EMBRYO AND SPERM DEEPFREEZING ON INDIGENOUS SHEEP BREEDS IN ÜLLÖ

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The Research Institute for University of Veterinary Sciences has been used the modern biotechnological methods in the field work. Using our experiences on deep-freezing the semen on different breeds and embryotransfer on cattle and sheep, we think that we have to join the work, which is connected the saving of genes of the Hungarian indigenous breeds. The Institute founded a collection of indigenous domestic breeds, and started this work. Among this collection we have Hungarian Grey Cattle, Racka Sheep, Cigaja Sheep, and Cikta Sheep. Because of economical problems the number of those animals are less and less, they are threatened by extinction. The Hungarian Grey cattle serves as a good sample for us, how to save the genes for the future. For this program we have collected nearly hundred embryos and more than ten thousand dose semen are stored.

Sperm deepfreezing

The Gene conservation, to store the semen from the indigenous sheep breed has been continuously done at our Institute. We have rams from all of the three threatened breed. The semen collection happens using artificial vagina or using electroejaculator. We found that the Racks and Cigaja are willing to ejaculate into the artificial vagina, but a teaser ewe is necessary. The Cikta is so wild that he is not mating at the presence of the human. It is interesting that if the breeder is in a pen where the ewe is in heat, the ram wait until the men disappears. Immediately after he mates the ewe. At the Cikta so we have to use electroejaculator. This method have a lot of disadvantages. The volume of the sperm is less and in some cases the urine can flow into the semen. This urine occurs problem during the deepfreezing. We can clear the semen from the urine by centrifugation but this harmful for the sperm, also. Collecting the sperm by electroejaculator is not a humane method but without this we can not saving the sperm. After collection the sperm we do the first dilution, using the Tris- base puffer. Then we evaluate the sperm, with Eosin-Nigrosin vital stain. After half an hours we reach the final dilution, dropping the puffer slowly until 5°C, where the equilibrization happens. After that time the chilled semen is dropped on the CO_2 ice, makes pellet form. We found that this puffer gives the better result as the straw form. The semen is stored under LN2. A sample from every cooling is evaluated before storing. (Fig. 1)

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The doses of semen is listed in Table 1.

Figure 1 METHOD OF DEEPFREEZING

Fresh semen → evaluation (motility and morphology) → first dilution with puffer at 37°C → final dilution until 1:8 ratio during 1/2 hours → put into fridge in plastic tube → equilibrization time is 2 hours at 5°C - make 0.2 ml pellets on CO_2 ice → merge into LN2 → evaluation - storing

Table 1 - LIST OF SEMEN STORED FROM DIFFERENT BREEDS

Racka Sheep	765 doses
Cigaja Sheep	393 doses
Cikta Sheep	426 doses

Embryotransfer

The Institute has great experience on sheep embryotransfer. We have flushed hundreds of sheep embryos. Using our facilities we have started the gene-save work. Generally, when we chose the donors for superovulation, we request a lot of healthy and reproductional demand. At the case when we superovulate the indigenous breeds, we can only use all the animals, which are available.

We can use the Hungarian indigenous breeds during the high season only. Out of high season we get unfertilized eggs or degenerated embryos.

So, after some disappoint results we resigned to do this only in the high season. The biggest problem was the low number of donors which are available and the poor response for superovulation, except the Cigaja Sheep.

The schedule of superovulation based on synchronization, hormonal treatment, insemination and embryo collection. We use intravaginal sponge (Chrono-gest, Intervet) for synchronization. This remains for 12 days in the ewe. Two days before the withdrawal of the sponge is the superovulation treatment, with application of PMSG (Folligon, Intervet). In the field condition it is more easy than the FSH-based one, which needs daily two injection. We found that the effectivity of PMSG as good as FSH. For forcing the oestrus is enough to pull the sponge away and two days later we inseminate the ewes. (Fig. 3) At the beginning time, when the ET was started, in the eighty, we inseminated the donors after laparotomy, immediately into the uterus. After six or seven days we had to make an other laparotomy, because the embryo collection can not happens through the vagina. Nowadays we inseminate the donors laparoscopically, without mentionable harm of the abdomen wall. Unfortunately, the flushing can be carried out only after laparotomy. This is the black point of the ET on sheep, because of the abdomen, the uterus and the surrounding tissues joint tightly with adhesions. After counting and recording the number of Corpus Luteum and Corpus Luteum Cysts

we flush the embryos from the uterus, through the oviduct. The flushing medium is Dulbecco-phosphate puffer. The embryos then searched, evaluated and deepfreezed. The number of embryos are stored is seen on the Table 2.

Table 2 - LIST OF EMBRYOS STORED FROM DIFFERENT BREEDS

Racka sheep	53
Cigaja sheep	122
Cikta sheep	16

Figure 2 - COMPONENTS OF THE PUFFER

Glucose	0.5 g
Citric acid	1,990 g
Tris	3,534 g
Glycerol	7 ml
Egg yolk	15 ml

Figure 3 - SCHEME OF SUPEROVULATION

1/ Synchronization of donor ewes	day 0
Insert of Chrono-gest B sponge (Intervet)	
2/ Superovulation of donor ewes	day 12
Treatment 1200-1500 IE Folligon (Intervet)	
Withdraw of sponge	day 14
Insemination	day 16
3/ Embryo flushing	day 22-23