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Treatment of suckling beef cattle with a progestagen sponge and oestradiol benzoate or equine chorionic gonadotrophin

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The ovarian responses of anoestrus beef cows to a combined treatment with medroxy-progesterone acetate (MAP) sponges and oestradiol benzoate or equine chorionic gonadotrophin (eCG) were evaluated. Forty-five suckling Hereford cows were allocated to three equal groups. Group 1 received a MAP sponge for seven days plus an injection of 2 mg oestradiol benzoate when the sponge was inserted (day 0) and 1 mg when the sponge was withdrawn; group 2 received identical treatment until day 7, when a dose of 400 iu of eCG was administered, and group 3 were left untreated as control animals. From day 0 to day 11 the cows' ovaries were examined daily by transrectal ultrasonography, and their oestrous behaviour was observed from 24 hours to 96 hours after the sponge was removed. Data from cows that had a corpus luteum present before the sponge was withdrawn were not used in subsequent analyses; there were four in group 1, five in group 2 and four in group 3. In 19 of the 21 cows in groups 1 and 2 a new follicular wave was observed to emerge at a mean (sd) interval of 3·9 (0·3) days after the insertion of the sponge, whereas in group 3 it occurred in all 11 cows after 3·4 (0·6) days. Only the six cows that had a follicle of 9 mm or larger in diameter ovulated (P≤0·001). Nine of the 11 cows in group 1 came into oestrus, compared with two of the 10 in group 2 and none of the control cows (P≤0·001). Ovulation was observed in four, two and none of the cows in groups 1, 2 and 3, respectively.

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PROLONGED postpartum anoestrus or anovulation limits reproductive efficiency by delaying or preventing conception. Follicular growth resumes soon after calving, with the formation of the first dominant follicle, which can be detected morphologically by ultrasound within 10·2 days (Murphy and others 1990). Thus, a prolonged anoestrus period in beef cows is due to the failure of dominant follicles to ovulate, rather than to a delay in the development of follicles (Crowe and others 1993). High concentrations of progesterone during the luteal phase prime the behavioural centres in the brain, so that oestrous behaviour can be induced by the rise in oestradiol during the follicular phase. In anoestrus cattle, pretreatment with progesterone might be useful, not only to induce an artificial luteal phase to facilitate oestrous behaviour, but also to improve the pregnancy rate, because the number of luteal phases before mating are closely related to fertility at mating (Britt 1992).

In cattle, systems of oestrus synchronisation need to consider not only the end of the luteal phase to promote oestrous

behaviour, but also the ovulation of a healthy follicle to optimise fertility (Macmillan and Burke 1996, Thatcher and others 1996, Roche and others 1999). If a follicle remains dominant for more than nine days, it deteriorates and its fertilisation results in early embryonic death (Mihm and others 1999). The dependence of the dominant follicle on support from gonadotrophins has resulted in the use of steroids to suppress follicle-stimulating hormone (FSH) and luteinising hormone (LH), in order to reset follicular development. A combined treatment, using a progestagen-containing device plus oestradiol 17-β, suppresses dominance more consistently than either hormone administered alone (Bo and others 1994, Caccia and Bo 1998). Treatment with a progesterone sponge for five days was associated with a transient initial decrease in the frequency of LH pulses and a reduction in mean LH concentrations after the device was inserted (Nation and others 2000), and oestradiol suppressed FSH shortly after treatment (O'Rourke and others 2000). The combined treatment promoted the emergence of a new wave of follicles 4·3 days later (Bo and others 1994). Bo and others (1995) concluded that the interval to the emergence of a new wave was consistent, regardless of the stage of growth of the dominant follicle when the oestradiol was administered. O'Rourke and others (1998) proposed that the ability of exogenous oestradiol to control the dynamics of a wave of follicles is dependent on the stage of the wave when the treatment with progesterone begins.

Oestradiol can also be used to synchronise the events of oestrus and ovulation after the withdrawal of the progestin. A low dose of oestradiol at the end of the treatment is important for inducing oestrous behaviour and ovulation successfully (McDougall and others 1992). Nevertheless, the use of oestradiol at the end of the treatment has been characterised by great variability in the conception rate to first mating (Rhodes and others 1998, Alberio and others 1999, De Castro and others 2001). As an alternative treatment to promote ovulation, the administration of equine chorionic gonadotrophin (eCG) when a progestagen implant is removed, has been evaluated by Humbolt and others (1996). The hormonal treatments available to synchronise the emergence of a new follicular wave and promote the ovulation of a growing follicle have proved to be successful in heifers. However, suckling anoestrus cows are in a different physiological state and their response to hormonal treatments is variable.

