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Supplementation of L-carnitine during *in vitro* maturation improves embryo development from less competent bovine oocytes

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ABSTRACT

The present study was designed to define the impact of L-carnitine, supplemented during maturation, on bovine oocytes with different meiotic competence in terms of their IVF outcomes. Meiotically more competent (MMC) and less competent (MLC) oocytes were obtained separately from differently sized follicles at selected phases of folliculogenesis. The oocytes were matured with or without L-carnitine, fertilized and cultured to the blastocyst stage. The oocytes were examined for nuclear maturation, mitochondrial cluster formation, lipid consumption, fertilization and embryo development. The proportion of oocytes at metaphase II was significantly higher in the L-carnitine-treated MMC than that in the L-carnitine-treated MLC oocytes. However in comparison with the untreated controls, the proportion of MII oocytes with mitochondrial clusters was significantly higher only in the L-carnitine-treated MLC oocytes, which also showed a significantly lower mean lipid content. The L-carnitine-treated MLC oocytes showed significantly higher fertilization and syngamy rates than the untreated MLC oocytes. On the other hand, in the L-carnitine-treated MMC oocytes, the fertilization rate was similar to that of the untreated controls and the syngamy rate was significantly delayed. Although no significant differences in cleavage on Day 2 were found among all oocyte categories, L-carnitine treatment resulted in a significantly higher blastocyst yield in the MLC oocytes on Day 7 and Day 8 and a significantly higher proportion of expanded blastocysts in relation to the total number of blastocysts in MMC oocytes on Day 8. It can be concluded that L-carnitine supplementation during maturation improves the development of bovine embryos from meiotically less competent oocytes and accelerates blastocyst formation from more competent oocytes.

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1. Introduction

For effective fertilization and embryo production in mammals, both the nuclear maturation as well as cytoplasmic maturation of oocytes are important. The activation of pathways involved in protein synthesis and phosphorylation is indispensable for oocyte cytoplasmic maturation and subsequent embryo development [1]. It is generally known that deficiencies in cytoplasmic maturation are responsible for reducing the development of embryos from prepubertal oocytes. Differences in the number and distribution of cytoplasmic organelles between prepubertal and adult oocytes matured under *in vitro* conditions have been reported. Prepubertal calf and lamb oocytes have mitochondria of lower volume density, fewer in number and smaller in size, compared with their adult counterparts [2,3].

In adult cyclic cows, the developmental potential of oocytes is changing in accordance with follicular waves emerging during the ovarian cycle. In each wave, the developmental competence of oocytes rises with an increasing size of follicles, stagnates due to dominant follicle (DF) selection and subsequenly decreases during DF growth. This influences the developmental competence of other oocytes from subordinate follicles [4–6]. Bovine oocytes undergo changes in the number and distribution of cytoplasmic organelles in a manner specific for each phase of follicular development. While in the growth phase, oocytes show little contact of mitochondria with lipid droplets, both sparsely distributed in the oocyte periphery, in the static phase they exhibit an increased number of

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mitochondria associated with lipid droplets, also in the periphery. In the regression phase, mitochondria in tight contact with lipid droplets are evenly distributed in the whole oocyte [7].

Mitochondria and lipids are crucial to energy production and supplementation of energy resources of oocyte. The ability of mitochondria to balance ATP supply is considered the most critical factor in relation to oocyte fertilization and embryo development [8,9].

In vitro matured bovine oocytes differ in mitochondrial patterns and ATP production [10]. More competent oocytes from medium follicles activate mitochondria twice during maturation, before meiosis resumption and before completion of maturation, while less competent oocytes do it only once, before completion of maturation [11].

Lipid metabolism regulators added to culture media can influence the expresion of lipid metabolism-regulating genes and thus can improve the developmental competence and quality of embryos [12,13]. L-carnitine is intrinsically involved in mitochondrial function and lipid metabolism via the transport of fatty acids to mitochondria and its involvement in fatty acid β-oxidation. It also participates in the regulation of vital cellular functions such as apoptosis. Although positive effects of L-carnitine on oocytes and embryos have been described in cattle [14,15], sheep [16], pigs [17,18] and mice [19–21], no information is available about L-carnitine impact on bovine oocytes with different meiotic and developmental competences. It is generally known that oocytes recovered from larger follicles have a greater capability to complete nuclear and cytoplasmic maturation, undergo successful fertilization and develop to the blastocyst stage than have oocytes from smaller follicles.

Therefore, the aim of this study was to investigate the effect of L-carnitine, supplemented during maturation, on subpopulations of bovine oocytes differing in their meiotic and developmental competence. We hypothesized that a) a response of more competent oocytes to L-carnitine is different from that of less competent oocytes; b) less competent oocytes utilize L-carnitine more effectively than do more competent oocytes; c) L-carnitine supplementation during maturation improves embryo development chiefly from less competent oocytes.

