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Ovarian response to hCG treatment during the oestrous cycle in heifers*

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Summary. The aims of this study were to investigate whether treatment with a single ovulatory dose of hCG, between the day of oestrus and the end of the luteal phase, could induce extra ovulations in heifers and whether the presence of an existing corpus luteum (CL) affected the response. Heifers (N = 32) were injected with 1500 i.u. hCG or saline on a given day of the oestrous cycle. Treatments were repeated during subsequent cycles to provide a total of 71 observations, 57 of which followed an injection of hCG, given between Day 0 (oestrus) and Day 16, and 14 of which followed saline injections as controls. Ovulatory responses were noted by laparoscopy 2 days after hCG treatment. No heifers injected with saline produced additional CL. Of the hCG-treated cycles, 23 resulted in the formation of an additional CL, and this was significantly affected by the stage of the oestrous cycle when hCG was given; a greater response was observed during the early (Days 4–7) and late (Days 14–16) stages of the luteal phase than at the mid-luteal phase of the oestrous cycle. Two heifers were also treated with hCG on Days 17 or 18 of the oestrous cycle, but before oestrus; both had induced CL. There were no significant differences between the left–right orientation of the existing CL or the hCG-induced CL.

These results demonstrate that the large, luteal-phase follicle of the cow is capable of ovulating in response to hCG and that the induced CL is not affected by the presence of an existing CL.

Keywords: cattle; ovulation; hCG; follicle; luteal phase

Introduction

The hypothesis that the corpus luteum (CL) plays a role in the control of ovarian follicular growth in ruminants is controversial. Most studies have examined changes in follicular populations in ovaries with or without a CL, either by dissection or by ultrasonography, rather than investigating the functional ability of large follicles present during the luteal phase.

It is known that large luteal-phase follicles have receptors for both LH and FSH on the granulosa cell layer (sheep: England *et al.*, 1981; cattle: Ireland & Roche, 1983), and are capable of secreting significant quantities of oestradiol (sheep: Baird *et al.*, 1976; cattle: Skyer *et al.*, 1987); in this, they are similar to preovulatory follicles present during the follicular phase. However, whilst removal or regression of the CL leads to ovulation, the follicles that ovulate may not necessarily be the large follicles present at the time of luteolysis (Ireland & Roche, 1982; Driancourt & Cahill, 1984).

Previous studies have been reviewed to demonstrate that there are at least three major periods of increased oestradiol secretion, primarily from mature follicles (Ireland & Roche, 1987). In

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addition, daily ultrasound examinations have shown that there are usually three periods during the oestrous cycle when dominant follicles are present (Savio *et al.*, 1988; Sirois & Fortune, 1988). These data are corroborated by the finding that the concentration of receptors for LH on granulosa cells is higher during the early luteal phase, Day 7, than during the mid-luteal phase, Day 13 (Ireland & Roche, 1983).

The aim of the present study was to assess the ability of large follicles in cattle to ovulate in response to hCG, and to determine whether this response was affected by the presence of an existing CL or by stage of the oestrous cycle.

Materials and Methods

Animals. The 32 Hereford Friesian crossbred heifers, ~2 years of age, used in this study were kept on Institute farms in Scotland. The heifers were maintained outdoors under standard husbandry conditions. Observations for behavioural oestrus were made at least twice daily, aided by a heat detection device (Kamar Inc., Steamboat Springs, CO, USA).

