Abstract

The embryonal development could be negatively disrupted by exogenous factors, which could cause developmental defect. These factors are called teratogens and amongst them there are not only physical and biological but also chemical substances including some of commonly used drugs. Those substances are recommended not to be use in pregnancy. In case of women with chronical disease, for example diabetes mellitus, permanent medication is necessary also during pregnancy. Therefore it is important to ensure medicament and dose, which are safe for use during pregnancy.

For finding out the embryotoxicity of drugs the prospective and retrospective epidemiological studies are used, which are supplemented by results from experimental studies. According to OECD (organization for economic co-operation and development) recommendations the classical testing is performed on two different mammalian species. Those experiments are limited by different pharmacokinetics and biotransformation, which affects substances in maternal organism. Therefore for alternative embryotoxicity testing are used such methods, that exclude this impact. One of these model organisms that enable this is chicken embryo. Chicken embryo, unlike cellular and tissue cultures, can provide complex information about effect of tested substance on the whole organism. For this purpose new *in vitro* SANDWICH method was developed in our laboratory, which also allows chronical exposition of tested substance, which increases testing sensitivity.

Offsprings of mothers with diabetes have higher risk of developmental defects, especially of cardiovascular and neural structures. These defects appear by these offsprings 4,5 respectively three times more often. One of the defects with highest risk is syndrome of caudal regression, which is in comparison to physiological pregnancies in diabetic up to 26 times more frequent.

Aim of this work was to evaluate the effect of humane insulin on developing chicken embryo by using the *in vitro* method SANDWICH and to confirm or disprove the possibility of causing the observed developmental defects.

Our results uncovered embryotoxic effect of insulin on exposed chick embryo in concentrations from 10^{-6} to 10^{-4} mg/ml which exhibited letality, growth retardation and malformations. Observed developmental defects were in creation of vitelline vessels and connections on *area vasculosa*, creation of blood clots in neural tube and lower limb buds,

neural tube defects of caudal part of embryo and defects of growth of caudal part of embryo. Insulin exposed individuals showed a shortening of it. We also observed apoptotic elements in lower limb buds that were not present by the control embryos.

These results suggest that one of the factors causing developmental defects of cardiovascular and neural systems could be application of therapeutical human insulin as well as in the case of development of syndrome of caudal regression. For this reason it is necessary to evaluate every risks and benefits of medication and physicians should carefully consider using of human insulin and optimize its dose in case of application of therapeutical insulin.

Keywords: Diabetes, birth defects, pregnancy, insulin