INCREASING THE USE OF AI IN SUCKLER HERDS

Authors

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The work summarised in this report was carried out at Belclare and Athenry Research Centres as part of the Teagasc Beef Research Programme.

Teagasc acknowledges support from the Operational Programme for Agriculture, Rural Development and Forestry, 1994-1999.
Measure 5 (b), Research Stimulus Fund

Project No. 4361
Beef Production Series No. 33

Agriculture and Food Development Authority

Teagasc,
19 Sandymount Avenue,
Ballsbridge,
Dublin 4.

May 2001

ISBN No. 1 84170 192 0

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Acknowledgements

The authors acknowledge the skilled technical assistance of P. Joyce, W. Connolly, J. Nally, G. Morris, Ms A. Glynn, Ms N. Hynes (UCD) and P. Reilly, P. Creaven, G. Burke and G. McHugh for care of animals. The authors also acknowledge South Western Cattle Breeding Station, Bandon Co. Cork and North Eastern Cattle Breeding Station, Ballyhaise, Co. Cavan for assistance with the farm-trial studies. M. O'Rourke was a recipient of a Teagasc Walsh Fellow.

SUMMARY

Reproductive efficiency is a major factor affecting production and economic efficiency of beef herds. For herds using artificial insemination (AI) heat detection rate and calving rate are the two major determinants of compactness of calving and ultimately to the calving-to-calving interval. Heat detection rate is hugely variable from herd-to-herd but for most herds only 40 to 70% of cows that exhibit heat are actually detected in heat by the stockman. The objective of the this project was to develop an improved cost effective hormonal method to control the time of ovulation to allow beef cows be bred by AI without the need for heat detection. A total of 3 studies were carried out. The main results are summarised in this publication.

The administration of exogenous oestradiol by injection, in the form of oestradiol benzoate (ODB), resulted in dose-dependent increase in peak plasma oestradiol and a dose-dependent transient decrease in follicle stimulating hormone (FSH) in ovariectomised heifers. Administration of higher doses (5mg and 10mg) of oestradiol by capsule attached to a progesterone releasing intravaginal devise generated much lower plasma concentrations of oestradiol compared with the lowest dose (0.75mg) of ODB administered intramuscularly. Both the maximum % decline in FSH and interval to FSH nadir were related to peak concentrations of oestradiol. Plasma concentrations of FSH began to increase 1-2 days after ODB administration while concentrations of oestradiol were declining but still high.

The results of Experiment 2 clearly showed that the administration of ODB, causes a dose-dependent increased in plasma concentrations of oestradiol and a dose-dependent decrease in plasma FSH in intact heifers, and, in a high proportion of animals causes atresia of the follicle wave present at treatment initiation followed by the emergence of new follicle wave 3 to 6 days later. This study also clearly demonstrated that the administration of oestradiol, by a capsule attached to the progesterone releasing device, was the least effective way of administering oestradiol in terms of its ability to generate plasma concentrations of 5-

15 pg/ml which, would appear to be required to terminate the current follicle wave and cause new wave emergence. Plasma concentrations of 3-5 pg/ml generated by the gelatine capsule attached to the progesterone releasing device were insufficient to consistently ensure new emergence but were sufficient to shorten the lifespan of the corpus luteum (CL). This is the first study to demonstrate that a lower dose of oestradiol is required to shorten the lifespan of the CL than is required to alter follicle wave dynamics.

The results of this study highlight the difficulty of obtaining a tight synchrony of heat in animals that are at the early stages of their oestrous cycles at time of treatment initiation. The use of oestradiol at the beginning of a progesterone synchronisation treatment, even when the duration of the treatment is extended to 12 days, does not ensure that corpus luteum regression is complete in all animals at the time of progesterone withdrawal, thus allowing animals to exhibit a synchronous heat. Consequently, there is a requirement to administer a luteolytic agent like prostaglandin $F_{2\alpha}$ or one of its analogues at or before progesterone withdrawal to ensure complete corpus luteum regression.

While there was clear evidence that administration of oestradiol by injection was superior to administration by a capsule, attached the progesterone releasing device, there was no evidence of any beneficial effects of increasing the dose of oestradiol from 0.75 mg/animal to 5 mg/animal in terms of controlling follicle wave dynamics, shortening the lifespan of the corpus luteum, or in terms of synchrony of heat or overall heat response.

In the farm trial studies (Experiment 3) there was no effect of progesterone duration (8-day versus 12-day) on oestrous response in either beef cows or heifers though in the latter oestrous response tended to be higher following an 8-day treatment. Similarly, duration of treatment had no effect on the synchrony of the oestrous response following withdrawal of the progesterone though there was some evidence of a more highly synchronised oestrous response following the 8-day treatment.

The use of 1.0-mg oestradiol 24 hours after progesterone removal significantly increased synchronised oestrous response in beef cows. However, in beef heifers oestrous response at 91% in the non-ODB treated heifers was already high and would have been difficult to improve upon.

The use of ODB improved the synchrony of the oestrous response with almost all cows exhibiting oestrus on Day 2 following withdrawal of the progesterone. This highly synchronised oestrous would allow one fixed-timed AI at about 54-56 hours after withdrawal of the progesterone. In cows there was a tendency for the pregnancy rate to be higher following an 8-day than a 12-day duration progesterone treatment. The administration of ODB had no effect on pregnancy rate in beef cows but significantly reduced pregnancy rate in heifers. It would appear that the dosage used in heifers was excessive and that a lower dose might be more appropriate. However, the high and synchronous oestrous response recorded in heifers would obviate the need to use ODB 24 hours after progesterone removal in heifers.

In cows the overall benefit of using ODB is evident by the higher conception rate (+7 percentage points) at the synchronised oestrus. This results from a small increase in both the oestrous response and pregnancy rate in ODB-treated cows.

The insemination of animals, preferably once at a specific time without recourse to oestrous detection and with repeatable high pregnancy rates, would allow for greater use of proven AI sires on beef cows thereby increasing the quality of cattle from the beef cow herd.

