

## Regulation of follicle waves to maximize fertility in cattle

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Cattle have recurrent follicular waves every 7–10 days in most physiological situations; an FSH increase is associated with emergence of the wave and LH pulse frequency determines the fate of the dominant follicle. To control oestrus with hormones it is necessary to ensure that either induced corpus luteum regression or the termination of a progestogen treatment coincides with the selection of the dominant follicle during the wave, to give a precise onset of oestrus and high fertility. The exogenous administration of progesterone or progestagen blocks the normal turnover of the dominant follicle once the corpus luteum regresses. Thus, the effects of duration of dominance of the preovulatory follicle on onset of oestrus and fertility were examined. The variation in onset of oestrus was reduced but occurred 5–9 h later after 4 versus 8 days of dominance; pregnancy rate was also affected with dominance periods of 2–4, 4–8 and > 10 days resulting in 0, 10–15% or 20–50% reduction in pregnancy rates, respectively. The necessity for short duration of dominance of the preovulatory follicle means that to ensure high fertility the follicular wave needs to be regulated when using hormones to control oestrus. Two approaches were examined, namely the use of GnRH or oestradiol at time of progesterone intravaginal releasing device insertion. The effect of 250 µg of synthetic GnRH on the fate of an existing follicle wave was to ovulate the dominant follicle (20/20 cows) and a new wave emerged  $1.6 \pm 0.3$  days later; however, there was no effect of GnRH on the wave if administered before dominant follicle selection. The effect of oestradiol concentrations on suppression of FSH in ovariectomized heifers showed that increasing oestradiol to  $10\text{--}15 \text{ pg ml}^{-1}$  caused a  $37 \pm 6.9\%$  decrease in FSH for 24 h, with a subsequent increase to pretreatment values by  $57 \pm 13$  h. In cyclic heifers, increasing oestradiol to  $> 10 \text{ pg ml}^{-1}$  in conjunction with progesterone treatment at emergence of the first wave of the cycle affected the current follicle wave by either preventing dominant follicle selection or decreasing diameter of the dominant follicle, without consistently affecting the interval to new wave emergence. Increase of oestradiol after dominance, however, delayed new wave emergence by 2–5 days. A better understanding of the hormonal control of follicle waves will lead to development of improved hormonal regimens to control oestrus sufficiently to give high pregnancy rates to a single AI without recourse to detection of oestrus.

### Introduction

The critical requirements for effective synchronization of oestrus in cattle are a predictable high oestrous response during a specified 12–24 h period and high pregnancy rates to a single breeding after treatment. To date, these two requirements have not been met successfully, despite our increasing knowledge of follicular development (Ginther *et al.*, 1996). Research in the early 1960s showed that progesterone–progestagen treatments of > 14 days duration resulted in good synchrony

of oestrus but low pregnancy rates. Subsequently, the treatment period was reduced to 9–12 days by administration of oestradiol as a luteolytic agent at the start of the progestagen treatment (Wiltbank and Kasson, 1968; Roche, 1974); this procedure resulted in normal pregnancy rates but greater variability in onset of oestrus. The discovery of prostaglandin  $F_{2\alpha}$  ( $PGF_{2\alpha}$ ) as the endogenous luteolysin led to its use at or near the end of progestagen treatments; its use increased the oestrous response in animals with a corpus luteum. However, it failed to give sufficient precision of onset of oestrus to allow high pregnancy rates to a single planned AI (Odde, 1990). With the advent of ovarian ultrasonography, it became clear that follicular status at the end of a progestagen treatment or at the time of induced luteolysis affected the interval to oestrus. Thus, effective hormonal regulation of the ovarian cycle is now known to require both the strategic induction of premature regression of the corpus luteum and the presence of a recently selected dominant follicle at the end of the treatment period.

Antral follicle growth in cattle occurs in distinct wave-like patterns during the ovarian cycle and the postpartum period (reviewed in Roche *et al.*, 1998). The emergence of each new wave is stimulated by a transient (1–2 day) increase in FSH (Adams *et al.*, 1992; Sunderland *et al.*, 1994). Selection of the dominant follicle occurs during declining FSH concentrations, and the dominant follicle then maintains FSH at nadir concentrations until it either ovulates or succumbs to atresia, depending on the pattern of LH secretion. During the final stage of selection of the dominant follicle, there appears to be a transition from mainly FSH to LH dependency, but the cause and specific time frame of this change are unknown. The consequences are that continued growth and oestradiol production by the dominant follicle are dependent on increased LH pulse frequency, which, if prolonged, can lead to persistence of the dominant follicle (Stock and Fortune, 1993; Savio *et al.*, 1993; Taft *et al.*, 1996). Therefore, hormonal treatments that modify both FSH and LH clearly affect the fate of the follicular wave. Manipulation of the follicular wave, in turn, may alter systemic hormone concentrations, the intrafollicular environment, and the competency of the oocyte to be ovulated. However, successful hormonal regulation of oestrus must not compromise oocyte competency, ovulation, fertilization, early embryonic development or subsequent luteal function.