Treatments. The animals were randomly assigned to receive a single 5 ml intravenous injection of 1500 i.u. hCG (Chorulon; Intervet, Cambridge, UK) or saline (0·154 M-NaCl) on a specified day of the oestrous cycle. This dose was chosen because 1500–2000 i.u. hCG has been commonly used to control the time of ovulation in cattle (see Hunter, 1980). The ovulatory response was examined by sublumbar laparoscopy 2 days after hCG injection; the location of the existing CL, and the number and location of any induced CL were recorded. For laparoscopy the heifers were fasted for up to 48 h. Animals were then lightly sedated (0·75 ml i.m. Rompun; Baker UK Ltd, Bury St Edmunds, Suffolk, UK) and the area of incision was locally anaesthetized by infiltration with 5 ml 2% lignocaine (Lignocaine-A; Univet 2 Ltd, Bicester, Oxon, UK). After observations, a topical antibiotic was applied to the incision. After the first treatment cycle, animals then underwent an oestrous cycle without treatment before being randomly reassigned to receive a second dose of 1500 i.u. hCG or saline on an alternative day of the third oestrous cycle. Finally, some of the heifers were treated during the fourth oestrous cycle of the experiment. Overall, 14 heifers were treated 3 times, 16 heifers twice, and 2 animals were treated once only. On three occasions the ovaries were not observed due to the extension of the rumen into the peritoneal cavity, but of the remaining 73 observations, 59 were after hCG treatment and 14 were after saline injections.

Data analysis. The data were analysed by contingency table and χ^2 tests, with Yates' correction where necessary. The results were grouped into the following stages of the oestrous cycle: peri-oestrous phase, Days 0–3; early luteal phase, Days 4–7; mid-luteal phase, Days 8–13 and the late-luteal phase, Days 14–16. The divisions used in the present study were based on published hormonal and morphological data. The period from the day of oestrus to Day 3 has been termed the peri-oestrous phase since the animal is neither follicular nor truly luteal. The early luteal phase covers the period when plasma progesterone concentrations have started to rise, but have not reached a plateau (Wetteman *et al.*, 1972; Milvae & Hansel, 1983). A 'wave' of follicular growth has been reported at this time (Matton *et al.*, 1981), and also an elevation in mean FSH concentrations (Schams & Schallenberger, 1976). The mid-luteal phase has been characterized as having a fairly stable follicle population (Matton *et al.*, 1981) and high circulating progesterone concentrations (Schams & Schallenberger, 1976; Milvae & Hansel, 1983). The late-luteal phase can be said to start when the CL begins to regress (Hafez & Sugie, 1963), but whilst progesterone concentrations remain high (Schams *et al.*, 1977). A fresh wave of follicle growth was reported at this time (Matton *et al.*, 1981; Pierson & Ginther, 1987a). Of less importance to the present study is the follicular phase, which occurs from luteolysis until ovulation. Two of the heifers were treated with hCG on Days 17 or 18 of the cycle, but before oestrus, and both had induced CL. The results of these 2 animals were not included in the data analysis.

Results

No corpora lutea were induced in heifers injected with saline at any stage of the oestrous cycle, as judged by the age of the single CL observed at laparoscopy (Ireland *et al.*, 1980). Of the 57 cycles treated with hCG from Days 0 to 16 of the oestrous cycle, 23 resulted in the formation of induced CL (Table 1). The induced CL were easily distinguished from the existing CL during the luteal phase, as being bright red in colour and usually smaller in size. Ovulation points were also clearly visible. However, 2 of the heifers treated during the peri-oestrous period (1–2 days after oestrus) had two CL, but it was not possible to distinguish which CL had been induced by the hCG treatment and which had been induced by the preovulatory LH surge. It is likely that one of the CL had

been produced by the treatment, firstly because none of the controls had double ovulations and, secondly, the twinning rate in cattle is naturally low.