GENERAL INTRODUCTION

Reproductive efficiency is a major factor affecting production and economic efficiency of beef herds. For herds using artificial insemination (AI) heat detection rate and calving rate are the two major determinants of compactness of calving and ultimately the calving-to-calving interval. Heat detection is a time consuming repetitive chore that must be carried out up to 5-times each day for as long as AI is used. Heat detection rate, usually measured as submission rate, is hugely variable from herd-to-herd but for most herds only between 40% and 70% of cows that exhibit heat are actually detected by the stockman. Despite an increased understanding of the endocrine control of the oestrous cycle the goal of fixed-time insemination is not yet consistently achievable in either cows or heifers treated at different stages of the oestrous cycle and in different physiological states. The overall objective of the this project was to develop an improved cost effective hormonal method to control the time of ovulation to allow beef cows be bred by AI without the need for heat detection. A total of 3 studies were carried out and the results are summarised in this report.

Study 1.
The effect of dose and route of oestradiol benzoate administration on plasma concentrations of oestradiol and follicle stimulating hormone in long-term ovariectomised heifers

Background

Follicle growth in cattle is a highly regulated process under the control of FSH, LH and local intra-follicular growth factors. There are recurrent waves of follicular growth throughout the oestrous cycle and most of pregnancy, and they resume early in the postpartum period. Each wave begins with the emergence of a cohort of oestrogen active follicles of 3-5 mm in diameter, preceded by an increase in concentrations of FSH. This is followed by the selection generally of one dominant follicle (DF) which occurs in association with declining FSH concentrations. Finally the fate of the DF is determined by LH pulse frequency, where infrequent pulses (1 every 3-4 hours) lead to DF atresia while more frequent pulses (1 per hour) lead to ovulation. Thus, each wave has an inherent life span, determined by the interval from wave emergence to dominance and the duration of dominance. The duration of dominance is dependent on the number of waves (2 or 3) during the oestrous cycle, and on the influence of nutrition, lactation and suckling during the postpartum period.

The factors that regulate the recurrent FSH increases are mainly of ovarian origin, particularly from the DF, with oestradiol (E_2) one of the key intra-follicular components involved. The relationship between systemic E_2 and FSH concentrations however, is unclear and the reported effects of E_2 on FSH are inconsistent. The ability to exogenously regulate FSH concentrations in cattle is important to synchronise follicle wave development in both, cyclic and anoestrous cattle. It is necessary to control follicle wave dynamics as well as luteal function in order to have a 'healthy' DF present at the end of treatment. Both oestradiol and progesterone used together can synchronise follicle waves, but with variable success. However, the optimum dose of oestradiol necessary to suppress FSH is not clear, neither is the effect of route of administration of oestradiol on plasma concentrations of oestradiol known. The specific objective of this experiment was to determine the effect of dose and route of oestradiol administration at time of progesterone administration on plasma concentrations of FSH and oestradiol in order to select the optimum dose to manipulate follicle waves in subsequent studies. In this experiment, ovariectomised animals were used to prevent possible confounding effects of endogenous oestradiol on plasma concentrations generated by the different routes and doses used.

Materials and methods

Thirty-four long-term ovariectomised crossbred beef heifers; 18-24 months of age and weighing 350-480 kg were used. All heifers received a progesterone releasing intravaginal device for 7 days in order to simulate luteal phase concentrations of progesterone before treatment. Four days after removal of the first progesterone-releasing device, a new device was inserted for a further 7 days. Animals were randomly allocated to receive one of eight oestradiol benzoate (ODB) treatments at this time; 1) 0.0 mg (Control; n = 3), 2) 0.5 mg (n = 4), 3) 1 mg (n = 4), 4) 2.5 mg (n = 6), 5) 5 mg (n = 4), 6) 10 mg (n = 4) all administered by intramuscular injection in 2 ml of corn oil and 7) 5 mg (n = 4) and 8) 10 mg (n = 5) ODB in powder form administered by gelatine capsule attached to the progesterone releasing device.

Blood sampling and hormone assay

Frequent blood samples were collected by jugular venipuncture for subsequent oestradiol and FSH determinations. Plasma concentrations of FSH and oestradiol were determined by radioimmunoassays.

Statistical analyses

The E_2 and FSH data were subjected to repeated measures analyses of variance followed by regression analyses. Because of the between animal variability, concentrations of FSH were analysed as % changes from pre-treatment concentrations.

Results

Plasma concentrations of E_2

All animals had similar and non-detectable concentrations of E_2 pre-treatment. Peak concentrations of E_2 following all treatments (Table 1) were significantly higher than pre-treatment concentrations and higher than those in untreated control animals. The interval (hours) from administration of ODB to peak E_2 concentration (Table 1) was similar ($P>0.1$) in heifers given intramuscular injections (mean 13.4±1.24 hours), and was longer ($P<0.05$) in heifers given the 5 mg and 10 mg ODB by intravaginal capsules (mean 25.5±2.84 hours). Peak plasma concentration of E_2 increased with dose of ODB injected, (plasma E_2 = $-0.237+16.109(\text{dose})-0.74(\text{dose})^2$, $R^2=0.75$; $P<0.05$) but not when given topically by the intravaginal route using the capsule. Peak concentrations of E_2 following use of the 5 and 10mg intravaginal capsules were similar to each other and to those following the 0.5 mg injection ($P>0.05$), but were lower than the concentrations in heifers given 1 mg, 2.5 mg, 5 mg and 10 mg ODB intramuscularly ($P<0.05$). The increase in plasma concentrations of E_2 in animals following the 5 mg and 10 mg injections was prolonged and slower to return to pre-treatment concentrations than in animals following all other treatments (Table 1).

Plasma concentration of FSH

The effects of ODB administration on plasma concentrations of FSH are presented in Table 2. There was a significant decline in FSH concentration following all ODB treatments ($P<0.05$) and the magnitude of this decline was greater as the blood concentration of E_2 increased. The maximum decline in FSH concentration expressed as percentage of the pre-treatment concentrations, was significantly greater following the 5 mg and 10 mg injections than following all other ODB treatments. Across all treatments, both the maximum percentage decline in FSH and the interval to reach that decline were related to the peak plasma concentrations of E_2 (maximum % decline in FSH = $11.17+1.564(\text{peak } E_2)-0.009(\text{peak } E_2)^2$, $R^2=0.75$; $P<0.005$), (hours to FSH nadir = $10.628+1.486(\text{hours to peak } E_2)-0.0282(\text{hours to peak } E_2)^2$, $R^2=0.22$; $P<0.05$). Following nadir concentrations, FSH increased as the E_2 concentration declined from peak value, irrespective of treatment. However, at the higher injected doses, concentrations of FSH begin to rise again in the face of declining but still elevated plasma concentrations of E_2. Concentrations of FSH following the 5 mg and 10 mg injections took significantly longer to return to pre-treatment concentrations than in animals assigned to all other treatments ($P<0.05$).