The aim of this study was to evaluate the ovarian response and reproductive performance of anoestrus beef cows given a combined initial treatment with oestradiol benzoate and an intravaginal sponge impregnated with medroxy-progesterone acetate followed by either oestradiol benzoate or eCG when the sponge was withdrawn.

MATERIALS AND METHODS

During December (late spring/early summer in the southern hemisphere), 45 suckling Hereford cows were allocated to three groups of 15. The cows were randomly selected according to their mean (se) interval from parturition (52·5 [2·3] days) and mean bodyweight (385 [51·8] kg) and body condition score (4·0 [0·6] units, on a scale from 1 [emaciated] to 8 [obese]) at calving. The cows in group 1 had an intravaginal sponge containing 250 mg of medroxy-progesterone acetate (MAP) (INIA) inserted for seven days; when the sponge was inserted 2 mg of oestradiol benzoate (Intervet) was administered intramuscularly, and a second dose of 1 mg was administered when it was withdrawn. The cows in group 2 received identical treatment until the sponge was withdrawn, when 400 iu of eCG was administered. The cows in group 3 remained untreated as controls.

From the start of the treatment until four days after the sponge was withdrawn the cows' ovaries were examined ultrasonographically by the same operator with a 500 SSD machine (Aloka) equipped with a 5 MHz linear array transducer. The time of emergence of a follicular wave was defined as the day on which the largest follicle was 4 mm in diameter. If the follicle was first observed to have a diameter of 5 mm, it was assumed to have measured 4 mm on the previous day. A dominant follicle was defined as a follicle that was 9 mm or larger in diameter and was at least 2 mm larger than the second largest follicle. Ovulation was considered to have occurred when a dominant follicle, that had been observed in the same position on the ovarian surface for several days, was no longer visible and had been replaced by luteal tissue at the same site. The cows that had a corpus luteum before the sponge was withdrawn were excluded from the data analyses; four in group 1, five in group 2 and four in group 3.

Blood samples were taken twice a week and analysed for progesterone, from two weeks before the beginning of the experimental period until the end of the natural mating period, to monitor the incidence of ovulation. The concentrations were measured by a solid phase radioimmunoassay (Coat-a-Count; DPC). The intra-assay and interassay coefficients of variation were 4·8 and 8·6 per cent, respectively. A luteal phase was considered to have occurred when the progesterone concentrations were above 1 ng/ml, and they were considered to be an indicator of cyclic activity.

The cows were observed for oestrous behaviour for 40 minutes at 05.00 and 17.00 from 24 hours until 96 hours after the sponge was removed.

One-way analyses of variance were used to analyse the following end points: follicular size, the emergence of a follicular wave, the interval from parturition to the first increase in progesterone levels, and the interval from parturition to conception. Fisher's exact test was used to analyse frequencies (the numbers of cows in heat, ovulations and cyclic cows during the mating period). The data are presented as means (se) and the results were considered significant when $P \leq 0.05$.

RESULTS

In group 3, the emergence of a new follicular wave was observed in all 11 cows, on average 3·4 (0·6) days (range one to six days) after the beginning of the ultrasound studies (day 0); on day 7, the dominant follicle had a maximum diameter of 7·0 (0·5) mm (range 5 to 9 mm). The cows in groups 1 and 2 received identical treatment until the sponge was removed, and they were therefore pooled to analyse the effect of the combined treatment on the emergence of a new wave of follicles. In 19 of the 21 cows a new follicular wave emerged on average 3·9 (0·3) days (range two to seven days) after the treatment commenced. When the sponge was withdrawn, the mean diameter of the dominant follicle was 7·5 (0·5) mm (range 3 to 12 mm). To study the effect of the stage of development of the dominant follicle when the treatment began on the emergence of a new wave of follicles the cows were subdivided with respect to the diameter of the largest follicle on day 0. In the 14 cows in which the largest follicle on day 0 was at least 9 mm in diameter (10·8 [0·2] mm), a new dominant follicle emerged on day 3·6 (0·4). In the five cows in which the largest follicle on day 0 was less than 9 mm (7·0 [0·8] mm) ($P \leq 0.001$), a new dominant follicule emerged on day 4·3 (0·3) ($P = 0.15$). However, in the cows that had a follicle of 9 mm or more on day 0, the new follicle reached a larger diameter (8·5 [0·4] mm) by the time the sponge was removed than in the cows that had a follicle of less than 9 mm (6·0 [0·5] mm) ($P \leq 0.01$). Only the five cows in which the new dominant follicle emerged early (2·8 [0·2] days after the treatment began) and had reached a diameter of 9 mm or more when the sponge was removed, ovulated; for these five cows the mean diameter was 10·5 (0·5) mm, compared with 6·3 (0·4) mm for the 15

