New insights into the function of progesterone in early pregnancy

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Implications

• 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.

• Low concentrations of circulating P4 after ovulation have been associated with a reduction in conceptus growth and elongation, a decrease in interferon-tau production, and reduced pregnancy rates in cattle.

• 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, in some cases, increased pregnancy rates in cattle.

• Administration of P4 early in the oestrous cycle of the recipient can effectively advance uterine receptivity for the transfer of older asynchronous embryos.

• Despite the potential beneficial effects of exogenous P4 supplementation on fertility, results of supplementation studies have been inconsistent and may be related to the strategy used to achieve high P4, the type of animal (e.g., lactating or not) as well as endogenous concentrations in the animal.

Key words: conceptus, embryo, fertility, uterine endometrium

Introduction

In most mammals, fertilization of the ovulated oocyte occurs in the oviduct as do the first mitotic cleavage divisions. The bovine embryo enters the uterus at about the 16-cell stage on approximately Day 4 of pregnancy. It subsequently forms a tight ball of cells referred to as a morula, in which the first cell-to-cell tight junctions are formed. By Day 7, the embryo has formed a blastocyst consisting of an inner cell mass, which after further differentiation, gives rise to the embryo/fetus, and the trophoectoderm, which ultimately forms the placenta. After hatching from the zona pellucida on Days 9 to 10, the spherical blastocyst begins to grow and change in morphology from a spherical to ovoid shape during a transitory phase preceding the elongation or outgrowth of the trophoectoderm to a filamentous form that usually begins between Days 12 and 14. Around this time, the conceptus begins to secrete copious amounts of interferon-tau (IFNT), the pregnancy recognition factor in cattle, which suppresses the mechanisms that otherwise would bring about luteolysis. Elongated conceptuses can be readily recovered by standard uterine flushing techniques up to Day 19, after which time the fully elongated conceptus begins implantation with firm apposition and attachment of the trophoectoderm to endometrial luminal epithelium.

While fertilization success is high (~90%), a significant proportion of the resulting embryos fail to develop to term. The majority of embryos are lost between fertilization and maternal recognition of pregnancy, which in cattle, occurs around Day 16 post oestrus (Diskin and Morris, 2008). This embryonic mortality is an important factor affecting reproductive efficiency in cattle (Figure 1).

Progesterone (P4) is a steroid hormone primarily secreted by the corpus luteum (CL) and the placenta. Progesterone secretion by the CL is critical for the establishment and maintenance of pregnancy in mammals and plays a major role in regulating endometrial secretion, essential for stimulating and mediating changes in conceptus growth and differentiation throughout early pregnancy. Circulating concentrations of P4 represent a balance between production of P4 by the CL and the metabolism of P4, primarily by the liver. Production of P4 is regulated by development of the CL after the LH surge, the number of granulosa cells that luteinize into large luteal cells, and constitutive production of P4 by these cells. Metabolism of P4 is primarily related to the rate of blood flow to the liver. Thus, practical regulation of circulating P4 will be most productive by focusing on increasing luteal tissue volume to increase P4 production and/or limiting P4 metabolism (Wiltbank et al., 2014).

Many recent studies have attempted to unravel the mechanisms involved in the complex relationship between circulating concentrations of P4 and fertility. Several retrospective studies have indicated a positive relationship between circulating concentrations of P4 in the week after breeding and subsequent pregnancy rate (e.g., Stronge et al., 2005). Using a novel model of high- and low-fertility Holstein Friesian cows, Cummins et al. (2012) reported 34% greater circulating P4 in cows with high breeding values for fertility compared with those with low breeding values for fertility. Experiments described below show unequivocal evidence for a role of P4 in driving conceptus elongation; however, studies investigating pregnancy rates following supplementation with P4 have yielded variable results.

The role of elevated P4 before artificial insemination during the growth of the preovulatory follicular wave in optimizing fertility has been well reviewed recently (Pursley and Martins, 2011; Wiltbank et al., 2014). The role of P4 in maintaining pregnancy has been known for a long time. Recent studies continue to characterize the uterine effects of P4, inducing a uterine environment compatible with embryo growth, implantation, and maintenance of pregnancy (Spencer et al., 2007; Forde et al., 2009). The
main focus of this review is to summarize recent data on the effect of elevated P4 in the first week post oestrus on conceptus development in cattle.

**Interaction of the Pre-hatching Embryo and the Reproductive Tract**

Despite clear evidence of an interaction between the developing conceptus and the uterine endometrium in early pregnancy, the evidence for reciprocal cross-talk during the transit of the embryo through the oviduct is less clear. On the one hand, there is convincing evidence for a positive influence of the oviduct on the quality of the early embryo. For example, short-term culture of bovine zygotes produced in vitro in the oviducts of cattle (Tesfaye et al., 2007; Gad et al., 2012), sheep (Enright et al., 2000; Lazzari et al., 2002; Rizos et al., 2002), or even mice (Rizos et al., 2007) has been shown to improve embryo quality measured in terms of morphology, gene expression, cryotolerance, and pregnancy rate after transfer. However, evidence for a reciprocal communication from the embryo to the oviduct is less clear and mainly restricted to litter-bearing species, where any signal is likely to be amplified.

