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Bovine blastocyst development rate in vitro is influenced by selection of oocytes by brillant cresyl blue staining before IVM as indicator for glucose-6-phosphate dehydrogenase activity

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Abstract

The aim of this present study was to increase the efficiency of blastocyst production from cows after in vitro maturation/fertilization (IVM/IVF) by oocyte selection before maturation. Oocytes were selected on the basis of brillant cresyl blue (BCB) staining, used to indicate glucose-6-phosphate dehydrogenase (G6PDH) activity. To re-valuate the hypothesis that growing oocytes are expected to have a high level of active G6PDH, while mature oocytes have low G6PDH activity, cumulus oocyte complexes (COCs) were recovered from slaughterhouse ovaries by slicing the surface of the ovary. Only oocytes with a compact cumulus investment were used. Oocytes were placed into three groups: (1) control—placed immediately into culture; (2) holding control—COCs kept in PBS containing 0.4% BSA for 90 min before placement into culture; and (3) treatment—incubation with BCB for 90 min before culture. Treated oocytes were then divided into BCB− (colorless cytoplasm, increased G6PDH) and BCB+ (colored cytoplasm, low G6PDH) on their ability to metabolize the stain. Activity of G6PDH was determined via measurement of NADP reduction induced by G6P as substrate oxidized by G6PDH in the cytosol of control, BCB− and BCB+ groups; G6PDH activity was significant higher in BCB− COCs than in control and BCB+ COCs. After IVM, oocytes were fertilized in vitro. Embryos were cultured to day 8. The rate of maturation to metaphase II was

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significantly higher for control and BCB+ oocytes than for BCB− oocytes. The BCB+ oocytes yielded a significantly higher proportion of blastocysts (34.1%) than did control or holding control oocytes (18.3 and 19.2%); and both controls and BCB+ oocytes had significantly higher blastocyst development than did BCB− oocytes (3.9%).

These results show that the staining of bovine cumulus oocyte complexes with BCB before in vitro maturation may be used to select developmentally competent oocytes for IVF. In addition, G6PDH activity may be useful as a marker for oocyte quality in future studies on factors affecting developmental competence.

Keywords: Bovine oocytes; Staining test; Developmental competence; G6PDH

1. Introduction

Follicular oocytes recovered from ovaries of slaughtered cattle are commonly used to study the processes of maturation and fertilization and the technique of in vitro production of embryos. The relatively low level of efficiency achieved using in vitro embryo production, manifested by the frequent failure of up to 60% of recovered oocytes to reach the blastocyst stage after fertilization, is almost certainly related to the quality of the oocyte at the beginning of maturation.

It is recognized that immature oocytes, especially from cows with reduced reproductive performance or which are slaughtered at the end of their use, are heterogeneous in quality and developmental competence [1]. Mammalian immature oocytes are routinely selected for IVF on the basis of the visual assessment of morphological features such as thickness and compactness of the cumulus investment and the homogeneity of the ooplasm [1].

Although morphological criteria provide reasonable means of identifying maturity and fertilization potential [2], results reported by De Loos et al. [3] suggest that there is considerable morphological variability among oocytes capable of normal development and that morphological criteria have led to limited improvement in the identification of oocytes that will develop in vitro.

Oocyte diameter is a determinant factor in acquiring meiotic competence [4]. In a study with prepuberal goat oocytes it was shown that when oocytes were exposed to BCB, those that stained with BCB were larger than those that remained unstained (136.6 μm versus 125.5 μm in diameter). The percentage of M II oocytes after IVM of BCB+ oocytes was higher than that of BCB− or control oocytes [5]. Similarly, pig oocytes selected as blue after staining with BCB were significantly larger than those that remained colourless (113.1 μm versus 100.3 μm) [6].

Immature oocytes are known to synthesize a variety of proteins [7], among them, glucose-6-phosphate dehydrogenase (G6PDH). This enzyme is active in the growing oocyte [8], but has decreased activity in oocytes that have finished their growth phase [7]. Brilliant cresyl blue (BCB) has been used to measure G6PDH activity. The BCB test is based on the capability of the G6PDH to convert the BCB stain from blue to colourless [9]. This enzyme is synthesized within the oocytes during oogenesis, and is a component of the pentose phosphate cycle which provides ribose phosphate for nucleotide synthesis and much of the NADPH utilized as a hydrogen or electron donor in reductive biosynthetic