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DEVELOPMENTAL COMPETENCE OF CMV-FUT TRANSGENIC AND NON-TRANSGENIC PIG EMBRYOS CULTURED *IN VITRO**

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Abstract

Possible influence of a transgene on life functions of embryos makes it reasonable to confirm or deny it for a particular gene construct. *In vitro* development of an embryo is a widely used criterion of its competence. The aim of the study was to compare *in vitro* developmental capacity of transgenic and non-transgenic pig embryos. The results showed a statistically significant difference in *in vitro* developmental capacity of embryos obtained from transgenic and non-transgenic pigs. Developmental competence of embryos (morula and blastocyst stage) produced from zygotes obtained from transgenic sows decreased compared to that obtained from non-transgenic sows.

Key words: pig, CMV-Fut gene construct, transgenic embryos, non-transgenic embryos, *in vitro* culture

Production of transgenic animals is a complex process. Its efficiency is affected by many factors (Jura et al., 2007; Samiec and Skrzyszowska, 2011). The quality of transfected material, transfection method, the type of gene construct, and the type and quality of transfer method into foster mothers are the key factors affecting the efficiency of the process that results in generation of a transgenic animal with proven expression of a given gene (Lipiński et al., 2010; Jura et al., 2004). Standard DNA microinjection is an efficient method to obtain transgenic animals (Lipiński et al., 2010). The main limitation of this transfection method is the low number of animals with an integrated transgene. Therefore, in order to multiply the number of transgenic animals that will serve further research on their application, reproduction methods

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such as crossbreeding and cloning should be applied. In both methods mentioned above, the gene construct used has an influence on their efficiency. Therefore, assessing the influence of a gene construct on developmental competence of embryos has a significant role (Skrzyszowska et al., 2008; Bryła et al., 2010). This role is even more important once systemic modifications are supposed to be achieved through the genetic modification. CMV-Fut gene construct applied in our study to pig transgenesis for xenotransplantation purposes modifies their immunological system towards a partial elimination of a human-pig immunological barrier (Lipiński et al., 2010). Assessment of a potential influence of the gene construct on developmental capacity of transgenic embryos was one of the aspects of the study.

Material and methods

Zygotes for *in vitro* culture were obtained from superovulated CMV-Fut transgenic F1 sows and from superovulated non-transgenic sows (control group). In order to obtain zygotes for *in vitro* culture, sows were inseminated with the semen of a CMV-Fut transgenic boar or a non-transgenic boar. Thirteen transgenic sows (group I-Fut) were inseminated with semen of a transgenic boar (CMV-Fut), 8 transgenic sows (group II-Fut) were inseminated with semen of a non-transgenic boar and 7 non-transgenic sows (control group) were inseminated with semen of the non-transgenic boar. Zygotes were collected from donor sows under full anaesthesia by flushing the oviducts with PBS supplemented with 1% bovine albumin (Sigma, Germany) 26 hours after insemination. Flushed zygotes were transferred into PBS supplemented with 20% of FCS (Sigma, Germany) and their morphology was evaluated under a stereoscopic microscope. Zygotes with morphologically normal cytoplasm and visible polar bodies were transferred into NCSU-23 medium (North Carolina State University) and transported to the laboratory at 39ºC. Prior to the culture, zygotes were washed twice in a NCSU-23 medium and then placed into culture dishes. *In vitro* culture in NCSU-23 medium (North Carolina State University) was carried out for 6 days in 5% CO_2 at 39ºC (Gajda, 2009). Afterwards, the number of embryos that reached either morula or early blastocyst stage, both in experimental and control groups, was assessed. A total of 156 zygotes were cultured in group I-Fut, 81 zygotes were cultured in group II-Fut and 73 zygotes were cultured in control group. Statistical analysis was performed using χ^2 test.

Results

Out of 156 zygotes cultured in group I-Fut (obtained from Fut transgenic superovulated sows inseminated with the semen of Fut transgenic boar), 141 were found to cleave. The morula stage was reached by 126 embryos and 109 embryos reached the blastocyst stage. In group II-Fut (where semen of the non-transgenic boar was used for inseminations) 78 out of 81 zygotes cleaved. Seventy-six embryos developed to

morula and 69 reached the blastocyst stage. Out of 73 non-transgenic zygotes of the control group, 69 cleaved. Sixty-six and 59 embryos reached the morula or blastocyst stages, respectively.

