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Short communication

THE POTENTIAL USE OF CHICKEN BLASTODERMAL CELLS OF ORAVKA BREED

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ABSTRACT

This study provides information about the method of isolation as well as evaluation of morfology and viability of chicken blastodermal cells (BCs) of Oravka breed. Oravka breed belongs to a critical endangered breed under the conditions of the Slovak Republic. Due to this fact, it is necessary to preserve genetic material of Oravka breed. Frozen blastodermal cells are the potential tool for long-term storage. Chicken blastodermal cells were derived from X stage of embryos (blastoderm stage) using filter paper ring method. To detect blastodermal cells viability specific nuclear fluorochrome Yo-Pro-1 in combination with propidium iodide and DAPI were used. Under the light microscope the isolated blastodermal cells were seen as a large round cells with diameter from 13 to 18 μm and acentric nucleus of 9 μm in diameter. Fluorescent analysis revealed only viable, DAPI-positive cells in the samples. Totally, 38000 blastodermal cells of Oravka breed from each blastoderm were obtained.

Key words: Oravka breed; blastodermal cells; cryopreservation; viability

INTRODUCTION

Conservation of avian genetic resources in the gene bank is necessary for the preservation of endangered species. Cryopreserved biological material can be used for recovering of the lost variation within breeds and restoring of the breeds which have become endangered as a result of destruction of their natural conditions (Sawicka *et al.*, 2011).

Domestic chicken (*Gallus gallus domesticus*) has become an important subject of biotechnological research, particularly in relation to cryopreservation. Compared to mammals, avians have advantages, such as short reproductive cycle, small gap between generations, high laying performance and easy breeding (Li and Lu, 2010). In regards of this facts, avian embryos are a powerful model to study developmental and stem cell biology.

One of the wonderful advantages of the avian embryo is its accessibility, since the avian embryo is subjected to genetic manipulation (Ishii *et al*., 2004). Free access to bird eggs (Ishii *et al*., 2004) allows subjected birds to genetic manipulation Cyriac *et al*., 2012). This feature has been utilized to produce somatic chimeras of various types through tissue grafts, chorioallantoic grafts, parabiosis, yolk sac chimeras and neural tube chimeras (Le Douarin and McLaren, 1984). However, in order to utilize any potential of avian embryonic stem line for manipulation of the avian genome, it was necessary to develop reliable means of producing germ line chimeras analogous to that produced by blastocyst injection of murine embryos.

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National Agricultural and Food Centre, Research Institute for Animal Production Nitra (NAFC, RIAP) deals with the genetic manipulations of Oravka the local poultry breed. Oravka breed was created by crossbreeding of the local hens in the Orava region with Rhode Island, Wyandotte and New Hampshire breeds (Hanusová *et al.*, 2014) and was recognized in 1990 (Chmelničná, 2004). Weis *et al.* (2010) categorized Oravka breed as a critical endangered breed under the conditions of the Slovak Republic.

The objective of this preliminary study was to optimize the method for blastodermal cells (BCs) isolation in Oravka breed and to evaluate their morphology and viability.

MATERIAL AND METHODS

In our study, chicken eggs of Oravka breed were used as a biological material. X stage blastoderms were collected from 20 freshly laid non-incubated fertilized eggs. The eggs were collected immediately after laying and transferred to the laboratory.

X stage blastoderm was prepared with filter paper ring, the perivitelline membrane was cut around the paper and the blastoderm was removed (Petitte *et al.* 1990). Blastoderm was isolated from yolk and washed several times in calcium-magnesium-free PBS (CMF-PBS) (Applichem, Darmstadt, Germany) to remove as much yolk as possible. Then, the cells were washed and centrifuged in CMF-PBS for 3 min at 300 x g, and supernatant was discarded. The cell concentration was determined using automated cell counter (Z1 Automated Cell Counter, Beckman-Coulter, Germany).

To determine the proportion of viable, apoptotic and dead cells fluorescent staining was used. The total number of cells was determined using 4‘, 6-diamidino-2-phenylindole (DAPI) fluorescent dye (blue signal; Vector Laboratories, Burlingame, CA, USA). The number of apoptotic cells was determined using Yo-Pro-1 (green signal; Molecular Probes, Lucerne, Switzerland) and the proportion of dead cells using propidium iodide (PI) staining (red signal; Molecular Probes, Eugene, Oregon, USA). Fluorescent dyes were prepared as follows: 1 µl Yo-Pro-1 (1mmol/l) was diluted with 5 µl PI and 194 µl CMF-PBS; 3 µl of cell suspension was transferred to a tube by gentle pipetting and stained with 3µl Yo-Pro-1/PI by incubation in the dark at room temperature for 20 min. After incubation, sample was washed two times in CMF-PBS. Three µl of the stained sample were placed on a microslide into 3 µl of the Vectashield anti-fade medium containing DAPI fluorescent dye and observed under a LEICA DMI 6000 B fluorescent microscope equipped with DCF 345 FX camera.

