Interferon-tau and fertility in ruminants

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Abstract

Establishment of pregnancy in domestic ruminants includes pregnancy recognition signalling by the conceptus, implantation and placentation. Despite the high fertilisation success rate in ruminants, a significant amount of embryo loss occurs, primarily during early gestation. Interferon-tau (IFNT), a type I interferon that is exclusively secreted by the cells of the trophectoderm of the ruminant conceptus, has been recognised as the primary agent for maternal recognition of pregnancy in ruminants. It produces its antiluteolytic effect on the corpus luteum by inhibiting the expression of oxytocin receptors in the uterine epithelial cells, which prevents pulsatile, luteolytic secretion of progstaglandin F2\(_{α}\) by the uterine endometrium. While the importance of IFNT in maternal recognition of pregnancy and prevention of luteolysis in ruminants is unequivocal, important questions, for example, relating to the threshold level of IFNT required for pregnancy maintenance, remain unanswered. This paper reviews data linking IFNT with measures of fertility in ruminants.

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Introduction

Reproduction in ruminants, as in all mammals, is a highly complex biological process requiring a dialogue between the developing conceptus (embryo/foetus and associated placental membranes) and the maternal uterus, which must be established during the peri-implantation period for pregnancy recognition signalling and regulation of gene expression by uterine epithelial and stromal cells.

Establishing successful early pregnancy in domestic ruminants begins at the conceptus stage of development with three distinct stages of pregnancy recognition signalling, implantation and subsequent placentation required (Guillomot 1995, Spencer et al. 2007). Following ovulation, if the oocyte undergoes successful fertilisation in the oviduct, the resulting embryo enters the uterus approximately 4 days later, forming a blastocyst by Day 6–7. This blastocyst contains two-cell layers, the inner cell mass and a layer of trophodermal cells, which surround a blastocoel cavity. After hatching from the zona pellucida the blastocyst develops into an ovoid, then tubular conceptus (containing the embryo proper and the extraembrionic membranes), which begins to elongate on Day 12–14, forming a filamentous conceptus (Degrelle et al. 2005).

Although blastocysts can be produced entirely in vitro using in vitro fertilisation (IVF) and embryo culture technology in the absence of any interaction with the female reproductive tract, they must be transferred to a receptive uterus in order for the hatched blastocyst to undergo the growth and development required to form an elongated filamentous conceptus (Flechon et al. 1986, Gray et al. 2002). The uterine endometrium secretes and actively transports substances, collectively termed histotroph, that support the process of conceptus elongation via effects on trophodermal proliferation and migration, as well as attachment and adhesion to the luminal epithelium of the uterine endometrium (Spencer et al. 2007, Bazer et al. 2011).

Despite the high fertilisation success rate in ruminants (approximately 90%) after mating or artificial insemination, birth rates are significantly lower indicating the occurrence of embryonic death and fetal losses during pregnancy. Most losses occur during the peri-implantation stage of pregnancy, likely due to deficiencies in oocyte quality and early embryo development with additional losses attributed to uterine dysfunction or failure of the conceptus to develop appropriately, signal pregnancy recognition and/or undergo implantation and placentation (reviewed by Willbank et al. 2016).

Maternal recognition of pregnancy can be defined as the physiological process whereby the conceptus signals its presence to the mother and prevents the initiation of the molecular mechanisms that bring about regression of the CL. Interferon-tau (IFNT) has been recognised as the primary agent for maternal recognition of pregnancy in ruminant species (see other papers in this special...
IFNT production by the blastocyst in vitro

The expression of IFNT in bovine embryos is initiated at the time of blastocoel formation (Hernandez-Ledezma et al. 1992, 1993). Although cultured blastocysts produced by IVF continued to secrete IFNT for a few days, they failed to attach to the substratum and form outgrowths, and soon lost structural integrity. However, when Day 8 blastocysts were transferred to the uteri of synchronised cows, recovered 4 days later and individually cultured, they attached and formed outgrowths that produced large amounts of IFNT (Hernandez-Ledezma et al. 1992, 1993). Embryos thus first express IFNT when a functional trophectoderm first formed, and induction did not require a period of in vivo development. However, continued viability of the blastocyst and IFNT production were not sustained in vitro, consistent with the fact that exposure to the uterine environment is required for conceptus elongation.