Therefore, up to the blastocyst stage, the embryo can be considered somewhat autonomous (i.e., does not need contact with the maternal reproductive tract). For example, using in vitro fertilization (IVF) technology, it is possible to produce large numbers of bovine blastocysts in vitro by aspirating ovarian follicles, maturing and fertilizing oocytes in vitro, and culturing the resulting zygotes for about 7 d (Figure 2). These embryos have never been in an oviduct but are nonetheless capable of establishing a pregnancy after transfer to the uterus of a synchronized recipient. This demonstrates that the early embryo does not absolutely require exposure to the oviduct and also that the oviduct does not require exposure to the embryo for pregnancy to be established. In a similar vein, in commercial bovine embryo transfer practice, early (~7 d old) embryos are typically recovered from superovulated donors and transferred to the uterus of non-pregnant synchronized recipients, which up to that stage, have not seen an embryo. This again suggests that the reproductive tract (of the recipient, in this case) does not need exposure to an embryo before Day 7 to establish a pregnancy. Indeed, taken to its extreme, it is possible to establish a pregnancy in a cow by transferring embryos as old as 16 d (Betteridge et al., 1980), i.e., up to the time when the luteolytic mechanisms would normally be initiated, although this would not be practical due to the filamentous nature of the conceptus at this stage.

**Effect of Progesterone on the Pre-hatching Embryo**

There is little or no convincing evidence that P4 has a direct effect on the early embryo. Culture of embryos in vitro in the presence of P4 did not affect the proportion developing to the blastocyst stage in the presence or absence of oviductal epithelial cells (Clemente et al., 2009). This is consistent with the observations of Larson et al. (2011) who failed to observe a direct effect of P4 either from Days 1 to 3 or 4 to 7 after fertilization. In two other in vivo studies, we failed to demonstrate an effect of elevated P4 on blastocyst development. In Carter et al. (2008), no difference in embryo
development on Day 5 or Day 7 was observed when beef heifers were supplemented with exogenous P4 from Day 3, despite dramatic effects on post-hatching development on Days 13 and 16. In a follow-up study, embryos produced in vitro were transferred to the oviduct of beef heifers that did or did not receive a P4 insert on Day 3 after oestrus. There was no detectable effect of P4 on the proportion of embryos that developed to the blastocyst stage by Day 7 (Carter et al., 2010).

Interaction between the Post-hatching Embryo and the Uterus

As mentioned above, despite the importance of the oviduct as the site of fertilization and early cleavage divisions in mammals, it can be readily bypassed using IVF and embryo transfer (ET) technology. In contrast, the development of the post-hatching and pre-implantation conceptus is entirely driven by the uterine environment. The protracted period of implantation characteristic of ruminants and pigs involves rapid proliferation of the trophoderm cells, which depends on substances in the uterine lumen fluid (or histotroph) that are derived from the endometrium, particularly the uterine glands, for growth and development. This is clearly demonstrated by the fact that: i) post-hatching elongation does not occur in vitro (Flechon et al., 1986; Brandao 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; Figure 3).

Figure 2. Bovine blastocysts produced in vitro. The ability to produce embryos in vitro suggests that the early stages are somewhat autonomous.

Figure 3. Day 14 bovine conceptuses recovered from the uterus at slaughter following transfer of Day 7 blastocysts produced in vitro. Note the variation in conceptus length. Multiple embryo transfer studies allow repeated measures of the ability of the uterine environment to support conceptus development but also highlight that there is an intrinsic embryo component to elongation, as reflected in the variation in conceptus size from the same uterus; conceptus elongation is maternally driven but embryo dependent.
Earlier studies in ewes (Wilmut and Sales, 1981; Lawson et al., 1983) and cows (Garrett et al., 1988) suggested that maternal P4 regulates early conceptus growth and development. More recent studies have confirmed these findings and begun to unravel the underlying biology (e.g., Satterfield et al., 2006; Forde et al., 2009, 2012). In recent years, significant progress has been made in clarifying the role of diestrus P4 in the successful establishment of pregnancy in cattle, with particular emphasis on how P4 affects conceptus elongation. 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 beef recipients (Clemente et al., 2009). Most convincingly, the embryo does not need to be present in the uterus during the period of P4 elevation to benefit from it (Clemente et al., 2009; O’Hara et al., 2014a), strongly suggesting that the effect of P4 is via advancement of the normal temporal changes that occur in the endometrial transcriptome (Forde et al., 2009; Satterfield et al., 2009), resulting in advanced conceptus elongation (Carter et al., 2008; Clemente et al., 2009). In addition, reducing the output of P4 from the CL results in a delay in the temporal changes in the endometrial transcriptome, resulting in delayed conceptus elongation in vivo (Forde et al., 2011a, 2012; Figure 4).

