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Programming of the preimplantation embryo by the embryokine colony stimulating factor 2☆

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ABSTRACT

Events in the preimplantation period can have long-term consequences that affect embryo competence to establish and maintain pregnancy and which can extend into fetal and postnatal life. One of the molecules responsible for maternal modulation of embryonic development during this time is colony stimulating factor 2, also termed granulocyte-macrophage colony stimulating factor. This cytokine is produced by the oviduct and endometrium and can act on the preimplantation embryo to improve competence of the embryo to establish pregnancy and develop to term. Actions of CSF2 on the embryo include changes in gene expression (particularly for genes related to apoptosis and differentiation), inhibition of apoptosis, and an increase in numbers of cells in the inner cell mass. Female embryos respond to CSF2 differently than male embryos. Alterations in maternal environment during the preimplantation period can affect subsequent development in a sex-specific manner and CSF2 may be one of the maternal signals responsible for this phenomenon.

1. Introduction

The mammalian embryo spends only a few days in its passage from being a totipotent zygote to a newly-differentiated blastocyst. In cattle, the blastocyst, consisting of inner cell mass (ICM) and trophectoderm (TE), forms by Day 6–7 in vivo (Betteridge and Fléchon, 1988). The hypoblast forms about 48 h later (Maddox-Hyttel et al., 2003). Although development of the zygote to the blastocyst stage can occur in the absence of maternal signals (i.e., in culture medium), the maternal environment is important for ensuring optimal development. Indeed, bovine embryos produced in vitro have several characteristics that are altered compared to embryos that develop in vivo, including gene expression (Corcoran et al., 2006; Gad et al., 2012), lipid content (Crosier et al., 2000; Sudano et al., 2012), ultrastructure (Rizos et al., 2002), DNA methylation (Niemann et al., 2010), competence to establish pregnancy after transfer into recipients (Lonergan et al., 2007) and properties of the resultant calf (Farin et al., 2006). Features of the maternal environment during the preimplantation period can affect embryonic growth and survival, as has been shown in cattle for lactation (Maillo et al., 2012), circulating concentrations of progesterone (Lonergan et al., 2007; Kenyon et al., 2013), parity (Berg et al., 2010) and exposure to supplemental somatotropin (Moreira et al., 2002).

One of the mechanisms by which the maternal environment affects development during the preimplantation period is through the secretion of hormones, growth

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factors, and cytokines that act on the embryo to regulate its physiology and differentiation. Here we propose the term "embryokine" to describe regulatory molecules produced by the reproductive tract that modulate embryonic development. Several embryokines have been identified that can alter one or more aspects of preimplantation development. In the cow, these include FGF2 (Fields et al., 2011), IGF1 (Block and Hansen, 2007), ILB1 (Paula-Lopes et al., 1998), LIF (Neira et al., 2010), and TGFB (Neira et al., 2010).

The best-studied embryokine is CSF2, which can improve competence of the preimplantation embryo to develop to term after transfer into recipients in mice (Sjöblom et al., 2005), cows (Loureiro et al., 2009; Denicol et al., 2014) and humans (Ziebe et al., 2013). CSF2 can be identified in uterine fluid (de Moraes et al., 1999) and is produced by both the oviduct and endometrium, with greatest localization of protein being found in the luminal epithelium (de Moraes et al., 1999; Emond et al., 2004; Nahar et al., 2013). Environmental alteration in CSF2 expression in the reproductive tract is likely to be one of the mechanisms by which maternal environment affects embryonic development. In the mouse, seminal plasma increases CSF2 production by uterine epithelial cells in the mouse (Tremellen et al., 1998) and *CSF2* expression in the mouse oviduct (Tremellen et al., 1998; Bromfield et al., 2014) and porcine endometrium (O'Leary et al., 2004). Obesity has been reported to decrease protein and mRNA for *CSF2* in the oviduct of the cow (Nahar et al., 2013).