Each follicular wave has an inherent life span of 7–10 days as it progresses through the different stages of development, namely emergence, selection, dominance and atresia or ovulation. Thus, a dominant follicle capable of ovulation is present only at specific times during each wave. Therefore, the interval from exogenous luteolysis or withdrawal of a progestagen treatment to oestrus depends on the stage of the follicular wave at the end of treatment: cattle with a selected dominant follicle will be in oestrus within 2–3 days, but those before dominant follicle selection will not be in oestrus for 3–7 days. Thus, induced luteolysis needs to be synchronized with selection of the preovulatory dominant follicle. Current progestagen strategies to synchronize oestrus in cattle are reviewed, with reference to regulation of follicular wave and duration of dominance of the preovulatory follicle, onset of oestrus and fertility.

### Precision of Onset of Oestrus

The variability in onset of oestrus after the end of a synchronization treatment will determine whether animals can be inseminated at pre-arranged times or at a detected oestrus to obtain high pregnancy rates to AI. In order to achieve maximum precision in oestrous onset, it is necessary to have a recently selected dominant follicle present at the end of treatment. New wave emergence needs to be synchronized during the treatment period, because both the stage of the follicular wave and the duration of dominance cause variation of 24 h in the duration of the follicular phase. Oestradiol concentrations of heifers with a dominant follicle of > 4 days of dominance are higher at the end of a progestagen treatment; subsequent follicular phases are short and onset of oestrus is 6–12 h earlier than in heifers with a dominant follicle of short (2–3 days) duration of dominance (Austin *et al.*, 1999). Synchrony of oestrus is optimal when the duration of dominance is either consistently short (< 4 days) or very long (10–12 days).

### Duration of Dominance of the Preovulatory Follicle

The duration of dominance of the preovulatory follicle can affect pregnancy rate. Thus, it is necessary that all animals have a recently selected dominant follicle at the end of a progestagen treatment or at the time of induced luteolysis using PGF<sub>2α</sub>. Recurrent follicular waves are regulated systemically by FSH and LH, and locally by specific growth factors namely inhibins, activins, insulin-like growth factor I (IGF-I) and their respective binding proteins.

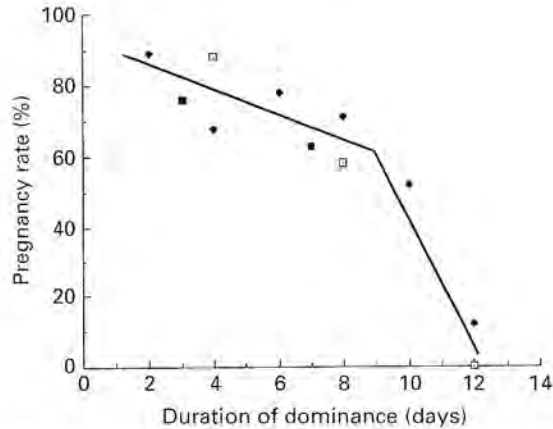
#### *Persistent dominant follicle*

Turnover of the dominant follicle is regulated by LH pulse frequency. During the luteal phase (Cupp *et al.*, 1995) or early postpartum anoestrous period (Stagg *et al.*, 1998), an LH pulse frequency of 1 pulse every 3–6 h results in loss of dominance and atresia of the dominant follicle. During the follicular phase, the high frequency–low amplitude LH pulse pattern stimulates sufficient oestradiol synthesis to induce oestrus and the preovulatory gonadotrophin surge (Rahe *et al.*, 1980). However, an intermediate LH pulse frequency of 1 pulse every 1–2 h causes an extension of the period of dominance (persistence) (Savio *et al.*, 1993; Stock and Fortune, 1993; Taylor *et al.*, 1993; Mihm *et al.*, 1999), which maintains not only continued growth of the dominant follicle (Taft *et al.*, 1996), but also its biochemical health, based on its high oestrogen activity, reduced amounts of the 34 kDa form of dimeric inhibin, and decreased amounts of the lower molecular weight forms of IGF-binding proteins (de la Sota *et al.*, 1994, 1996). During an extended period of dominance, the persistent dominant follicle suppresses emergence of a new follicular wave (functional dominance; Sirois and Fortune, 1990; Savio *et al.*, 1993; Stock and Fortune, 1993). Any dominant follicle becomes persistent in the presence of either subluteal concentrations (40–50% of normal luteal phase concentrations) of progesterone (which occur after endogenous luteolysis and more than 3–4 days after insertion of an intravaginal progesterone releasing device) or after use of a norgestomet ear implant combined with luteolysis. Neither protocol suppresses LH pulse frequency to the same extent as that achieved during the luteal phase (Kojima *et al.*, 1992).

