The latest research indicates that cellular metabolism and its regulation may be crucial for the proper development of the embryo prior to implantation and for oocyte maturation. Changes in the cellular metabolism of embryos are strictly timed and depend on their developmental stage. Heme plays a key role in the proper functioning of mitochondria, but its synthesis consumes glycine and succinyl-CoA. Although the level of free heme in oocytes and embryos may play a fundamental role in their biology, the role of heme in developmental biology has largely been overlooked. Our preliminary data show that δ-aminolevulinic acid, a precursor of heme, inhibits pre-implantation embryonic development. On the other hand, stimulation with heme accelerates embryonic development. Increased levels of heme or porphyrin may lead to changes in DNA replication and cell cycle regulation, also through the influence of heme oxygenase-1 (HO1) on the activity of PARP1 protein.

Based on our preliminary data and the available literature, we hypothesize that free heme is a significant regulator of embryo metabolism and the embryonic cell cycle. The main objective of this project is to verify this hypothesis and answer the following questions:

1. Which stages of heme synthesis are limiting during embryonic development, and which metabolites of heme synthesis and degradation are embryotoxic?
2. Does heme stabilize G-quadruplexes in embryos? Do G4 structures affect embryonic development?
3. How do levels of free heme in developing mouse embryos affect cell cycle progression and levels of DNA damage?
4. Does and how does heme oxygenase-1 form a complex with PARP1 protein?

To answer these questions, we will use time-lapse microscopy, bright-field, and fluorescence microscopy. Cell metabolism analysis using the SeaHorse method, as well as glucose and glycine uptake tests, will be employed to examine cellular metabolism in developing embryos. Electron microscopy will be used to determine the interaction between HO1 and PARP1 proteins. Finally, we will check whether accelerated cleavage of blastomeres results from PARP1 dysfunction and contributes to increased levels of DNA damage.

Understanding the molecular mechanisms responsible for embryonic development is crucial for developing new methods in reproductive medicine. We hypothesize that free heme affects cellular metabolism, regulates the functions of pre-implantation embryos, thereby enabling them to mature or grow more rapidly. This project will help fill gaps in knowledge regarding the role of heme in embryonic development and assess whether maintaining a steady level of heme in embryos is more important for their development than rapidly removing excess of heme