Genome reprogramming during the first cell cycle of embryonic development

I. Barnetova, dissertation abstract

The sperm head contains highly compacted genome. This compaction is mediated by protamines. Sperm protamines are replaced by cytoplasmic histones after the sperm entry into the oocyte. Beside the proteins replacement, also some epigenetic remodeling occurs. One of the most studied epigenetic remodeling in early zygotes is DNA demethylation. This phenomenon was observed in some mammals (mouse, rat, monkey) but not in some other mammals (sheep) and what is more, in some of them quite inconsistent data were published (pig, human, goat, rabbit).

In our work we were mostly concentrated on porcine zygotes and attempted to explain the reasons of inconsistency in observed data. Three factors were evaluated in our work – the technique of embryo production, sperm factors, and the oocyte quality. In the first part of the study (the technique of embryo production) we compared the zygotes produced by conventional *in vitro* fertilization and zygotes produced by intracytoplasmic sperm injection. The epigenetic remodeling was evaluated by immunolabeling. There were no differences between zygotes produced by the two mentioned techniques. The paternal genome was not demethylated in any of zygotes. The labeling with anti-H3/K9-me2 (anti dimethyl group on lysine 9 of histone 3) showed the positive labeling of both pronuclei in about half of zygotes. In the second part of the study (sperm factors) we aimed at sperm factors. The technique of interspecific intracytoplasmic sperm injection (iICSI) was used to experimentally separate the oocyte and sperm factors. We injected boar spermatozoa into mouse oocytes, human spermatozoa into mouse oocytes, and mouse sperm heads into porcine oocytes. All the injected spermatozoa (or sperm heads) formed the paternal pronucleus in oocytes of different species. The paternal genome of porcine and human origin was demethylated in mouse oocytes. In contrast, the paternal genome of mouse origin was not demethylated in porcine oocytes. From these results it is clear that oocytes cytoplasm mainly affects the paternal genome remodeling. Moreover, boar spermatozoa, which are not demethylated in porcine zygotes, are able to undergo the active demethylation in cytoplasm of other specie. In the third part of the study (the oocyte quality) we compared mouse and porcine ovulated and *in vitro* matured oocytes. The oocytes were used for embryo production by intracytoplasmic sperm injection techniques (ICSI and iICSI). We observed a difference between ovulated and *in vitro* matured oocytes in both species. The difference was more evident in activation capabilities of oocytes then in epigenetic remodeling capabilities. The mouse ovulated oocytes are able to form the paternal pronucleus of porcine origin. In contrast, the mouse *in vitro* matured oocytes were not able to form the paternal pronucleus of porcine origin. Similarly, the paternal pronucleus of mouse origin was formed in porcine ovulated oocytes, but the *in vitro* matured oocytes formed the paternal pronucleus only after additional activation with the electrical pulse. Only a small difference was observed in epigenetic remodeling in mouse control zygotes (intraspecies) produced from ovulated and *in vitro* matured oocytes (extent of DNA demethylation). In addition, no difference was observed in porcine control zygotes (intraspecies) produced from ovulated and *in vitro* matured oocytes. Moreover, we did not observe the DNA demethylation of paternal pronucleus in porcine zygotes which is agreement with some reported studies but in contrast with some other studies.

From these results we concluded that the technique used for embryo production, sperm factors, or the quality of oocytes did not affect the final epigenetic remodeling in zygotes. We suppose that the problem of inconsistent data observed in pig is much more complicated. It seems that the reason is not mainly in the quality of oocyte, as is often quoted, but in some other not defined factors.