#### *Fertility*

There is an association between persistency of the ovulatory follicle and decreased fertility in progestagen synchronized heifers (Savio *et al.*, 1993; Sanchez *et al.*, 1993; Co-operative Regional Research Project, 1996). However, the duration of dominance necessary to initiate this fertility decline, and whether the decline is gradual or abrupt, requires elucidation. Hence, the association between the duration of dominance of the ovulatory follicle and subsequent fertility to AI was examined in our laboratory using progestagen-treated heifers (Mihm *et al.*, 1994; Austin *et al.*, 1999). The duration of dominance of the second dominant follicle was controlled by causing corpus luteum regression with PGF<sub>2α</sub> in heifers and insertion of a 3 mg norgestomet implant (Intervet Ltd, Boxmeer, Netherlands) at emergence or first day of dominance for different durations to maintain the dominant follicle. Dominance periods of up to 8 days resulted in high pregnancy rates, which were reduced as the period of dominance was increased: 89, 68, 78, 71, 52 and 12% for periods of dominance of 2, 4, 6, 8, 10 and 12 days at oestrus, respectively (Fig. 1).

The factors that cause these low pregnancy rates are not known but neither the ovulatory ability of a persistent dominant follicle, nor subsequent luteal function seem impaired (Stock and Fortune, 1993; Mihm *et al.*, 1994; Co-operative Regional Research Project, 1996). Prolonged increase of circulating oestradiol concentrations before oestrus may compromise uterine function, and thus reduce subsequent implantation rates (Butcher and Pope, 1979; Bryner *et al.*, 1990). However, pregnancy rates of heifers after embryo transfer in the presence or absence of a persistent dominant follicle were similar (Wehrman *et al.*, 1997); likewise, fertility was normal after AI when the subsequent dominant follicle after the persistent follicle was allowed to ovulate (Fike *et al.*, 1997). Thus, the absence of detrimental effects of the increased oestradiol from a persistent dominant follicle on embryo survival indicates that intrafollicular biochemical alterations or oocyte



**Fig. 1.** Pregnancy rates in heifers after ovulation of follicles of different durations of dominance. The data are derived from Mihm *et al.* 1994 (□); and Austin *et al.*, 1998 (◆ and ■). Heifers were given a single injection of a PGF<sub>2α</sub> analogue (PG) at second wave emergence and inseminated at a detected oestrus ( $n = 9$ ) to achieve a duration of dominance of 1–2 days. Heifers were given PG as described above, but to achieve longer periods of dominance implants of norgestomet were also inserted at the time of PG injection for different periods as follows: 2 days ( $n = 44$ ), 4 days ( $n = 18$ ), 6 days ( $n = 67$ ), 8 days ( $n = 23$ ) and 12 days ( $n = 31$ ), which resulted in durations of dominance of the preovulatory follicle of 4, 6, 8, 10 and 12 days, respectively. In all cases heifers detected in oestrus subsequent to implant removal were inseminated approximately 12 h later.

incompetency are potentially important factors in the decreased fertility after ovulation of the persistent dominant follicle (Ahmad *et al.*, 1995).

Changes may occur within the oocyte of a persistent dominant follicle after continued exposure to prolonged high LH pulse frequency. In rats, dissociation of the resumption of meiosis from the LH surge was induced by exogenous LH pulses, leading to the ovulation of excessively 'aged' oocytes (Mattheij *et al.*, 1994). In cattle, premature germinal vesicle breakdown and progression to metaphase I occurred in six of eight oocytes recovered from preovulatory follicles with a dominance period of 12 days (associated with severely reduced fertility) that were collected before the predicted onset of the gonadotrophin surge (Mihm *et al.*, 1999). This finding is in agreement with the findings of Revah and Butler (1996), who reported the dispersion of nuclear material in oocytes from single or multiple persistent follicles. Oocytes within persistent follicles may, therefore, experience alterations in both the timing and nature of nuclear and cytoplasmic maturation, leading to potential inhibition of normal polar body extrusion, sperm decondensation, or normal transgression to the activation of the embryonic genome. Such asynchrony between oocyte and follicular maturation may be one factor involved in the reduction of pregnancy rates after ovulation of follicles with an extended duration of dominance (Mihm *et al.*, 1999).