cows that did not ovulate (P≤0·001), irrespective of whether they received oestradiol benzoate or eCG treatment (Fig 1). In one additional cow that ovulated after the treatment, the dominant follicle had already emerged on day 0 and it continued growing to reach 10 mm in diameter on the day before ovulation. A higher proportion of the cows in group 1 (nine of 11) came into oestrus than of those in group 2 (two of 10) and group 3 (none of 11) (P≤0·001). Ovulations were observed ultrasonographically in four of the 11 cows in group 1, two of the 10 in group 2, and none of the 11 cows in group 3.

Two cows in group 1 died before the end of the mating period and were excluded from the subsequent analyses. The interval from parturition to the first increase in progesterone was not significantly different between the groups (81·3 [5·8] days, 88·9 [4·5] days and 95·5 [3·6] days in groups 1, 2 and 3 respectively). The numbers of cows that re-initiated cyclic activity during the mating period were similar: eight of nine in group 1, eight of 10 in group 2 and six of 11 in group 3, and the intervals from parturition to conception were also similar: 82·4 (4·6) days in group 1, 88·3 (5·0) days in group 2 and 88·3 (3·2) days in group 3.

DISCUSSION

The injection of oestradiol benzoate at the beginning of the treatment with an MAP sponge was not effective in synchronising a new follicular wave, the emergence of which was very variable. The control exerted by the exogenous administration of oestradiol (O'Rourke and others 1998) or progesterone (Nation and others 2000) on follicular dynamics depends upon the stage of development of the dominant follicle at the time of treatment. These results suggest that the presence or absence of a large follicle did not account for the variation in the interval from the treatment to the emergence of a new wave. However, the presence of a follicle larger than 9 mm in diameter was associated with a larger new dominant follicle by the time the sponge was removed.

The ability of oestradiol to induce a new follicular wave is also dependent on high concentrations of progesterone 48 hours after its administration (Day and Burke 2000). It is possible that the MAP was not absorbed from the sponge quickly enough to promote this beneficial effect. The length of the progestin treatment controls the timing of the new follicular wave and the subsequent period required for the new dominant follicle to attain the capacity to ovulate (Day and Burke 2000). However, in this experiment the emergence of a new wave was not synchronised, and only in those animals in which the new wave emerged early did the dominant follicle have enough time to reach maturity. The ovulatory response was determined by the size of the dominant follicle when the sponge was withdrawn. In potentially ovulatory follicles, a diameter of 8 mm or more is highly related with their oestrogenic capacity, and their having granulosa and theca cell LH receptors (Ireland and Roche 1982, Ginther and others 1996). An injection of oestradiol benzoate or eCG when the sponge is withdrawn would induce a preovulatory release of LH. Nevertheless, the presence of a follicle capable of ovulating in response to the LH surge is essential, and it has been reported that follicles less than 10 mm in diameter did not ovulate in response to a gonadotrophin-releasing hormone-induced release of LH (Garverick and others 1980). In the present study, only follicles that reached a diameter larger than 9 mm ovulated after the injection of either oestradiol benzoate or eCG.

The administration of exogenous oestradiol after the termination of progesterone treatment was developed as a method for inducing oestrus in anoestrus cows (McDougall and others 1992). Much of the improvement was attributed to the elimination of silent ovulations. In this study, although

