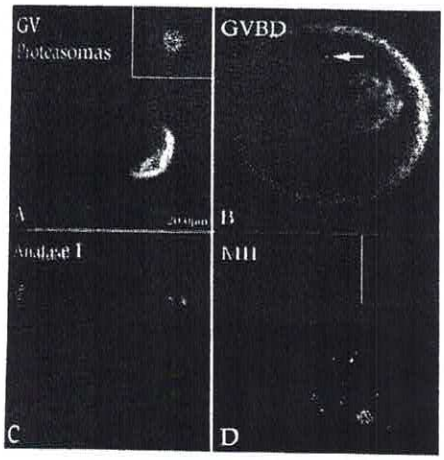

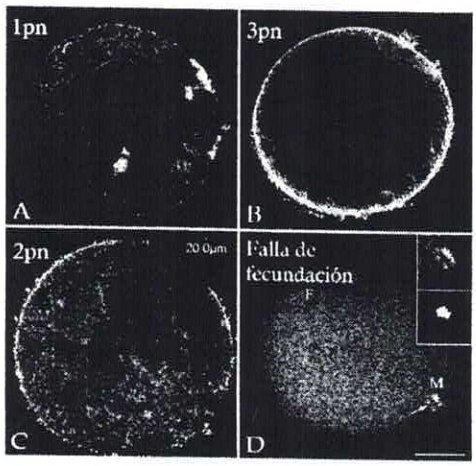

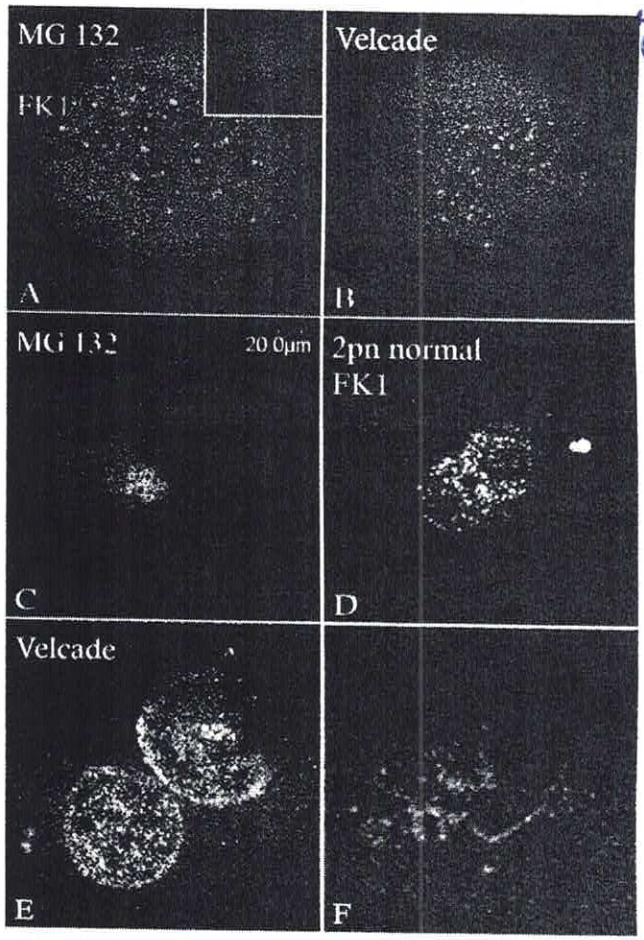

RED-T05 Expression of Hsp-70 and ban genes in blastocysts from bovine oocytes matures in chemically defined medium

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INTRODUCTION

Analysis of expression patterns of developmentally important genes essential in early developmental provides a useful tool to assess the normality of produced embryos and a tool to optimize assisted reproduction technologies. Growing evidence indicates that cattle are a useful model for analyzing human preimplantation development. Bovine and human embryos are similar with respect to microtubule patterns during fertilization, the timing of genome activation, metabolic requirements, interactions with the culture medium, and duration of preimplantation development. In vitro procedures, such as IVP (in vitro production) and sNT (somatic nuclear transfer) of bovine embryos have been shown to be correlated with significant up- or downregulation, *de novo* ind of genes critical for undisturbe atal development. Bax and Hsp-7 gly transcripts in *in vitro* produce heir products are involved in proce osis and stress adaptation, respe ture environment affects both the m onic expression of genes, and is like cyte and embryo developmental c for better and less variable culture ting the *in vivo* environment has lea nt of

defined culture media, with lower impact on the molecular reprogramming of oocytes and embryos. This study aimed to quantify Hsp-70 and Bax gene expression in *in vitro* fertilized bovine blastocysts produced from oocytes matured in chemically defined IVM system with a-MEM medium supplemented with IGF-I, Insulin and either synthetic macromolecules polyvinyl alcohol (PVA) or polyvinylpyrrolidone (PVP-40).

