DOI: 10.21451/1984-3143-AR867

Anim. Reprod., v.13, n.3, p.143-152, Jul./Sept. 2016

Progesterone and conceptus-derived factors important for conceptus survival and growth

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Abstract

Progesterone (P4) from the corpus luteum (CL) is critical for the establishment and maintenance of pregnancy and plays a major role in regulating endometrial secretions essential for stimulating and mediating changes in conceptus growth and differentiation throughout early pregnancy in ruminants. Numerous studies have demonstrated an association between elevated P4 and acceleration in conceptus elongation. A combination of *in vivo* and *in vitro* experiments found that the effects of P4 on conceptus elongation are indirect and mediated through P4-induced effects in the endometrium. Despite effects on elongation, data on the impact of post-insemination supplementation of P4 on pregnancy rates are conflicting and typically only result in a modest improvement, if any, in fertility. Differences in conceptus length on the same day of gestation would suggest that factors intrinsic to the blastocysts transferred regulate development, at least in part, and would be consistent with the hypothesis that the quality of the oocyte regulates developmental competence. This paper will review recent knowledge on the effect of P4 on conceptus development in cattle and summarize strategies that have been undertaken to manipulate post fertilization P4 concentrations to increase fertility.

Keywords: cattle, conceptus, embryo, pregnancy, progesterone.

Introduction

Most embryonic loss in cattle occurs in the first few weeks after conception. Fertilisation success is typically high (~90%) but a significant proportion of the resulting embryos fail to develop to term. The majority of these embryos are lost between fertilisation and maternal recognition of pregnancy, which in cattle occurs around day 16 post-mating (Diskin and Morris, 2008; Wiltbank *et al*., 2016). Wiltbank *et al*. (2016) described four pivotal periods for pregnancy loss during the first trimester of gestation and discussed possible causes for pregnancy failure during these periods. Despite a relatively high fertilization rate (>85%), 20-50% of high-producing lactating dairy cows experience pregnancy loss during the first week of gestation. From days 8 to 27, concomitant with embryo elongation and maternal recognition of pregnancy, losses average approximately 30%. From days 28 to 60, losses of approximately 12% occur while in the fourth period, during the third month of pregnancy, pregnancy losses are reduced (approximately 2%), but may be elevated in some cows, particularly in those carrying twins in the same uterine horn (Wiltbank *et al*., 2016).

Optimal dialogue between the developing embryo and its mother is essential for successful pregnancy recognition and maintenance of pregnancy during the critical peri-implantation period of pregnancy when the stage is set for implantation and placentation that precedes fetal development (Guillomot, 1995; Hue *et al*., 2012; Spencer *et al*., 2015). However, this dialogue really only becomes absolutely essential as the time of pregnancy recognition approaches as evidenced by the fact that embryos are routinely transferred to a synchronous uterus up to about day 8 of development in commercial embryo transfer with good success. Indeed, pregnancies have been achieved following transfer of embryos as late as day 16 (Betteridge *et al*., 1980), although due to the filamentous nature by that time, it is impractical to do so.

Uterine epithelial cells secrete and/or transport a wide range of molecules, including nutrients, collectively referred to as histotroph that are transported into the fetal-placental vascular system to support growth and development of the conceptus (embryo/fetus and associated membranes). In turn, molecules secreted by conceptuses, in particular interferon tau (IFNT), the maternal recognition of pregnancy signal in ruminants, but also prostaglandins (PGs; Dorniak *et al*., 2011, 2012; Spencer *et al*., 2013), induce changes in the uterine endometrium which are essential if pregnancy is to be maintained.

There is a strong positive association between the post-ovulatory rise in concentrations of progesterone (P4) and embryonic development in sheep and cattle (Satterfield *et al*., 2006; Carter *et al*., 2008). Much has been written about the role of P4 in the establishment and maintenance of pregnancy. Many researchers have tried to manipulate P4 concentrations during the first two weeks after mating in an attempt to achieve higher pregnancy rates. Rather than repeat in detail what has already been written, the reader is directed to several other recent comprehensive reviews on the subject (Lonergan, 2011, 2015; Wiltbank *et al*., 2014; Spencer *et al*., 2015).