Attempts have been made to correlate the amount of IFNT that is secreted by individual blastocysts to their morphological quality, but these have remained inconclusive (Hernandez-Ledezma et al. 1993, Kubisch et al. 1998). A number of factors have been shown to affect the secretion of IFNT. For example, culturing blastocysts in vitro in the absence of a protein source results in elevated mRNA levels (Wrenzycki et al. 1999), while others have reported the effects of the composition of serum-enriched media on IFNT release by blastocyst outgrowths (Stojkovic et al. 1995). Similarly, the density in which embryos are cultured, the paternal genotype and embryo manipulations, such as nuclear transfer, have been shown to affect production of IFNT (Larson & Kubisch 1999, Stojkovic et al. 1999). Blastocysts derived from embryos that had reached the eight- or four-cell stage 44 h after insemination produced significantly more IFNT than those derived from two-cell embryos (Kubisch et al. 1998). Furthermore, the day at which blastocyst formation occurs had a significant effect on IFNT secretion during the subsequent 2 days, with early blastocysts (Days 7 and 8) producing significantly less IFNT than blastocysts, which appear later in culture (Days 9 and 10) (Kubisch et al. 1998). This may seem counter-intuitive given that other studies have clearly shown the importance of the kinetics of early development and that the earliest cleaving embryos give rise to the greatest proportion of blastocysts. There may be a negative relationship between early interferon-tau production and competence.

Stojkovic and coworkers demonstrated that viable trophectodermal tissue can be maintained entirely in vitro and secretes high amounts of biologically active IFNT (Stojkovic et al. 1995). The same group (Stojkovic et al. 1999) subsequently studied trophectodermal growth and IFNT secretion of embryos with different developmental potential (in vivo-derived and in vitro-produced embryos, cloned embryos and demi-embryos) to evaluate the differences in IFNT secretion, which might explain the differences in pregnancy rates after transfer.

Thus, in vitro-derived bovine embryos secrete IFNT at the time of blastocyst formation (Hernandez-Ledezma et al. 1992), and their ability to secrete IFNT may be related to developmental competence (Kubisch et al. 1998) Studies investigating the correlation between morphological quality and IFNT secretion of blastocysts have led to conflicting results. Hernandez-Ledezma and coworkers concluded that high-quality hatched blastocysts secrete more IFNT than hatched blastocysts of poor quality (Hernandez-Ledezma et al. 1993). These results are consistent with those of Stojkovic and coworkers that hatched Day 8 blastocysts secrete significantly more IFNT than non-hatched Day 8 blastocysts (Stojkovic et al. 1995). However, Kubisch and coworkers reported that the secretion of IFNT was
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Uterine receptivity in ruminants

During pregnancy recognition, IFNT exerts its antiluteolytic effect via inhibition of transcription of the oestrogen receptor alpha gene in sheep and oxtocin receptor gene in both sheep and cattle, specifically in the endometrial luminal epithelium and superficial glandular epithelium. The absence of oxytocin receptor expression in the cells of the endometrium prevents the release of luteolytic pulses of PGF2α, thereby maintaining the CL and adequate progesterone (P4) production. In addition to this antiluteolytic action on the CL, IFNT acts in a paracrine manner to induce or enhance endometrial expression of both classical and non-classical interferon-stimulated genes (ISGs) in different cell compartments of the endometrium to facilitate uterine receptivity to implantation as well as further stimulate conceptus elongation and IFNT production (reviewed by Bazer et al. 2011, Brooks et al. 2014). Carefully orchestrated spatiotemporal alterations in gene expression within the endometrium are required for attachment of trophectoderm and implantation in all studied mammals, including ruminants. Progesterone plays a critical role in this process by stimulating and maintaining endometrial functions necessary for conceptus growth, elongation, implantation, placentation and development to term. Similar changes occur in endometrial gene expression in both nonpregnant (cyclic) and confirmed pregnant females up to the time of pregnancy recognition, suggesting that the default mechanism in the uterine endometrium is to prepare for successful early pregnancy (Forde et al. 2011b, Bauersachs et al. 2012). It is only during pregnancy recognition period, which occurs by approximately Day 16 in cattle (Forde et al. 2011b, Bauersachs et al. 2012) and Day 14 in sheep (Gray et al. 2006), that significant changes in the transcriptomic profile are detectable between cyclic and pregnant endometria. The majority, but not all (Bauersachs et al. 2012), of the changes in the endometrial transcriptome at this time are driven by increasing amounts of IFNT secreted by the filamentous conceptus.