Table 1. *In vitro* developmental competence of pig zygotes obtained from CMV-Fut transgenic sows (Fut) inseminated with semen of the CMV-Fut transgenic (Fut) or non-transgenic (ntg) boar

Group	AI	Zygotes obtained	Cleaved (%)	Morulae (%)	Blastocysts (%)
I-Fut	Fut	156	141 (90.3%)	126 (80.7%) a	109 (69.9%) a
II-Fut	ntg	81	78 (96.3%)	76 (93.8%) b	69 (85.2%) b
Control	ntg	73	69 (94.5%)	66 (90.4%) b	59 (80.8%)

b, b – values with identical letters within the same column do not differ significantly ($P \geq 0.05$).
a, b – values with different letters within the same column differ significantly ($P < 0.05$).

Discussion

In vitro development of an embryo is widely used as a basic criterion of its developmental competence (Dang-Nguyen et al., 2010; Petters and Wells, 1993). By comparing the developmental potential of embryos obtained from transgenic sows inseminated with the semen of boars transgenic for the same gene with the developmental potential of embryos obtained from transgenic sows inseminated with the semen of non-transgenic boars as well as in relation to the non-transgenic embryos (obtained from non-transgenic sows inseminated with the semen of non-transgenic boars), it is possible to examine the influence of the genetic modification on the developmental capacity of embryos. What makes the study more interesting is the fact that the DNA microinjection method used for zygote transfection results in random integration of a transgene into the recipient's genome (Lipiński et al., 2010). Because transgene integration is random, it is difficult to predict its influence on neighbouring genes. During the integration of introduced genetic material interruption or suppression of developmentally important genes may occur. This may cause cleavage arrest or delay the development of the embryos at more advanced developmental stages, before hatching. It will result in a reduced number of implantations and, as a consequence, in a reduced number of potentially transgenic offspring produced by crossbreeding of animals obtained with use of the DNA microinjection method. As a result of our study, where CMV-Fut gene construct was applied, one transgenic boar (Tg1154) was obtained. The Tg1154 boar became the founder of a transgenic Fut pig line. Furthermore, next generations of transgenic individuals with Fut gene expression were obtained via crossbreeding. Based on generation of transgenic sows and boar with proven Fut gene expression, an influence on developmental competence of embryos obtained from transgenic sows was investigated. For this purpose transgenic Fut sows were inseminated with the semen of Fut transgenic boar or with the semen of non-transgenic boar that intentionally led to obtaining 50% presence of the transgene in the pool of the embryos obtained. The control group consisted of non-transgenic embryos. Our results showed that cleavage ratio of zygotes was

comparable in both experimental and control groups. Developmental competence of embryos (morula and blastocyst stage) produced from zygotes collected from transgenic sows was decreased compared to that obtained from non-transgenic ones. This was probably due to the fact that microinjection method was used for transfection. The use of DNA microinjection method causes random integration of a transgene. The random integration in the immediate vicinity or inside the sequence of the gene essential for the development can lead to its dysfunction. This being the case, the percentage of transgenic embryos obtained at morula and blastocysts stage was slightly lower than what was obtained in the control group.

Biotechnological safety is an important aspect of transgenesis. Assessment of the embryo developmental potential in the process of transgenesis allows evaluating the influence of the gene construct used for transfection on the development of zygotes obtained from transgenic sows. If the percentage of embryos developing from zygotes obtained from transgenic sows inseminated with the semen of boars transgenic for the same gene is significantly lower than in a control group (non-transgenic embryos), this shows clearly the negative impact on the vital functions of gene construct. However, in the present study the negative impact of the transgene on the developmental competence of embryos obtained from Fut transgenic females inseminated with the semen of Fut transgenic boars was not observed. Based on this it may be concluded that Fut gene expression has no effect on functions important for the development at embryo stage and therefore does not pose any biotechnological threat. A complete picture of the impact of introduced gene on the developmental potential of embryos will be possible after the molecular analysis. The material obtained has been frozen and will be the subject of further research.

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Potencjał rozwojowy CMV-Fut transgenicznych i nietransgenicznych zarodków świni hodowanych *in vitro*

STRESZCZENIE

Ekspresja określonego transgenu jest czynnikiem, który może mieć wpływ na potencjał rozwojowy zarodków. Rozwój zarodków w warunkach *in vitro* jest szeroko stosowanym kryterium oceny ich kompetencji życiowych. Celem badań było porównanie zdolności rozwojowych transgenicznych i nietransgenicznych zarodków świni hodowanych pozaustrojowo. Uzyskane rezultaty wykazały statystycznie istotną różnicę w kompetencjach rozwojowych zarodków uzyskanych od transgenicznych i nietransgenicznych loch. Potencjał rozwojowy zarodków wyhodowanych z zygot uzyskanych od transgenicznych loch był obniżony w odniesieniu do potencjału rozwojowego zarodków wyhodowanych z zygot uzyskanych od loch nietransgenicznych.