Preparation of the uterine luminal epithelium for attachment of trophectoderm and implantation in all studied mammals, including ruminants, involves carefully orchestrated spatiotemporal alterations in gene and protein expression and localization within the endometrium. In both cyclic and pregnant heifers, 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 d after oestrus and establish a pregnancy, as is routine in commercial bovine embryo transfer, and even up to Day 16. 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 amounts of IFNT secreted by the filamentous conceptus.

### Asynchronous Embryo Transfer

Embryo transfer studies in sheep and cattle have clearly demonstrated a need for close synchrony (±24 h) between embryo and the uterine environment of the recipient. Previous studies have established that pregnancy rates are reduced when embryos are >48 h from synchrony with the recipient’s uterine environment. The dramatic regulatory effect of the uterus on bovine conceptus development, and the role played by P4, is nicely illustrated in studies comparing the outcome of synchronous and asynchronous embryo transfer. For example, transfer of Day 7 bovine embryos to Day 5 or Day 9 uteri resulted in retarded (5.4 ± 0.4 mm) or advanced (50.4 ± 5.2 mm) conceptuses on Day 14, respectively, compared with synchronous controls (Day 7 to Day 7: 15.7 ± 1.5 mm) or conceptuses derived from AI (12.0 ± 3.3 mm; Ledgard et al., 2012). Geisert et al. (1991) demonstrated that administration of exogenous P4 on Days 1 to 4 of the recipient’s oestrus cycle allowed “older” (+3 d) bovine embryos to establish pregnancy, consistent with the successful maintenance of pregnancy with advanced (+4 d) embryos transferred to recipient ewes that received progesterone shortly after oestrus (Lawson et al., 1983). Without treatment with progesterone, only 4.8% (1/21) of asynchronous recipient cows maintained pregnancy with advanced embryos. Together, these studies indicate that progesterone stimulates changes within the uterine environment, which regulates receptivity and promotes embryo survival and conceptus elongation.

### Strategies to Increase Concentrations of Progesterone

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., 2014). Several approaches can be taken to increase peripheral concentrations of P4 after AI, including those that: i) increase endogenous function of the existing CL (e.g., strategies that promote growth of the dominant follicle before ovulation resulting in a larger CL, or luteotrophic treatments that stimulate CL development, e.g., hCG); ii) induce ovulation of a dominant follicle and formation of accessory CL formation (e.g., hCG or GnRH administration); or iii) 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) the timing of treatment; ii) that only a proportion of animals with inherently low P4 may benefit from such treatment; iii) that P4 supplementation may be less effective in high-producing dairy cows due to liver metabolism (Wiltbank et al., 2014); or iv) 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). A larger CL secretes more P4, and this has, in some
studies, been associated with improved pregnancy rates. Therefore, strategies that 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) incorporated into synchronization protocols has been reported to improve pregnancy rates following fixed-time AI/ET, although results in lactating dairy cows have been less promising than heifers or beef cows (Bo et al., 2011).

Administration of hCG 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 (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 Days 4 to 9 post-AI in lactating dairy cows. Overall, hCG administration increased pregnancies per artificial insemination by 3% points [34% (752/2,213) vs. 37% (808/2,184)]. In a subsequent field trial, lactating Holstein cows (n = 2979) 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.

Although the use of exogenous P4 administration to improve synchrony of embryo transfer and/or advance conceptus elongation as described above is encouraging, caution is warranted. Paradoxically, depending on the timing of administration, exogenous P4 can have a negative effect on CL lifespan resulting in short interoestrous periods due to premature CL regression (Ginther, 1970; Garrett et al., 1988; Burke et al., 1994) while at the same time advancing conceptus development due to the changes induced in the endometrium (O’Hara et al., 2014a). This situation is clearly not compatible with successful maintenance of pregnancy. We have recently shown that a single intramuscular injection of hCG as early as Day 2 or Day 3 after oestrus resulted in a larger CL and increased circulating concentrations of progesterone compared with controls (Maillo et al., 2014). It is possible that a combination of exogenous progesterone, to induce the required stimulation of the endometrium and conceptus, and luteotropic support, such as that provided by hCG, to avoid early CL regression, would provide a means of optimizing maternal recognition of pregnancy. Indeed, administration of hCG at the time of progesterone injections on Days 1 to 4 overcame the negative effect on CL lifespan (Ginther, 1970). In support of this notion, in a recent study (O’Hara et al., 2014b), administration of eCG, a glycoprotein secreted by the endometrial cups of pregnant mares with a relatively long half-life of about 2 to 3 d and with both LH- and FSH-like properties in cattle, to beef heifers on Day 3 post oestrus in association with an intravaginal P4 insert reduced the number of short cycles and increased mean luteal tissue weight and circulating P4. However, the numbers of heifers involved was small, and this area requires further study.

**Conclusion**

The role of P4 in optimizing uterine receptivity is unequivocal. Innovative strategies aimed at optimizing circulating concentrations of P4 post-conception have the potential to improve embryo survival in cattle. It is clear that elevated P4 early post oestrus induces changes in the uterine environment that promote conceptus elongation. Whether a P4-induced increase in conceptus size can improve fertility continues to be an active area of investigation.

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