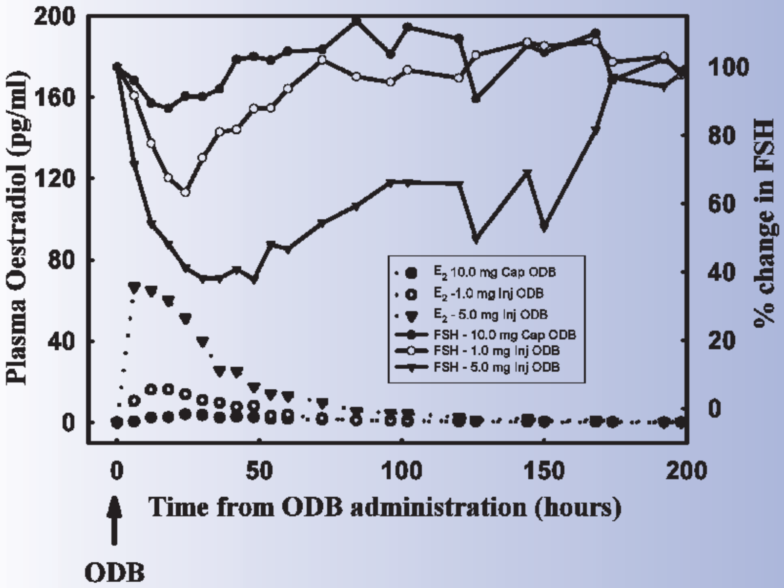


Figure 1. Effect of different doses of oestradiol benzoate (ODB) administered either by gelatine capsule (10 mg dose) attached to progesterone releasing device or two doses (1.0 and 10.0 mg) dissolved in 2 ml of corn oil and administered by intramuscular injection to ovariectomised heifers at the time of progesterone releasing device insertion on mean plasma concentrations of oestradiol (E2 – dotted lines) and FSH (solid lines) in ovariectomised heifers (n=4 heifers per dose).

Table 1. **Effect of ODB administration using different doses and routes of administration on characteristics of plasma concentrations of oestradiol (Mean ± S.E.) in ovariectomised heifers given a progesterone intravaginal device.**

ODB Treatment (mg)	No. Of heifers (n)	Pre-treatment Concentrations of E_2	Peak Concentration Of E_2 (pg/ml)	No. of hours to h maximum concentration of E_2	No. of hours to reach < 3 pg/ml of E_2	No. of hours to reach < 1 pg/ml of E_2
Controls						
0.0	3	0.1	0.1 ± 0.05^{a}			
Topical administration by intravaginal capsule						
5.0	4	0.1	3.8 ± 7.1^{b}	25.5 ± 4.2^{ad}	54.0 ± 23.8^{a}	72.0 ± 19.4^{a}
10.0	5	0.1	4.1 ± 6.3^{b}	27.6 ± 3.7^{bd}	43.5 ± 16.8^{a}	87.6 ± 17.3^{a}
Intramuscular injection in 2ml corn-oil						
0.5	4	0.1	11.9 ± 7.1^{bc}	13.5 ± 4.2^{ac}	44.0 ± 19.4^{a}	57.0 ± 19.4^{a}
1.0	4	0.1	18.0 ± 7.1^{c}	15.0 ± 4.2^{ac}	70.5 ± 16.8^{a}	97.5 ± 19.4^{ac}
2.5	6	0.1	24.3 ± 5.8^{c}	11.0 ± 3.4^{c}	82.0 ± 13.7^{a}	125.0 ± 15.8^{bc}
5.0	4	0.1	72.4 ± 7.1^{d}	12.0 ± 4.2^{c}	135.0 ± 16.8^{b}	163.5 ± 19.4^{bd}
10.0	4	0.1	84.6 ± 8.2^{d}	18.0 ± 4.8^{ac}	158.0 ± 19.4^{b}	188.0 ± 22.4^{d}

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

Table 2. Effect of ODB administration using different doses and routes of administration on characteristics of FSH concentrations (Mean ± S.E.) in ovariectomised heifers given a progesterone intravaginal device.

ODB Treatment (mg)	No. Of heifers (n)	Pre-treatment Concentrations of FSH (ng/ml)	Mean Concentration of FSH at nadir (ng/ml)	No. of hours to reach FSH nadir	Maximum decline in FSH expressed as % of pre-treatment values	No. of hours to reach first FSH rise following nadir	No. of hours for FSH to return to pre-treatment concentrations
Controls							
0.0	3	95.9 ± 16.96^{ab}	$(102.9 \pm 13.1)^{a}$		0.0^{a}		
Topical administration by intravaginal capsule							
5.0	4	110.4 ± 13.45^{ab}	81.5 ± 11.3^{b}	24.0 ± 4.2^{ad}	26.6 ± 6.9^{b}	30.0 ± 14.9^{a}	46.5 ± 16.92^{a}
10.0	5	97.5 ± 12.70^{ab}	78.6 ± 10.1^{b}	24.0 ± 3.8^{a}	18.9 ± 6.2^{b}	33.6 ± 13.3^{a}	57.6 ± 15.13^{a}
Intramuscular injection in 2ml corn-oil							
0.5	4	100.8 ± 9.55^{ab}	78.1 ± 11.3^{b}	19.5 ± 4.2^{a}	24.3 ± 6.9^{b}	25.5 ± 14.9^{a}	38.0 ± 19.54^{a}
1.0	4	129.4 ± 3.47^{a}	82.5 ± 11.3^{b}	24.0 ± 4.3^{a}	36.6 ± 6.9^{b}	31.5 ± 14.9^{ac}	64.5 ± 16.92^{a}
2.5	6	116.5 ± 8.37^{ab}	71.2 ± 9.2^{b}	24.0 ± 3.5^{ac}	40.0 ± 5.7^{b}	34.0 ± 12.2^{ad}	57.0 ± 13.82^{a}
5.0	4	108.4 ± 18.5^{ab}	31.3 ± 11.3^{c}	37.5 ± 4.2^{b}	69.7 ± 6.9^{c}	45.0 ± 14.9^{b}	183.0 ± 16.92^{b}
10.0	4	75.2 ± 14.11^{b}	22.3 ± 13.1^{c}	34.0 ± 4.9^{bcd}	69.5 ± 8.1^{c}	104.0 ± 17.2^{bcd}	168.0 ± 19.54^{b}

