CRYOCONSERVATION OF BLASTODERMAL CELLS AS A TOOL FOR PRESERVATION OF POULTRY BIODIVERSITY

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ABSTRACT

The major threat to genetic resources are the rapid structural changes and intensification of poultry production. Currently, 30% of the breeds are threatened with extinction and 9% have already extinct. These data indicate necessity of searching the methods which may be useful for preservation of poultry biodiversity . Our objective was cryoconservation of chicken blastodermal cells. Chicken blastodermal cells (BCs) was isolated from blastodisc in X stage of development. BCs were frozen using of 3 electronically controlled programs with addition of 10% DMSO. The 10% DMSO allowed to obtain viability of BCs in the range 79-85%. The frozen-thawed blastodermal cells viability was determined by trypan blue dye. Frozen-thawed BCs may be used to produce sex chimeras and through appropriate mating enable to restore a species of poultry threatened with extinction. Elaboration an effective techniques for cryoconservation of BCs gives a broad perspective of virtually unlimited storage of genetic material.

Key words: genetic resources, blastodermal cells, cryoconservation technique, sex chimeras

INTRODUCTION

The major threat for genetic resources are the rapid structural changes and intensification of poultry production. Currently, 30% of avian breeds are threatened with extinction and 9% have already extinct (**Hoffman 2009**). These data indicate necessity of searching the methods, which may be useful for preservation of endangered breeds. At present, there are used two strategies for poultry biodiversity conservation: *in situ* and *ex situ*. The *in situ* strategy is based on protection of the natural environment. That strategy is the most often used in case of wild species (**Pisenti *et al.*1999**). The *ex situ* strategy includes the storage of the genetic material under the deep-freezing in liquid nitrogen. Materials used to cryoconservation are fragments of DNA, tissues, blood, semen, oocytes, embryonic stem cells and embryos. In mammals for preserving of endangered breeds are used gametes and embryos. In case of birds cryoconservation of semen does not guarantee survival of avian species, because the males are homogametic. Due to large size and structure of female gametes their isolation and conserving is not possible. The solution to overcome problems is to isolate and conserve the embryonic cells : blastodermal cells (BCs) and primordial germ cells (PGCs). These cells isolated from embryos of endangered breeds at the appropriate stage of development may be used to produce sex chimeras and through appropriate mating enable to restore a endangered species of poultry (**Kino et al. 1997**). The aim of our study was elaboration an effective method for cryoconservation of chicken blastodermal cells.

MATERIAL AND METHODOLOGY

As the model was used chicken blastodermal cells (Fig.1) isolated from blastodisc in X stage of development of chicken Gallus Gallus domesticus .The suspension of blastodermal cells with addition of 10% DMSO was placed in straws (0,25ml) and frozen using 3 electronically controlled programs (CL- 8800 system, CryoLogic, Australia). The frozen-thawed BCs viability was determined by trypan blue dye.

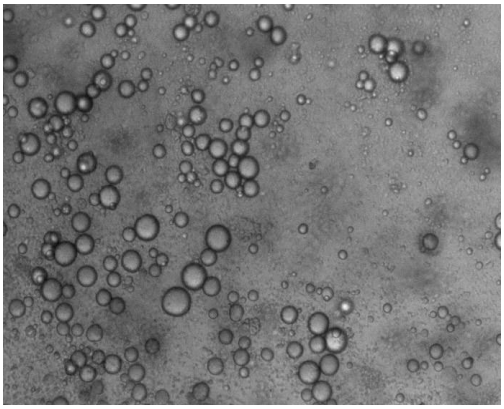

Fig. 1. Living chicken BCs isolated from blastodisc in X stage of development (according to Hamburger–Hamilton). (10x20)

RESULTS AND DISCUSSION

The basis of applying cryoconservation of blastodermal cells for preservation of endangered breeds are properties of BCs. Blastodermal cells well tolerate the deep-freezing and after thawing are alive and show the potential ability to produce chimeras (**Naito *et al.* 1992**). Both of fresh and frozen-thawed BCs may be used to produce chimeras. Chimera is a organism made up of cells from two or more donors which differ in their genetic background.

Injected donor's blastodermal cells can incorporate in gonads, breast muscle, femoral muscle, heart and liver of recipient embryos (**Siwek et al. 2010**). Some of these embryos will be germline chimeras. Sex chimeras through appropriate mating can be used for restore a species of poultry threatened with extinction (**Kino et al. 1997**).

In Chełmońska *et al.* (1997) studies, suspension of BCs with 10% DMSO was equilibrated for 10 min and frozen at a cooling rate 1°C/min by means of electronically controlled program (Kriomedpol, Warsaw, Poland). The frozen-thawed blastodermal cells viability was determined by trypan blue dye. Survival of BCs in this experiment was 83 percent (**Chełmońska *et al.* 1997**).Conducted experiments (**Tereshchenko *et al.* 1994**) and (**Chełmońska**

***et al.* 1997**) show, that applying of 10% DMSO and the cooling rate 1 °C/min allows to receive the greatest number of live chicken BCs. The BCs survival after thawing depends, inter alia, on the type and concentration of applied cryoprotectants and also decrease of temperature rate during freezing, which is associated with exposing the cells to damages during dehydration and hydration process (**Mazur, 1963**). In our study the best program for freezing in connection with 10% DMSO allowed to obtain viability of BCs in the range 79-85%. These data indicate that applied technique for cryoconservation of BCs is effective and may be used for preservation of endangered breeds .

CONCLUSION

Applying for cryoconservation of quails BCs the same method like in case of chicken allowed to obtain only 62.7% of alive cells (**Naito *et al.* 1992**). Elaboration an effective freezing techniques of BCs from different species of poultry gives a broad perspective of virtually unlimited storage of genetic material which may be used in a future to restore an extinct breeds of poultry.

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