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Developmental potential of bovine oocytes cultured in different maturation and culture conditions

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Abstract

Diverse groups of chemicals in culture media are needed for successful bovine oocyte maturation and embryo development during which dramatic cytoplasmic and nuclear reprogramming events take place. In vitro embryo production (IVP) procedures frequently include supplements such as serum and/or co-culture with various types of somatic cells. However, the presence of undefined serum in culture media introduces a variation from batch to batch, increases viral or prion contamination risk, and leads to problems during fetal development. The aim of the present study was to investigate the possibility of using chemically defined-synthetic serum substitute (SSS) in place of fetal calf serum (FCS) during maturation and long-term culture to stimulate in vitro maturation (IVM), fertilization (IVF) and subsequent embryo development. In Experiment I, the effect of the protein source on in vitro maturation was tested by maturing oocytes in culture media supplemented with 10% FCS (Control Group), 10% SSS (Group I) and 10% SSS + 10 ng/ml epidermal growth factor (EGF) (Group II). In Experiment II, effects of SSS on both oocyte maturation and embryo development during in vitro culture (IVC) were tested by maturing oocytes in media supplemented with 10% FCS (FCS Group) or 10% SSS + 10 ng/ml EGF (SSS Group), followed by IVF and IVC in SOF media supplemented with 10% FCS and 10% SSS on day 4 for FCS and SSS Groups, respectively. Even though rates for cleavage and development to blastocyst stage were not different, blastocyst cell numbers were higher in Group II containing SSS and EGF. The SSS supplementation group had higher apoptotic nuclei as compared to the FCS Group in Experiment II. Transcripts for heat shock protein 70 (Hsp70), interferon tau (IF-τ), DNA methyltransferase 3a (Dnmt3a), desmosomal glycoprotein desmocollin III (DcIII) and insulin-like growth factor II receptor (Igf-2r) were altered in different culture conditions in Experiment I. However, only glucose transporter-1 (Glut-1) mRNA was different in the SSS and FCS Groups in the

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second experiment. In summary, SSS and EGF in maturation medium and replacement of FCS with SSS alone in culture medium on day 4 of IVC support oocyte maturation and embryo development in vitro. However, significance of culture condition induced changes on the genome-wide abundance of messenger ribonucleic acid and the significance of the apoptotic nuclei during fetal development still remain to be determined.

Keywords: Early development; Gametogenesis; Oocyte development; Embryo; Developmental biology

1. Introduction

In vitro maturation of bovine oocytes, followed by in vitro fertilization for the production of bovine embryos in the laboratory, is rapidly increasing (Zeuner et al., 2003). These in vitro techniques have important values both in studying the basic biological events occurring during oocyte maturation, fertilization and early embryonic development, and in providing an inexpensive and readily available source of preimplantation bovine embryos. Consistently successful and reliable oocyte maturation (both cytoplasmic and nuclear maturation) would dramatically improve the efficiency of preimplantation embryonic development as well as fetal development. However, current methods include supplements such as serum and/or co-culture with various types of somatic cells because of the limited understanding of embryo metabolism and growth requirements (Mastromonaco et al., 2004). This causes undefined and variable culture conditions. One of the most commonly used protein sources in the culture media has been serum, which has a biphasic effect. Including serum in the culture media can inhibit early cleavage divisions, while it can improve the development later in culture (Lonergan et al., 1999; Thompson et al., 1998). Its beneficial effect is especially important during the development of morulae into blastocyst (Gordon, 1994). In addition, it was reported that the effectiveness of serum in in vitro embryo production (IVP) might change considerably from one batch to another (Kane, 1987) and ingredients of serum, such as amino acids, hormones, growth factors, cytokines, vitamins and many other substances exhibit wide variations (Gordon, 1994). Even though a higher development rate to the blastocyst stage is obtained from media supported with serum, these variations could cause some alterations in the ultrastructure of embryos, impaired compaction, abnormal blastulation, large calf syndrome, aberrant mRNA expression profiles, and greater incidences of stillbirths and deaths after birth (Abe et al., 1999; Holm et al., 2002; Wrenzycki et al., 1999, 2004). Moreover, bovine-derived sera or proteins have recently been avoided especially in human IVP systems because of the appearance of bovine spongiform encephalopathy (BSE) and a viral or prion contamination risk. Therefore, even more completely defined culture conditions supporting high developmental rates are important not only to obtain consistent results but also to eradicate the contamination risk of BSE and other diseases. Because of these reasons, there has been a trend to use more defined proteins, such as bovine serum albumin (BSA), human serum albumin (HSA) and synthetic serum preparations instead of undefined natural serum preparations like fetal calf serum (FCS) and estrus cow serum (OCS) (Chanson et al., 2001; Russell et al., 1997; Sagirkaya et al., 2004).

Morphological characteristics such as appearance of cumulus cells and cytoplasm, oocyte size and time of polar body extrusion are related to the ability of oocytes to be fertilized and develop into viable embryos (Dominko and First, 1997). However, these are not reliable enough to act as the sole criteria for the evaluation of embryo developmental potential in vivo. Recently, there