There was a significant effect of the stage of the oestrous cycle on the proportion of heifers producing induced CL (Table 1; $\chi^2 = 18{\cdot}2$, d.f. = 3; $P < 0{\cdot}001$), and this effect remained significant even when only the different stages of the luteal phase (early, mid- and late) were compared ($\chi^2 = 7{\cdot}5$, d.f. = 2; $P < 0{\cdot}05$). Before treatment all existing ovulations were singles, whereas two induced ovulations were doubles, one unilateral (late luteal phase) and one bilateral (early luteal phase). There appeared to be no carry-over effect of repeated hCG injections as treatment between the 2nd and 3rd treatment cycles had no significant effect on oestrous cycle length (controls: $20{\cdot}2 \pm 0{\cdot}5$ days (N = 4); hCG treatment with induced CL: $21{\cdot}3 \pm 1{\cdot}6$ days (N = 12); hCG treatment with no induced CL: $19{\cdot}2 \pm 0{\cdot}9$ days (N = 9)). Furthermore, 8 heifers, given saline after treatment with hCG during a previous cycle, had single ovulations. Although the first and second treatment oestrous cycles were separated by an oestrous cycle to reduce any possible carry-over effect of successive treatments, 16 animals were treated during succeeding cycles (oestrous cycles 3 and 4). In these 16 heifers there was no obvious trend as 3 heifers had induced CL in both cycles; 5 heifers had induced CL during the 3rd cycle, but no induced CL in the 4th cycle; 4 heifers had induced CL during the 4th cycle, but none on the 3rd cycle; and 4 heifers had no induced CL in either cycle. Moreover, in all subsequent oestrous cycles after the first treatment with hCG, regardless of the treatment of that cycle, all heifers had shown single, morphologically healthy corpora lutea produced at natural oestrus and ovulations; this indicates that previous hCG treatment did not affect the growth of preovulatory follicles.

Table 1. Effect of the stage of the oestrous cycle* on the number of heifers ovulating in response to 1500 i.u. hCG

	Peri-oestrus (Days 0–3)	Early luteal (Days 4–7)	Mid-luteal (Days 8–13)	Late luteal (Days 14–16)
No. of control heifers treated with saline†	6	3	3	2
No. of heifers treated with hCG	19	12	20	6
No. (%) of hCG-treated heifers with induced CL	2[a] (11)	10[b] (83)	7[a] (35)	4[b] (66)
Mean no. of induced CL per heifer responding to hCG	1·0	1·1	1·0	1·1

*Day 0 = oestrus.
†All control heifers had a single ovulation.
a *versus* b are significantly different ($P < 0{\cdot}001$).

Existing CL were equally distributed between right and left ovaries (42·3% left, 57·7% right; $P > 0{\cdot}05$). Similarly, CL induced by hCG treatment were equally distributed between right and left ovaries (40·7% left, 59·3% right; $P > 0{\cdot}05$). There was no significant effect of (i) the left/right orientation of the existing CL on the number of heifers responding to the hCG ($P = 0{\cdot}7$; d.f. = 1); (ii) the left/right orientation of the existing CL on that of the induced CL ($P = 0{\cdot}87$; d.f. = 1); (iii) the left/right orientation of the existing CL on the tendency of the induced CL to occur in the same ovary ($P = 0{\cdot}34$; d.f. = 1); (iv) the stage of the oestrous cycle on the occurrence of induced CL in the existing CL-bearing ovary ($P = 0{\cdot}29$; d.f. = 3); or (v) the stage of the oestrous cycle on the left/right orientation of the induced CL ($P = 0{\cdot}69$; d.f. = 3).

Discussion

These results show that the large, luteal-phase follicle of the cow is capable of responding to hCG to produce a luteinized structure which at the level of the laparoscope is not distinguishable from a

normal CL. Presumably, therefore, the large luteal-phase follicle is capable of ovulating in response to an LH surge, for although follicular status was not assessed before hCG injection, previous studies of cattle follicular populations suggest that large follicles are present throughout most of the luteal phase of the oestrous cycle (Matton *et al.*, 1981; Pierson & Ginther, 1987a; Savio *et al.*, 1988; Sirois & Fortune, 1988). The functional ability of the hCG-induced CL was not assessed in the present experiment. The hCG-induced CL appeared to be smaller than the existing CL, but this may have been an effect of the age of the CL. Peripheral progesterone concentrations were not measured in this study, because the functional ability of the CL would have to be assessed by in-vitro progesterone production since there are no significant differences in peripheral progesterone concentrations between single and twin-ovulating animals (Morris *et al.*, 1987). However, the presence of an hCG-induced CL, particularly during the late-luteal phase, did not extend the length of the oestrous cycle compared with either control heifers or treated heifers with no extra ovulations. Usually, the CL of cattle are unresponsive to luteolysis until at least Day 5 after ovulation (Rowson *et al.*, 1972; Hafs & Manns, 1975), and this indicates that the induced CL were not actually fully functional.