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

Discussion

This experiment clearly shows that the route of administration and dose of ODB affect subsequent concentrations of E_2 and FSH in blood. The concentration of E_2 in blood was elevated faster following ODB administered i.m. compared with topical application using gelatine capsule attached to the progesterone-releasing device. Plasma concentrations of oestradiol in heifers given ODB topically took over ten hours longer to reach maximum concentration than in heifers given a similar dose by i.m. Peak concentrations of oestradiol in heifers were twenty times greater for the 5 and 10-mg injections than similar doses administered in powder form using the intravaginal capsule. While peak plasma concentration of E_2 was not reached for a further ten hours in these heifers, some of the oestradiol may have been lost before being absorbed, given that the gelatine capsule dissolves within 6 hours. This experiment demonstrates more efficient absorption following intramuscular injection in oil than following intravaginal capsule administration of ODB. The rate of absorption of ODB from an oil solvent is slower than from an aqueous solvent, but the rate of absorption is still faster than topical administration. Retrograde loss in vaginal secretions may also be a significant source of loss from the vagina.

Peak E_2 increased in a dose-dependent manner with dose injected but not following capsule administration. Once peak plasma concentrations were reached, the rate of decline of E_2 was similar for all treatments. This is the first experiment to show the difference between peak plasma concentrations of E_2 following administration of ODB by intramuscular injection and intravaginal gelatine capsule. Some synchronisation regimes have included the administration of 10-mg ODB in oil by intramuscular injection, which would elevate concentrations of E_2 to pharmacological levels and result in slow return of E_2 to basal cyclical values. While there is a dose-dependent elevation of E_2 following intramuscular injection, the same is not true following administration by intravaginal capsule. Peak plasma concentrations were similar following both 5 and 10-mg intravaginal doses (Table 1), demonstrating the inefficiency of absorption of ODB by topical administration to vaginal epithelium following dissolution of the gelatine capsule.

Increasing concentrations of E_2 transiently suppress FSH concentration in ovariectomised heifers that were given progesterone. The magnitude of the decline in FSH was affected by the peak concentrations of E_2 achieved. The duration of the transient suppression was dose-dependent and with E_2 blood concentrations of 80 pg/ml in animals following 10mg ODB intramuscularly in oil, the FSH suppression lasted considerably longer, viz. up to 150 hours. This is the first report showing an increase in FSH concentrations while plasma concentrations of E_2 were still very high demonstrating that E_2 is only one part of the negative feedback mechanism involved in the control of FSH.

Study 2.
The effect of oestradiol administered during the first follicle wave in association with progesterone administration on follicle wave dynamics and oestrous response in heifers

Background

Oestradiol given at the start of a progesterone treatment in cattle affects follicle dynamics, however, the effectiveness of different doses on terminating the existing follicle wave, on the interval to new wave emergence, on follicle wave status at progesterone withdrawal and on the lifespan of the corpus luteum are not known. Furthermore, there is evidence that it is more difficult to induce a highly synchronised heat and ovulation in animals that are the early stages of their oestrous cycles at time of treatment initiation. The objective of this study was to determine if the follicle and oestrous response to exogenous oestradiol was dose- and follicle status-dependent.

Materials and Methods

Ninety-one crossbred beef heifers, 18-24 months of age, weighing 310-480 kg were used. Heifers were synchronised with prostaglandin. Heifers (n=81) were given a progesterone releasing intravaginal device during distinct stages of development of the first follicle wave following the synchronous heat. At progesterone insertion, heifers were randomly assigned to receive one of four plasma concentrations of oestradiol (E_2) as follows: 1) 0, 2) low 2-4 pg/ml by use of 10mg ODB by gelatine capsule, 3) medium 15-20 pg/ml by 0.75mg ODB i.m. injection in 2ml corn oil or 4) high 40-60 pg/ml by 5mg ODB i.m. in oil. These plasma concentrations of oestradiol and doses of ODB were derived from the results of Study 1. The treatments were administered at i) emergence (E), ii) 1st day of dominance (DOM1) and iii) 4th day of dominance (DOM4) of the 1st follicle wave (see Figure 2). The design was, therefore, a 4x3 factorial with four dose of ODB administered at three distinct follicle stages. The progesterone releasing devices were left in place for 12 days. Ten heifers remained untreated (controls). Heifers remained on the experiment until heat and ovulation had occurred following treatment. Heifers were observed for heat four times daily from 36 hours following progesterone removal.

Ovarian Ultrasonography

Ovarian follicle dynamics of all heifers were monitored once daily from the day following the second PG injection until ovulation following treatment. The emergence of a follicle wave was defined as the development of a cohort of small follicles that measured between 4 - 5 mm in diameter. Dominance was determined as the 1st day a follicle became dominant. A dominant follicle was defined as the largest follicle present on either ovary, that was at least 8.5 mm in diameter and at least 2 mm larger than all other follicles on either ovary, and suppressed the growth of other follicles. Duration of dominance of a dominant follicle was defined as the interval in days from the 1st day on dominance to the emergence of the subsequent wave. Ovulation was taken as the day in which a dominant follicle was no longer detectable, followed by the development of a corpus luteum in the position previously occupied by the ovulatory follicle.

Blood sampling and hormone assays

Blood samples were collected for progesterone, FSH and oestradiol determination. Plasma concentrations of progesterone, FSH and oestradiol were determined by radioimmunoassays.

