Evaluation of Sheep Embryo Quality by Morphologic Methods – Advantages and Disadvantages

S.Angela G. Prefac T. Paul Ş. Alexandru T. Dana
The University of Agronomical Sciences and Veterinary Medicine, Faculty of Animal Science
Bucharest, Romania

The present study is focused on one of the most important steps in embryo technology evaluating embryo quality. During three years, we have conducted embryo transfer experiments on sheep. The evaluation of embryo quality was made using morphologic methods.

We have evaluated embryos immediately after recovery (surgical methods recovery) or after throwing of frozen embryo under a optical microscope were used for evaluation. International Embryo Transfer Society standards for embryo quality classification. There are four grades of quality: excellent, good, medium and poor.

The morphological method's advantages are: it is very fast, not so expensive and does not require excessive embryo manipulation.

Disadvantages of this method are: grading is subjective, depending on the experience of the evaluator, metabolic, genetic or epigenetic disorders of embryos are not detectable.

Keywords: embryo, quality, sheep, morphological evaluation.

Koyun Embriyolarının Morfolojik Metotlar İle Değerlendirilmesi – Avantaj ve Dezavantajları

Bu çalışma embriyo teknolojisi sahasında en önemli konulardan biri olan embriyo kalitesinin belirlenmesi üzerine yapılmıştır. Yıllar boyunca koyunlar üzerinde embriyo transfer çalışmaları yapılmıştır. Embriyo kalitesinin belirlenmesinde morfolojik metotlar kullanılmıştır.

Embriyolar cerrahi metotla alındıktan sonra süratle kalite değerlendirilmesi yapılmış ve dondurulmuştur. Çalışmada optik mikroskop kullanılmış ve Uluslar arası Embriyo Transfer Birliği standartları kullanılarak embriyoların kaliteleri sınıflandırılmıştır. Bu sistemde embriyo kalite sınıflandırmasında dört grup vardır. Bunlar mükemmel, iyi, orta ve kötüdür.

Morfolojik metotların avantajı çok hızlı, ucuz ve embriyo manüplasyonu gerektirmemesidir. Bu metotların dezavantajı ise tecrübeye bağlı olarak öznel olması ve metabolik, genetik ve epigenetik hastalıkları belirleyememesidir.

Anahtar kelimeler: embriyo, kalite, koyun, morfolojik değerlendirme

Introduction

Embryo transfer is a heavily used technique in many fields of science, from biology to veterinary and human medicine. One of the most important steps in this embryo technology is the evaluation of embryo quality. Various procedures are available, depending on the species and the economic or scientific value of the animals involved. One of the most simple, quick and inexpensive method is evaluating by morphological methods. But how accurate is it and how can influence the result of embryo transfer? This question we had to answer, evaluating the results on four groups of sheep.

Materials and Methods

The researches were carried out at the Palas Institute for Sheep and Goat Research, Constanta and the Ruşeţu Research Institute, Buzău. We used four groups of embryo donors, every one of them with different hormonal treatment for super ovulation. The embryos

were recovered by surgical methods, using sterile instruments and washing fluid.

Once recovered from the uterus, the washing fluid was stored into a Petri dish. A stereo glass magnifier was used to identify every embryo in the fluid. This is not a difficult operation, the only problem that can appear is the presence of cell ravage and various scams, even unfecundated ovules, that can be erroneous evaluated as embryos. Even so, this problem is overcome at the next step of embryo detection and seclusion.

For this next step, we have used Petri dishes containing between 4 and 8 drops of nutritive culture liquid. Using a small glass pipette, the embryo is taken from the washing fluid and transferred into a small drop of nutritive culture liquid.

Each drop of liquid is then searched using an optical microscope, x 80 magnifier. The embryo can be easily moved with a glass micromanipulator, in order to evaluate from different views and sides. If the Zona Pellucida is undamaged, it will ensure embryo protection during this procedure. It is now possible to evaluate the overall morphologic status and developmental stage, according to the time of fertilization.

For our 6 day embryos, we have considered as excellent and good those with intact Zona Pellucida, perfect spherical shape and the developmental stage of morula or early blastocys.

Medium or poor embryos were those with 16 cells morula (retarded), Zona Pellucida injuries, asymmetrical and dispersed blastomeres.

After the recovery and quality evaluation, the embryos were transferred to sheep with synchronized oestrus, also by surgical methods. Then we have evaluated pregnancy rates and number of live offspring obtained from embryo transfer.

Results and Discussion

The results we have obtained from the four groups of sheep, after different hormonal treatments, are presented in the next tables and figures. We show the number of embryos obtained from the left an right uterine horn for each group, and also the quality after evaluation.

As we can see from the Table 1. We generally had more embryos recovered from the right uterine horns, and that is because the right ovaries have developed a better response at poly ovulation treatment.

For lots 1, 2 and 4, at least 75% of the embryos were evaluated as excellent or good, with high chances for obtaining viable offspring. Quality value “poor” was assigned for only 6 embryos out of 63, ant that counts for less than 10%.

Table 1. Constanta and Buzau, Romania, July 2003

LOT	RECOVERED EMBRYOS		EMBRYO QUALITY			
	Right horn	Left horn	Excellent	Good	Medium	Poor
1	12	4	6	6	2	2
2	15	13	9	14	3	2
3	-	2	-	2	-	-
4	10	7	7	8	-	2
Total	37	26	22	30	5	6

Table 2. Constanta and Buzau March 2004

Quality	Number of embryos	Number of offspring	Efficiency (%)
Excellent	22	14	63.63
Good	30	13	43.33
Medium	5	2	40

We have transferred only excellent, good and medium embryos, 2 embryos per sheep. The off springs obtained transfers are presented in Table 2. It also shows the efficiency of embryo transfer, according to the quality as evaluated by morphological methods. We can see a very good percentage for excellent and good classes. As far as medium quality class is concerning, the percentage is also good, but their number is too small to draw a conclusion from here.

Following the results in table 2, classify embryos as excellent and good quality embryos resulted 20% difference in pregnant rate. This result show that morphological quality determination of embryos is an efficient way of embryo quality evaluation

Conclusion

1. The morphological method has the advantage of being quick and not invasive.
2. The experience of the quality evaluator is very important; different evaluators can make different analysis for the same embryo, so it is a subjective method;
3. It is not a expensive method;
4. The morphologic method has its disadvantages: metabolic, genetic or epigenetic disorders of embryos are not detectable.

References

Gavrilă L., 2003. Genomica ,Ed. Enciclopedica, Bucharest, Romania

Kanka Jiri, 2003. Gene expression and chromatin structure in preimplantation embryo, International Embryo Transfer Society Annual Meeting 2003

Prefac, G., Tăpăloagă, P.R., Şonea, Al., 2004. Impact of different hormonal treatments on follicular growth and polyovulation rates in sheep, The 33rd International Scientific Session of the Faculty of Animal Science, Bucharest, Romania.

Prefac, G., 2004. Researches on embryo transfer in sheep. License Thesis Faculty of Veterinary Medicine, Bucharest, Romania.

Stoica Maria-Angela, Şonea, Al., Tăpăloagă, P.R., Drăguţ Paula, 2003. Methods of intensive reproduction in mammals and birds. Ed. Granada, Bucharest, Romania.