In ruminants, high concentrations of circulating P4 in the immediate post-ovulatory period have been associated with advanced conceptus elongation (cattle: Garrett et al. 1988, Carter et al. 2008; sheep: Satterfield et al. 2006), increased IFNT production, and thus an increased likelihood of pregnancy success (cattle: Lamming & Royal 1999, Mann & Lamming 2001, Stronge et al. 2005, McNeill et al. 2006; sheep: Ashworth et al. 1989, Satterfield et al. 2006). In contrast, low P4 concentrations in the early luteal phase delay the elongation process in sheep (Nephew et al. 1991) and cattle (Forde et al. 2011a, 2012b; Fig. 1). Interestingly, however, supplementation with exogenous P4 in an attempt to accelerate conceptus development and lead to a consequent increase in IFNT production have met with modest and variable success in terms of improved pregnancy rate (reviewed by Wilbank et al. 2014, Lonergan 2015, Lonergan et al. 2016). Indeed, in a recent study, temporarily increasing P4 after timed AI decreased embryo growth during early pregnancy in lactating Holstein cows but did not affect pregnancy rate or embryo loss (Carvalho et al. 2017).

The outcome of the carefully orchestrated changes in the endometrial transcriptome as the oestrous cycle progresses is modification to the secretions and transport of substances (e.g., glucose, amino acids, proteins) from the endometrium into the uterine lumen, which regulates conceptus survival and elongation via effects on trophoblast proliferation, migration, attachment and adhesion. Some, but not all of these mechanisms are conserved between cattle and sheep (Forde & Lonergan 2012).

Actions of IFNT on CL lifespan

While IFNT is secreted in appreciable amounts over a period of a week or so, acute exposure of the uterus is sufficient in some cases to establish pregnancy. Both cattle (Betteridge et al. 1980) and sheep (Moor & Rowson 1966b) conceptuses are capable of preventing luteolysis within a day or so of transfer.
Embryo removal studies in sheep (Moor & Rowson 1966a) and cattle (Northey & French 1980) highlighted the period of maternal recognition as a critical stage in embryo-uterine-ovarian axis. Studies utilizing embryo removal and transfer established that ovine embryos affect luteal lifespan by Day 13 after mating (Moor & Rowson 1966a). Furthermore, homogenates of Day 13 and 14 ovine conceptuses contained substances that prolonged luteal lifespan in nonpregnant ewes (Rowson & Moor 1967). Similarly, in cattle, Northey and French reported that removal of conceptuses at Day 17, 18 or 19 prolonged luteal lifespan compared to nonbred controls or when conceptuses were removed on Days 13 or 15 of pregnancy (Northey and French 1980). Furthermore, intrauterine infusions of homogenised 17- and 18-day-old conceptuses lengthened the inter-oestrus interval by delaying regression of the CL. The authors concluded that between Days 15 and 17 of pregnancy, the bovine conceptus produces a substance, which we now know to be IFNT, that prolongs the functional lifespan of the CL. Betteridge and coworkers described the collection of bovine embryos between Days 10 and 16 after oestrus (Day 0) and their transfer to recipients between Days 10 and 17 (Betteridge et al. 1980). Although numbers of recipients per group were low, the data are nonetheless informative. Transfer of 2 embryos (to maximise the luteotrophic or antiluteolytic effects of the conceptus) as late as Day 16 after oestrus led to normal pregnancy. While Day 17 transfers could prolong the luteal phase in some recipients, none of the Day 17 recipients was pregnant at Day 42.

