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Role of diestrus progesterone on endometrial function and conceptus development in cattle

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Abstract

Successful growth and development of the post-hatching blastocyst and pregnancy establishment are a result of the interaction between a competent embryo and a receptive uterine environment. Progesterone (P4) plays a key role in reproductive events associated with establishment and maintenance of pregnancy through its action on the uterine endometrium. Elevated concentrations of circulating P4 in the immediate post-conception period have been associated with an advancement of conceptus elongation, an increase in interferon-tau production and higher pregnancy rates in cattle. The potential beneficial effects of exogenous P4 supplementation on fertility have been acknowledged for a long time but results of supplementation have been inconsistent and may be related to the strategy used to achieve high P4 endogenous concentrations in the animal. This review summarizes recent data highlighting the role of progesterone in regulating uterine function and embryo development in cattle.

Keywords: conceptus elongation, cow fertility, embryo mortality, maternal recognition of pregnancy.

Introduction

The steroid hormone progesterone (P4) plays a key role in reproductive events associated with establishment and maintenance of pregnancy. Conceptus growth and development require the action of P4 on the uterus to regulate endometrial function, including conceptus-maternal interactions, pregnancy recognition, and uterine receptivity to implantation. A considerable proportion of embryo loss may be attributable to inadequate circulating P4 concentrations and the subsequent downstream consequences on endometrial gene expression and histotroph secretion into the uterine lumen. Indeed, low P4 concentrations have been implicated as a causative factor in the low pregnancy rates observed in high-yielding dairy cows (Diskin and Morris, 2008). Elevated concentrations of circulating P4 in the immediate post-conception period have been associated with an advancement of conceptus elongation (Garrett *et al.*, 1988; Satterfield *et al.*, 2006; Carter *et al.*, 2008), an increase in interferon-tau production (Mann and Lamming, 2001), and higher pregnancy rates in cattle and sheep (Ashworth *et al.*, 1989; Stronge *et al.*, 2005; McNeill *et al.*, 2006). This review summarizes recent data highlighting the role of progesterone in regulating uterine function and embryo development in cattle. The reader is also referred to other recent reviews of the topic (Inskeep, 2004; Spencer *et al.*, 2007; Lonergan, 2011; Wiltbank *et al.*, 2011; Forde and Lonergan, 2012).

Interaction between the developing embryo and the oviduct

Despite, clear evidence of an interaction between the developing conceptus and the uterine endometrium in early pregnancy (see below), the evidence for reciprocal cross-talk during the transit of the embryo through the oviduct is less clear. Temporal changes occur in the oviduct epithelium gene expression during the estrous cycle (Bauersachs *et al.*, 2004) reflecting the changing requirements of the embryo. There is very convincing evidence for an effect of the oviduct on the quality of the early embryo. For example, short term culture of in vitro produced zygotes in the oviducts of sheep (Enright *et al.*, 2000; Rizos *et al.*, 2002), cattle (Tesfaye *et al.*, 2007) or even mice (Rizos *et al.*, 2007; Rizos *et al.*, 2010b) has been shown to improve embryo quality measured in terms of morphology, gene expression, cryotolerance and pregnancy rate after transfer. In contrast, relatively little evidence exists of an effect going the other way (embryo to oviduct). The limited data reporting an effect of gametes on the oviduct come from litter-bearing species, where any effect is likely to be amplified (Lee *et al.*, 2002; Fazeli *et al.*, 2004; Alminana *et al.*, 2012). We have recently characterized the transcriptome of the bovine oviduct epithelium at the initiation of embryonic genomic activation on day 3 post estrus in pregnant and cyclic heifers (Maillo *et al.*, 2013, School of Agriculture and Food Science, Dublin; unpublished results). The isthmic region, from which all 8-cell embryos and unfertilized oocytes were collected were compared. While large differences in gene expression were observed between the isthmus and ampulla, preliminary data suggest that the presence of an 8-cell embryo had no effect on the transcriptome of the isthmus, although a local effect at the precise position of the embryo cannot be ruled out.

Hugentobler *et al.* (2010) characterized the effects of changes in systemic P4 (achieved by infusion

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of P4) on amino acid, ion and energy substrate composition of oviduct and uterine fluids on days 3 and 6, respectively, of the estrous cycle in cattle. Progesterone increased uterine glucose, decreased oviduct sulphate and, to a lesser degree, oviduct sodium, but had no effect on any of the ions in the uterus. The most marked effect of P4 was on oviducal amino acid concentrations; 9 of 20 amino acids increased following supplementation, with glycine showing the largest increase of approximately two-old whereas in the uterus only valine was increased.

