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 progestogen 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 ± 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 10–15 pg ml⁻¹ caused a 37 ± 6.9% decrease in FSH for 24 h, with a subsequent increase to pretreatment values by 57 ± 13 h. In cyclic heifers, increasing oestradiol to > 10 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\textsubscript{2\alpha}. 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 \textit{et al.}, 1995) or early postpartum anoestrous period (Stagg \textit{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 \textit{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 \textit{et al.}, 1993; Stock and Fortune, 1993; Taylor \textit{et al.}, 1993; Mihm \textit{et al.}, 1999), which maintains not only continued growth of the dominant follicle (Taft \textit{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 \textit{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 \textit{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 \textit{et al.}, 1992).

Fertility

There is an association between persistency of the ovulatory follicle and decreased fertility in progestagen synchronized heifers (Savio \textit{et al.}, 1993; Sanchez \textit{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 \textit{et al.}, 1994; Austin \textit{et al.}, 1999). The duration of dominance of the second dominant follicle was controlled by causing corpus luteum regression with PGF\textsubscript{2\alpha} 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 \textit{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 \textit{et al.}, 1990). However, pregnancy rates of heifers after embryo transfer in the presence or absence of a persistent dominant follicle were similar (Wehrman \textit{et al.}, 1997); likewise, fertility was normal after AI when the subsequent dominant follicle after the persistent follicle was allowed to ovulate (Fike \textit{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 PGF₂α 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 PGF₂α 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 PGF2α (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⁻¹. 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 PGF2α synchronizing regimens. The subsequent use of PGF2α 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 (± SEM) peak plasma concentrations of oestradiol (E₂) 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 E₂ (pg ml⁻¹) | Time (h) to reach maximum concentrations of E₂ | Time (h) to reach E₂ of < 3 pg ml⁻¹ | Maximum decline of FSH expressed as % from pretreatment concentrations | Time (h) for FSH to return to pretreatment concentrations |
|-------------------|-----------------------|-----------------------------------|-----------------------------------------------|---------------------------------|---------------------------------------------------------------------|-----------------------------------------------------|
| Controls          | 0.0                   | 3                                 | 0.1 ± 0.05a                                   | 0.0a                           | 0.0a                                                                | 0.0a                                                |
| Injection (mg)    |                       |                                   |                                               |                                 |                                                                     |                                                     |
| 1.0               | 4                     | 18 ± 7.1b                         | 15 ± 4.2                                      | 97 ± 19.4a                     | 37 ± 6.9b                                                           | 32 ± 14.9a                                         |
| 2.5               | 6                     | 24 ± 5.8b                         | 11 ± 3.4                                      | 125 ± 15.8a                    | 40 ± 5.7b                                                           | 34 ± 12.2a                                         |
| 5.0               | 4                     | 72 ± 7.1c                         | 12 ± 4.2                                      | 163.5 ± 19.4a                  | 70 ± 6.9c                                                           | 45 ± 14.9b                                         |

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

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⁻¹ using a 10 mg oestradiol benzoate capsule), medium (15-20 pg ml⁻¹ using a 0.75 mg oestradiol benzoate injection) or high (40-60 pg ml⁻¹ 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 ovariectomized heifers pretreated with progesterone releasing devices (n = 4 heifers per dose). The range in SEM for oestradiol was 0.05–3.5 pg ml⁻¹ and for FSH was 0.5–5.5 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
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 PGF2α 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 PGF2α 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 proestrous 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 \((8 \text{ days} 61/114 (0.54) \text{ and } 12 \text{ days} 47/112 (0.42); P < 0.10)\) 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 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, progesteragen treatments of < 12 days will also require the use of PGF\(_{2\alpha}\) 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 progesteragen 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.

Part of the research work in this paper was funded by Department of Agriculture EU Stimulus Fund and Sanofi Sante Nutrition Animale, Libourne, France.

**References**

Adams GP, Matteri RL, Kastelic JP, Ko JCH and Ginther OJ (1992) Association between surges of follicle-stimulating hormone and the emergence of follicle waves in heifers Journal of Reproduction and Fertility 94 177–188

Ahmad N, Schrick FN, Butcher RL and Inskeep EK (1995) Effect of persistent follicles on early embryonic losses in beef cows Biology of Reproduction 52 1129–1135

Austin BJ, Milhm M, Ryan MP, Williams DH and Roche JE (1999)
Effect of duration of dominance of the ovulatory follicle on onset of oestrus and fertility in heifers Journal of Animal Science 77 (in press)

Bergfelt DR, Plata-Madrid H and Ginther OJ (1994) Counteraction of the follicular inhibitory effect of follicular fluid by administration of FSH in heifers Canadian Journal of Animal Science 74 633–639