### Synchronization of New Wave Emergence

It is necessary to have a recently selected dominant follicle of short duration of dominance at the end of a progestagen or PGF<sub>2α</sub> treatment regimen to give a precise onset of oestrus and high pregnancy

rates to a single timed AI. The changing dependency of follicles on gonadotrophins during the wave and the intricacies of local control mechanisms that play a key role in regulating the sequential progression of follicles through different physiological stages of the wave make it difficult to develop a simple exogenous hormonal treatment that gives predictable new wave emergence in > 95% of treated animals irrespective of stage of wave at time of treatment (Bo *et al.*, 1995; Roche *et al.*, 1998). Thus, the induction of new wave emergence using exogenous hormonal treatments imposed at different stages of the follicular wave requires the predictable induction of a transient increase in FSH to give new wave emergence and normal growth of dominant follicle after its selection.

### GnRH

Hormones to control the follicular wave are usually administered at the start of synchrony treatments to cause demise of the existing wave, and subsequent predictable new wave emergence. Removal of the dominant follicle or antral cohort follicles before selection (either by cauterization or ultrasound-guided transvaginal follicle aspiration) leads to a transient FSH increase and consistent new wave emergence within 1–2 days (Bergfelt *et al.*, 1994). However, such approaches are impractical on the farm. Functional removal of the dominant follicle or cohort follicles by either inducing ovulation or their rapid luteinization with exogenously induced gonadotrophin release is an alternative approach. Thus, GnRH has been used to cause predictable new wave emergence in some cattle synchronization protocols, that is, either in combination with PGF<sub>2α</sub> (Twagiramungu *et al.*, 1995; Schmidt *et al.*, 1996) or as part of a progesterone regimen (Ryan *et al.*, 1995). The GnRH-induced release of LH and FSH is acute, and its magnitude may be affected by the endocrine status of the animal or the stage of the follicular wave at the time of injection. Accordingly, Ryan *et al.* (1998) examined the effects of injection of dairy cows with 250 µg of a synthetic GnRH (gonadorelin, Sanofi, France) on the gonadotrophin release, fate of existing follicular wave and interval to new wave emergence (i) before or after selection of the dominant follicle and (ii) when progesterone concentrations were above or below 1 ng ml<sup>-1</sup>. GnRH induced a coincident LH and FSH surge, the magnitude of which was independent of days postpartum, progesterone concentration or stage of follicular wave. However, the effect of GnRH on the existing follicular wave was dependent on the presence or absence of a dominant follicle. GnRH administered after dominant follicle selection caused its ovulation (20/20) and predictable new wave emergence 1.6 ± 0.3 days later; if GnRH was administered before selection, it had no effect on the progression of the existing follicular wave and a dominant follicle was formed 3.6 ± 0.5 days later. In all cows treated after dominant follicle selection, the induced gonadotrophin surge was followed by a transient increase in FSH but not LH, which was associated with new wave emergence. It was concluded that GnRH synchronizes new wave emergence only when administered in the presence of a functional dominant follicle. Furthermore, smaller doses of gonadorelin (25 or 100 µg) are only partially effective (100 µg) or incapable (25 µg) of ovulating a luteal phase dominant follicle, and do not affect the progression of the wave when administered before formation of the dominant follicle (Mihm *et al.*, 1998). The dichotomy of GnRH effects on the progression of a follicular wave is a limitation that needs to be considered when using it as a treatment to synchronize new wave emergence at the start of progesterone or PGF<sub>2α</sub> synchronizing regimens. The subsequent use of PGF<sub>2α</sub> to cause regression of induced corpus luteum is mandatory when GnRH is used to synchronize follicle waves.

### The use of oestradiol and progesterone alone and in combination

The primary dependence of the follicular wave on gonadotrophin support has resulted in the use of steroids to suppress FSH and LH and thus, terminate the existing wave. However, FSH and LH are differentially regulated. In the case of FSH, the dominant follicle is the key regulator of the recurrent increases that take place, but the specific roles of oestradiol, different inhibin forms and other putative FSH regulators are not known. LH is regulated by GnRH; hence, administration of progesterone is important in the regulation of LH pulse frequency in the cyclic cow, although changes in LH pulse frequency occur during the luteal phase that are difficult to explain by changes

**Table 1.** Effect of oestradiol benzoate (ODB) administration at the time of progesterone-releasing intravaginal device (PRID) insertion on mean ( $\pm$  SEM) peak plasma concentrations of oestradiol ( $E_2$ ) and FSH, duration of oestradiol increase and rate of decline of concentrations of oestradiol and FSH in ovariectomized heifers pretreated with PRID.

