

# The impact of temperature conditions of incubation on mouse embryonic development during *in vitro* fertilization

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**Motivation and Aim:** Nowadays, *in vitro* fertilization (IVF) is used not only in IVF clinics, but also when replicating farm animals, and creating and preservation of genetic collections of laboratory animals. The effects of incubation conditions on the destabilization of individual development of embryo at the stage from fertilization to transplantation to surrogate mothers remain unclear. The aim of the work was to investigate the degree of destabilization of the pre-implantation development of mouse embryos depending on the incubation conditions.

**Methods and Algorithms:** The experiments were carried out on 12-14-week-old CD-1 mice of SPF-status on the basis of Centre for Collective Use “SPF-vivarium” of the Institute of Cytology and Genetics, SB RAS. After superovulation procedure, IVF was performed at 37 °C. Zygotes were collected and transferred to incubators with certain temperature: 35, 37, and 39 °C for 24 hours. A day later, only 2-cell embryo were selected and transferred to an incubator with a temperature of 37 °C, where further embryonic development took place up to the morula stage. The state of the embryos was fixed every 2 hours using an automatic microscope Lionheart FX. To analyze the global level of DNA methylation, embryos were stained with antibodies to 5mC (anti-5-methylcytosine) and propidium iodide.

**Results:** The passage of the first division under different temperature conditions is reflected on certain developmental parameters. Lowering the temperature affects the fusion of pronuclei and the first cell division, so they occur more slowly. 24-hours incubation at different temperatures also affects the rate of cell division. Embryos of 35 °C group divide more slowly up to the 4-cell stage. By the morula stage, the rate of cell divisions aligns in all groups. Blastomere size is affected by factors such as incubation temperature, cell numbers, and interplay of factors. Sizes increase from minimal at 35 °C to larger at 37 and 39 °C. The proportion of synchronously dividing embryos increases with the rise in the temperature of the first division. Synchronously dividing embryos are more likely to develop to a morula compared to asynchronously dividing embryos. Incubation temperature during the first 24 hours of development affects both methylation parameters: mean and fluctuation. At 39 °C, both mean methylation and inter-individual fluctuation in methylation are comparable to control. At 35 and 37 °C both of these parameters increase.

**Conclusion:** The results obtained in the experiments suggest that modification of the temperature conditions of the embryonic development during IVF as in *in vivo* fertilization, namely, incubation in the first 24 hours of development at 39 °C, followed by a decrease to 37 °C, shows that these embryos have a higher percentage of synchronous divisions, which provides them with a greater likelihood of successful development and a significantly lower level of methylation.

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