Establishment of pregnancy in cattle

Following fertilization in the oviduct, the early

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Received: July 4, 2016
Accepted: July 11, 2016

embryo undergoes the first mitotic cleavage divisions before entering the uterus at about the 16-cell stage on approximately day 4 after ovulation. It soon forms a morula and, by day 7, a blastocyst containing an inner cell mass and a single layer of trophectoderm surrounding a fluid-filled blastocoel cavity. After hatching from the zona pellucida on approximately days 8 to 9, the spherical blastocyst grows and changes in morphology from a spherical to ovoid shape during a transitory phase preceding the elongation of the trophectoderm to a filamentous form that usually begins between days 12 and 14. The conceptus continues to grow and secrete IFNT, which prevents prostaglandin-induced luteolysis and maintains the pregnancy. Unlike primate and rodent embryos which invade the endometrium soon after hatching, ruminant conceptuses do not invade the endometrium during implantation, which commences at approximately day 19 in cattle (Guillomot, 1995), but rather undergo an extended free-floating period of development during which they exibit rapid elongtion of the extra-embryonic tissues.

In vitro fertilization studies have demonstrated that contact with the female reproductive tract is not necessary in order for the embryo to reach the hatched blastocyst stage. However, the characteristic elongation of the conceptus prior to implantation is dependent on secretions from the uterus. This elongation does not occur *in vitro* (Flechon *et al*., 1986) and does not occur *in vivo* in the absence of uterine glands (Gray *et al*., 2002; Spencer and Gray, 2006). Several authors have attempted to induce elongation *in vitro* by growing blastocysts in confined spaces (Brandão *et al*., 2004; Alexopoulos *et al*., 2005; Zhao *et al*., 2015) but while lateral expansion occurs, events as they occur *in vivo* are not recapitulated.

Thus, exposure to the uterine environment is required for conceptus elongation. Uterine luminal fluid (ULF) contains embryotrophic substances, collectively termed histotroph, that drive elongation of the conceptus via effects on trophectoderm proliferation and migration as well as attachment and adhesion to the endometrial luminal epithelium (LE; Gray *et al*., 2001; Spencer *et al*., 2008; Bazer *et al*., 2010; Forde *et al*., 2014a). The ULF is derived primarily from transport and (or) synthesis and secretion of substances by the endometrial LE and glandular epithelium (GE), but also by the conceptus (Forde *et al*., 2015), and it is a complex and rather undefined mixture of proteins, lipids, amino acids, sugars (glucose, fructose), ions, and exosomes/microvesicles (Bazer, 1975; Gray *et al*., 2001; Bazer *et al*., 2012; Burns *et al*., 2014; Forde *et al*., 2014b). P4 induces the expression of a number of genes in the endometrial epithelium that are then further stimulated by factors from the conceptus (e.g., IFNT and PGs) and the endometrium itself (Dorniak *et al*., 2013; Brooks *et al*., 2014; Lonergan and Forde, 2014). In turn, the genes and functions regulated by these hormones and factors in the endometrial epithelia cause specific changes in the uterine histotroph that govern conceptus survival and elongation (Faulkner *et al*., 2013; Forde *et al*., 2014a, 2015).

Progesterone and the endometrium

A prerequisite for establishing uterine receptivity to implantation in all species studied thus far is loss of expression of P4 receptors (PGR) from uterine LE and then GE (Bazer *et al*., 2010). Paradoxically, it is sustained exposure of the endometrium to circulating concentrations of P4 that leads to this down-regulation of PGR as the luteal phase of the estrous cycle progresses. The concentrations of P4 in circulation modify the loss of expression of PGR in the endometrium such that, in animals in which P4 is high there is early loss of the PGR (Okumu *et al*., 2010) i.e. uterine receptivity to implantation is established earlier. Conversely, low or sub-optimal concentrations of P4 delay loss of the PGR and thus delay establishing uterine receptivity to implantation (Forde *et al*., 2011a). Thus, in simple terms, it would appear that elevating P4 immediately after estrus or mating simply advances the changes in endometrial gene expression which normally occur (Forde *et al*., 2009).

