CRYOPRESERVATION OF EARLY EMBRYONIC CELLS OF GOOSE

Patakiné Várkonyi, E., Végi, B., Váradi, É., Szőke, Zs., Liptói, K., Barna, J.

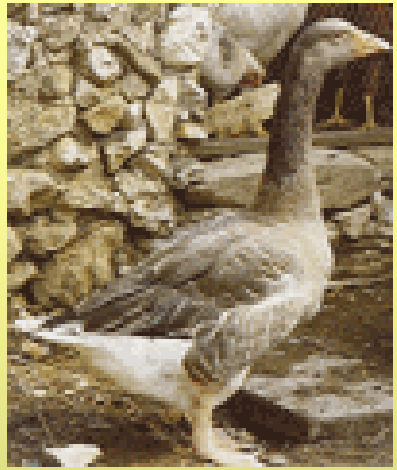
Research Institute for Animal Breeding and Nutrition
Gödöllő
2006

INTRODUCTION

- Present investigation was a part of a large-scale research project with the goal of improvement of reproduction traits in goose.

- At the same time, trials on cryopreservation of goose embryonic cells support the planned Hungarian poultry gene bank's establishment as well, since one of the main tasks of the Institute is the *ex situ* and *in situ* conservation of the Hungarian poultry genetic materials.

MATERIALS AND METHODS



Technique of BCs isolation

Blastodermal cells were collected from freshly laid unincubated fertile eggs. For isolation of BCs the perivitelline membrane was cut around, the GD removed and the cells were dispersed by pipetting in DMEM medium.

Steps of cryopreservation procedure

- After dissociation of BCs, they were centrifuged (11 min., 200 G) and resuspended in 1 ml DMEM.
- After the 2. centrifugation (11 min., 200 G), the pellet was resuspended in 2 ml DMEM containing 200 µl 20 % DMSO cryoprotectant.
- The cell suspension was put either into straws or ampoules. (250 µl/straw, 200 µl/ampoule)
- The final cell concentration was 2-4.000.000 cells/straw (ampoule).

Programable freezing machine (PLANER CRYO 10)

The cooling rate protocol

(modified after Pokorny, 2002)

From →		to
+20°C	- 4°C/min.	+4°C
At +4°C 10 min. equilibration		
+4°C	- 1°C/min.	-40°C
-40°C	- 15°C/min.	-70°C

Thawing procedure

- The samples were put into water-bath (20°C) from LN (straw: 10 sec., ampoule 2 min.).
- After thawing samples were centrifuged (11 min., 200 G).
- The supernatant was removed and the embryonic cells were resuspended in 0.2 ml DMEM again.

Examination on viability of BCs at each steps of freezing protocol

1. After the 1. centrifugation (cell concentration and ratio of viable cells)
2. After the 2. centrifugation (cell concentration and ratio of viable cells)
3. After the addition DMSO (only ratio of viable cells)
4. During the deep-freezing, at +4°C (only ratio of viable cells)
5. Immediately after thawing (cell concentration and ratio of viable cells)
6. After the 3. centrifugation (cell concentration and ratio of viable cells)

Results of succesful deep-freezing trials of goose embryonic cells (1.)

1. trial Viability	Vital %	Dead %	2.trial Viability	Vital%	Dead%
After 1. centr.	96	4	After 1. centr	95	5
After 2. centr.	78	22	After 2. centr.	91	9
After DMSO	69	31	After DMSO	87	13
At 4°C	55	45	At 4°C	83	17
Frozen/ thawed	26	74	Frozen/ thawed	45	55
			After 3. centr.	19	81

Results of succesful deep-freezing of goose embrionic cells (2.)

3. trial Viability	Vital %	Dead %	4. trial Viability	Vital%	Dead%
After 1. centr.			After 1. centr	95	5
After 2. centr.	95	5	After 2. centr.	93	7
After DMSO	87	13	After DMSO	89	11
At 4°C	86	14	At 4°C	87	13
Frozen/ thawed	27	73	Frozen/ thawed	44	56
After 3. centr.	30	70	After 3. centr.	11	89

Results of succesful deep-freezing of goose embrionic cells (3.)

5. trial Viability	Vital %	Dead %	6. trial Viability	Vital%	Dead%
After 1. centr.	97	3	After 1. centr	90	10
After 2. centr.	96	4	After 2. centr.	88	12
After DMSO	93	7	After DMSO	84	16
At 4°C	86	14	At 4°C	74	26
Frozen/thawed	58	42	Frozen/thawed	30	70
After 3. centr.	**24**	76	After 3. centr.	**28**	72

RESULTS (1)

1. Average cell concentration was: 32.805 cell/µl
2. Decrease in *cell concentration* during the steps of deep-freezing procedure:
   - After the first two centrifugation the decrement of cells was 14,6% .
   - After thawing, when the samples was dropped into centrifuge-tube, the decrement of cells were 14,25%.
   - After the third centrifugation another 14,7% was the decrement.

Total decrease in cell concentration was 43,55% during the whole procedure.

RESULTS (2)

3. The best result was 30% survival after the freezing/thawing procedure (3. trial). The present data can be compared only to the chicken derived results (40-60%), since there are no available data from goose species in this respect.

4. Strong significant difference (*t-test*, P≤0,01) was found between the usage of two type of containers for freezing: the average survival rate after the freezing procedure in ampoules was 25 %, while in straws only 15.5 %.

Conclusion

- The adopted method - with some modification - for freezing goose BCs produced acceptable result (25 % survival of cells in ampoule container).

- However, for producing of chimeras from frozen-thawed goose BCs further improvement of procedure is necessary.


THANK YOU FOR YOUR ATTENTION