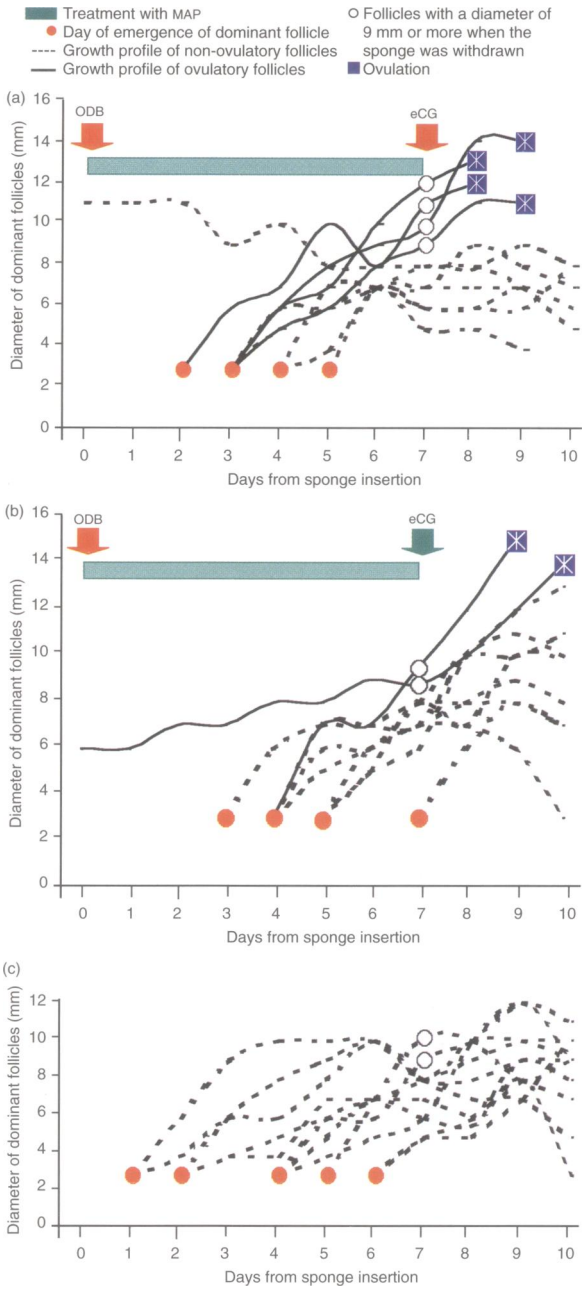

FIG 1: Follicular dynamics in cows that received a combined treatment with an intravaginal sponge impregnated with 250 mg of medroxyprogesterone acetate (MAP) and 2 mg of oestradiol benzoate (ODB). When the sponge was withdrawn they received either (a) 1 mg of ODB (group 1), (b) 400 iu of equine chorionic gonadotrophin (eCG) (group 2) or (c) remained as untreated controls (group 3)

a larger proportion of the cows in group 1 showed oestrus, six of the 11 did not ovulate. This result differs from the results of previous studies in which 82 per cent of the cows showed oestrus followed by ovulation and only 6 per cent of the animals exhibited a non-ovulatory oestrus (MacMillan and others 1995). It is probable that the oestrous behaviour in the cows of group 1 was promoted by the pharmacological effect of the oestradiol benzoate, rather than the presence of an oestrogen-active follicle. In group 1, the first increase in progesterone concentrations occurred on average 14·2 days before the increase in the cows in group 3, and 7·6 days before that in the cows in group 2, although the differences were not statistically different. At the end of the mating period there was no difference between the numbers of cows that had recommenced cyclic activity. The large number of cows in heat in group 1 and the introduction of the bulls to the herd might have had a biostimulatory effect on the anoestrus cows (Azzam and others 1991).

It was concluded first, that the administration of oestradiol benzoate at the beginning of a seven-day period of treat-

ment with a MAP sponge was not effective in synchronising the emergence of a new follicular wave. Secondly, the size of the dominant follicle when the oestradiol benzoate or eCG was administered determined the ovulatory response. Thirdly, treatment with oestradiol benzoate when the sponge was withdrawn was more effective in inducing oestrous behaviour than treatment with eCG, but in half the animals the oestrus was not followed by ovulation. Finally, the administration of the combined hormonal treatment did not restore the reproductive endocrine axis of anoestrus cows to that of cyclic cows.

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ABSTRACT

Diagnostic arthroscopy of dogs with traumatic lesions of the stifle joint

THE medical records of 70 dogs that had remained lame after traumatic injuries to their stifle joints, including ruptures of the cranial cruciate ligament and meniscal tears, had been repaired surgically, were examined to determine whether pre-existing lesions in the joints might have been responsible. Arthroscopic examinations of the damaged joints had revealed that 68 of the dogs had already had osteoarthritic lesions, rather than any failure of the surgical procedures, were responsible for the dogs' persistent lameness.

ADAMIAK, Z. & NOWICKI, M. (2003) Diagnostic arthroscopy in dogs with traumatic lesions of the stifle joint. *Irish Veterinary Journal* **56,** 517-518

Treatment of suckling beef cattle with a progestagen sponge and oestradiol benzoate or equine chorionic gonadotrophin

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