Statistical analyses

Follicle data were analysed using analyses of variance (SAS, 1998). Hormone data were analysed using repeated measures analyses of variance followed by regression analyses (SAS, 1988). Because of the between animal variability, concentrations of FSH were analysed as % changes from pre-treatment concentrations.

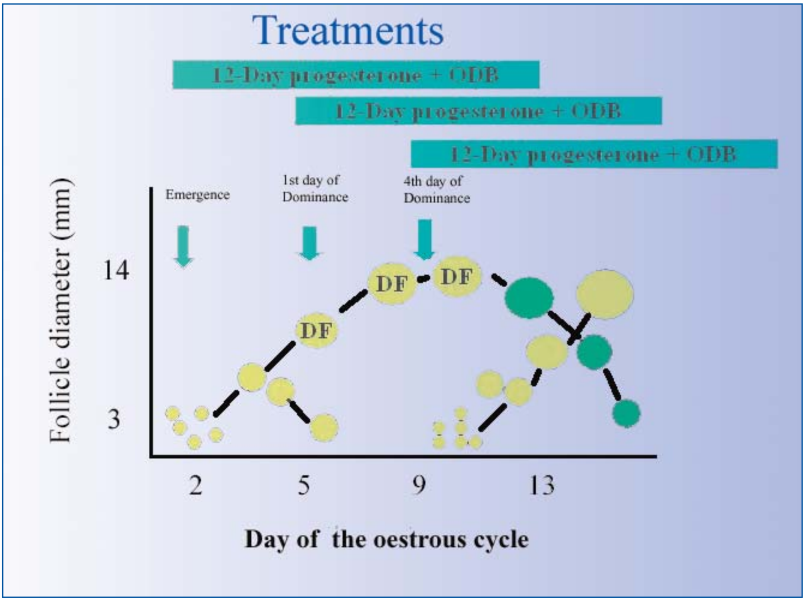


Figure 2. Schematic representation of treatments relative to stage of follicle wave follicle wave.

Results

Effect of different dose of ODB on plasma concentrations of oestradiol

Different ODB treatments generated significantly different ($P<0.05$) and distinct peak plasma concentration of E_2, irrespective of the stage of the follicle wave at which treatment was initiated (see Figure 3). Peak concentrations of E_2 were higher ($P<0.01$) at all stages of the follicle wave following the administration of 5-mg ODB by injection, than all other doses used. A dose 0.75 mg of ODB generated peak plasma concentrations of 8-15 pg/ml which, were higher than concentrations of 3-5 pg/ml generated by the 10mg capsule of ODB attached to the progesterone releasing device.

Effect of dose ODB on fate of first follicle wave

The administration of progesterone, irrespective of accompanying dose of ODB, at emergence of the first follicle wave altered the course of the first follicle wave with fewer (P<0.05) ODB-treated (11/26; 42%) forming a 1^{st} DF compared with control heifers (10/10;100%). More animals that received ODB by injection (10/13; 77%) formed a new follicle wave compared with animals receiving progesterone only or progesterone + ODB by capsule (3/11;27%; P<0.05)

Effect of exogenous progesterone and different doses of ODB on plasma concentrations of progesterone

Plasma concentrations of progesterone increased rapidly following progesterone administration with highest concentrations recorded in animals treated on the 1^{st} (DOM1) and 4^{th} day (DOM4) of dominance (Figure 4). At 24 hours after progesterone withdrawal (Day 13, Figure 4) plasma concentrations of progesterone were lowest (P<0.05) in animals receiving ODB irrespective of dose compared with control or animals not receiving ODB. The lower concentrations of progesterone on Day 13 was reflected in the shorter interval to onset of heat following withdrawal of the exogenous progesterone (Table 4)

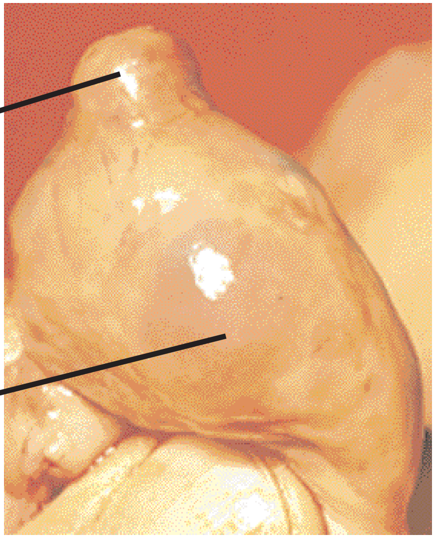
Effect of exogenous progesterone and different doses of ODB on plasma concentrations of FSH

Irrespective of the stage of the follicle wave at treatment initiation the administration of progesterone only or progesterone + ODB caused a transitory decrease in plasma FSH (Figure 5 and Table 3.). The greatest % decline in FSH was caused by the administration of 5.0-mg ODB by injection.

Heat response and synchrony of heat

The overall heat response was high and similar for all treatments. The synchrony of heat, measured as the proportion of animals observed in heat on days 2 and 3 following progesterone removal was similar (P>0.05) for heifers receiving progesterone only (45%) or progesterone + ODB by capsule (55%). Similarly, the synchrony of heat was similar (P >0.05) for animals which received 0.75 mg (78%) or the 5.0 mg

(60%) ODB by injection. The overall synchrony of heat was higher (P<0.05) for animals receiving ODB by injection (27/39;69%) compared with animals receiving no ODB or ODB by gelatine capsule (50%).



External appearance of bovine ovary showing corpus luteum (CL) and large dominant follicle. For good synchrony of heat and good fertility it is necessary to control the life span of the CL and to ensure that there is a "healthy" oestrogen active follicle present at the time of progesterone withdrawal.