Interdependency of the embryo and reproductive tract

Up to the blastocyst stage, the embryo is somewhat autonomous (i.e., does not need contact with the maternal reproductive tract) as evidenced by the fact that blastocysts can be successfully developed in vitro in large numbers using IVF technology and transferred to synchronized recipients. Furthermore, based on the same evidence and the fact that embryos recovered from superovulated donors are typically transferred to non-pregnant synchronized recipients, the reproductive tract does not need exposure to the embryo prior to day 7 (and even up to day 16; Betteridge *et al.*, 1980) in order for a pregnancy to be established. In contrast, the post-hatching and pre-implantation conceptus is dependent on substances in the uterine lumen, termed histotroph, that are derived from the endometrium, particularly the uterine glands, for growth and development. This is demonstrated by the fact that: (i) post-hatching elongation does not occur in vitro (Brandão *et al.*, 2004; Alexopoulos *et al.*, 2005); and (ii) the absence of uterine glands in vivo results in a failure of blastocysts to elongate (Gray *et al.*, 2002; Spencer and Gray, 2006).

On the maternal side, preparation of the uterine luminal epithelium for attachment of trophectoderm and implantation in all studied mammals, including ruminants, involves carefully orchestrated spatio-temporal alterations in gene expression within the endometrium. In both cyclic and pregnant animals, similar changes occur in endometrial gene expression up to initiation of conceptus elongation (approximately day 13), suggesting that the default mechanism in the uterus is to prepare for, and expect, pregnancy (Forde *et al.*, 2011b). Indeed, as mentioned above, it is possible to transfer an embryo to a synchronous uterus 7 days after estrus and establish a pregnancy, as is routine in commercial bovine embryo transfer. It is only in association with maternal recognition of pregnancy, which occurs on approximately day 16 in cattle, that significant changes in the transcriptomic profile are detectable between cyclic and pregnant endometria (Forde *et al.*, 2011b; Bauersachs *et al.*, 2012), when the endometrium responds to increasing interferon-tau (IFNT) secreted by the filamentous conceptus.

Effect of progesterone on the endometrium and consequences for the embryo

In recent years we and others have made significant progress in clarifying the role of the maternal environment, in particular the role of diestrus progesterone, in the successful establishment of pregnancy in cattle. We have demonstrated that:

- Significant changes occur in the endometrial transcriptome during both the estrous cycle and early pregnancy in cattle (Forde *et al.*, 2009, 2011a, b). As mentioned above, these temporal changes occur irrespective of pregnancy status until the time of maternal recognition of pregnancy when conceptus-induced changes in endometrial gene expression are detectable (Forde *et al.*, 2011b; Bauersachs *et al.*, 2012).
- Elevated P4 results in advancement in the normal temporal changes that occur in the endometrial transcriptome (Forde *et al.*, 2009) and in the timing of P4 receptor downregulation in the luminal epithelium (Okumu *et al.*, 2010), the consequence of which is advancement in conceptus elongation (Carter *et al.*, 2008) that is associated with greater embryonic survival.
- Using a combination of *in vitro* embryo production and *in vivo* embryo transfer techniques, we have shown that the effect of P4 on conceptus development is mediated exclusively via the endometrium (Clemente *et al.*, 2009). Addition of P4 to culture medium had no effect on blastocyst formation (Clemente *et al.*, 2009; Larson *et al.*, 2011) or elongation after transfer to synchronized recipients (Clemente *et al.*, 2009). Most convincingly, the embryo does not need to be present in the uterus during the period of P4 elevation in order to benefit from it, strongly suggesting that the effect of P4 is via the endometrium and altered histotroph composition (Clemente *et al.*, 2009).
- Reducing circulating concentrations of P4 results in an alteration in endometrial transcriptome and retarded embryonic development (Forde *et al.*, 2011a, 2012).
- Follicle aspiration just prior to ovulation results in a reduction in CL size and P4 output, decreased expression of *LHCGR* in luteal tissue and a compromised uterine capacity to support conceptus elongation after transfer of in vitro produced blastocysts (O'Hara *et al.*, 2012)
- The ability of the oviduct/uterus of the postpartum lactating dairy cow to support early embryonic development is impaired compared to that of the nonlactating heifer (Rizos *et al.*, 2010a) and postpartum nonlactating cow (Maillo *et al.*, 2012) and this is likely due to low concentrations of progesterone in blood and an inadequate luminal environment.

