UDK 636.082.454

Izlaganje sa znanstvenog skupa

Conference paper

EMBRYOSPLITTING - A WAY TO INCREASE THE EFFICIENCY OF EMBRYO - TRANSFER IN SHEEP

I. Vintila, I. Babusik, L. Kulickova, I. Bencsik, N. Pacala, N. Corin

Abstract

In has been compared the gestation results and the number of lambs obtained from 40 unsplitted integral embryos collected from 31 superovulated sheep, to the same parameters in the situation when 40 demi -embryos were used, in order to estimate the embryosplitting advantage to increase the efficiency or embryo- transfer in sheep. The gestation percentage obtained by the recipient sheep in the case of integral embryos, was 57,5% and in case of demiembryos was 47,5%, only. Also, the proportion of lambs obtained from the total number of prelevated embryos was 85% in the case of splitting method and 50% only, in the case of integral embryos transfer. The results obtained support the statement that the embryosplitting used in embryo-transfer is a way to increase the efficiency of this unconventional technology in sheep reproduction.

Introduction

Superovulation and embryo-transfer at monotocyc animal species are efficient "tools" in accelerating the modification of the genetic structure of population.

According to this, it is possible to obtain from a female subject more descendants than by natural way. In consequence, it leads to the increasement of the selection intensity (i) and the decreasement of the generation interval (y). Finaly, all these, improve the selection effect, because $\Delta g = i. \sigma. h^2/y$

This, it is possible to obtain groups of 3-4 natural sisters which may be used to find the genetic additional value of their brother. Information received from natural sisters or demi- sisters added to information from their descendants could be sufficient to abandon the "progeny-test" in estimating the amelioration value of male subjects.

In this way, even with a decreasing accuracy of selection, we are able to improve the economic efficiency in the selection activity.

In sheep, the embryo-transfer is not too much used in production because:

a) collection and transfer of the embryos from the superovulated females are still realized in surgical way the multiple adherents following this surgery operation, make the female animal unable to repeat the superovulation;

Rad je priopćen na 46th Meeting European Association for Animal Production 4-7 September, Prague, 1995.
I. Vintila, I. Bencsik, N. Pacala, N. Corin, Departement of Genetics, Banat's University of Agicultural Sciences, Calea Aradului 119, 1900 Timisoara, Romania; I. Babusik, L. Kulickova, Research Institute of Animal Production, Holohovska 2, 949 92 Nitra, Slovakia

b) the increased variability of the ovary reaction against the treatment of superovulation induction and the small number of embryos adequate to the transfer in one collection (50%) make this technology to become very expensive.

Only the increasement of embryos number per collection adequate to the transfer could make the embryo-transfer technology more attractive for sheep breeders.

From literature, it is know that trying to stop by different ways the selection and growing of the dominant follicle and the synchronisation of 2 or 3 follicles waves and adding to this the blastomers splitting, it is possible to increase the number of lambs per embryos collection.

This study aims to estimate the degree of positive influence of embryosplitting in sheep embryo-transfer.

Materials and experimental methods

The experiment has been done on 111 sheep Merinos breed and we have chosen the embryos donors from a group of pedigree animals, haring one anterior lamb, at least, without any parturition complication or post-partum infections.

The superovulation and synchronisation of the ovarian cycle were induced in september-october by introducing progesteron sponge into the vagina, keeping them in for 12 days. One day before the removing operation the sheep were injected with PMSG intramuscular, 1500 U. I. per dose for donors and 400 U. I. for recipient animals. The donor sheep in heat, were inseminated intercervical by raw sperm. Six days later, we collected embryos from donors, by laparatomy. In order to obtain this, we perfuzed the uterine horns with the buffer medium Dulbeco PBS + penicillin + streptomicin + 2% bovine foetus serum. The perfusion of uterine horns was done by pricking the uterine horn right under the uterotubal junction, using a syringe needle attached to the syringe by a plastic tube. The perfusion liquid flushed into the horn lumen was collected into a test tube by pricking the wall, using a syringe needle at the distal edge of it. Before starting perfusion, the uterine horn was obturated by intestinal pincer set at a few centimetres distance from the edge of the uterine horn to base.

From this liquid, after having been examinated by stereomi - croscope the embryos found were put in Dulbeco PBS medium riched in calf foetal serum 20%, and 0,2 mmol / l Na piruvate, in order to be splitted.

The embryosplitting (morulas and incipient and expanded blastocysts) has been made by using 2 Leitz micromanipulators, a "holding" dropper for fixing the embryo and for the splitting, the sharp edge of a shaving blade attached to a metal stick.

We splitted the morula and blastocyst embryos only.Then, the splitted embryos were still kept in the same medium, at room temperature. After having been allowed to settle for 20 min., pair of 2 bisected embryos + a littre bit of medium were drown in a Pasteur dropper and transfered into the uterine horn of the recipient sheep, beeing in reproductiv synchronisation to donor animals.