In sheep, a delay in recovering embryos after Day 12 extended the CL lifespan (Moor & Rowson 1966b). Transfer of Days 5–9 embryos into uteri of cyclic ewes on Day 5 of the oestrous cycle extended luteal life span (Moor & Rowson 1966b). If conceptuses from Day 12–13 were transferred into recipient ewes later than Day 12 post oestrus, the chances of establishing and maintaining pregnancy were drastically reduced (Moor & Rowson 1966a,b). Thus, the uterus is programmed to receive a pregnancy recognition signal by Day 12 in order to abrogate the mechanisms that bring about luteolysis in sheep. Hansen et al. (1985) reported an inter-oestrous interval of 19 days when conceptuses were flushed on Day 13 and an extension of this interval to 35 days when conceptuses were flushed from the pregnant uterus on Day 17, suggesting that exposure to the conceptus-derived IFNT is necessary until Day 17 for significant extension of the oestrous cycle.

Twice-daily infusions or injections of IFNT (Vallet et al. 1988) or recombinant ovine (ro) IFNT (Martal et al. 1990) into the uterus of sheep for 2–8 days extends luteal life span to 22–27 days post oestrus. Similarly, uterine vein infusion of roIFNT extends luteal life span in ewes (Bott et al. 2010). This transient extension of the oestrous cycle was thought to be regulated by paracrine actions of IFNT on the endometrium and until recently, conceptus-derived IFNT was thought to act exclusively within the uterus. However, interferon-stimulated genes (ISG) are expressed in peripheral blood mononuclear cells (PBMC) after initial IFNT signalling in pregnant ruminants (Han et al. 2006, Gifford et al. 2007) and pregnancy and IFNT also induce ISG within the CL (Oliveira et al. 2008). In support of this notion of an acute action of IFNT, transcriptomic studies have shown that while there is a dynamic temporal pattern to the endometrial transcriptome in cattle up to the time of pregnancy recognition, few if any differences exist in the gene expression pattern of the endometrium of pregnant and cyclic heifers (Forde et al. 2011b, 2012a). However, from Day 15 (Bauersachs et al. 2012) to Day 16 (Forde et al. 2011b) onwards, significant conceptus-derived deviations occur, predominantly but not exclusively driven by IFNT. Similar data are available in sheep (reviewed by Brooks et al. 2014).

**Interest in trophoblastic vesicles**

As mentioned previously, homogenates of 17- to 18-day-old bovine conceptuses injected into the uteri of heifers on Day 14–15 of the oestrous cycle prolong the cycle by about 3–4 days by delaying regression of the CL (Northey & French 1980). Furthermore, the bovine embryo must be present in the uterus before Day 16 to prevent luteolysis (Betteridge et al. 1980). Martal et al. (1979) showed that homogenates of 14- to 16-day-old entire conceptuses injected into the uteri of cyclic ewes were able to extend the lifespan of the CL for >1 month, in agreement with earlier report of Rowson and Moor (1967). They suggested that an antiluteolytic substance extracted from the homogenate, called trophoblastin, likely corresponding to the protein X described by Godkin and coworkers, might be secreted by the trophoblast rather than the embryonic disc cells (Godkin et al. 1982).

