Influence of DNA concentration on the efficiency of bovine blastocyst production after WAP-bGH microinjection into germinal vesicle of immature oocytes, zygotes and embryos obtained *in vivo* or *in vitro*

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1. Introduction

How to increase the efficiency of production of transgenic farm animals is one of the major research tasks in the field (1,2,3).

By far microinjection has been the most commonly and successfully used method to generate transgenic animals (4,5). Microinjection is the direct introduction of foreign DNA into the pronucleus of fertilized mammalian egg, into its cytoplasm and/or into both nuclei of a 2-cell embryo. Direct microinjection into the pronucleus (usually male) of a zygote has been known as the most effective method.

Often when superovulation treatment is used to produce a number of zygotes *in vivo*, the obtained material is not embryologically homogeneous. Within the pool of collected eggs, there are many zygotes in different stages of organization, e.g., eggs before pronuclei formation or after syngamy where pronuclei are not visible after centrifugation (a method to visualize pronuclei in cattle and pigs) and 2-cell embryos are also found. Therefore, direct injection of DNA into one of the pronuclei, where the integration rate of the

foreign gene is the most effective, is not possible. The problem with heterogeneity of the cattle material can be partly overcome by employment of the *in vitro* maturation and fertilization technologies. Zygotes obtained using these technologies are almost in the same phase of development. Still, there are a few zygotes where only microinjection into the cytoplasm is possible. It is because of naturally occurring diversity in the time of fertilization (pronuclei formation and syngamy process). Moreover, the *in vitro* produced cattle zygotes are more sensitive to the microinjection procedure and their ability to propagate in the *in vitro* culture is significantly decreased in comparison to zygotes obtained *in vivo* and cultured *in vitro*.

The use of bovine ephitelium cells co-culture system to support growth of bovine zygotes in the *in vitro* culture had positive influence on the number of developing embryos. With the use of this technique, many more co-cultured bovine zygotes can pass through the 2-cell stage block, also a larger number of them can develop to the blastocyst stage, in comparison to those cultured only in a simple medium (6,7,8).

The idea to use immature bovine oocytes for production of transgenic cattle is promising. Non-matured bovine oocytes are available in a large number from ovaries of slaughtered heifers and cows. Our previous experiments clearly showed that in most morphologically normal oocytes germinal vesicle (GV), the target of exogenous DNA injection, is well visible without centrifugation (9,10). We also proved that one third of the microinjected oocytes can be successfully matured and fertilized *in vitro* and propagated in *in vitro* culture to the blastocyst stage (9).

Irrespective of the described opportunities, up to date, the productivity of transgenic cattle has been far from satisfactory, being the lowest among the farm animal species.

The quality and concentration of the vector used for microinjection (1,11) play a very important role in the transgenesis including embryological aspects. A vector construction, mainly the introduction into it new elements responsible for the effective integration into a host genome are difficult, time and labor consuming. It also dramatically increases the costs of production of transgenic farm animals. Prior to being used for microinjection, each new vector has to be tested in the *in vitro* tissue culture to check its expression ability.

DNA purity is not a problem. There are many commercially available kits for DNA purification giving very high purity and yield. The least known factor, especially in the production of transgenic cattle, is the influence of exogenous DNA concentration on developmental abilities of oocytes, zygotes and embryos after microinjection. The aim of the presented experiments was to find how the microinjection of DNA at different concentrations influences the number of bovine blastocysts produced *in vitro* from: 1) GV microinjected non-mature bovine oocytes; 2) zygotes and 2-cell *in vivo* obtained embryos, microinjected into pronucleus or both nuclei; 3) zygotes produced *in vitro* and microinjected into cytoplasm or into one of the pronuclei.

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Summary

Microinjection is one of the most successfully used methods to produce transgenic farm animals. But the effectiveness of transgenesis with the use of microinjection, specially in cattle is still low. Many steps of the transgenesis has been found to influence its effectiveness; DNA purity, the site of its injection, the culture system. There are no reports on the influence of a DNA vector concentration influence on the developmental ability of transformed bovine eggs to the blastocyst stage in the *in vitro* culture. In presented experiments we investigated the influence of different DNA concentrations on the developmental rate of microinjected immature bovine oocytes, zygotes and 2-cell embryos to the blastocyst stage.

Key words:
DNA concentration, microinjetion, bovine immature oocytes, zygotes, 2-cell embryos, co-culture.

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