The transcriptome of the bovine endometrium has been described under a variety of physiological and experimental conditions (Forde *et al*., 2009, 2011a, b; Sandra *et al*., 2011; Bauersachs *et al*., 2012; Binelli *et al*., 2015). Temporal changes in gene expression in the uterus occur irrespective of whether the cow is pregnant or not and it is really only at the time of maternal recognition of pregnancy at around day 16 that major changes in gene expression between pregnant and cyclic endometrium are detectable (Forde *et al*., 2011b; Bauersachs *et al*., 2012). Forde *et al*. (2009) described the global transcriptome of the endometrium from day 5 to day 16 in pregnant and cyclic cattle under conditions of normal and elevated P4 and revealed how circulating concentrations of P4 regulate endometrial genes. This study found that P4 supplementation advanced the normal temporal changes in endometrial gene expression, particularly for genes associated with energy sources or contributors to histotroph, which may contribute to advanced conceptus development on day 13 and day 16.

Progesterone and conceptus elongation

Elongation of the ruminant conceptus is essential for normal pregnancy recognition and implantation. Mamo *et al*. (2011) described the global transcriptome profile of the bovine conceptus at five key stages of its pre- and peri-implantation growth (days 7, 10, 13, 16, and 19). Analysis identified differentially regulated genes organized in nine gene clusters forming a sequential transcript dynamics across these developmental stages. These data have been expanded upon by more recent studies (Valour *et al*., 2014; Barnwell *et al*., 2015, 2016; Ribeiro *et al*., 2016a, b).

Flechon *et al*. (1986) cut day 12 ovine blastocysts into pieces and cultured them *in vitro* for 24 h, to produce structures called trophoblastic vesicles (TV, blastocysts without the embryonic disc). Such TV survived *in vitro* for up to 10 days but failed to elongate. In contrast, TVs elongated *in vivo* after transfer to

recipients demonstrating that trophoblast elongation does not depend necessarily on the presence of the embryo proper, but can occur in TV composed only of the trophectoderm and the extraembryonic endoderm.

Earlier studies in ewes (Wilmut and Sales, 1981; Lawson and Cahill, 1983) and cows (Garrett *et al*., 1988b) suggested that maternal P4 regulates early conceptus growth and development. More recent studies have confirmed those findings and began to unravel the underlying biology. In particular, significant progress has been made in clarifying the role of P4 in the successful establishment of pregnancy in sheep and cattle, with particular emphasis on how P4 affects endometrial gene expression and conceptus elongation.

The stimulatory effect of P4 on trophoblast elongation is unequivocal. As mentioned earlier, however, this effect is likely a result of downstream effects of P4-induced changes in gene expression in cells of the endometrium (Satterfield *et al*., 2006; Forde *et al*., 2009, 2011a) resulting in changes in the composition of ULF or histotroph to which the developing embryo is exposed (Faulkner *et al*., 2013). Whether any of the effects of P4 are directly on the embryo has been assessed by experiments in which P4 was added to medium during the *in vitro* culture of embryos. Results of such studies have been varied and contradictory with some authors reporting positive effects of P4 (Ferguson *et al*., 2005, 2011; Merlo *et al*., 2007) while others have reported no effect (Reggio *et al*., 1997; Goff and Smith, 1998). Overall, however, despite the presence of PGR mRNA on embryos (Clemente *et al*., 2009), there is little convincing evidence that P4 has a direct effect on the early embryo. In our own laboratory, 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 finding 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 fertilisation. Furthermore, addition of P4 to culture medium had no effect on conceptus elongation after transfer to synchronised recipients (Clemente *et al*., 2009). In two other *in vivo* studies, we failed to demonstrate an effect of elevated P4 on blastocyst development. In the study of Carter *et al*. (2008), no differences in embryonic development on day 5 or day 7 were observed when beef heifers were supplemented with exogenous P4 from day 3, despite dramatic effects on post-hatching elongation between days 13 and 16 of pregnancy. In a follow-up study, multiple *in vitro* produced embryos were transferred to the oviduct of beef heifers that did or did not receive a P4 insert on day 3 after onset of oestrus. There was no effect of P4 on the proportion of embryos that developed to the blastocyst stage by day 7 (Carter *et al*., 2010).