Collectively, these results highlight the importance of an optimal uterine environment to support successful development of the conceptus. However, the role of the developing conceptus itself in eliciting appropriate temporal and spatial changes in the endometrial functions should not be underestimated. For example, two recent key papers provide strong evidence that the endometrium of the cow reacts differently depending on the type of embryo present (Bauersachs *et al.*, 2009; Mansouri-Attia *et al.*, 2009). In other words, embryos of different quality (i.e., with divergent developmental fates) signal differently to the endometrium and in turn elicit a different response in terms of the endometrial transcriptome. In this way, the endometrium can be considered as a biological sensor that is able to fine-tune its physiology in response to the presence of embryos whose development will become altered much later after the implantation process (Mansouri-Attia *et al.*, 2009).

Strategies for manipulating diestrus progesterone to improve fertility

The potential beneficial effects of exogenous P4 supplementation on fertility have been acknowledged for a long time (see reviews by Inskeep, 2004; Lonergan, 2011; Wiltbank *et al.*, 2011). Several treatments can be used to increase peripheral concentrations of P4 after AI, including those that (i) increase endogenous function of the existing CL (e.g., strategies which promote growth of the dominant follicle before ovulation resulting in a larger CL), (ii) induce accessory CL formation (e.g., hCG or GnRH administration), or those which supplement progesterone directly (e.g., via injection or intravaginal devices). However, data on outcome in terms of pregnancy rate are often conflicting or inconclusive, and may reflect (i) timing of treatment, (ii) that only a proportion of animals with inherently low P4 may benefit from such treatment, or (iii) the lack of sufficient animal numbers and statistical power in many studies.

Dominant follicle size is associated with subsequent CL size (Vasconcelos *et al.*, 2001). Larger CLs secrete more P4 and this has, in some studies, been associated with improved pregnancy rates. Therefore strategies which promote growth of the dominant follicle before ovulation and/or stimulate CL development are likely to increase pregnancy rate (Baruselli *et al.*, 2010). Equine chorionic hormone (eCG) has been incorporated in synchronization protocols in South America for some time and have been reported to improve pregnancy rates following fixed time AI/ET, although results in lactating cows have been less promising than heifers or beef cows (Bo *et al.*, 2011).

Human chorionic gonadotropin (hCG) administration to ovulate a dominant follicle and form an accessory CL has been widely used in an attempt to improve pregnancy rates, albeit with variable results. These data have been summarized by Lonergan, 2011. In a recent large study Nascimento *et al.* (2013) reported the results of two separate analyses that evaluated the effect of hCG treatment post-AI on fertility in lactating dairy cows. The first study used meta-analysis to combine the results from 10 different published studies that used hCG treatment on day 4 to 9 post-AI in lactating dairy cows. Overall, hCG administration increased pregnancies per artificial insemination (P/AI) by 3.0% (34%, 752/2,213 *vs*. 37%, 808/2,184). In a subsequent field trial lactating Holstein cows (n = 2,979) from six commercial dairy herds received hCG or not on day 5 after a timed AI; pregnancies per AI were greater in cows treated with hCG (40.8%, 596/1,460) than control (37.3%, 566/1,519) cows. Surprisingly, the positive effect of hCG was restricted to first-lactation cows.

Despite positive effects of administration of exogenous P4 using intravaginal P4 devices on conceptus development (Carter *et al.*, 2008; Clemente *et al.*, 2009) recent studies suggest that this may not translate into improved pregnancy rates (Beltman *et al.*, 2009; Parr *et al.*, School of Agriculture and Food Science, Dublin; unpublished results), possibly due to its potentially negative effects on CL lifespan (O'Hara *et al.*, 2012). More work in this area is required.

Conclusion

Progesterone is critical for the establishment and maintenance of pregnancy. It has a crucial role in creating an optimal uterine environment in which the embryo can develop, through its actions on the uterine endometrium, and in turn, the composition of the uterine lumen fluid. Strategies aimed at elevating P4 in the early luteal phase have led to variable results in terms of improving pregnancy rates; these variable results may be due to the type of animal treated (nonlactating heifer, lactating dairy cow, beef cow), the endogenous P4 concentrations in such animals and the mode of achieving elevated P4.

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