Heyman and coworkers described a method for obtaining so-called trophoblastic vesicles (TVs, conceptuses from which the embryonic disc had been removed) derived from Day 14 bovine or Day 11–13 ovine conceptuses and showed that this tissue was able to develop in vitro for at least 5 days, maintaining some of its physiological activity (e.g., luteolysis inhibition) after transfer (Heyman et al. 1984). In cows, the CL was maintained in 8 of 12 recipients, which had 25- to 37-day cycles, while in ewes, the CL was maintained in 7 of 12 recipients, which had 20- to 54-day cycles. Camous and coworkers cultured embryos for 3–4 days in the absence or presence of TV and showed that TV promoted the in vitro development of early bovine embryos (Camous et al. 1984). Subsequently, support for the development of bovine embryos by secretions from TVs was shown by Stojkovic and coworkers who reported that serum-free
TV-conditioned medium contains potent embryotrophic factors, which support high blastocyst rates in vitro (Stojkovic et al. 1997).

To produce TV in sheep, Flechon and coworkers dissected 12-day-old ovine blastocysts into pieces and cultured them in vitro for 24 h (Flechon et al. 1986). While these TV survived in vitro for up to 10 days, successful elongation did not occur. In contrast, after they were transferred surgically into the uterine horn ipsilateral to the CL on Day 12 of the oestrous cycle, these TV elongated in 5 out of 7 recipient ewes slaughtered on Day 17. These data demonstrate that trophoblast elongation, in the sheep at least, is not necessarily dependent on the presence of the embryo proper, but can occur in TV composed only of the trophectoderm and the extraembryonic endoderm.

Heyman and coworkers determined if the loss of embryo viability due to freezing could be overcome by supplementation with trophoblastic tissue at the time of embryo transfer (Heyman et al. 1987). Forty-nine recipient heifers each received one frozen blastocyst plus two frozen TV. Pregnancy rates by Day 45, 60 and 90 were 73, 61 and 57% respectively. In a control group of 53 recipients that received only a frozen blastocyst, pregnancy rates for the same periods were 43, 42 and 40% respectively. The authors concluded that addition of TV to frozen embryos contributed to luteal maintenance in recipients and likely magnified the intensity of embryonic signals resulting in improved pregnancy rates.

Stojkovic and coworkers studied trophoblast growth and IFNT secretion from embryos with different developmental potential (in vivo-derived, in vitro-produced, cloned and semi-embryos) to evaluate if the ability to secrete IFNT might be responsible for differences in pregnancy rate after transfer of such embryos (Stojkovic et al. 1999). Incidentally, more recent studies have shown that such conceptsuses at around Day 18–20 elicit differential responses from the endometrial transcriptome, which may reflect their subsequent viability (Bauersachs et al. 2009, Mansouri-Attia et al. 2009).

### Conceptus size and progesterone studies

As mentioned earlier, IVF studies have demonstrated that contact with the female reproductive tract is not necessary for the embryo to develop to the hatched blastocyst stage. However, the characteristic post-hatching elongation of the conceptus, which occurs prior to maternal recognition of pregnancy and implantation is dependent on secretions from the uterus as demonstrated by the fact that it does not occur in vitro (Flechon et al. 1986) and does not occur in vivo in the absence of uterine glands and reduced luminal epithelial layer (Gray et al. 2002, Spencer & Gray 2006).

Attempts to induce elongation in vitro, for example, by culturing blastocysts in confined spaces (Brandao et al. 2004, Alexopoulos et al. 2005, Zhao et al. 2015), have thus far failed to recapitulate events exactly as they occur in vivo.

The importance of maternal P4 in the regulation of conceptus growth and development has been appreciated for some time based on earlier studies in ewes (Wilmut & Sales 1981, Lawson & Cahill 1983) and cows (Garrett et al. 1988). More recent studies (Satterfield et al. 2006, Forde et al. 2009) have reaffirmed its importance and made significant progress in unravelling the biology underlying this process. In particular, significant progress has been made in our understanding of how P4 modifies the endometrial transcriptome and the resulting effects on the ability of the uterus to drive the elongation process (see reviews by Spencer et al. 2015, Lonergan et al. 2016). The stimulatory effect of P4 on trophoblast elongation, although indirect via the endometrium, is unequivocal. This effect is 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, 2012b) resulting in changes in the composition of ULF to which the developing embryo is exposed (Faulkner et al. 2013) (Fig. 1).