The effects of elevated P4 shortly after conception on the advancement of conceptus elongation have been convincingly demonstrated in cattle and sheep. Garrett *et al*. (1988b) administered 100 mg P4 on days 1, 2, 3 and 4 of pregnancy which increased concentrations of P4 in peripheral plasma on days 2 to 5 and significantly larger conceptuses on day 14. Using a P4 implant on day 3 of pregnancy, Carter *et al*. (2008) significantly elevated concentrations of P4 in plasma until day 8 and this was associated with larger conceptuses recovered at slaughter on day 16. Similarly, when ewes received daily injections of 25 mg P4 from 36 h post-mating, blastocyst diameter increased by 220% on day 9 and at the time of initiation of elongation of blastocysts to a filamentous conceptus on day 12 was advanced (Satterfield *et al*., 2006); these effects of P4 treatment on blastocyst development were blocked by administration of RU486, a PGR antagonist.

As mentioned above, 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). Interestingly, the embryo does not need to be present in the uterus during the period of P4 elevation in order to benefit from it (Clemente *et al*., 2009), 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) resulting in advanced conceptus elongation. In addition, reducing the output of P4 from the CL, for example, by treatment with prostaglandin F2α (Beltman *et al*., 2009b; Forde *et al*., 2011a, 2012) or by aspirating the contents of the preovulatory follicle just before the expected time of ovulation (O'Hara *et al*., 2012) results in a delay in the temporal changes in the endometrial transcriptome resulting in delayed conceptus elongation *in vivo*.

Barnwell *et al*. (2015) examined the effect of embryo source (*in vitro vs. in vivo* derived) and recipient P4 concentration at the time of embryo transfer on conceptus development on day 17. They reported no relationship between P4 concentration on day 7 at the time of embryo transfer and conceptus length on day 17. Strangely, when only longer conceptuses were considered, heifers with *in vitro* produced embryos had lower P4 than those with *in vivo* derived embryos. In contrast, Frade *et al*. (2014) reported that higher plasma P4 concentration at timed embryo transfer was associated with increased pregnancy rate in *in vitro*-produced embryo recipients.

Asynchronous embryo transfer

The regulatory effect of the uterus on bovine conceptus development, and the role played by P4, has been beautifully illustrated in studies comparing the outcome of synchronous and asynchronous embryo transfer. Such synchrony between the needs of the developing embryo and uterine secretions has long been recognized as being critical to the successful establishment of pregnancy (Pope, 1988). Indeed, embryo transfer studies in sheep and cattle have clearly demonstrated a need for close synchrony between embryo and the uterine environment of the recipient. Previous studies have established that pregnancy rates are reduced when embryos are greater than 48 h from synchrony with the recipient's uterine environment (Moore and Shelton, 1964; Rowson and Moor, 1966;

Rowson *et al*., 1972).

Asynchronous transfer of day 7 bovine blastocysts to the uteri of day 5 or day 9 recipients resulted in retarded (5.4 ± 0.4 mm) or advanced (50.4 ± 5.2 mm) conceptuses on day 14, respectively, compared to 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). Consistent with these observations, Geisert *et al*. (1991) reported that only 1 of 21 (4.8%) day 8 bovine blastocysts transferred to a day 5 uterus established pregnancy compared to 50% in synchronous controls.