ODB treatment (mg)	Number of heifers (n)	Peak concentration of $E_2$ (pg ml <sup>-1</sup> )	Time (h) to reach maximum concentrations of $E_2$	Time (h) to reach concentration of $E_2$ of < 3 pg ml <sup>-1</sup>	Maximum decline of FSH expressed as % decline from pretreatment values	Time (h) from ODB treatment to first FSH increase after nadir	Time (h) for FSH to return to pretreatment concentrations
Controls							
0.0	3	0.1 $\pm$ 0.05 <sup>a</sup>			0.0 <sup>a</sup>		
Injection (mg)							
1.0	4	18 $\pm$ 7.1 <sup>b</sup>	15 $\pm$ 4.2	97 $\pm$ 19.4 <sup>a</sup>	37 $\pm$ 6.9 <sup>b</sup>	32 $\pm$ 14.9 <sup>a</sup>	65 $\pm$ 16.9 <sup>a</sup>
2.5	6	24 $\pm$ 5.8 <sup>b</sup>	11 $\pm$ 3.4	125 $\pm$ 15.8 <sup>ab</sup>	40 $\pm$ 5.7 <sup>b</sup>	34 $\pm$ 12.2 <sup>a</sup>	57 $\pm$ 13.8 <sup>a</sup>
5.0	4	72 $\pm$ 7.1 <sup>c</sup>	12 $\pm$ 4.2	163.5 $\pm$ 19.4 <sup>b</sup>	70 $\pm$ 6.9 <sup>c</sup>	45 $\pm$ 14.9 <sup>b</sup>	183 $\pm$ 16.9 <sup>b</sup>

Within column, means with different superscripts are significantly different ( $P < 0.05$ ).

in progesterone or oestradiol concentrations (Cupp *et al.*, 1995). Oestradiol and progesterone administered together can affect the progression of an existing wave, and hence new wave emergence (Bo *et al.*, 1995; Caccia and Bo, 1998). However, the optimum dose of exogenous oestradiol to suppress FSH consistently for a specific time period is not clear in cattle. Thus, an experiment was carried out in ovariectomized heifers to determine the effect of different blood plasma concentrations of oestradiol at the time of progesterone administration on FSH suppression, to select effective doses for manipulation of follicular waves (O'Rourke *et al.*, 1997). The results (Table 1) show that (i) blood plasma concentrations of oestradiol reached maximum concentrations within 11–15 h after intramuscular injection of oestradiol benzoate in oil, irrespective of dose administered, and (ii) administration of 5 mg of oestradiol benzoate resulted in higher ( $P < 0.05$ ) blood plasma concentrations for longer periods than 1.0 or 2.5 mg doses (Table 1). The resulting increased concentrations of oestradiol in blood plasma caused a significant reduction in FSH concentrations (Fig. 2). However, the extent of the decline in FSH was greater and the time for FSH to reach pretreatment concentrations was longer ( $P < 0.05$ ) for heifers administered 5.0 mg oestradiol benzoate compared with those administered 1.0 or 2.5 mg oestradiol benzoate (O'Rourke *et al.*, 1997). Blood plasma concentrations of oestradiol 2–3 times greater than pro-oestrous concentrations (1.0 mg ODB injection group) resulted in a 37  $\pm$  6.9% decrease in FSH, which was maintained for 32  $\pm$  14.9 h. FSH concentrations returned to pretreatment values by 57  $\pm$  13.8 h. Thus, high concentrations of oestradiol have only a transitory suppressive effect on FSH, and concentrations begin to increase in the presence of high but declining blood plasma concentrations of oestradiol.