Bo GA, Adams GP, Pierson RA and Mapleton RJ (1995) Exogenous control of follicular wave emergence in cattle Theriogenology 43 31–40

Breyner RW, Garcia-Winder M, Lewis PE, Inskeep EK and Butcher RL (1990) Changes in hormonal profiles during the estrous cycle in old lactating beef cows Domestic Animal Endocrinology 7 181–190

Butcher RL and Pope RS (1979) Role of estrogen during prolonged estrous cycles of the rat on subsequent embryonic death or development Biology of Reproduction 21 491–495

Caccia M and Bo GA (1998) Follicle wave emergence following treatment of CIDR-B implanted beef cows with estradiol benzoate and progesterone Theriogenology 49 341 (Abstract)

Cooperative Regional Research Project, NE-161 (1996) Relationship of fertility to patterns of ovarian follicular development and associated hormonal profiles in dairy cows and heifers Journal of Animal Science 74 1943–1952

Cupp AS, Stumpf TT, Koijima FN, Werth LA, Wolfe MW, Roberson MS, Kittok RJ and Kinder JE (1995) Secretion of gonadotrophins change during the luteal phase of the bovine estrous cycle in the absence of corresponding changes in progesterone or 17β-estradiol Animal Reproduction Science 37 109–119

de la Sota RL, Good TM, Ireland JL, Ireland JJ, Simmen FA and Thatcher WW (1994) Analysis of different forms of intrafollicular inhibin and inhibin a-subunits during ovarian follicular dominance in cattle Biology of Reproduction 50 Abstract 162

de la Sota RL, Simmen FA, Diaz T and Thatcher WW (1996) Insulin-like growth factor system in bovine first-wave dominant and subordinate follicles Biology of Reproduction 55 603–612

Fike KE, Wehrman ME, Bergfeld EG, Koijima FN and Kinder JE (1997) Prolonged increased concentrations of 17β-estradiol associated with development of persistent ovarian follicles do not influence conception rates in beef cattle Journal of Animal Science 75 1363–1367

Ginther OJ, Willbank MG, Fricke PM, Gibbons JR and Kot K (1996) Selection of the dominant follicle in cattle Biology of Reproduction 55 1187–1194

Koijima N, Stumpf TT, Cupp AS, Werth LA, Roberson MS, Wolfe MW, Kittok RJ and Kinder JE (1992) Exogenous progesterone and progesterone as used in estrous synchrony regimes do not mimic the corpus luteum in regulation of luteinizing hormone and 17β-estradiol in circulation of cows Biology of Reproduction 47 1009–1017

Lemon M (1975) The effect of oestrogens alone or in association with progesterogens on the formation and regression of the corpus luteum of the cyclic cow Annales de Biologie Animale Biochimie Biophysique 15 243–253

McDougall S, Burke CR, Macmillan KL and Williamson NB (1992) The effect of pretreatment with progesterone on the oestrous response to oestradiol-17β benzoate in the postpartum dairy cow Proceedings of the New Zealand Society of Animal Production 52 157–160

Macmillan KL and Peterson AJ (1993) A new intravaginal progesterone releasing device for cattle (CIDR-B) for oestrous synchronisation, increasing pregnancy rates and the treatment of post-partum anoestrous Animal Reproduction Science 33 1–25

Mattheij JAM, Swarts JIM, Hurvis HMM and Mulder K (1994) Advancement of meiotic resumption in Graafian follicles by LH in relation to preovulatory ageing of rat oocytes Journal of Reproduction and Fertility 100 65–70

Mihm M, Bagais A, Boland MP and Roche JF (1994) Association between the duration of dominance of the ovulatory follicle and pregnancy rate in beef heifers Journal of Reproduction and Fertility 102 123–130

Mihm M, Curran N, Hyltet F, Knight PG, Boland MP and Roche JF (1999) Effect of dominant follicle persistence on follicular fluid oestriadiol and inhibin and on oocyte maturation in heifers Journal of Reproduction and Fertility 117 (in press)

Mihm M, Deletang F and Roche JF (1998) The gonadotrophin and ovarian response to an intermediate or low dose of gonadorelin in beef heifers: influences of dose, follicle status and progesterone environment Journal of Reproduction and Fertility Abstract Series 21 Abstract 74

Mihm M, Curran N, Hyltet F, Knight PG, Boland MP and Roche JF (1999) Effect of dominant follicle persistence on follicular fluid oestriadiol and inhibin and on oocyte maturation in heifers Journal of Reproduction and Fertility 117 (in press)

