Acta agriculturae slovenica, suplement 1(avgust 2004), 169–171.

http://www.bfro.uni-lj.si/zoo/publikacije/zbornik/suplementi/index.htm

Original scientific article
Izvirni znanstveni prispevek

GENE PRESERVATION IN DEER SPECIES

Zoltán ZOMBORSZKY [a)], Miklós SZABARI [b)], Éva KANGYALICS [c)] and Róbert TÓTH [c)]

[a)] Univ. of Kaposvár, Fac. of Animal Science, Guba Sándor u. 40, H-7400 Kaposvár, Hungary, Assist.Prof., Ph.D., e-mail: zzombors@mail.atk.u-kaposvar.hu.
[b)] Same address, M.Sc.
[c)] Same address.

ABSTRACT

Trophy value and body mass of the red and fallow deer (*Cervus elaphus*, *Dama dama*) living in Hungary is well known all over the world. In this study we researched the bovine in vitro fertilisation (IVF) method for saving the genetic material of killed males and females in order to establish a deer gene bank. First of all we studied wether it is possible to collect oocytes from females after shooting. In IVF experiments these oocytes and post mortem collected spermatozoa from epididymides were used. Our results show it is possible to obtain oocytes which are able to maturate after death, and that the bovine IVF protocol requires more exams.

Key words: deer / gene bank / biotechnology / Hungary

OHRANJANJE GENETSKE PESTROSTI PRI DAMJAKIH

IZVLEČEK

Vrednost trofeje in telesna masa jelenov in damjakov (*Cervus elaphus*, *Dama dama*), živečih na Madžarskem, je po vsem svetu dobro poznana. V opravljeni študiji smo testirali primernost metode za in vitro oploditev (IVF) pri govedu za shranjevanje genetskega materiala ubitih moških in ženskih živali jelenov in damjakov. Predvsem smo želeli ugotoviti, ali je možno zbrati oocite ustreljenih ženskih živali. V tem IVF poskusu smo uporabili tako zbrane oocite in po smrti zbrane spermije. Rezultati so pokazali, da je mogoče zbrati po ustrelitvi oocite, ki so sposobne zoreti in da je potrebno nadaljnje testiranje protokola za govejo IVF.

Ključne besede: divjad / genska banka / biotehnologija / Madžarska

INTRODUCTION

The deer farming is a chance to maintain and preservation the valuable genetic potential with the biotechnologies and traditional methods. These biotechnology's methods like the artificial insemination, (Willard *et al.*, 1996), embryo-transfer (Fennessy *et al.,* 1994) and in vitro fertilisation (Chapman *et al.*, 1999).

In vitro fertilisation (IVF) was elaborated in human program (Steptoe and Edwards, 1978) at the same time started the experiments in domestic animals. The first bovine oocyte was in vitro fertilisated by Brackett *et al.* in 1977 (Brackett *et al.*, 1977).

The IVF method in deer has been developed in New Zealand (Berg *et al.*, 2002a,b,c; Berg and Asher, 2003).

The aim of this study was collected oocytes and used it for IVF in deer species (*Cervus elaphus, Dama dama*).

MATERIAL AND METHODS

IVF preliminary experiments in deer species was used bovine protocol (http.//sperm.abc.hu).

Oocyte source and *in vitro* maturation (IVM)

Ovaries were collected from killed pregnant females in the hunting season 2–6 hours after the shooting. It was transported to the lab in PBS (38 °C). The contents of follicle's liquor were gently transpired with a needle (18G Luer) into a syringe. Medium for harvesting was TALP-HEPES.

The first occasion we investigated the viability of oocytes (n=10) by Trypan-blue (2%) staining for ten minutes. The result of the viability of gametes was evaluated by the colour. Blue colour shows the death oocytes. It was repeated it after 38 hours storing.

The collected cumulus-oocytes complexes were washed two-time in TALP-HEPES and once in IVM-medium. Then 25–30 oocytes were transferred to a drop (400 μl). The drops were covered with paraffin oil (300 μl). These plates were incubated for 24 h at 39 °C in 5% CO_2 in humidified air.

Sperm collection and preparation

Sperm were collected from *epididymides* in the matting seasons (September and November) from killed red stags and fallow bucks. Sperm was treated method of Zomborszky *et al*. (1999).

In vitro fertilisation (IVF)

Frozen straw was thawed in a water bath (37 °C, 12 sec.). The sperm samples were washed. In this method the sperm cells were cleaned from the diluting. The sperm sample of one straw was mixed with 5 mL SPERM-TALP, and centrifugationed 300 G at 10 minutes. After this, the upper part was removed, mixed with 5 mL SPERM-TALP and centrifugationed again. The fertilisation concentration was determined in Bürkher-chamber, from cells.

