

Studies on The Impact of Hyaluronidases and Low Molecular Weight Hyaluronan on Preimplantation Embryo Development, Morphokinetics, Pregnancy and Live birth rates in Sheep

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There is increasing interest in understanding the role of the hyaluronan (HA) system in mammalian embryo development and implantation. We have previously shown size-specific effect of HA on embryo development and the beneficial effects of hyaluronidase-2 (hyal-2) on improving blastocyst rate and quality. The effect of HA is mediated through binding to its receptors CD44 and RHAMM present in bovine embryos.

Here we compared the effect of hyal-2 and sperm hyaluronidase (Spam-1; PH20) and the corresponding low molecular size HA fragments produced by these enzymes [LMWHA (15-40kDa) and very LMWHA (<10kDa)] respectively, on sheep embryo development in vitro. Survival rate after vitrification, pregnancy and live birth rate after blastocyst transfer to oestrous synchronised recipient donors were compared. Pregnancy was assessed by ultrasound scanning on day 35, and number and normality of lambs were recorded. In addition, impact of hyal2 on morphokinetic of cell division in the bovine embryos was analysed. Cleaved 2-cell embryos were cultured in the absence or presence of 300IU/ml hyal-2 in an Embryoscope time-lapse imaging incubator.

For each experiment, sheep embryos were produced in four independent repeats after in vitro maturation and in vitro fertilisation of abattoir-derived oocytes. Hyal2 supplementation increased blastocyst rate (58% v 28% in control) ($p < 0.001$). This effect was abrogated in the presence anti- CD44 and RHAMM and no blastocyst could be produced in the presence of anti-RHAMM even in the presence of hyal-2. No difference was observed in blastocyst rate between control and Hyal-1 treatment groups. VLMWHA at 2 ug/ml significantly increased blastocyst and hatching rates ($p < 0.001$). Higher percentage of the blastocysts produced in VLMWHA survived after vitrification as observed by re-expansion and hatching after re-culture (80% v. 52.2%, $P < 0.05$). Higher number of pregnancies and live birth was observed in ewes received hyal-2 [83% (10/12)] and VLMWHA [75% (9/12)] treated blastocysts versus control (6/11; 55%). No abnormality was observed in the lambs' weight, behaviour and survival. Presence of hyal-2 accelerated cell division to different stages of the embryos with and overall average of 16h faster development of blastocyst as compared to control group.

The findings of these studies provide significant results leading to the potential use of VLMWHA or hyal-2 as part of the preimplantation culture media.