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**THE USE OF NEUTRAL RED AS A VIABILITY INDICATOR HAMPERS *IN VITRO*
DEVELOPMENT OF SEMI-NUDE BOVINE OOCYTES TO THE BLASTOCYST STAGE**

BAETENS E., ANDRIES S., BUS A., DE PORTE H.F.M., LEROY J.L.M.R., BOLS P.E.J.

Gamete Research Centre, Veterinary Physiology and Biochemistry, Department of Veterinary Sciences, University of Antwerp, Universiteitsplein 1, 2610 Wilrijk, Belgium

Women suffering from premature ovarian failure due to cancer treatment can appeal to oocyte vitrification to preserve their fertility. An important factor to increase the effectiveness of the procedure is viability assessment of the cryopreserved oocytes after warming. To date, survival is predominantly assessed on the basis of morphological criteria by conventional light microscopy, a subjective assessment method that depends largely on the expertise of the observer. Therefore, there is a great need for an objective method to assess viability in a fast and non-invasive way. Oocytes can be cryopreserved at the immature or mature stage. After choosing to use mature oocytes, based on literature, this experiment examined whether the relative non-toxic stain Neutral Red (NR) can be used as an oocyte viability marker without affecting subsequent development to blastocysts. NR is taken up by lysosomes of metabolically active cells. Briefly, immature cumulus-oocyte-complexes (COCs) were subjected to routine *in vitro* maturation (IVM) for 21 or 24 hours, whereupon the 270 mature COCs were divided into 3 groups (2 replicates). A control group with an intact cumulus oophorus (24h IVM; LAB CTRL) and 2 groups of COCs with only the corona radiata (21h IVM), the semi-nude (SN) and Neutral Red group (NR) respectively. In view of future vitrification and IVF, cumulus cells were partially removed (semi-nude) by pipetting to facilitate oocyte handling and future cryoprotectant penetration. Following 30 minutes incubation with 15µg NR/ml maturation medium and a subsequent 1h washout period (NR group), all 3 groups were subjected to routine IVP (cultured under oil for 8 days). Cleavage and blastocyst rate were observed at respectively 2 and 8 days post-insemination. Developmental competence data were analyzed using a binary logistic regression including treatment as fixed factor and replicate as random factor (IBM SPSS version 22). Although there is a significant difference in cleavage (75 vs 55,8%) and blastocyst (36 vs 20,9%) ratio between the LAB CTRL and SN group, our results demonstrate that semi-nude oocytes still have an acceptable fertilization rate that can definitely be improved. However, oocytes from the NR-group significantly failed to cleave (42,9%) and develop to the blastocyst stage (2,4%) as compared to the CTRL and SN group. In conclusion, Neutral Red clearly affects cleavage and blastocyst formation of semi-nude oocytes in the above used conditions and therefore is not suitable for semi-nude oocyte viability assessment.

KEYWORDS: Neutral Red, oocyte viability, semi-nude oocytes