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Effect of bovine blastocyst size at embryo transfer on day 7 on conceptus length on day 14: Can supplementary progesterone rescue small embryos?

Lydia O'Hara, Niamh Forde, Alan K. Kelly, Patrick Lonergan*

School of Agriculture and Food Science, University College Dublin, Belfield, Dublin, Ireland

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ABSTRACT

Conceptus size on Day 14 after multiple embryo transfer of Day 7 *in vitro*–produced blastocysts varies greatly within animal. One explanation for this variation may be related to blastocyst cell number at the time of transfer. The aim of this study was to examine the effect of Day 7 blastocyst cell number on Day 14 conceptus size and to examine the effect of progesterone (P4) supplementation on embryo development after the transfer of Day 7 blastocysts containing a low total cell number. The estrous cycles of crossbred beef heifers were synchronized using an 8-day progesterone (P4)–releasing intravaginal device (PRID) with the administration of a prostaglandin $F_{2\alpha}$ analog on the day before device removal. Only those heifers recorded in standing estrus (Day 0) were used. Heifers were randomly assigned to one of four treatment groups: (1) control: large blastocysts (high total cell number), (2) control: small blastocysts (low total cell number), (3) small blastocysts plus a single intramuscular injection of 3000 IU human chorionic gonadotropin (hCG) on Day 2 after estrus, or (4) small blastocysts plus insertion of a vaginal P4 insert (PRID, 1.55 g P4) between Days 3 and 5 after estrus. *In vitro*–produced blastocysts were transferred to each heifer on Day 7 ($n = 10$ blastocysts per heifer), and conceptuses were recovered at slaughter on Day 14. Daily blood samples were collected from Day 0 to 14 to measure serum P4 concentrations. Data were analyzed using the PROC MIXED procedure of SAS. Total cell number on Day 7 was significantly lower in small versus large blastocysts (72.4 ± 3.93 vs. 144.8 ± 3.90, $P < 0.05$). Conceptus recovery rate was 53.8% overall (140 of 260) and was highest in the large blastocyst group (68.3%, 41 of 60) compared with the other groups (45.7%–55.0%). Concentrations of serum P4 were similar in the two unmanipulated recipient groups but were significantly elevated ($P < 0.05$) by Day 8 in the hCG-treated heifers and on Days 4 and 5 in the PRID group ($P < 0.003$). In the absence of supplemental P4, Day 14 conceptuses resulting from the transfer of small blastocysts (2.48 ± 0.54 mm) were smaller than those from large blastocysts (3.32 ± 0.52 mm). Administration of hCG on Day 2 approximately doubled conceptus length on Day 14 (4.94 ± 1.15 mm; $P < 0.05$), whereas insertion of a PRID from Days 3 to 5 increased conceptus length approximately fivefold (13.09 ± 2.11 mm; $P < 0.05$) compared with controls. In conclusion, results indicate that supplemental P4 is capable of "rescuing" poor-quality blastocysts, presumably via the now well-described actions on the endometrium and consequent effects on uterine lumen fluid composition.

1. Introduction

Elevated progesterone (P4) concentrations in the first to second weeks postconception stimulate conceptus

* Corresponding author. Tel.: +353 1 601 2147; fax: +353 1 628 8421.
E-mail address: pat.lonergan@ucd.ie (P. Lonergan).

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elongation, leading to increased interferon-τ (IFNT) production, and in some cases, are associated with higher pregnancy rates in cattle and sheep [1–3]. Elevated P4 through insertion of a P4-releasing intravaginal device (PRID) on Day 3 after estrus results in an earlier loss of the P4 receptor [4] from the luminal epithelium and an advancement in the normal temporal changes that occur in the endometrial transcriptome during early pregnancy [5], the consequence of which is an advancement in conceptus elongation [1]. The embryo does not need to be present in the uterus during the period of P4 elevation to benefit from it, indicating that the effect of P4 on conceptus development is mediated exclusively through the endometrium, presumably via altered uterine lumen fluid composition [6]. Recently, we have shown that P4 supplementation by insertion of a PRID for as little as 2 days is sufficient to elevate circulating P4 concentrations and increase conceptus elongation and IFNT production after artificial insemination or embryo transfer [7].

An alternative strategy to increase P4 is to stimulate the corpus luteum (CL) using a luteotropic agent. Administration of human chorionic gonadotropin (hCG) on Day 5 postestrus induces ovulation of a dominant follicle forming an accessory CL, leading to an increase in circulating P4 concentrations [8,9]. We have recently shown that administration of a single injection of hCG as early as Day 2 leads to an increase in the volume of luteal tissue in the endogenous CL, resulting in increased P4 concentrations form Day 6 onward, which may be of benefit to the development of the early embryo [10].