RESULTS AND DISCUSSION

Stem cell biology is a rapidly expanding field that includes also the manipulation with stem cells. Chick stem cells can be obtained from embryos and maintained in culture. They can be derived from different sources at various stages of embryonic development. It was demonstrated that in regards to their pluripotency they can form embryoid bodies, which can differentiate into all cell types including embryonic germ layers and contribute to somatic and germline lineages in chimaeras. They are, therefore, comparable to mammalian stem cells, offering a model for studying stem cell biology, as well as being a tool for many applications.

Proper techniques of cell isolation, culture and freezing will give a broad perspective for unlimited storage of genetic recourses.

In our study we isolated, cultured and evaluated under the light microscope BCs from freshly laid non-incubated fertilized eggs of Oravka breed. For isolation we used the same filter paper ring method of BCs isolation as Pettite *et. al.* (1990) and Hamidu *et al.* (2010). Our preliminary results showed that this method of chicken BCs isolation is proper and can be used in our subsequent experiments.

Morphological characterization of freshly isolated BCs revealed large round cells with diameter from 13 to 18 µm with acentric nucleus 9 µm in diameter. Furthermore, we determined total number of viable blastodermal cells. Blastoderm of Oravka breed consists of 38000 blastodermal cells. Fluorescent microscopy revealed only living, DAPI positive BCs in the samples (blue signal). Figure 1 (a, b) represents morphology and viability of freshly isolated BCs.

However, for the future most important is the preservation of isolated BCs for long-term storage in liquid nitrogen. Published reports on this topic indicate that BCs tolerate deep-freezing and have ability to produce chicken chimeras (Naito *et al*., 1992; Bednarczyk *et al*., 2002).

Chimeras can be used to restore breeds threatened with fate to extinction. Similar studies were conducted by Kino *et al.* (1997) and Naito *et al.* (1994), where injection of BCs into recipient embryos allowed obtaining sex chimeras, and through appropriate mating enabled the restoration of the genotype of the donor. According to Carcience *et al*. (1993) and Naito *et al.* (1992) injected donor blastodermal cells can incorporate into recipient embryos and differentiate into the somatic tissues. The same method was used by Bednarczyk *et al.* (2000); blastoderm was prepared with paper filter rings and thereafter BCs were injected into the sub-germinal cavity of recipient embryo to create transgenic chickens.

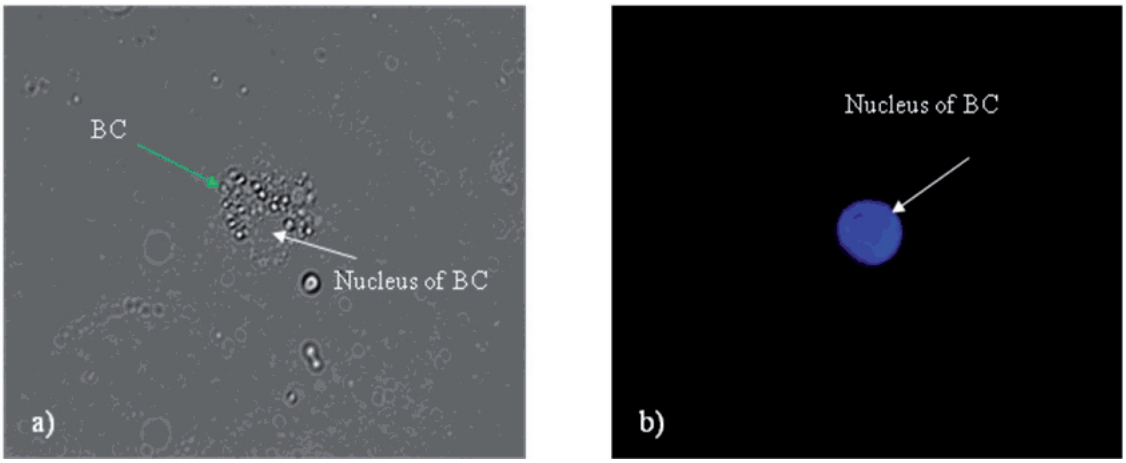

Fig. 1: Morphology and viability of freshly isolated BCs. a) light microscope; b) fluorescent microscope – DAPI staining (magnification x 20)

CONCLUSION

Our preliminary study represent only *in vitro* experiments. However, the evaluation of the BCs viability after cryoconservation should be conducted also *in vivo*. The application of isolated BCs for cryopreservation requires further development to expand its potential for avian genetic resources.

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