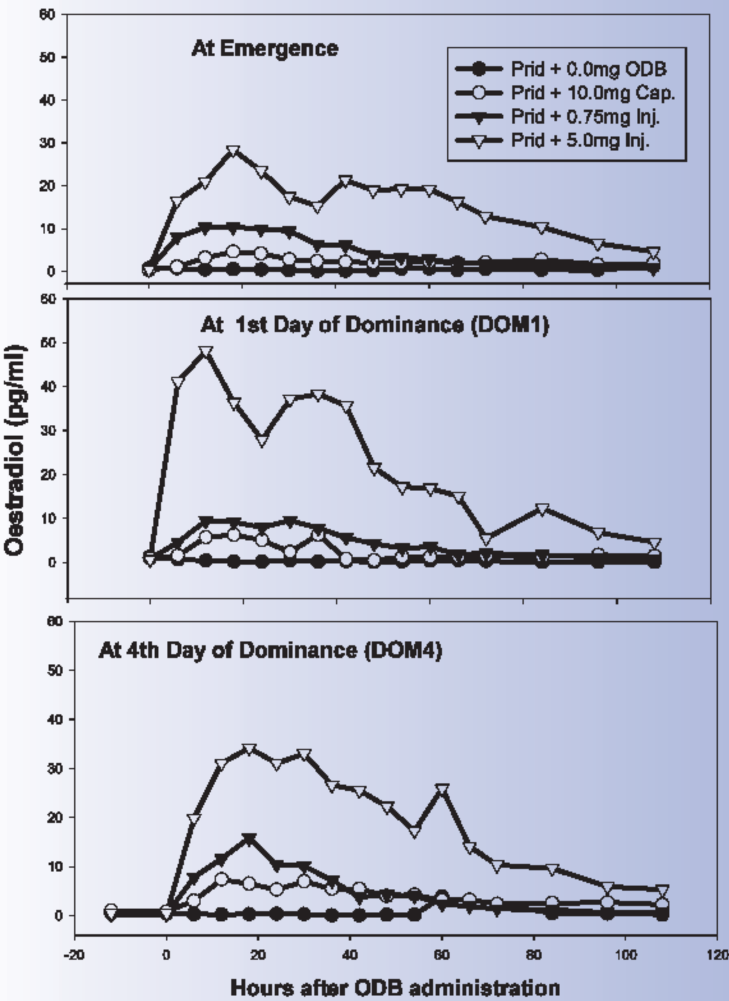


Figure 3. Plasma concentrations of oestradiol generated by different doses and routes of oestradiol administration in conjuncton with exogenous progesterone at three distinct stages of the first follicle wave.

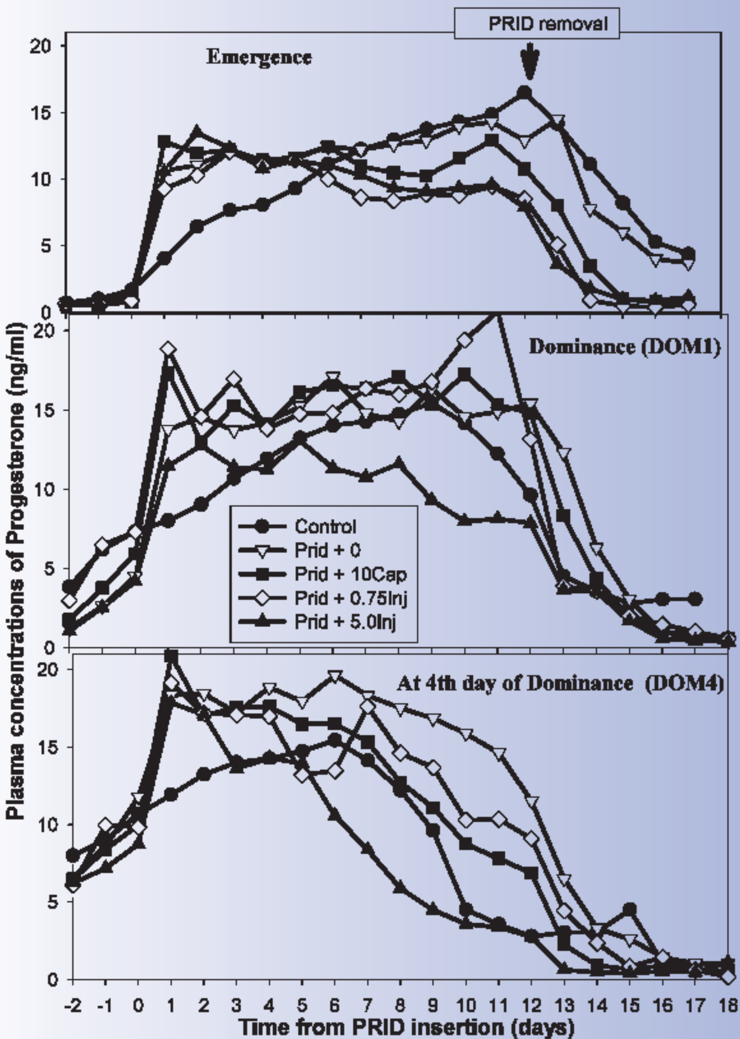


Figure 4. Plasma concentration of progesterone in heifers receiving exogenous progesterone and different doses of oestradiol benzoate at three distinct stages of the first follicle wave of the oestrous cycle

