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Expression of IGF receptors and its ligands in bovine oocytes and preimplantation embryos

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ABSTRACT

The objectives of this study were to assess the mRNA expression and protein location of IGF receptors and its ligands in bovine oocytes and different stages of preimplantation embryos, and then evaluate the effect of different concentrations of IGF-II when added to either the maturation or culture medium on *in vitro* embryo development. For the assessment of mRNA expression by RT-PCR three replicates each of 100 oocytes, and 60 embryos at each of the 2-cell, 8-cell, morula and blastocyst stages of development were used. Immunocytochemical techniques were used to study the location of IGFs and their receptors for COC, oocytes, and embryos at the same stages of development ($n = 25$). The effect of supplementing maturation medium with IGF-II was examined using groups of 20 oocytes exposed to 0 (control), 10, 20, 50 or 100 ng IGF-II/ml medium. Each treatment was replicated five times. To study the effect of IGF-II added to culture medium, groups of 10 zygotes were cultured in the presence of 0 (control), 50, 100 or 150 ng IGF-II/ml medium and the treatments replicated four times. The results showed that IGF-I mRNA could not be detected but IGF-II, IGF-IR and IGF-IIR mRNA existed in bovine preimplantation embryos. Proteins for IGF-II, IGF-IR and IGF-IIR were detected on the cell plasma membrane of cumulus cells of COC, immature and mature oocytes, and 2-cell stage embryos. They were observed in blastomere cytoplasm of 8-cell and morula stage embryos. In blastocysts, the IGF proteins were

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distributed in the trophectoderm but not in the inner cell mass. Adding 20 ng/ml IGF-II to maturation medium resulted in higher rates of post-fertilization development than control at 8-cell (58.2% versus 44.5%; $p < 0.05$) and blastocyst (37.0% versus 25.0%; $p < 0.05$) stages of development; and the number of viable cells per blastocyst were significantly higher (126 ± 6 versus 103 ± 5; $p < 0.05$). When IGF-II was added to the culture medium, no significant treatment differences were observed at 8-cell embryo stage but the development rate of zygotes cultured in the presence of 100 ng IGF-II/ml medium to blastocysts was significantly higher than that of control (30.0% versus 19.2%; $p < 0.05$). It was concluded that supplementation of *in vitro* maturation or culture media with IGF-II affects the development of bovine embryos and could be used to improve *in vitro* embryo production.

1. Introduction

The insulin-like growth factor (IGF) system is composed of two ligands (IGF-I, IGF-II), two type of receptors (IGF-IR, IGF-IIR) and six IGF binding proteins (IGFBPs) (Pavelić et al., 2007). As we know, IGFs are important growth factors during preimplantation embryo development, as they can enhance cell proliferation, mitogenesis and regulate apoptosis (Adashi et al., 1985; Kamada et al., 1992). Biological functions of IGFs are mediated by high-affinity IGF-IR during preimplantation embryo development (Matsui et al., 1997). The addition of exogenous IGF-I to developmental medium has been known to improve bovine oocyte maturation and embryonic development (Matsui et al., 1995; Palma et al., 1997; Pawshe et al., 1998). IGF-I mRNA has been detected in bovine preimplantation embryos *in vitro* (Schultz et al., 1992; Watson et al., 1992; Yoshida et al., 1998; Lonergan et al., 2000), but other researchers have concluded that the mRNA levels were below detectable levels (Winger et al., 1997; Watson et al., 1999; Yaseen et al., 2001; Moore et al., 2007; Warzych et al., 2007).

In the mouse, there is no expression of IGF-I protein or mRNA, with IGF-II functioning during preimplantation stages (Rappolee et al., 1992). IGF-I is proven to have a beneficial effect on preimplantation embryo development by decreasing apoptosis and increasing cell proliferation in the mouse (Doherty et al., 1994), rabbit (Herrler et al., 1998) and human (Lighten et al., 1998). IGF-II is believed to mediate growth through the heterologous type I IGF receptor, whereas the IGF-II/M6P receptor is believed to act as a negative regulator of somatic growth by limiting the availability of excess levels of IGF-II.

Until now, there have been no reports regarding the effects of IGF-II on bovine oocyte maturation and preimplantation embryo development. Although IGF-II, IGF-IR and IGF-IIR protein expression has been reported, the description about their localization at specific stages in preimplantation embryos is limited.

The objectives of this study were to assess the mRNA expression of IGF-I, IGF-II, IGF-IR and IGF-IIR in bovine oocytes and different stages of preimplantation embryos. Based on this, location of IGF's proteins in bovine oocytes and the preimplantation embryos was examined. Then the effect of different concentrations of IGF-II when added to either the maturation or culture medium on *in vitro* embryo development was evaluated. These results might be helpful to improve the *in vitro* culture system and contribute to understanding the mechanism of mammalian preimplantation embryo development.

2. Materials and methods

2.1. Oocyte collection, maturation and embryo culture

2.1.1. Oocyte collection and in vitro maturation

Bovine ovaries were obtained from a local abattoir and transported to the laboratory within 2 h of retrieval in sterile saline (9 g NaCl/l) and maintained at 35–37 °C. Immature COCs were retrieved from obvious follicles (2–8 mm in diameter) with an 18-gauge needle. All oocytes that were completely