PRELIMINARY RESULTS OF CRYOPRESERVATION OF EARLY EMBRYONIC CELLS OF GOOSE.

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Summary

Cryopreservation of semen is only applicable for single-gene traits and does not allow for full reconstitution of the genetic material of the original line. In the last 15 year, advances in the manipulation of the early chick-embryo suggests that cryopreservation of blastodermal cells might offer a means to preserve the entire genome of endangered or rare-breed stock of poultry. Present investigation was a trial on cryopreservation of goose embryonic cells, which supports the planned Hungarian poultry gene bank's establishment as well, since one of the main tasks of the Research Institute for Animal Breeding and Nutrition in Hugary is the *ex situ* and *in situ* conservation of the Hungarian poultry genetic materials.
Blastodermal cells were collected from freshly laid unincubated fertile goose eggs. The cryopreservation of blastodermal cells was carried out in a programmable freezing machine (PLANER CRYO 10). The cooling protocol elaborated for chicken embryonic cells by *Pokorny (2002)* was adapted for goose. The concentration and viability of blastodermal cells were examined at each steps of freezing protocol.
The best result was 30% survival of cells after the freezing/thawing procedure. 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. The cell concentration continuously decreased at all steps of deep-freezing procedure. Following the first two centrifugations the decrement of cells was 14,6 %, while after thawing it was 14,25 % additionally. Following the third centrifugation another 14,7 % dead cells was find. Total decrease in live cell concentration was 43,55 % during the whole procedure.
Strong significant difference (*t-test*, $P \leq 0{,}01$) was found between the usage of two type of containers for freezing: an average survival rate after the freezing procedure in ampoules was 25 %, while in straws only 15.5 %.
The adopted method - with some modifications - for freezing goose embryonic blastodermal cells produced acceptable result (25 % survival of cells in ampoule container). However, for producing of chimeras from frozen-thawed goose blastodermal cells further improvement of protocol is necessary.

Introduction

Preserving of avian gametes or fertilized eggs is effective way for conserving endangered avian species as well as maintaining of special poultry genetic stocks. Besides maintaining live populations or instead of it, biotechnological methods have been being under development for conservation of early embryonic cells as a complete genetic material.
Techniques for producing offspring from embryonic cells are only a few years old**,** limited to chicken, and technically demanding. By the time the egg is laid, embryonic development in the chicken has progressed to produce a blastoderm of around 50.000 cells *(Eyal-Giladi and Kochav, 1976).* Cryopreservation of the avian egg *in situ* is impractical therefore the principle

for production of progeny from cryopreserved embryonic cells is to remove them from donor embryos and return them - after freezing/thawing - in a form of simple cell suspension to a host embryo. The cells, which have been successfully used for this technology, can be either blastodermal or primordial germ cells. In the present study goose blastodermal cells were used from laid, unincubated eggs.
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 blastodermal cells isolation

Blastodermal cells were collected from freshly laid unincubated fertile White Embden and Grey Landes goose eggs. The eggs were stored at 15 °C. Prior to using they were swabbed with 70% alcohol. For isolation of blastodermal cells the perivitellin membrane was cut around, the germinal disc removed and the cells were dispersed mechanically by pipetting in DMEM medium. After dissociation of blastodermal cells, they were centrifuged (11 min., 200 G) and resuspended in 1 ml DMEM. The viability and concentration of cells were determined.

Steps of freezing procedure

After the 2. centrifugation (11 min., 200 G), the pellet was resuspended in 2 ml DMEM containing 200 µl 20 % DMSO as 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).

The cryopreservation of blastodermal cells was performed in programmable freezing machine (PLANER CRYO 10). The cooling protocol elaborated for chicken embryonic cells by *Pokorny (2002)* was adapted for goose.

The cooling rate was the following:

from	cooling rate	to
+20ºC	4ºC/min.	+4ºC
at +4ºC 10 min. equilibration		
+4ºC	1ºC/min.	–40ºC
–40ºC	15ºC/min.	–70ºC

After freezing the samples to –70ºC the straws and ampoules were plunged into liquid nitrogen (–196ºC).

Thawing procedure

From liquid nitrogen the samples were put into water-bath (20ºC) for 10 sec in the case of straws and for 2 min in the case of ampoules. 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 blastodermal cells

The concentration and viability of blastodermal cells were examined at each steps of the freezing protocol. Tripan blue staining *(McLimans et al., 1957)* were applied for the determination of ratio of viable/dead cells.

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 +4ºC (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

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.

The cell concentration continuously decreased 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 dead cells were 14,25 %. After the third centrifugation another 14,7 % dead cells were found. Total decrease in live cell concentration was **43,55 %** during the whole procedure.

1. Trial Viability	**Vital %**	**Dead%**	**2. Trial Viability**	**Vital %**	**Dead%**
After 1. centrifugation	96	4	After 1. centrifugation	95	5
After 2. centrifugation	78	22	After 2. centrifugation	91	9
After DMSO	69	31	After DMSO	87	13
At 4ºC	55	45	At 4ºC	83	17
Frozen/thawed	**26**	**74**	Frozen/thawed	45	55
			After 3. centrifugation	**19**	**81**
3. Trial Viability	**Vital %**	**Dead%**	**4. Trial Viability**	**Vital %**	**Dead%**
After 1. centrifugation			After 1. centrifugation	95	5
After 2. centrifugation	95	5	After 2. centrifugation	93	7
After DMSO	87	13	After DMSO	89	11
At 4ºC	86	14	At 4ºC	87	13
Frozen/thawed	27	73	Frozen/thawed	44	56
After 3. centrifugation	**30**	**70**	After 3. centrifugation	**11**	**89**
5. Trial Viability	**Vital %**	**Dead%**	**6. Trial Viability**	**Vital %**	**Dead%**
After 1. centrifugation	97	3	After 1. centrifugation	90	10
After 2. centrifugation	96	4	After 2. centrifugation	88	12
After DMSO	93	7	After DMSO	84	16
At 4ºC	86	14	At 4ºC	74	26
Frozen/thawed	58	42	Frozen/thawed	30	70
After 3. centrifugation	**24**	**76**	After 3. centrifugation	**28**	**72**

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 embryonic blastodermal cells produced acceptable result (25 % survival of cells in ampoule container). However, for producing of chimeras from frozen-thawed goose blastodermal cells further improvement of procedure is necessary.

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