In cyclic heifers, oestradiol administered at the start of progesterone treatment will transiently affect FSH and consequently follicular wave dynamics. Suppression of the peri-ovulatory FSH increase delays new wave emergence (Turzillo and Fortune, 1993), but the effects of decreasing FSH at other stages of the follicular wave on current wave or new wave emergence are unknown. For selecting optimum doses of oestradiol benzoate to manipulate transient increases in FSH and hence, new wave emergence in cyclic cattle, it is essential to determine the optimum blood plasma concentrations of oestradiol required at different stages of the follicular wave to give predictable new wave emergence. Three different oestradiol benzoate treatments were administered to cyclic heifers treated with a progesterone intravaginal releasing device (PRID, Sanofi Ltd, France) for 12 days to achieve low (2–4 pg ml<sup>-1</sup> using a 10 mg oestradiol benzoate capsule), medium (15–20 pg ml<sup>-1</sup> using a 0.75 mg oestradiol benzoate injection) or high (40–60 pg ml<sup>-1</sup> using a 5 mg oestradiol benzoate injection) blood plasma concentrations. Heifers were treated at specific stages of development of the first follicular wave of the cycle, that is, on the day of wave emergence, and the

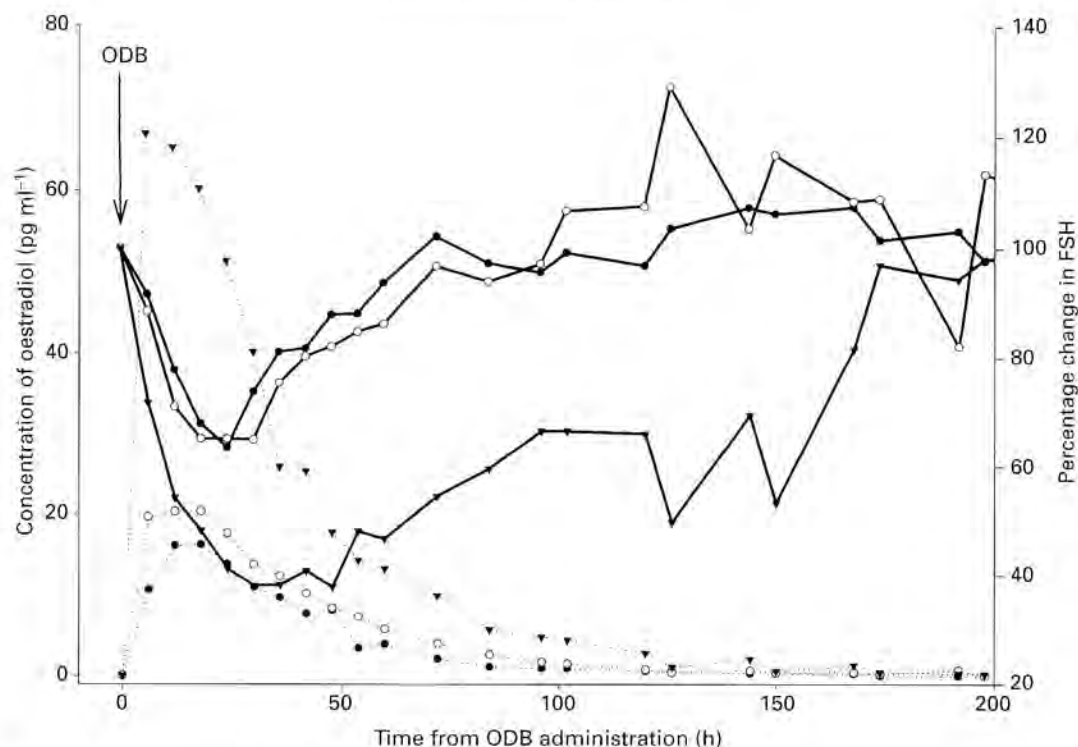


Fig. 2. Effect of injection of 1.0 (●), 2.5 (○) or 5.0 mg (▼) of oestradiol benzoate (ODB) in oil at time of progesterone releasing device (PRID) insertion on mean plasma concentrations of oestradiol (dotted lines) and percentage change in FSH (solid lines) in ovarietomized heifers pretreated with progesterone releasing devices ( $n = 4$  heifers per dose). The range in SEM for oestradiol was 0.05–3.5  $\text{pg ml}^{-1}$  and for FSH was 0.5–5.5  $\text{ng ml}^{-1}$ .

first or fourth day of dominance before next new wave emergence. The results indicate that (i) progesterone with or without oestradiol benzoate (intravaginal capsule, or 0.75 or 5 mg intramuscular injections) administered in early metoestrus can either prevent selection of the first dominant follicle, or when selection has occurred, this treatment results in a decrease in dominant follicle diameter without consistently changing the timing of next new wave emergence, and (ii) all oestradiol benzoate treatments administered before the next predicted transient FSH increase delayed new wave emergence by 4–5 days (Table 2; O'Rourke *et al.*, 1998).

