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Effects of time of progesterone supplementation on embryo development and interferon-τ production in the cow

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Abstract

We have investigated the effects of the timing of progesterone supplementation on early embryo development in mature, non-lactating Holstein–Friesian cows. Animals were inseminated 72 h (day 1) and 96 h following prostaglandin injection and were either left as untreated controls ($n = 6$) or received progesterone supplementation from either days 5 to 9 (early; $n = 6$) or from days 12 to 16 (late; $n = 6$). Daily plasma samples were collected until day 16, when cows were slaughtered and reproductive tracts recovered and flushed to collect embryos and to measure interferon-τ activity. Both early and later progesterone supplementation resulted in marked increases in plasma progesterone ($P < 0.01$). Early, but not late, progesterone supplementation resulted in a fourfold increase in trophoblast length ($P < 0.01$) and a sixfold increase in uterine concentration of interferon-τ ($P < 0.05$). The results demonstrate that progesterone supplementation during the postovulatory rise, but not later in the luteal phase, increases embryo development and interferon-τ production.

Keywords: Cow; Progesterone; Embryo; Pregnancy; Interferon

1. Introduction

In the cow, the successful establishment of pregnancy depends on the embryo developing sufficiently well to produce adequate quantities of the anti-luteolytic protein, interferon-τ (for reviews see Thatcher et al., 1995; Mann et al., 1999). One of the key hormones in the control of embryo development is progesterone, which stimulates the production of the endometrial secretions necessary for the successful development of the embryo (see Geisert et al., 1992, for review). Low progesterone has been linked to early pregnancy failure (see Mann et al., 1999, for review) and poor embryo development (Walton et al., 1990) while supplementing cows with progesterone has been shown to enhance conceptus development (Garrett et al., 1988).

Studies in which progesterone has been administered in an attempt to improve pregnancy rates have yielded variable results. In these studies, progesterone supplementation initiated at the time of onset of the postovulatory rise (between days 4 and 5) has resulted in consistent increases in pregnancy rate. However, when supplementation has been initiated later than this, consistent improvements have not been reported (for review see Mann and Lamming, 1999). Furthermore, in a recent study we found a close association between the specific pattern of maternal progesterone secretion and the development of the early embryo, poor embryo development being associated with both a delayed postovulatory progesterone rise and a low subsequent luteal phase concentrations (Mann and Lamming, 2001).

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In the present study, we have investigated the importance of the time of progesterone supplementation on the development of the embryo in the cow by treating cows with exogenous progesterone at two stages of the luteal phase. The principle aim was to determine whether different effects on embryo development support reported differences in the effectiveness of different times of progesterone supplementation on pregnancy rates.

2. Materials and methods

2.1. Experimental protocol

The study was performed in 18 mature non-lactating Holstein–Friesian cows. All cows had calved at least once and were obtained from the University of Nottingham commercial herd at the end of lactation. Throughout the study, cows were maintained on a diet of hay and concentrate pellets. All experimental procedures were carried out under an appropriate United Kingdom Home Office project licence.

The oestrous cycles of all cows were synchronised by two injections of the prostaglandin (PG) $F_{2\alpha}$ analogue, cloprostenol (Estrumate; Schering-Plough Animal Health) administered 11–13 days apart. All cows were inseminated 72 and 96 h following the second injection of cloprostenol by Genus technicians (Genus plc) using semen from beef bulls. Cows were then allocated to receive no progesterone supplementation (control, $n = 6$), progesterone supplementation from days 5 to 9 (early, $n = 6$) or progesterone supplementation from days 12 to 16 (late, $n = 6$). Progesterone supplementation was via an intravaginal CIDR-B device containing 0.95 g progesterone (InterAG, New Zealand) inserted at 0900 h on the day of treatment initiation and withdrawn at 0900 h on the day treatment ceased. Prior to the second PG injection all cows underwent jugular vein cannulation to allow collection of daily blood samples.

On day 16, cows were slaughtered by captive bolt and exsanguination and the reproductive tract collected and transported to the laboratory. The uterine horns were dissected free from surrounding tissues and separated past the bifurcation. The horn adjacent to the corpus luteum was then flushed with 20 mL saline into a single dish and the embryo collected and its length measured. Uterine flushings were then frozen at −20 °C until subsequent analysis for interferon-τ content.

2.2. Hormone assays

Progesterone was measured in plasma by previously described radioimmunoassay (Law et al., 1992). Intra- and inter-assay coefficients of variation were 6.3% and 9.8% and a sensitivity of the assay was 0.5 ng/mL. Interferon-τ activity of samples was measured by an Mx/CAT reporter gene assay based on Madin Darby Bovine Kidney cells transfected with plasmid, p123-intron containing a chloramphenicol acetyltransferase (CAT) expression unit linked to a human MxA promoter (Fray et al., 2001). The potencies of the unknown samples were calculated relative to recombinant bovine IFN-α_1, the anti-viral activity of which had previously been determined in an anti-viral assay and compared against the first international reference preparation of human leucocyte IFN (69/019). CAT expression was determined using a commercial ELISA kit (Roche Molecular Biochemicals). Sensitivity was 0.5 anti-viral units (avu) interferon-τ/mL and the inter assay coefficient of variation 9.5%.

2.3. Statistical analysis

Plasma concentrations of progesterone were analysed by repeated sample analysis of variance. Differences between groups in embryo length and level of interferon-τ were analysed by analysis of variance. The relationship between embryo length and interferon-τ production was analysed by regression analysis.

3. Results

During the periods when progesterone was not supplemented, plasma concentrations were similar between groups (Fig. 1). During supplementation, progesterone was elevated ($P < 0.01$) in the early group on days

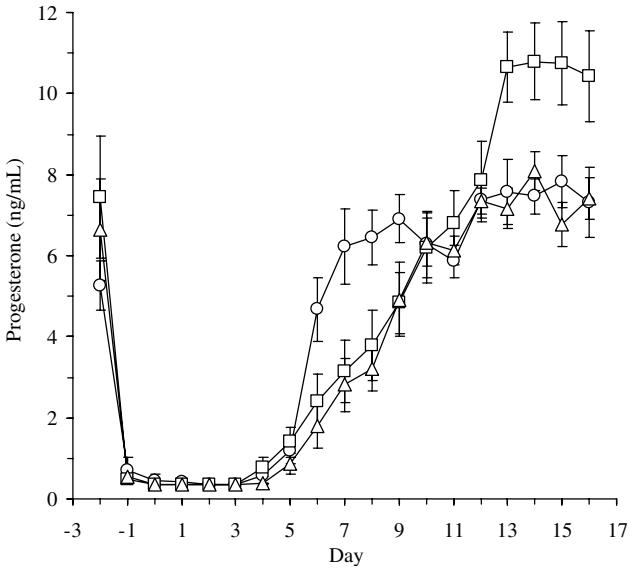

Fig. 1. Means (±SEM) plasma concentration of progesterone in inseminated cows either untreated (control; △; $n = 6$) or supplemented with progesterone via an intravaginal CIDR from days 5 to 9 (early; ○: $n = 6$) or from days 12 to 16 (late; □; $n = 6$).