COMPARISON OF CLASSIC FREEZING AND VITRIFICATION FOR EMBRYO CRYOPRESERVATION OF SANTA INES SHEEP: PRELIMINARY RESULTS

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In reproduction technology, cryopreservation of mammalian embryos has many advantages: lower costs of long distance transport, enhancement of genetic evaluation in selection programs, elimination of the need for synchronous recipients after superovulatory treatment, and decreased risk of disease transmission. Vitrification has been proposed as a method for sheep embryo cryopreservation. The Santa Ines sheep is a local breed of Northeast Brazil that presents elevated productive rates. A simple and efficient method for embryo cryopreservation could improve the genetic programs of this breed in this region. The aim of this study was to compare the efficiency of standard freezing versus vitrification procedures for the cryopreservation of the Santa Ines sheep in vivo derived embryos. Thirty adult Santa Ines ewes were used as donors. The estrus synchronization treatment was achieved using intravaginal sponges impregnated with 60 mg of medroxiprogesterone acetate during 14 days. The superovulation was performed at days 12, 13, and 14 of treatment (day 0 = sponge insertion) using a total dose of 200 iu pFSH, divided in six decreasing doses (im) at each 12 h. Estrus was observed each 12 h interval from 12 h after sponge removal. The ewes were naturally mated by two Santa Ines rams using hand mating. Six to seven days after estrus the embryo recovery was performed using the surgical method (laparotomy). The recovered embryos were evaluated in stereomicroscope (20-40 X) according to quality and development stage. The viable embryos were randomly divided in two groups. The first was cryopreserved using classic freezing according to previously cited method (Baril G et al., FAO 1993; 183p). The second group was cryopreserved by vitrification method (Mermillod et al., XIII meeting AETE, 1997). Twenty embryos were cryopreserved by the classic method, and 29 by vitrification. The embryos were transferred, by semilaparoscopic method, for recipients previously synchronized with intravaginal sponges impregnated with 60 mg of medroxiprogesterone acetate for 14 days. At Day 14 of progestagen treatment, ewes received im injections of 200 iu eCG (Chrono-gest; Intervet, France). The recipients received one or two embryos in the uterine horn ipsilateral to the ovary with one or more corpora lutea. The fertility rate was determined by non-return to estrus using a teaser until 20 days after estrus. The comparison between the fertility rate was performed by chi-square test. Others results were presented as mean $\pm$ SD. In this experiment, mean ovulation rate was 7.7 $\pm$ 1.0. The structure recovery rate was 80.1%. It was observed 2.6 $\pm$ 0.6 fecundated structures and 2.3 $\pm$ 0.7 not fecundated structures. Embryos freezing were transferred to 11 recipients and vitrified embryos to 15 recipients. No significant difference ($P < 0.05$) was observed between the non return to estrus percentages: 27.3% (3/11) vs 46.7% (7/15), for classic method and vitrification, respectively. In conclusion, the results show that the vitrification is a competitive method for cryopreservation of Santa Ines embryos, when compared to classic freezing.