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Storage of bovine isolated follicles: A new alternative way to improve the recovery rate of viable embryos from ovarian follicles of slaughtered cows

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Abstract

The vitality of bovine oocytes stored in isolated follicles was examined. The aim of this work was to prolong the time of in vitro manipulation of oocytes before their maturation and develop a new alternative of oocyte "capacitation" to improve the quality of in vitro produced embryos. Follicles were dissected from the ovaries of slaughtered cows; subsequently, follicles were divided according to their diameter into three categories (2–3, 3–4 and 4–6 mm), and stored at 17–18 °C for 24 or 48 h in a modified tissue culture medium-199 (TCM-199) with reduced pH. After that time, the cumulus–oocyte complexes (COCs) were isolated, matured, fertilized, and embryos cultured in vitro for a total of 9 days. The percentage of total blastocysts, and hatched blastocysts developed from oocytes, initially kept ("capacitated") for 24 h at 17–18 °C, within follicles of 3–6 mm size categories, were significantly higher than that oocytes of the control [of control oocytes] (44.9 and 30.3% versus 36.2 and 20.4%, respectively). The oocytes of follicles stored for 48 h at 17–18 °C already had decreased developmental capacity. Interesting data were obtained when COCs of the 3–4 and 4–6 categories were additionally divided into two subgroups according to their presumed developmental history (originating from the supposed growing "fit" in contrast to the supposed regressing "unfit" follicles). The higher improvement in the rate of hatched blastocysts from 24 h stored oocytes was observed only in the subgroup originated from "fit" COCs (15.3 versus 25.0%, and 20.0 versus 34.4%, in the 3–4 and 4–6 mm categories, respectively). The transfer of 26 blastocysts (developed of follicles kept for 24 h at 17–18 °C) to 26 recipient heifers resulted in 18 pregnancies. Storage of follicles at 17–18 °C in

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vitro resulted not only in recovery of higher numbers of blastocysts of better quality but also facilitated the safe transport of follicles for a long distance. The extended, time of follicle storage before the proper oocyte maturation allowed also the synchronization of an appropriate number of recipient animals according to the number of isolated follicles.

Keywords: Bovine follicle storage; In vitro fertilization; Embryo culture; Embryo transfer

1. Introduction

The meiotic, fertilization and developmental capacity of bovine oocytes isolated from follicles of slaughtered or living animals (by OPU), it depends on a number of factors studied and previously reported in many papers (Bavister, 1995; Lonergan et al., 2003; Fair, 2003). To increase the oocyte developmental capacity, some approaches were oriented to a different hormonal treatment in vivo (Dieleman et al., 2002; Blondin et al., 2002), or by choosing the most optimal day of the cycle (Machatková et al., 2000, 2004). The improvement of COCs' selection according to some morphological criteria (DeWit et al., 2000; Bilodeau-Goeseels and Panish, 2002) could improve the recovery rate of blastocysts, but not their total yield per animal.

The situation is considerably different when the oocytes need to be isolated from the slaughtered animals in a not determined stage of the cycle and without possibility to stimulate their growth in vivo. In these animals, the lower developmental competence of in vitro matured oocytes is related not only to an unknown stage of their oestrous cycle, but also to some reproductive defects caused by the slaughter of the animals (Peter, 2004). For these reasons, many attempts have been made to improve the developmental competence of oocytes from growing follicles by improving the in vitro maturation protocol. The philosophy of this attempt is to extend the duration of oocyte growth in the germinal vesicle (GV) stage, enabling the completion of all transcriptional and translational activities indispensable for acquiring their full meiotic and developmental capacity. A special protocol of a prolonged culture has been used for oocytes of very small follicles from adult heifers or cows and even fetal calves requiring even a longer time of growth (Hirao et al., 2003; Chohan and Hunter, 2004). More attention has been paid to increase the developmental capacity of oocytes already more or less competent to finish their nuclear but not cytoplasmic maturation and final embryo development (Lonergan et al., 1997; Marmillod et al., 2000; Ponderato et al., 2002; Pavlok et al., 2005). However, at present all these efforts have not been very effective in practical applications. One of the most serious obstacles is probably the heterogeneity of the history of antral follicles permanently going through the stages of their recruitment, growing and regression (for review see Driancourt, 1991).

The positive effect of storage of oocytes before their maturation was described earlier and this phenomenon has been called "capacitation" as an analogy to sperm "capacitation" (Hyttel et al., 1997). The application of the proposed in vitro maturation protocol is suitable mainly for animals with previous long time top (milk) production, as the last opportunity for reproducing their genome. Most ovaries of such animals could also possess (beside different cysts and other anomalies) a number of follicles with meiotically and developmentally competent oocytes. The storage of the follicles for an extended time under in vitro conditions also allows transporting them for long distances.

The aim of the present experiments was to improve the developmental capacity of oocytes by an alternate to their "capacitation" in isolated follicles. The rationale of this