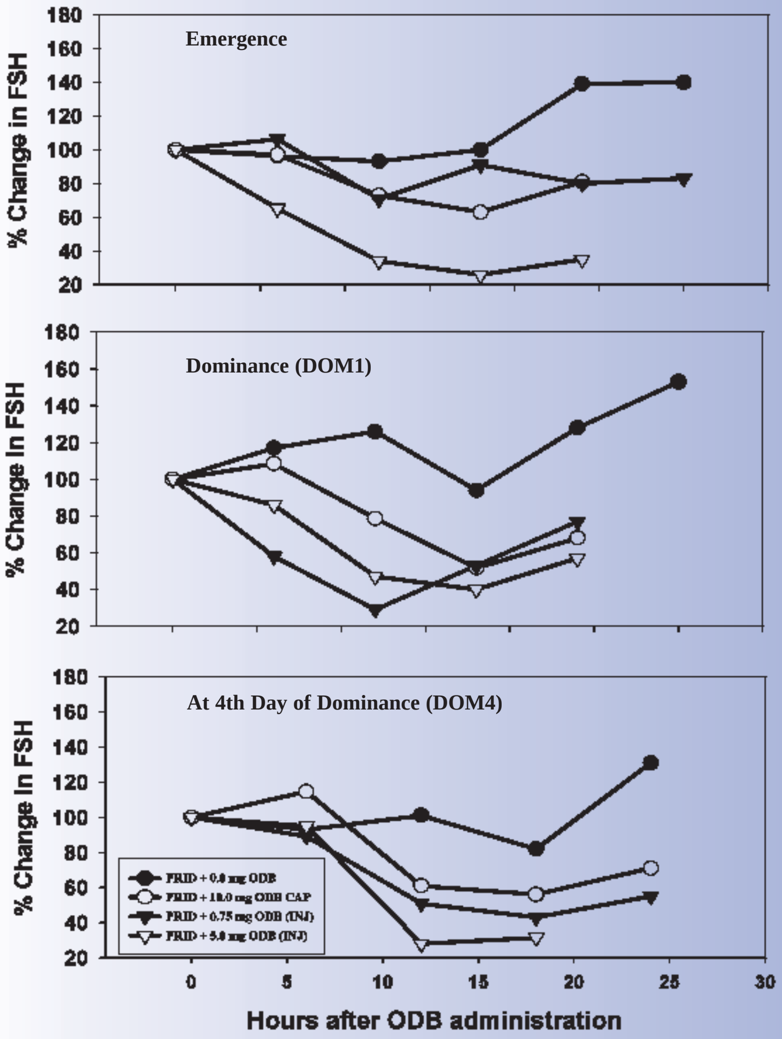


Figure 5. Relative changes in plasma concentrations of FSH following the administration of exogenous progesterone and different doses of ODB at different stages of the first follicle wave of the oestrous cycle

Table 3. The effect of dose and route of oestradiol benzoate (ODB) on plasma concentrations of FSH (ng/ml) when administered at different stages of development of the first dominant follicle in beef heifers (Mean ± S.E.)

Parameter	Follicle stage	Treatment				
		Control	Progesterone alone	Progesterone + 10 mg cap.	Progesterone + 0.75 mg i.m.	Progesterone + 5.0 mg i.m.
Pre-treatment concentrations of FSH (ng/ml) (3 values)	**Emergence**	11.7±2.5	6.6±2.5^{a}	14.5±2.2^{b}	12.5±2.2	11.8±2.2
	DOM1	7.2±2.5	4.5±2.2	7.0±2.5	6.2±2.5	8.7±2.5
	DOM4	17.4±2.5	9.6±2.5^{b}	18.6±2.2^{a}	11.5±2.2^{b}	9.0±2.0^{b}
% decline in FSH (ng) from pre-treatment values	**Emergence**	70±12.7^{a}	16±12.7^{b}	48±11.0^{a}	44±11.0^{a}	84±11.0^{a}
	DOM1	0.0±1.8^{a}	29±11.0^{ab}	41±12.7^{bc}	56±12.7^{bc}	66±12.7^{c}
	DOM4	46±12.7^{ab}	23±12.7^{a}	53±11.0^{bc}	58±11.0^{bc}	80±9.8^{c}
Duration of decline in FSH (days)	**Emergence**		0.5±0.3	0.9±0.3	0.6±0.3	0.9±0.3
	DOM1		0.3±0.8	0.7±0.3	0.6±0.3	0.6±0.3
	DOM4		0.2±0.3^{a}	0.6±0.3	0.8±0.3	1.1±0.2^{b}
Mean maximum conc. (ng/ml) of FSH achieved following nadir (ie.+ Peak of next wave)	**Emergence**	20.9±3.8	20.9±4.4	16.8±3.8	27.2±3.8	23.2±3.8
	DOM1	20.7±4.4	17.2±3.8	26.7±4.4	20.0±4.4	24.0±4.4
	DOM 4	36.0±4.4^{a}	20.4±4.4	27.9±3.8^{a}	21.0±3.8^{b}	14.9±3.4^{b}
Interval from treatment to peak of wave 2 (days) independent of N.W.E.	**Emergence**	5.2±1.0	4.0±1.2	4.6±1.0	2.9±1.0	4.5±1.0
	DOM1	3.2±1.2	4.0±1.0	3.0±1.2	5.7±1.2	5.8±1.2
	DOM4	9.7±1.2^{a}	4.0±1.2^{b}	6.2±1.0^{b}	4.8±1.0^{b}	5.9±0.9^{b}
Interval from treatment to peak of next wave (days) associated with N.W.E.	**Emergence**	6.8±1.1^{a}	4.0±1.1^{ab}	4.9±0.9^{ab}	3.6±0.9	6.4±0.9^{a}
	DOM1	3.3±1.1	4.0±0.9	3.0±1.1^{a}	5.7±1.1	6.0±1.1^{b}
	DOM4	0.4±1.1^{a}	1.8±1.1^{a}	4.6±0.9^{b}	5.1±0.9^{b}	5.9±0.8^{b}
Inter - peak – interval (days)	**Emergence**	7.3±1.2	6.0±1.2	5.8±1.1	4.2±1.1	5.5±1.1
	DOM1	7.3±1.2	8.1±1.2	7.2±1.2	9.5±1.2	9.5±1.4
	DOM4	7.3±1.2^{a}	10.6±1.2^{ab}	12.5±1.1^{bc}	11.5±1.5^{bc}	14.2±0.9^{c}

Within row, treatments with different superscripts are significantly different ($P < 0.05$).