2. Materials and methods

All the chemicals used in this study were purchased from Sigma-Aldrich Chemical Co. (Prague, Czech Republic) unless otherwise stated.

2.1. Oocyte collection

Slaughtered Holstein dairy cows (n = 181), aged 4–6 years, with a checked ovarian cycle stage, served as donors. Ovaries in the growth, stagnation and regression phases, after dominant phase elimination, defined by follicle population and corpus luteum morphology, were used for oocyte recovery. Meiotically more competent (MMC) oocytes were collected from medium follicles (6–10 mm) by aspiration and meiotically less competent (MLC) oocytes were subsequently collected from small follicles (2–5 mm) by slicing of the ovarian cortex. Only healthy cumulus-oocyte complexes with the homogenous ooplasm, surrounded by compact multiple layers of cumulus cells, were selected from each oocyte category and used in experiments.

2.2. Oocyte maturation

In each category, one half of the oocytes was matured in 500 μL of TCM-199 medium (M4530; Earle's salts), with 20 mM sodium pyruvate, 50 IU/mL penicillin, 50 μg/mL streptomycin, 5% oestrus cow serum (ECS; Sevapharma, Prague, Czech Republic) and gonadotropins (P.G. 600 15 IU/mL; Intervet, Boxmeer, Holland) supplemented with 2.5 mM L-carnitine (C0283). The concentration of 2.5 mM L-carnitine was selected as being most effective for maturation of bovine oocytes on the basis of our unpublished results. The other half oocytes was matured in the same medium, but without L-carnitine, and served as a control. Maturation took place in four-well plates (Nunclon Intermed, Roskilde, Denmark) under a humidified atmosphere of 5% CO_2 in air at 38.8 °C for 24 h. Aliquot parts of L-carnitine-treated and untreated matured oocytes, in which the first polar body had been extruded, were examined. Before their examination, the oocytes were denuded from cumulus cells by vortexing in TCM-199 medium containing 0.1% (w/v) hyaluronidase.

2.3. Maturation assessment

For chromatin and mitochondria evaluation, the oocytes were first stained in PBS supplemented with 0.4% BSA and 200 nM MitoTracker Orange CMTMRos dye (Molecular Probes, Eugene, OR, USA) for 30 min at 38.8 °C. Subsequently, the oocytes were washed in PBS, fixed in 3.7% paraformaldehyde for 60 min at room temperature and washed again. Avoiding compression, the oocytes were mounted on slides using Vectashield medium (Vector Lab, Burlingame, CA, USA) containing l μM of DNA dye (SYTOX Green; Invitrogen; Carlsbad, CA, USA). They were examined with a laser scanning confocal microscope (Leica TCS SP2 AOBS; Leica, Heidelberg, Germany) equipped with Ar and DPSS lasers. The 40 × HCX PL APO CS objective, pinhole, offsets, gain and AOBS were adjusted. The 488 nm excitation band and a 490–515 nm detector and the 561 nm excitation band and a 565–600 nm detector were used for chromatin and mitochondria detection, respectively. The oocytes were scanned in equatorial optical sections and processed by NIS-ELEMENTS AR 3.0 software (Laboratory Imaging, Prague, Czech Republic).

The oocytes at metaphase II (MII) and those with mitochondrial clusters (Fig. 1) were considered to have completed nuclear and cytoplasmic maturation, respectively [11]. The proportion of oocytes with completed maturation was expressed as a proportion of

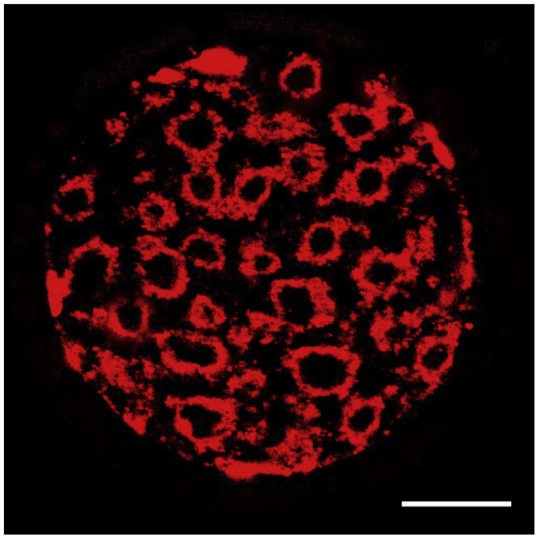

Fig. 1. Representative image of bovine oocyte with mitochondrial clusters after 24 h-maturation. Mitochondria were stained with MitoTracker Orange CMTMRos and oocytes were examined by confocal microscopy. Scale bar = 30 μm.

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