Thus, exogenous oestradiol administered with progesterone suppresses formation of or decreases the diameter of the dominant follicle when administered before or during emergence of the wave, presumably due to the suppression of FSH. However, once again the effects on the existing wave and interval to new wave emergence after administration of different oestradiol concentrations at the time of PRID insertion were variable, and dependent on stage of wave at time of treatment. In addition, oestradiol benzoate administered with progesterone in early metoestrus appeared to advance corpus luteum regression by 3–5 days as detected by ultrasound, thus confirming the luteolytic function of oestradiol when administered during early corpus luteum formation (Lemon, 1975), but the mechanism is not clear.

#### Synchronization of Pro-oestrous Oestradiol Rise after Treatment

The pattern and precision of onset of the synchronized oestrus are dependent on the time of luteolysis in relation to dominant follicle development, and the efficacy of hormones used to

**Table 2.** The effect of dose and route (intravaginal application in gelatin capsule versus intramuscular injection in oil) of oestradiol benzoate (ODB) given at the time of PRID insertion on wave dynamics when administered at different stages of development of the first dominant follicle (DF) in cyclic beef heifers

Parameter	Follicle stage at treatment <sup>†</sup>	Treatment				
		Control	PRID alone	PRID + ODB capsule 10 mg	PRID + ODB injection 0.75 mg	PRID + ODB injection 5.0 mg
Number forming first dominant follicle	Emergence	10/10 <sup>a</sup>	4/7 <sup>bc</sup>	4/6 <sup>ac</sup>	1/6 <sup>bc</sup>	2/7 <sup>bc</sup>
Maximum size of first dominant follicle (mm)	Dominance	13.1 ± 0.5 <sup>a</sup>	11.4 ± 0.6 <sup>b</sup>	10.8 ± 0.60 <sup>b</sup>		
Interval from treatment to new wave emergence (days)	Emergence	8.3 ± 0.7 <sup>a</sup>	6.6 ± 0.8 <sup>ab</sup>	6.5 ± 1.1 <sup>ab</sup>	5.5 ± 0.5 <sup>b</sup>	8.2 ± 0.8 <sup>a</sup>
	Dominance	5.0 ± 0.6 <sup>a</sup>	5.6 ± 0.8 <sup>ab</sup>	5.3 ± 0.8 <sup>ab</sup>	7.0 ± 0.9 <sup>ab</sup>	7.3 ± 0.8 <sup>b</sup>
	Dominance + 3	2.0 ± 0.5 <sup>a</sup>	3.0 ± 0.7 <sup>a</sup>	5.9 ± 0.7 <sup>b</sup>	6.1 ± 0.7 <sup>b</sup>	7.0 ± 0.7 <sup>b</sup>
Interwave-interval (days)	Emergence	8.3 ± 0.7 <sup>a</sup>	6.6 ± 0.8 <sup>ab</sup>	6.5 ± 1.1 <sup>ab</sup>	5.5 ± 0.9 <sup>b</sup>	8.3 ± 0.8 <sup>a</sup>
	Dominance	8.3 ± 0.7 <sup>a</sup>	8.6 ± 0.8 <sup>a</sup>	8.2 ± 0.9 <sup>a</sup>	10.0 ± 0.9 <sup>b</sup>	10.3 ± 0.9 <sup>b</sup>
	Dominance + 3	8.3 ± 0.7 <sup>a</sup>	8.6 ± 0.8 <sup>a</sup>	11.7 ± 0.8 <sup>b</sup>	12.3 ± 0.8 <sup>b</sup>	13.1 ± 0.8 <sup>b</sup>

<sup>a,b,c</sup> Within row, means with different superscripts are significantly different ( $P < 0.05$ ).

<sup>†</sup>Determined by daily ultrasound examination.

synchronize follicular waves. Fertility depends on the duration of persistency of the dominant follicle during progestagen treatments. High fertility to AI after progesterone or progestagen administration requires a short duration of treatment (7–12 days) because it has been shown that treatment for 14 or more days results in decreased pregnancy rates to AI (Roche, 1978; Macmillan and Peterson, 1993). However, with the known relationship between duration of dominance of the ovulatory dominant follicle and fertility in heifers, it is important to re-examine the effect of duration of treatment on fertility, especially because recent results indicate that progesterone treatment for 8 days results in higher fertility (Ryan *et al.*, 1995) than treatment for 12 days. Shortening the duration of progesterone treatment to 7–8 days requires the use of PGF<sub>2α</sub> as a luteolysin at or near the end of treatment, because oestradiol administered at the start of a progesterone treatment only shortens the duration of the ovarian cycle by 2–6 days when administered in early metoestrus. Thus, the time of occurrence and precision of the onset of oestrus are affected by the duration of progesterone treatment, the use and timing of PGF<sub>2α</sub> administration and the specific hormone(s) used to synchronize follicular waves.