Results and discussions

The treatment done to induce superovulation in 31 embryos donor sheep, was efficient. (see table 1.)

Table 1. - RESULTS OBTAINED IN SUPEROVULATION AND EMBRYOS COLLECTING

Dose PMSG (I. U.)	N	Super ovulated donors		Cor pora lutea	Collected embryos				
		n	%	X	Compac-ted morulae %	Blasto-cysts %	Unfertilized ovules and degenerated embryos %	Total	X
1500	31	28	90,3	12,3	19,9	32,2	47,9	286	10,2

I has been observed that superovulation was done at 90% of the experimental-group of sheep. It has been registered an average of 12,3 follicles grown and ovulated on each sheep ovary. It has been collected an average of 10 embryos from each donor sheep. Among these, 32% were in blastocyst stage and 20% were compacted morulae. This observation is suggesting that ovulation doesn't happen simultaneously in all De Graff follicles and it lasts for 15-24 hours, only, otherwise it is impossible to explain the high proportion (50%) of unfertilized ovules found into the liquid.

Our results demonstrate that intercervical insemination in super ovulated sheep, is not very efficient. There are many unfertilized ovules because of the missing spermatozoa in the oviduct ampoule or because of a too late ovulstion due to the follicles beeing lazy in their growing on the ovary.

Here these are (see table 2) the results obtained in the transfer of integral normaly grown embryos into the uterine horn ipsilateral to corpus lutea, in recipient sheep.

Table 2. - RESULTS OBTAINED IN THE TRANSFER OF INTEGRAL EMBRYOS

Recipient female seep	Integral embryos transfered	Pregnant females sheep		Lambs obtained	
n	n	n	%	n	% of total number of transfered embryos
40	40	23	57,5	22	55,0

By corroborating table 1 and table 2 results, it is evident that from an average of 10 embryos per donor sheep, only 5 of them were adequate to be transfered. After their implant into the ipsilateral uterine horn of the recipient sheep, at the and of gestation interval, it was obtained from each superovulated donor sheep 2,5 lambs, only. In consequence, these results do not justify all economic and physic effort that has been done (repeated embryo-collectings by laparatomy can not be expected because of the adherences).

An increased efficiency in surgical embryo-transfer in sheep is realized by embryosplitting. According to our data and other author s data, it is clearly demonstrate that the splitting of normaly grown embryos, especially in incipient blastocyst, blastocyst and expanded blastocyststage, and the demi-embryos transfer in adoptive mothers, seriously improve the results of the experiment.

Here are the results obtained in our experiment.

Table 3. - RESULTS OBTAINED IN EMBRYOSPLITTING TRANSFER

Splitted transferable embryos	Obtained splitted embryos	Recipient females	Pregnant recipient females		Lambs obtained	
n	n	n	n	%	n	% of total number of integral embryos used
40	80	40	19	47,5	34	85,0

From this table it is evident that the splitting of integral embryos into demi-embryos by microsurgical methods and their transfer into adoptive mothers do not seriously influence the gestation percent. Even if the gestation percent using integral embryos is higher than the percentage in the case of splitted embryos (47,5%), this fact is statistically insignificant.

In consequence, the splitting of compacted morulae and blastocysts, followed by the transfer of splitted embryos obtained in adoptive mothers leads to a gain of 30 % lambs from a donor sheep, compared to the situation when normal transfered embryos are used.

If the 40 integral embryos had been transfered unsplitted into adoptive mothers at a gestation percent of 55%, we should have obtained 22 lambs only, but not 34 lambs as actually resulted following the splitting method. Our experiments show that by dividing compacted morula into 2 or 3 fragments, it considerably decreases the embryos chances to grow, till parturation time comes. It seems that the best results may be achieved when blastocysts and expanded blastocyst are splitted.

Chesne & team (1987) also demonstrated the advantage expressed by the number of lambs resulted by fresh embryosplitting transfer against integral embryos transfer. The proportion between the number of lambs and the number of unsplitted embryos obtained per collection, was 118% and 64% of them, were monozygotic twins. Chesne & team, experimentally prove that the optimal embryos age for splitting is above 7 days, better between 8-10 days, the number of lambs, increasing gradually according to the age of embryo as the examples show: (100% at 8 days, 118% at 9 days, 131% at 10 days). Similar results have been obtained by Smith & team 1991 (embryosplitting into the pellucida zone), Niroshi & team 1989; Herr & team 1990; Sheton 1992; J. M. Lewis 1994.

Conclusions

Our experiments finally conclude into this:

1. By embryosplitting in sheep, at blastocyst or expanded blastocyststage, it has been obtained the double of demi-embryos able to be transfered two by two, into the uterine horn ipsilateral to corpus lutea, in recipient animals beeing in reproductive synchronisation to the donor, withaut serious influence on gestation rate.

2. By embryosplitting transfer into the uterine horn of adoptive mothers it is possible to obtain more lambs (+ 30% at least) than using conventional integral embryo-transfer.

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