Administration of P4 early in the estrous cycle of the recipient has been shown in some cases to effectively advance uterine receptivity for the transfer of older asynchronous embryos. In sheep, day 6 recipients after early exposure to exogenous P4, supported development of transferred day 10 blastocysts (Lawson and Cahill, 1983). In cattle, embryo transfer to P4-treated recipients (100 mg/day from day 1 to day 4) which showed estrus 72 h after the donor cows (i.e., day 8 blastocysts transferred into a day 5 uterus) resulted in pregnancy rates at day 35 similar to those of synchronous (±12 h) recipients (42.1 *vs*. 50%), while, as mentioned above, only approximately 5% of day 5 asynchronous recipients became pregnant (Geisert *et al*., 1991).

Similar data have been reported recently by Randi *et al*. (2015) who transferred multiple day 7 bovine blastocysts to synchronous (day 7) or asynchronous (day 5 or day 9) recipients (n = 10 per recipient). Transfer of day 7 blastocysts to a day 5 uterus resulted in fewer conceptuses surviving (20%) and delayed elongation in those that were recovered. In contrast, transfer to an advanced day 9 uterine environment resulted in the same level of survival as synchronous controls (~50%), but conceptus elongation was markedly advanced, in agreement with the observations of (Ledgard *et al*., 2012). Supplementation of day 5 recipients with P4 from day 3 increased circulating concentrations of P4 and increased conceptus length compared to day 5 controls; however, supplementation with P4 reduced the length of estrous cycles in approximately 50% of heifers.

Together, these studies indicate that P4 stimulates changes within the uterine environment which regulate receptivity and promote embryo survival and conceptus elongation. Manipulating P4 may be one way of strategically regulating the temporal changes that normally occur in the uterine environment in order to allow flexibility in the timing of embryo transfer. Given the above results indicating that transfer to an advanced uterus (i.e., uterus ahead of the embryo), which has had longer exposure to P4 results in an advancement in conceptus elongation and that such advanced conceptuses produce more IFNT (Kerbler *et al*., 1997; Rizos *et al*., 2012), one could reasonably hypothesize that transfer to an advanced uterus would result in improved pregnancy rates. However, interrogation of data from commercial embryo transfer operations does not support that hypothesis (Wright, 1981; Donaldson, 1985; Hasler *et al*., 1987; Heyman, 1988; Hasler, 2001; Rodrigues *et al*., 2003; Randi *et al*., 2015). For example, in the study of Randi *et al*. (2015), 4749 recipients received a single *in vitro* produced fresh blastocyst. Overall pregnancy rate was 43.5%, which is about the norm in such commercial IVF operations. Transfer of a day 7 blastocyst to a synchronous day 7 uterus resulted in a pregnancy rate of 47.3%. Transfer to a uterus one day behind (day 6: 46.6%) did not affect pregnancy rate. However, transfer to a day 5 (40.8%) or a day 8 (41.3%) uterus moderately impacted pregnancy rate while transfer to a uterus 2 days in advance (day 9: 24.4%) or 3 days behind (day 4: 27.0%) dramatically reduced pregnancy rates compared to results from synchronous transfer of blastocysts. Taking results of all of these studies together, it is clear that the accelerated conceptus elongation associated with transfer of a blastocyst to an advanced uterus does not necessarily translate into an improved pregnancy rate; rather, once synchrony is exceeded by approximately 48 h, pregnancy rates decline appreciably.

