Morphological Assessment of Mature Oocytes Recovered from Transylvanian Merino Sheep during Breeding Season

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SUMMARY

In vitro maturation (IVM) of sheep oocytes is an important stage of embryo production. The aim of this research was to evaluate the degree of sheep oocytes maturation by morphological exam.

The research has been carried out during August – November 2010 (breeding season) on a total of 10 Transylvanian Merino sheep, aged between 1,3 and 6 years.

Oocytes collection involved median lapatomy technique after superovulatory treatment with intravaginal sponges (Chrono-Gest Sheep - Intervet) for 14 days, 750 I.U. PMSG (Folligon - Intervet) and 0.75 mg/sheep prostaglandin (Proliz - Pasteur Institute) administered immediately after sponges withdrawal. The COC were observed under stereoscopic microscope 30X, to determine the integrity and the degree expansion of cumulus. Oocytes were classified into the following categories: Class A- completely surrounded by cumulus cells; Class B - partially surrounded by cumulus cells; Class C- oocytes denuded, Herrera (2000). Once oocytes were located, they were transferred to a Petri dish containing Hepes buffered TCM 199 (Sigma) supplemented with 10% FCS and washed 2 times and one time in maturation medium (TCM 199 supplemented with 10% FCS, 5 µg/ml LH, 1 µg/ml FSH, 1 µg/ml 17 β-Estradiol and 100 µM Cysteamine). Maturation was performed in 4 well plastic dishes at 38,5°C in an humidified atmosphere of 5% CO₂ in air for 28 h. In order to put into practice the IVF protocol, the selection of ovine oocytes after maturation was based on morphologic exam. Sheep oocytes were classified into two quality categories according to morphological characters as: cumulus expansion, the size of perivitelin space integrity of pellucida zone, cytoplasm appearance.

After performing the aspiration technique 141 oocytes were obtained from 197 follicles, the recovery rate was 71.57% with an average of 7.05 oocytes/ovary. Morphological exam allowed classification of oocytes in three quality classes: 92 oocytes (65.25%) were included in class A, 29 oocytes (20.57%) were in class B and 20 oocytes (14.28%) were in class C. From 121 oocytes subjected to IVM 103 oocytes (85.12%) were included in the “mature” class (cumulus cell expansion, homogeneous or slightly granular cytoplasm, uniform perivitelin space and the integrity of pellucida zone) and the remaining 18 oocytes (14.88%) in “degenerated” class (total or partial loss of cumulus cells, highly granular cytoplasm, vacuolated or retracted, irregular perivitelin space and broken or degraded pellucida zone).
These results show that mature oocytes obtained by median laparatomy of poliovulated sheep have rates comparable to slaughterhouse-derived. We recommend for IVF only the oocytes that present: cumulus cell expansion, homogeneous or slightly granular cytoplasm, uniform perivitelin space and the integrity of pelucida zone.

REFERENCES