There is little convincing evidence that P4 has a direct effect on early embryo development. For example, in our own laboratory, culture of embryos in vitro in the presence of P4 did not affect the proportion that developed to the blastocyst stage (Clemente et al. 2009). This is consistent with the observations of others including Larson and coworkers who failed to observe a direct effect of P4 on embryo development when cultured with P4 either from Days 1 to 3 or 4 to 7 after fertilisation (Larson et al. 2011). Furthermore, embryo exposure to P4 in vitro had no effect on conceptus elongation after blastocysts were transferred 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. For example, no differences in embryo development on Day 5 or Day 7 were observed when beef heifers were supplemented with exogenous P4 from Day 3, despite evidence of advanced conceptus elongation between Days 13 and 16 of pregnancy (Carter et al. 2008). In a follow-up study in which multiple in vitro-produced embryos were transferred to the oviducts of beef heifers that did or did not receive a P4 insert on Day 3 after onset of oestrus, no effect of P4 on the proportion of embryos that developed to the blastocyst stage was noted (Carter et al. 2010).

Despite this lack of evidence for an effect of P4 on the early embryo, the stimulatory effects of elevated P4 shortly after conception on conceptus elongation have been convincingly demonstrated in cattle and sheep. For example, administration of 100 mg of P4 on Days 1, 2, 3 and 4 of pregnancy increased P4 concentrations in peripheral plasma on Days 2–5 and resulted in a 10-fold increase in the proportion of embryos that developed to the blastocyst stage (Stojkovic et al. 1999).
increase in mean conceptuses length on Day 14 (Garrett et al. 1988). Insertion of an intravaginal P4 pessary on Day 3 of pregnancy significantly elevated plasma P4 concentrations until Day 8, and this was associated with larger conceptuses on Day 16 (Carter et al. 2008). Similarly, when ewes received daily injections of 25 mg P4 from 36 h post mating, blastocyst diameter increased by 220% on Day 9 and accelerated conceptus elongation (Satterfield et al. 2006); these effects of P4 treatment on blastocyst development were blocked by administration of RU486, a P4 receptor antagonist.

The embryo does not need to be present in the uterus during the period of P4 elevation in order to benefit from it, as determined by accelerated conceptus elongation (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). In addition, reducing P4 production by the CL, for example, by treatment with PGF2α (Beltman et al. 2009, Forde et al. 2011a, 2012) or by aspiration of the preovulatory follicle just before the expected time of ovulation (O’Hara et al. 2012), results in a delay in the temporal changes that occur during the cycle in the endometrial transcriptome and subsequent delayed conceptus elongation.

Supplementation with exogenous P4 advances the temporal changes in endometrial gene expression that occur normally (Forde et al. 2009) such that conceptus elongation is advanced (Carter et al. 2008, Clemente et al. 2009, O’Hara et al. 2014a). Similarly, supplementation with P4 can advance uterine receptivity in recipients that are ‘out of sync’ with the embryo (e.g., Day 8 blastocyst transferred to a Day 5 uterus, Geisert et al. 1991). Likewise, transfer of embryos to an ‘advanced’ uterus (e.g., Day 7 blastocyst to a recipient on Day 9) results in accelerated conceptus elongation (Ledgard et al. 2012, Randi et al. 2015).

Many groups have attempted to supplement cows with exogenous P4 after AI in order to increase fertility, resulting in inconsistent outcomes. Results from various studies have reported an increase (Monteiro et al. 2014), no change (Stevenson et al. 2007, Monteiro et al. 2015) or a decrease (Parr et al. 2014) in pregnancy per AI (P/AI) for cows supplemented with exogenous P4 after ovulation compared with untreated controls. Although several studies evaluated the effect of decreasing P4 concentrations after ovulation on the uterine environment and embryo development (Forde et al. 2011, 2012), few studies have evaluated the effect of decreasing P4 after ovulation on establishment of pregnancy (Kenyon et al. 2013, Carvalho et al. 2017). In the latter study, no pregnancies occurred after transfer of embryos into the uterus of cows manipulated to have low P4 concentrations. Interestingly, decreasing P4 from 6 to 11 days after timed AI (TAI) did not have a major effect on fertility or pregnancy loss (Carvalho et al. 2017), challenging the notion that increasing P4 early after AI might be a practical strategy to increase fertility in lactating dairy cows.