Supplementation of progesterone and pregnancy rate

Results of several retrospective studies have indicated a positive relationship between circulating concentrations of P4 in the week after breeding and subsequent pregnancy rate (Stronge *et al*., 2005; Diskin *et al*., 2006; Parr *et al*., 2012). Interestingly, there is both a linear and quadratic component to this relationship; that is, too much P4 may lead to a decline in pregnancy rate. Thus, both sub-and supra-optimal concentrations of P4 from days 4 to 7 after AI or a sub-optimal rate of increase in the concentration of P4 during this interval are negatively associated with embryonic survival. Cummins *et al*. (2012) reported that circulating concentrations of P4 were 34% greater in cows with similar genetic merit for milk production traits, but with extremes of good (Fert+) or poor (Fert-) genetic merit for fertility traits. In a follow-up study, Moore *et al*. (2014) investigated the factors affecting circulating concentrations of P4 in those cows. Concentrations of P4 were measured from days 1 to 13. CL volume was 41% greater and mean circulating concentrations of P4 were 79% greater in Fert+ cows compared with Fert- cows. The results indicate that greater circulating concentrations of P4 were primarily due to a greater capacity of CL to secrete P4 rather than differences in clearance rate of P4 in this lactating cow genetic model of fertility.

Ultimately, circulating concentrations of P4 are determined by the balance between the rate of P4 production by the CL and the rate of P4 metabolism, mainly by the liver. Production of P4 is mainly regulated by the number of large luteal cells (LLC) and constitutive production of P4 by these cells which in turn is dependent on the provision of sufficient cholesterol substrate, mainly in the form of high-density lipoprotein (HDL). Increasing the number of granulosa cells and thereby the number of LLC , by ovulation of larger or multiple follicles, results in increased P4 output by the CL. Circulating HDL may be manipulated

by diet and this has been used as a strategy to increase P4 (Cordeiro *et al*., 2015). Metabolism of P4 is primarily related to the rate of blood flow to the liver (Sangsritavong *et al*., 2002) and is affected by the physiological condition of the cow. Therefore, practical strategies aimed at changing inherent CL capacity through genetic selection (Cummins *et al*., 2012; Butler, 2013; Moore *et al*., 2014) or the manipulation of circulating concentrations of P4 will be most productive by focussing on increasing luteal tissue volume to increase P4 production and/or limiting P4 metabolism (Wiltbank *et al*., 2014).

In a study in which inseminated cows were blood sampled on week 5 of presumed pregnancy, 50% of cows with P4 < 2.8 ng/ml aborted before week 9 of gestation and 95% of cows with P4 of 6.0 ng/ml on week 5 maintained pregnancy (Starbuck *et al*., 2004). Kenyon *et al*. (2013) determined P4 concentration from days 4 to 28 relative to presumptive estrus necessary for maintenance of pregnancy in lactating Holstein cows. An early rise in P4 from day 0 to 14 was associated with establishment of pregnancy after embryo transfer. Cows with P4 concentration <5 ng/ml on day 14 were more likely to lose pregnancy from day 28 to 63. Faster rise in P4 concentration during the metestrus and early diestrus are associated with pregnancy establishment following embryo transfer, which suggests that early rise in P4 concentration has an indirect effect on embryo development through modulation of uterine environment and secretion of histotroph. Furthermore, the positive effects of early rise in P4 concentration appear to go beyond the phase of maternal recognition of pregnancy through adhesion and placentation stages.

Given the importance of P4 for pregnancy establishment and the known effects on uterine receptivity and conceptus development many researchers have attempted to manipulate P4 using a variety of strategies in the days immediately post-conception in order to improve conception rates. Clearly, increasing concentrations of P4 after ovulation stimulates conceptus elongation in beef heifers, dairy cows, and sheep. However, supplementation of cattle with P4 during early pregnancy has resulted in mixed outcomes in terms of embryonic survival (Beltman *et al*., 2009a; Parr *et al*., 2014).