Groups of matured oocytes (25–30) were transferred and inseminated in a 400 μl IVF-TALP (supplemented with heparin) dropped covered with paraffin oil. The concentration of spermatozoa was 3 000/oocyte.

In vitro cultured (IVC)

Spermatozoa and oocytes were incubated (38 °C, 5% CO_2) for 24 hours. Oocytes were cleaned by vortexing, and were cultured in SOFaaci supplemented with 5% FCS for 5 days in 38 °C-on, 5% CO_2.

RESULTS AND DISCUSSION

The results of the viability of oocytes are shown in the 1. table.

Table 1. Survival of the oocytes evaluation with Trypan-blue (2%) staining

Ovaries	Oocytes	Fresh live/death	Living cells, %	38 hours live/death
2	10	**7/3**	70	0/10

The results of collecting of oocytes from shoot females are in the 2. and 3. tables.

Table 2. Harvest of oocytes and data in fallow deer (n=16)

Time	Ovaries	Oocytes	Means
2004. 01. 21.	18	56	3.1
2004. 01. 25.	14	48	3.43
Total	32	104	3.25

Table 3. Harvest of oocytes and data in red deer (n=4)

Time	Ovaries	Oocytes	Means
2003. 12. 18.	2	10	5
2004. 01. 12.	4	14	3.5
2004. 01. 13.	2	9	4.5
Total	8	33	4.1

Based on our IVF preliminary trials have not been successful, as we have not got embryos yet.

CONCLUSIONS

- Our results show that collecting oocytes from killed pregnant hinds and does are possible.
- These oocytes can be maturated but in vitro fertilisation experiments have to continue in order to get transferable embryos.
- These trials show that the genetic material of the females and most valuable killed wild males can be saved for the genetic maintenance or progress.

REFERENCES

Berg, D.K./ Asher, G.W. New developments reproductive technologies in deer. Theriogen, 59(2003), 189–205.

Berg, D.K./ Thompson, J.G./ Asher, G.W. Development of in vitro embryo production systems for red deer (*Cervus elaphus*), Part 1. Effect of epithelial oviductal monolayers and heparin on in vitro sperm motility and penetration of in vitro matured oocytes. Anim. Reprod. Sci., 70(2002a), 65–76.

Berg, D.K./ Thompson, J.G./ Asher, G.W. Development of in vitro embryo production systems for red deer (*Cervus elaphus*), Part 2. The timing of in vitro nuclear oocyte maturation. Anim. Reprod. Sci., 70(2002b), 77–84.

Berg, D.K./ Thompson, J.G./ Asher, G.W. Development of in vitro embryo production systems for red deer (*Cervus elaphus*), Part 3. In vitro fertilisation using sheep serum as a capacitating agent and the subsequent birth of calves. Anim. Reprod. Sci., 70(2002c), 85–98.

Brackett, B.G./ Oh, Y.K./ Evans, J.F./ Donawick, W.J. Bovine fertilization and early development in vivo and in vitro. In: Proceedings of 10th Ann. Meet. Soc. Study Reprod., Austin TX, 1977, 56

Chapman, S.A./ Keller, D.L./ Westhusin, M.E./ Drew, M.L./ Kraemer, D.C. In vitro production of axis deer (Axis axis) embryos, a preliminary study Theriogen, 50(1999), 208 (abstract).

Fennessy, P.F./ Asher, G.W./ Beatson, N.S./ Dixon, T.E./ Hunter, J.W./ Bringans, M.J. Embryo transfer in deer. Theriogen, 41(1994), 133–138.

Steptoe, P.C./ Edwards, R.G. Birth after the reimplantation of a human embryo. Lancet, 2(1978), 366.

Willard, S.T./ Jr. Hughes, D.M./ Bringans, M./ Sasser, R.G./ White, D.R./ Jaques, J.T./ Godfrey, R.W./ Jr. Welsh, T.H./ Randel, R.D. Artificial insemination, hybridization and pregnancy detection in sika deer (*Cervus nippon*). Theriogen, 46(1996), 779–789.

Zomborszky, Z./ Zubor, T./ Tóth, J./ Horn, P. Sperm collection from shot red deer stags (*Cervus elaphus*) and the utilisation of sperm frozen and subsequently thawed. Acta Vet. Hung., 47(1999), 263–270.