**Asynchronous embryo transfer**

Synchrony between the needs of the developing embryo and uterine secretions has long been recognised as being critical to the successful establishment of pregnancy (Pope 1988). Embryo transfer studies in sheep and cattle have clearly demonstrated the need for close synchrony between the embryo and the uterus environment of the recipient with pregnancy reduced rates when embryos are greater than 48 h from synchrony with the recipient’s uterus (Moore & Shelton 1964, Rowson & Moor 1966, Rowson et al. 1972).

Results of studies involving the transfer of embryos to a uterus that is ‘out of sync’ (i.e., asynchronous) demonstrate the regulatory effect of the uterus on conceptus development and the role played by P4. 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 and coworkers 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 (Geisert et al. 1991).

Administration of P4 early in the oestrous cycle of the recipient has been shown in some cases to advance uterine receptivity for the transfer of older embryos. For example, exposure of Day 6 recipient ewes to exogenous P4, prior to the transfer of Day 10 blastocysts supported development as in study by Lawson & Cahill (Lawson & Cahill 1983). In cattle, embryo transfer to P4-treated recipients, which showed oestrus 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 previously, only approximately 5% of Day 5 asynchronous recipients became pregnant (Geisert et al. 1991).

Our laboratory has generated similar data (Randi et al. 2015). 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 and coworkers (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 shortened the length of the oestrous cycle 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, and the data outlined above would support the notion 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 our own study (Randi et al. 2015), in which 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 the 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%) resulted in lower pregnancy rates compared to synchronous controls. In summary, the accelerated conceptus elongation, and presumably dramatically increased IFNT secretion, 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.

**Interferon-stimulated genes and pregnancy diagnosis**

As some IFNT escapes the uterus and can be detected in blood (Oliveira et al. 2008, Bott et al. 2010), the response of peripheral blood leukocytes to IFNT by expression of ISG has been used as an early pregnancy diagnosis (Han et al. 2006, Gifford et al. 2007, Stevenson et al. 2007, Green et al. 2010). In addition to its use for pregnancy detection, measurement of ISG expression has also been used for evaluating embryo survival/mortality (Han et al. 2006, Wijma et al. 2016).

Little is known regarding the amount of IFNT that is required for successful establishment of pregnancy. Matsuyama and coworkers reported a positive correlation between levels of ISG (ISG15, MX2) mRNA in PBMC and the quantity of IFNT (0, 500 or 1000 µg) infused into the uterine horn ipsilateral to the CL 16 or 17 days after oestrus (Matsuyama et al. 2012). This would suggest that the quantity of conceptus-produced IFNT could be monitored by measuring ISG mRNA in PBMC, potentially providing a tool to monitor embryo mortality. One issue with this is ISG in circulation can be induced by other type I interferons e.g. IFN alpha, which are expressed not due to the presence of the conceptus, but due to an inflammatory response, which can make interpretation of the ISG expression in circulation, at times, difficult.

The decline in P4 concentration is an immediate response to PGF2α and the embryo may survive longer than the CL on the basis of the extended period of ISG expression (Kose et al. 2016). This suggests that the absence of P4 could be the reason for embryonic loss rather than a direct effect of PGF2α. Kose and coworkers in sheep reported that the expression of ISG, including ISG15, RTP4 and MX1, but not B2-microglobulin, in PBMC may serve as a marker of embryonic loss (Kose et al. 2016).