Based on the demonstration that elevated P4 accelerates conceptus development and that larger conceptuses produce more IFNT, one could reasonably hypothesize that such advanced conceptuses would be more likely to establish pregnancy. However, data on the impact of post insemination supplementation of P4 on pregnancy rate are conflicting and, at best, indicate a modest positive response. For example, in one recent large study, Nascimento *et al*. (2013) reported the results of 2 separate analyses that evaluated the effect of hCG treatment post-AI on fertility in lactating dairy cows. The first study was a meta-analysis of 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 (P/AI) by 3 percentage points [34% (752/2,213) *vs*. 37% (808/2,184)]. In a subsequent field trial, lactating Holstein cows (n = 2,979) from 6 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%) than control (37.3%) cows. Interestingly, the positive effect of hCG (overall approximately 3.5%) was restricted to first-lactation cows.

A variety of strategies can be used to increase peripheral P4, ranging from those that stimulate endogenous production such as: (i) manipulation of follicular development to increase the size of the preovulatory follicle and hence the CL (Baruselli *et al*., 2012; Mesquita *et al*., 2014; Ramos *et al*., 2015); (ii) direct stimulation of CL development with luteotrophic agents (Maillo *et al*., 2014); (iii) induction of accessory CL using appropriately timed administration of GnRH or hCG (Santos *et al*., 2001; Stevenson *et al*., 2007; De Rensis *et al*., 2010; Lonergan, 2011; Torres *et al*., 2013); or (iv) direct supplementation with exogenous P4 through injections (Garrett *et al*., 1988b; Geisert *et al*., 1991; Pugliesi *et al*., 2014) or P4-containing devices (Stevenson *et al*., 2007; Carter *et al*., 2008; O'Hara *et al*., 2014b, c).

Paradoxically, depending on the timing of administration, exogenous P4 can have a negative effect on CL lifespan resulting in short inter-oestrous periods due to premature CL regression (Ginther, 1970; Garrett *et al*., 1988a; 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. It is possible that a combination of exogenous P4, to induce the required stimulation of the endometrium and conceptus, and luteotrophic 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 P4 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 2-3 days 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.

We have recently shown that a single i.m. injection of hCG as early as day 2 or day 3 after oestrus resulted in a larger CL and increased circulating concentrations of P4 compared to controls (Maillo *et al*., 2014). However, the results of Souza *et al*. (2015) examining the effect of administration of long-acting injectable P4 (LAP4) and/or hCG on luteal function and conception rate of high producing dairy cows (n = 982) would suggest that this does not translate into improved pregnancy rates. Cows were assigned to one of four groups: (i) control; (ii) 900 mg LAP4; (iii) 2000 IU hCG; (iv) a combination of LAP4 and hCG. While treatments resulted in elevated P4, conception rate after

30 days was higher in the LAP4 group, but not in the hCG or LAP4 + hCG groups. Conception rates at 60 days, as well as pregnancy loss between 30 and 60 days after TAI were not affected by treatment.

Final remarks

One consistent observation from the multiple embryo transfer studies we have carried out, involving the transfer of 10-20 day 7 blastocysts to the uterus of synchronized recipients and recovery on day 14, is the variation in conceptus size on day 14, even amongst those recovered from the same uterus. Such differences in conceptus length on the same day of gestation may be related to an inherent lack of developmental competency or may simply be a consequence of asynchrony with the maternal environment. It would suggest that factors intrinsic to the blastocysts transferred regulate development, at least in part, and would be consistent with the hypothesis that the quality of the oocyte regulates developmental competence (Rizos *et al*., 2002).

Our current studies are aiming to understand the underlying factors that regulate conceptus elongation and to attempt to separate those intrinsic to the conceptus from those intrinsic to the uterus. In this regard, Barnwell *et al*. (2016) recently characterized differential patterns of mRNA expression between short and long bovine conceptuses recovered on day 15 of gestation which may be indicative of conceptus survival.

Acknowledgments

The work of the authors described here was provided, in part, by grants from Science Foundation Ireland (07/SRC/B1156, 10/IN.1/B3011 and 13/IA/1983) and AFRI competitive grants 2009-01722 and 2012-67015-30173 from the USDA National Institute of Food and Agriculture and R01 HD072898 from the Eunice Kennedy Shriver National Institute of Child Health and Human Development.

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