The results in the present study have allowed a new approach to the examination of an old problem. Previous work has suggested that the ovary bearing the CL has a higher number of follicles than the contralateral ovary in sheep and cattle (Dufour *et al.*, 1971; Matton *et al.*, 1981; Pierson & Ginther, 1987b; Savio *et al.*, 1988), and that the diameter of the large luteal-phase follicle is greatest (Dufour *et al.*, 1971; Staigmiller & England, 1982), and the follicle more oestrogenic (England *et al.*, 1973) when positioned ipsilateral rather than contralateral to the CL. However, other authors have reported that the location of the CL has no effect upon antral follicle distribution (Ireland *et al.*, 1979), and that the diameter of the large follicle is smaller when in the CL-bearing ovary (Matton *et al.*, 1981). Hunter & Southee (1987) reported that progesterone has a direct effect upon the sheep ovary, decreasing oestradiol secretion from large follicles, but increasing the ability of hCG to bind to the thecal cells. The present data indicate that the existing CL has no significant effect upon the orientation or distribution of follicles capable of responding to hCG. This is in agreement with the frequency of bilateral and unilateral double ovulations observed in a herd of cattle selected for high twinning rate (Morris & Day, 1987), and may support the data of Al-Gubory & Martinet (1987), who suggest that the CL only affects preantral follicle distribution. Any progesterone effect (Hunter & Southee, 1987) would therefore have to be mediated via the peripheral circulation. Our results also suggest that the angiogenic activity of cattle CL (Redmer *et al.*, 1988) does not play a role in the development of large follicles.

The response of the heifers in this study was significantly affected by the period of the luteal phase at which the hCG was given; animals were more responsive during Days 4–7 and 14–16 than they were from Days 8 to 13 (Table 1). These data are corroborated by the higher concentration of receptors for LH on the granulosa cells during the early luteal phase (Day 7) than during the mid-luteal phase (Day 13) in cattle (Ireland & Roche, 1983); the concentrations of receptors for FSH appear to remain constant throughout the cycle (Staigmiller *et al.*, 1982; Ireland & Roche, 1983). It has also been reported that 'waves' of large follicle growth occur during the early and late luteal phases of the oestrous cycle (Matton *et al.*, 1981; Pierson & Ginther, 1987a; Savio *et al.*, 1988; Sirois & Fortune, 1988). The increases in the responsiveness to hCG at the different periods of the luteal phase observed in the present study therefore appear to coincide with the presence of large follicles that have been reported elsewhere. The lower response during the mid-luteal phase may reflect an absence of follicles at an adequate stage of maturity; for example, sufficient granulosa cell LH receptors (Ireland & Roche, 1983). In addition over 70% of cattle injected with a luteolytic dose of PGF-2α on Days 11 and 12 of the oestrous cycle took 4–5 days to exhibit oestrus, compared with 48–72 h in animals injected on Days 7 or 16 (MacMillan & Henderson, 1983/4). This response was interpreted as reflecting a wave-like pattern in ovarian follicle development, with the presence of less mature follicles during the mid-luteal phase. However, a lower proportion of cattle did have induced ovulations during the mid-luteal phase possibly due in part to the fact that 20–30% of

heifers have 1, 2 or 4 dominant follicles per cycle (Savio *et al.*, 1988; Sirois & Fortune, 1988), rather than 3. This may also, in part, account for the fact that not all heifers during the early or late luteal phases of the cycle had induced CL.

In conclusion, these results show that the large luteal-phase follicle is capable of responding to hCG, and that this response is lower when the hCG was given in the mid-luteal phase than in the early or late luteal phases. In addition, the data suggest that the corpus luteum *per se* does not exert a local constraint on the growth of 'potentially-ovulatory' follicles.

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