Ribeiro et al. (2016) evaluated the effect of disease on preimplantation conceptus development as well as secretion of IFNT and transcriptome and the effect of diseases before AI on the transcript expression of ISG in PBMC during peri-implantation stages of conceptus development after first AI postpartum. Inflammatory disease before breeding reduced fertilisation of oocytes and development to morula, and impaired early conceptus development to elongation stages and secretion of IFNT in the uterine lumen. Diseases...
caused inflammation-like changes in transcriptome of conceptus cells, increased risk of pregnancy loss and reduced pregnancy or calving per breeding. Reduced oocyte competence is a likely reason for carryover effects of diseases on developmental biology, but impaired uterine environment was also shown to be involved.

Variation in conceptus size
As mentioned earlier, conceptus development involves transition from a spherical blastocyst on Day 7 of gestation, through intermediate ovoid (Day 12–13) and tubular (Day 14–15) forms, to a filamentous conceptus on Day 16–17 (Guillomot 1995, Degrelle et al. 2005). During elongation, the conceptus increases more than 1000-fold in size (Betteridge et al. 1980, Grealy et al. 1996), associated with an increase in total protein content (Grealy et al. 1996, Morris et al. 2000). Trophoblast length doubles daily between Days 9 and 16, with a particularly sharp increase from Day 13 to 14 (Berg et al. 2010). As IFNT is produced solely by the conceptus trophectoderm, and there is a strong positive correlation between conceptus length and IFNT secretion (Kerbler et al. 1997, Rizos et al. 2012), conceptuses must therefore reach an adequate size in order to produce sufficient quantities of IFNT to prevent luteolysis. Disproportionally, short conceptus length may ultimately result in a failure of the pregnancy recognition response (Berg et al. 2010).

Numerous studies have reported a wide variation in length among conceptuses recovered on the same day, even from the same uterine environment (Clemente et al. 2009, O’Hara et al. 2014a,b). Such variation in trophoblast length (Fig. 2) among age-matched conceptuses is likely to reflect differences in developmental competence, although this is yet to be formally tested by re-transferring such conceptuses to examine pregnancy rates.

Blastocyst cell number at Day 7 is predictive of conceptus length at Day 14 (O’Hara et al. 2014b). In addition, there is a strong correlation between conceptus size and IFNT production in vitro (Rizos et al. 2012) and in vivo (Kerbler et al. 1997, Mann & Lamming 2001). Differences in conceptus length on the same day of gestation may be related to an inherent lack of developmental competence or may simply be a consequence of asynchrony with the maternal environment (Pope 1988). Barnwell and coworkers characterised differential patterns of mRNA expression between short (mean length of 4.2 ± 0.1 mm) and long (24.7 ± 1.9 mm) bovine conceptuses recovered on Day 15 of gestation (Barnwell et al. 2016). A total of 348 genes were differentially expressed (221 genes upregulated and 127 downregulated in long compared to short conceptuses) suggesting inherent differences exist between such conceptuses and not just differences in the conceptuses’s ability to produce IFNT.

Conclusions
An increased knowledge of conceptus–endometrial interactions during early pregnancy in ruminants is necessary to understand and elucidate the causes of infertility and early pregnancy loss and provide new strategies to improve reproductive efficiency. Questions remaining unanswered include how much IFNT is required to maintain a pregnancy. Given the fact that IFNT secretion is correlated with conceptus length, one would hypothesise that larger conceptuses are more developmentally competent. However, this has not been adequately tested and the limited data that exist relating to larger conceptuses would suggest that this may not be the case.

Thus, whether the ability of a ruminant conceptus to produce IFNT is reflective of its capacity to establish and maintain pregnancy has not been clarified. Studies from our group involving the transfer of multiple (10–20) blastocysts to synchronised recipients in order to generate elongating conceptuses have revealed a large variation in conceptus size on a given day, despite being in the same uterine environment. While much of this variation may be due to oocyte quality-related factors, whether small conceptuses are less developmentally competent than age-matched larger conceptuses remains to be tested.

Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research reported.

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