METHODS

Immature cumulus oocyte complexes from slaughtered cows were matured for 24h in either TCM-199 or a-MEM, supplemented as following. Three treatments were performed: T1) a-MEM + IGF-I + Insulin + 0.1% PVA; T2) a-MEM + IGF-I + Insulin + 0.1% PVP-40; T3) controi: TCM + FSH + 10% oestrous cow serum (OCS). *In vitro* fertilization took place in Fert-Talp droplets. Twenty two hours after fertilization, zygotes were cultured in CR2aa supplemented with 10% fetal calf serum under mineral oil in a humidified atmosphere of 5% CO2 at 38,5°C. Blastocysts on day 8 post-fertilization were frozen in liquid nitrogen and subsequently thawed for RNA extraction. Total RNA extraction was performed using Rneasy Micro kit (Qiagen, Valencia, CA, USA) and first strand synthesized using Superscript III First Strand Synthesis kit (Invitrogen, Chicago, IL, USA). Relative quantification was performed in duplicate using Real Time PCR (ABI Prism 7000 Applied Biosystem, Foster City, CA, USA) and reactions consisted of a mixture of iTaq SYBR Green Supermix with ROX (Bio-Rad, Waltham, MA, USA) with cDNA equivalent to 0.8 embryos and gene specific primers. Expression of H2a gene was used as endogenous reference. Calculations of relative quantification were performed by REST © (Relative Expression Software Tool), using the value found in TCM group as calibrator.

RESULTS

The blastocyst rates in T1, T2 and T3 were 40%, 48% and 38%, respectively. The relative expression of Hsp-70 and Bax transcripts were, respectively, 0.69±0.69 and 0.53±0.32 for T1, and 1.08±0.75 and 0.7±0.47 for T2 in comparison to the calibrator group (T3: control), with no significant differences between groups ($P>0.05$).

CONCLUSIONS

These results indicate that oocyte maturation in chemically defined media supplemented with PVA or PVP-40, without FCS and gonadotropins, do not alter the relative abundance of Hsp70-1 and Bax transcripts in resulting early embryos.

RED-T06 Meiotic spindle imaging in in vitro matured human oocytes

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INTRODUCTION

Since the first report of a childbirth after *in vitro* maturation (IVM) in 1991 (Cha *et al.*, 1991), there have been improvements in the pregnancy and birth rates with *in vitro* fertilization (IVF) (Piquette *et al.*, 2006) and at the present, IVM is one of the most important focus in assisted reproduction technology (ART). IVM does not require the use of larger doses of gonadotropin for follicular development and maturation, the cost and inconveniences of gonadotropin therapy are avoided and the risk of ovarian hyperstimulation syndrome (OHSS) is blocked (Jurema and Nogueira, 2006). Furthermore, during controlled ovarian stimulation (COS), some of the collected oocytes are immature and these oocytes are usually discarded due to concerns regarding the safety of IVM (Balakie *et al.*, 2004; Cha *et al.*, 2005). Nevertheless, in cases of poor responders and in patient with an unsynchronized cohort of follicles, the use of immature oocytes for IVF is important in order to increase the number of embryos obtained to be transferred (Strassburger *et al.*, 2004). IVM has become a potential choice in ART, with pregnancy rates of 22 to 35%; however, many studies reported that oocytes matured *in vivo*, methaphase II (MII-stage) at retrieval, result in highest pregnancy rates whereas immature oocytes germinal vesicle (GV-stage) and methaphase I (MI stage), subsequently matured in the laboratory rarely result in a viable pregnancy (Lanzendor, 2006; Son *et al.*, 2007). Li *et al.*, (2006) suggested that IVM could have deleterious effects on the organization of the spindle

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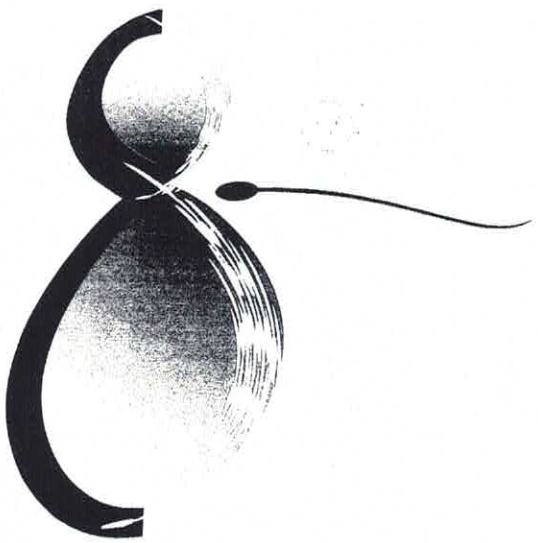

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