and epigenetic analyses in a three-generation mouse model.

We will determine whether and how APA and ART affect the development of autistic traits in offspring using a test battery for ASD-like behavior in the mouse. To evaluate whether autistic traits are transmitted to the next generation(s), we will evaluate the progeny of APA, ART and control groups, for 3 generations (F1, F2 and F3). To identify epigenetic signatures of APA and/or ART in tissues which are relevant for the programming of ASD, we will perform methylome analyses of mouse brains and placentae of the F1 generation as well as of paternal sperm. For F1 brains and placentae, we will also perform transcriptome analyses. Expression and methylation of the top 30 candidate genes will be more accurately quantified by targeted RNA Seq and bisulfite pyrosequencing in brains of F1, F2 and F3 generations. The resulting data base for APA- and ART-related epigenetic changes in mouse brain, placenta and sperm, correlated with phenotypic parameters on behavior and general health status will reveal the magnitude of risk and enable the development of epigenetic marker panels for ASD diagnosis and prognosis.

This is a pioneering project because the mechanisms shaping the risk for neurodevelopmental disorders and health outcome in CCT offspring and the underlying transgenerational inheritance are unknown. Observations of adult CCT offspring and subsequent generations are lacking. The present proposal will provide a proof-of-principle in the mouse model that behavioural and/or epigenetic changes can be caused by APA and/or ART. Moreover, we will identify genes and tissues susceptible to programming by CCT, sex-specific effects and, to which extent phenotypic and/or epigenetic traits are transmitted to subsequent generations. The arousal of ASD may be regulated either directly or indirectly through the influence on general growth during pre and post- natal development.

Although one cannot directly extrapolate from the mouse model to the situation in humans, the data produced will allow better understanding of the fundamentals of increasingly prevalent neurodevelopmental disorder, ASD, and will open up new scientific horizons for subsequent studies pinpointing mechanisms contributing to ASD using human placentae and sperm.