The route to achieve elevated P4 is of critical importance as supplementation with exogenous P4 (injection, insert) can have a detrimental effect on the life span of the CL, leading to short cycles [11–13] because of the effect of elevated P4 on luteinizing hormone (LH) pulsatility [13]. This leads to the anomalous situation in which exogenous P4 stimulates conceptus elongation and at the same time compromises the survival of the CL. Luteotropic treatments that stimulate the CL directly, such as hCG, are less likely to have a negative effect on CL life span; indeed, administration of hCG at the time of P4 injection negated the negative effect of P4 on CL life span [11].

In many of the studies examining the relationship between P4 and conceptus elongation, we have used a multiple embryo transfer model involving the transfer of multiple blastocysts (10–20 per recipient) on Day 7 and the recovery of Day 14 conceptuses at slaughter [6,14–16]. Although P4 treatment increases the mean size of the conceptus, there is still a significant variation in conceptus size within animal [6]. One possible explanation for this variation could be variation in blastocyst cell number at the time of transfer.

The present study was designed to test the hypothesis that supplemental P4, through hCG administration on Day 2 or insertion of a PRID between Days 3 and 5, would promote elongation of a small blastocyst with a low total cell number at the time of transfer, that is, P4 "priming" of the uterine environment can overcome the decreased developmental potential of embryos with small cell number.

2. Materials and methods

All experimental procedures involving animals were licensed by the Department of Health and Children, Ireland. Protocols were in accord with the Cruelty to Animals Act (Ireland 1876) and the European Community Directive 86/609/EC and were sanctioned by the Institutional Animal Research Ethics Committee.

The experimental design is illustrated in Figure 1. All animals were housed indoors on slats for the duration of the experiment and were fed a diet consisting of grass and maize silage supplemented with beef ration. The estrous cycles of crossbred beef heifers (n = 33, mean age 23.5 ± 0.39 months, mean weight 603.30 ± 5.68 kg) were synchronized using an 8-day PRID (1.55 g P4; Ceva Sante Animal, Libourne, France) with intramuscular administration of a prostaglandin F2 α analog (2 mL Estrumate; Schering-Plough Animal Health, Hertfordshire, UK, equivalent to 0.5 mg cloprostenol) given on the day before P4 device removal. Heifers were checked for signs of estrus four times per day commencing 30 hours after P4 device withdrawal. Only those seen in standing estrus (n = 26) were used in the experiment and randomly assigned to one of four treatment groups: (1) control A: large blastocysts (high total cell number), (2) control B: small blastocysts (low total cell number), (3) small blastocysts plus a single intramuscular injection of 3000 IU hCG (Chorulon; Intervet, Boxmeer, The Netherlands) on Day 2 after estrus [10], or (4) small blastocysts plus insertion of a vaginal P4 insert (PRID) between Days 3 and 5 after estrus. Jugular vein blood samples were collected daily from Day 0 (estrus) to slaughter on Day 14 to determine circulating P4 concentration.

After collection, blood samples were refrigerated (4 °C) for 12 to 24 hours before being centrifuged at 1500× *g* at 4 °C for 20 minutes. Serum was separated and stored at −20 °C until analysis of P4 concentration by solid-phase radioimmunoassay using a Coat-A-Count Progesterone kit (Siemens Medical Solutions Diagnostics, Los Angeles, CA, USA) as previously described [17]. The sensitivity of the

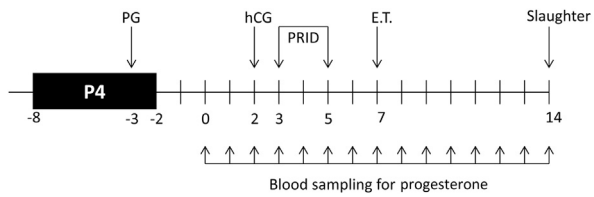

Fig. 1. Experimental design. The estrous cycles of crossbred beef heifers were synchronized using a progesterone (P4)–releasing intravaginal device with administration of a prostaglandin $F_{2\alpha}$ analog on the day before device removal. Heifers were randomly assigned to one of four treatment groups and received blastocysts as follows: (1) control: large blastocysts (high total cell number, n = 6), (2) control: small blastocysts (low total cell number, n = 6), (3) small blastocysts plus a single intramuscular injection of 3000 IU human chorionic gonadotropin on Day 2 after estrus (n = 7), or (4) small blastocysts plus insertion of a vaginal P4 insert (PRID, 1.55 g P4) between Days 3 and 5 after estrus (n = 7). Ten *in vitro*–produced blastocysts were transferred to each heifer on Day 7, and all heifers were slaughtered on Day 14 after estrus. Daily blood samples were collected between Day 0 and Day 14 to determine circulating P4 concentrations.