Owing to the variability in onset of oestrus caused by different developmental stages of the dominant follicle at the end of treatment (Austin *et al.*, 1998), another approach has been used to improve precision of onset of oestrus, that is to induce the synchronous occurrence of the prooestrous oestradiol increase (McDougall *et al.*, 1992) by its exogenous administration at a specific time after the end of treatment. The use of 1.0 mg oestradiol benzoate administered 12–24 h after the end of a progesterone treatment increased the number of cows in oestrus the second day after treatment (Ryan *et al.*, 1995). This earlier onset of oestrus in some cows could result in ovulation of a small dominant follicle shortly after emergence of a new wave, which raises the question of the fertility consequences of induction of early ovulation of the dominant follicle. Thus, a trial was carried out (212 beef cows and 85 heifers) with the following aims: (1) to determine whether duration of PRID treatment for either 8 or 12 days affects oestrous and pregnancy response and (2) to determine whether 1 mg oestradiol benzoate administered 24 h after PRID removal will affect synchrony of onset of oestrus or pregnancy rates.



The overall oestrous response was similar for treatment for 12 days (94/112; 0.84) and 8 days (100/114; 0.88), but was higher ( $P < 0.05$ ) after administration of 1.0 mg oestradiol benzoate 24 h after PRID removal (103/112; 0.92) compared with no oestradiol benzoate (91/114; 0.80) ( $P < 0.01$ ). There was no interaction between the post-treatment use of oestradiol and duration of treatment on overall pregnancy rate ( $P > 0.05$ ). The shorter duration of progesterone treatment tended to increase pregnancy rate (8 days 61/114 (0.54) and 12 days 47/112 (0.42);  $P < 0.10$ ) but the effect was not significant. It appears that (i) pregnancy rate may be better ( $P < 0.1$ ) after treatment for 8 days than after treatment for 12 days and (ii) oestradiol administered 24 h after PRID removal significantly increased the oestrous response without adversely affecting pregnancy rate. Thus, the use of oestradiol benzoate after treatment has the potential to increase the number of heifers or cows in oestrus and decrease the variation in onset of oestrus.

### Conclusions

Major progress has been made in our understanding of the dynamics and regulation of follicular waves. It is essential now to use this knowledge to develop more effective hormonal regimens to regulate the time of predictable occurrence of oestrus in farm animals. It is a major scientific challenge to develop a hormonal treatment to cause predictable new wave emergence when administered at different physiological stages of a follicular wave in cattle. The most predictable and reliable induction of new wave emergence is achieved by dominant follicle ablation or ovulation. However, high GnRH doses that cause ovulation (250 µg) or lower doses (25 or 100 µg) that may not do not affect the morphological development of the wave when administered before dominant follicle selection. The alternative use of a combination of oestradiol and progesterone is equally problematic, and the interval to new wave emergence depends on the stage of the follicular wave at the time of treatment. As this could be due to changes in the physiological status of small antral follicles during the progression of a wave, the controlled increase in FSH needs to be synchronized with antral follicle function for new wave emergence to occur. It is necessary to have a short duration of dominance of the preovulatory follicle for maximum fertility and decreased variability in the onset of oestrus. Persistence of the dominant follicle for 8 days causes a slight reduction in pregnancy rates, but after 10 days, this decline is abrupt. Thus, the shorter the duration of progesterone treatment, the better the chance of achieving high pregnancy rates; however, progestagen treatments of <12 days will also require the use of PGF<sub>2α</sub> at or towards the end of treatment, because of the variable but slow luteolytic effects of exogenous oestradiol when administered at the start of a progestagen treatment. Variability in onset of oestrus even when a dominant follicle is present can be overcome by obtaining a synchronous increase in oestradiol by exogenous administration of oestradiol benzoate 24 h after the end of treatment; this procedure decreases the variability in onset of oestrus without compromising fertility. This approach has the potential to give sufficiently good synchrony of oestrus and high pregnancy rates after a single predetermined AI without recourse to detection of oestrus. Thus, it is important to understand the mechanisms regulating follicular waves and oocyte competency, to develop better hormonal methods to breed cattle to high genetic merit sires with significantly decreased labour input, but maintaining the goal of overall high pregnancy rates to AI.

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