The focus of this review will be to delineate what is known about the mechanisms through which CSF2 enhances the competence of the preimplantation embryo to develop to term after transfer into recipients. The general picture that emerges is that CSF2 functions as a developmental programming agent[1] that acts during the preimplantation period to affect capacity for apoptosis, gene expression, cell number and differentiation status to modify the developmental trajectory of the embryo and affect critical events related to embryonic and fetal survival later in pregnancy.

2. Consequences of exposure to CSF2 during the preimplantation period on competence of the embryo to develop to term

In cattle (Loureiro et al., 2009; Denicol et al., 2014), humans (Ziebe et al., 2013) and mice (Sjöblom et al., 2005), treatment of cultured preimplantation embryos with CSF2 can exert long-term effects that increase the likelihood that the embryo gives rise to a live neonate after being transferred to a recipient female. A summary of some of the results demonstrating this idea is presented in Fig. 1. Examined collectively, it is apparent that the increase in live birth rate is the consequence of higher likelihood for the embryo to establish pregnancy, reduced frequency of embryonic and fetal loss after pregnancy is established, or both.

Effects of CSF2 on the competence of the embryo to establish pregnancy have been variable. In some experiments, CSF2 increased pregnancy rate at early stages of gestation whereas it was without effect in others. For example, treatment of bovine embryos with CSF2 from Day 5-7 of development increased the proportion that established pregnancy at Day 30-35 of gestation after transfer to recipients (Loureiro et al., 2009; Denicol et al., 2014). Similarly, CSF2 increased the proportion of transferred human embryos that were implanted at 7 wk of gestation (Ziebe et al., 2013). This effect of CSF2 occurred when culture was performed in a medium with low concentrations of albumin (i.e., low binding capacity for ligands) but not when culture was performed in a high-albumin medium. There was also no effect of CSF2 on early embryonic survival following transfer when bovine embryos were treated with CSF2 from Days 1 to 7 (Loureiro et al., 2009) or when mouse embryos were treated with CSF2 (Sjöblom et al., 2005). In the latter case, pregnancy establishment was assessed as the percent of transferred blastocysts forming implantation sites. As late as Day 18 of gestation, there was no difference in survival between mouse embryos cultured in the presence or absence of CSF2 (Sjöblom et al., 2005).

In other experiments, CSF2 reduced loss of pregnancies that were diagnosed earlier in gestation. This was observed for the two experiments in cattle by Loureiro et al. (2009) but not in the experiment with cattle by Denicol et al. (2014). In the human, there was a non-significant tendency for live birth rate to be greater for embryos treated with CSF2 in the presence of high albumin concentration even though embryo survival rates at 7 wk of gestation did not differ between treatments (Ziebe et al., 2013). In the mouse, live birth rate was greater for embryos produced by CSF2 even though there were no differences between groups in survival after transfer as late as Day 18 of gestation (Sjöblom et al., 2005). Increased survival late in gestation may reflect, at least partly, improved placental function because treatment with CSF2 increased trophoblast surface area for exchange at Day 18 of gestation (Sjöblom et al., 2005).

Other data in the mouse (Sjöblom et al., 2005) are supportive of the idea that programming of development by CSF2 during the preimplantation period can extend into postnatal life. In particular, male and female offspring derived from CSF2-treated embryos were smaller in weight (i.e., more similar to offspring from embryos produced in vivo) than male and female offspring from embryos cultured in control medium. When pregnant, female offspring that were derived from embryos cultured with CSF2 has smaller placenta more similar to that of offspring produced in vivo than placental weights of female offspring derived from embryos cultured without CSF2.

3. Regulation of blastocyst formation

Results of experiments described in the previous section are indicative that actions of CSF2 in the preimplantation period include changes that affect embryonic survival early in pregnancy as well as long-acting changes that reduce the likelihood of lethal errors in the late-embryonic or fetal period. Examples of modifications in embryonic

[1] Developmental programming refers here to the programming of various bodily systems and processes by a change in the maternal system during pregnancy (modified from Reynolds et al., 2010).