O’Rourke M, Diskin MG, Sreenan JM and Roche JF (1997) The effect of dose and method of oestriadiol administration on plasma concentrations of E2 and FSH in long-term ovariectomised beef heifers Journal of Reproduction and Fertility Abstract Series 19 Abstract 148

O’Rourke M, Diskin MG, Sreenan JM and Roche JF (1998) Effect of different concentrations of oestriadiol administered during the first follicle wave in association with PRD insertion on follicle wave dynamics and oestrous response in beef heifers Journal of Reproduction and Fertility Abstract Series 21 Abstract 15

Rahbe CI, Owens RE, Fleeger JL, Newton HJ and Harms PG (1989) Pattern of plasma luteinizing hormone in the cyclic cow: dependence upon the period of the cycle Endocrinology 107 498–503

Revel I and Butler WR (1996) Prolonged dominance of follicles and reduced viability of bovine oocytes Journal of Reproduction and Fertility 106 39–47

Roche JF, Mihm M, Diskin M and Ireland JJ (1998) A review of regulation of follicle growth in cattle Journal of Animal Science 76 (supplement 3; 16–23)

Roche JF (1974) Effect of short-term progesterone treatment on oestrous response and fertility in heifers Journal of Reproduction and Fertility 40 433–440

Roche JF (1978) Control of oestrus in cattle using progesterone coils Animal Reproduction Science 3 145–154

Ryan DP, Snijders S, Yakuub H and O’Farrell KJ (1995) An assessment of follicle waves in cattle by change in progesterone or 17β-oestradiol and ovarian response to an intermediate or low dose of gonadorelin in beef heifers: influence of dose, follicle status and progesterone environment Journal of Reproduction and Fertility Abstract Series 21 Abstract 74

Ryan M, Mihm M and Roche JF (1999) Effect of dominant follicle persistence on follicular fluid oestriadiol and inhibin and on oocyte maturation in heifers Journal of Reproduction and Fertility 117 (in press)

Sanchez T, Wehrman ME, Bergfeld EG, Peters KE, Koijima FN,
Cupp AS, Mariscal V, Kittok RJ, Rasby RJ and Kinder JE (1993) Pregnancy rate is greater when the corpus luteum is present during the period of progestin treatment to synchronize time of estrus in cows and heifers Biology of Reproduction 49 1102–1107

Savoio JD, Thatcher WW, Badinga L, de la Sota RL, and Wolfenson D (1993) Regulation of dominant follicle turnover during the oestrous cycle in cows Journal of Reproduction and Fertility 97 197–203

Sirois J and Fortune JE (1990) Lengthening of the bovine oestrous cycle with two levels of exogenous progesterone: a model for studying ovarian follicular dominance Endocrinology 127 916–925

Stagg K, Spicer LJ, Sreenan JM, Roche JF and Diskin MG (1998) Effect of calf isolation on follicular wave dynamics, gonadotrophin and metabolic hormone changes and interval to first ovulation in beef cows fed either of two levels of energy postpartum Biology of Reproduction 59 777–783

Stock AE and Fortune JE (1993) Ovarian follicular dominance in cattle: relationship between prolonged growth of the ovulatory follicle and endocrine parameters Endocrinology 132 1108–1114

Sunderland SJ, Crowe MA, Boland ME, Roche JF and Ireland JJ (1994) Selection, dominance and atresia of follicles during the oestrous cycle of heifers Journal of Reproduction and Fertility 101 547–555

Taft R, Ahmad N and Inskeep EK (1996) Exogenous pulses of luteinizing hormone cause persistence of the largest bovine ovarian follicle Journal of Animal Science 74 2985–2991

Taylor C, Rajamahendran R and Walton JS (1993) Ovarian follicular dynamics and plasma luteinizing hormone concentrations in norgestomet-treated heifers Animal Reproduction Science 32 173–184

Turzillo AM and JE Fortune (1993) Effects of suppressing plasma FSH on ovarian follicular dominance in cattle Journal of Reproduction and Fertility 98 113–119

Twagiramungu H, Guilbault LA and Dufour JJ (1995) Synchronization of ovarian follicular waves with gonadotropin-releasing hormone agonist to increase the precision of estrus in cattle: a review Journal of Animal Science 73 3141–3151

Wehrman ME, Fike KE, Melvin EJ, Kojima FN and Kinder JE (1997) Development of a persistent ovarian follicle and associated elevated concentrations of 17β-estradiol preceding ovulation does not alter the pregnancy rate after embryo transfer in cattle Theriogenology 47 1413–1421

Wiltbank JN and Kasson CW (1968) Synchronization of estrus in cattle with an oral progestational agent and an injection of an estrogen Journal of Animal Science 27 113–116.