Table 4. The effect of dose and route of oestradiol benzoate (ODB) on development of the first follicle wave, interval to CL regression, oestrous response and synchrony of oestrus when administered at different stages of the first dominant follicle in beef heifers (Mean ± SE)

Parameter	Follicle stage at Treatment initiation	Treatment				
		Control	Progesterone alone	Progesterone + 10 mg cap.	Progesterone + 0.75 mg i.m.	Progesterone + 5.0 mg i.m.
Number of heifers	Emergence	10	7	6	6	7
DOM1		10	7	7	6	6
DOM4		10	8	7	7	7
No. forming 1st DF	Emergence	10/10^{a}	4/7^{b}	4/6^{ab}	1/6^{bc}	2/7^{bc}
Interval from treatment to detected ultrasound CL regression (days)	Emergence	18.1±0.95^{a}	16.6^{c}±1.0^{ac}	14.8±1.1^{bc}	12.8±1.09^{b}	13.9±1.0^{bc}
	DOM1	14.9±0.72^{a}	15.0±1.01	16.5±1.09^{a}	12.4±1.19^{b}	14.8±1.09
	DOM43	11.9±0.85^{ac}	14.6±0.94^{b}	12.9±1.01^{c}	12.3±1.01	10.0±1.01^{a}
Interval from progesterone removal to onset of oestrus (hours)	Emergence		156±18.5^{a}	96±18.5	64±18.5^{b}	90±17.1
	DOM1		109±18.5	117±18.5	72±20.3	109±22.7
	DOM4		90±16.0	59±18.5	52±20.3	45±18.5
No. observed in heat after:						
2 days	Pooled across follicle stage		7/22	7/20	14/19	12/20
3 days			3/22	4/20	1/19	0/20
4+ days[1]			10/22	8/20	1/19	5/20
Overall Heat Reponse (%)		-	20/22 (91%)	20/22 (91%)	16/19 (84%)	17/20 (85%)

Within row, treatments with different superscripts are significantly different ($P < 0.05$). [1] Too few observations to include in analysis.
[2] Identical measurement due to definition.

Discussion

The results of this experiment clearly showed that the administration of exogenous oestradiol, in the form of oestradiol benzoate (ODB), causes a dose-dependent increased in plasma concentrations of oestradiol and a dose-dependent decrease in plasma FSH, and, in a high proportion of animals causes atresia of the follicle wave present at treatment initiation followed by the emergence of new follicle wave 3 to 6 days later. The results of this study are consistent with those of Study 1, where an ovariectomised heifer model was used to establish the circulating concentrations of oestradiol generated by different doses of exogenous ODB. This study also clearly demonstrated that the administration of oestradiol, by a capsule attached to the progesterone releasing device, was the least effective way of administering oestradiol in terms of its ability to generate plasma concentrations of 5-15 pg/ml which, would appear to be required in order to terminate the current follicle wave and cause new wave emergence 3-6 days later. It was also clear from the both plasma progesterone profiles, which represent endogenous and exogenous sources of progesterone, and from the shorter intervals from progesterone withdrawal to heat onset, that exogenous oestradiol significantly shortened the lifespan of the current corpus luteum. It would appear that while the low plasma concentrations of 3-5 pg/ml generated by the gelatine capsule attached to the progesterone releasing device were insufficient to consistently alter follicle wave dynamics and ensure new emergence they was sufficient to shorten the lifespan of the corpus luteum. This is the first study to demonstrate that a lower dose of oestradiol is required to shorten the lifespan of the CL than is required to alter follicle wave dynamics.

The results of this study clearly demonstrated the difficulty of obtaining a tight synchrony of heat in animals that are at early stages of their oestrous cycles at time of treatment initiation. Tight synchrony of oestrus will only occur when the CL is regressed and when a recently dominant, oestrogen-active follicle is present at time of progesterone withdrawal. The use of oestradiol at the beginning of a progesterone synchronisation treatment, even when the duration of the treatment is extended to 12 days, does not ensure that corpus luteum regression is complete in all animals at the time of, or, 24 hours after progesterone withdrawal, thus allowing animals to exhibit a synchronous heat. Consequently, there is a requirement to administer a luteolytic agent like prostaglandin $F_{2\alpha}$ or one of its analogues at or before progesterone withdrawal to ensure complete corpus luteum regression.

While there was clear evidence that administration of oestradiol by injection was superior to administration by a capsule attached the progesterone releasing device there was no evidence of any beneficial effects of increasing the dose of oestradiol from 0.75-mg /animal to 5-mg/animal in terms of controlling follicle wave dynamics, shortening the lifespan of the corpus luteum, or in terms of synchrony of heat or overall heat response. In fact there was a suggestion in the data that the synchrony of the heat response, measured in terms of the proportion of treated animals observed in heat on days 2 and 3 after progesterone withdrawal, was lower in animal receiving the 5 mg of ODB by injection. Numerically the tightest synchrony of heat was obtained in animals receiving the 0.75-mg of ODB by injection.

Study 3
Effect of duration of progesterone treatment, with or without oestradiol benzoate, on synchronised oestrous response and fertility in beef cows and heifers

Background
The critical requirements for successful synchronisation of the oestrous cycle in cattle are a consistent and high oestrous response during a specified 12- to 24-hour period and a high pregnancy rate following insemination. To date these requirements have not been generally met. Earlier studies have shown that progestagen treatments of more than 14 days duration resulted in good synchrony of oestrus but low pregnancy rates. When the treatment period was reduced to 9-12 days by the administration of oestradiol at the start of the progestagen treatment, this resulted in a normal pregnancy rate but a greater variability in the onset of oestrus. The use of prostaglandin (PG) at or near the end of the progestagen period increased the oestrous response in animals with a

corpus luteum, but failed to give a sufficiently precise onset of heat to allow high pregnancy rates to a single fixed-timed A.I. An alternative approach to improve the synchrony of oestrus is to induce the synchronous occurrence of the proestrous oestradiol rise by the exogenous administration of oestradiol after withdrawal of the progesterone. The aims of this experiment were to determine the effects of 1) the duration of progesterone treatment and 2) of administering 1.0 mg oestradiol benzoate (ODB) 24 hours after progesterone removal on oestrous response and on pregnancy rates in beef cows and heifers.

Material and Methods

A total of 297 beef cows and 163 beef heifers, in 15 herds, were used in a farm trial. The progesterone synchronisation treatments were either of 12- or 8-day duration. Prior to insertion, the oestradiol benzoate capsule, normally attached to the progesterone releasing device, was removed. All animals received 0.75 mg ODB by intra-muscular injection at the start of the synchronisation. All animals receiving progesterone for 8 days received PG 24 hours prior to the end of the progesterone treatment. Twenty four hours after removal of the progesterone half of the animals received 1.0 mg of ODB. Approximately 20% of each herd were left untreated and were inseminated at an observed heat. The treatments were:

1. 12-day progesterone
2. 12-day progesterone + 1.0 mg ODB 24 hours after progesterone removal.
3. 8-day progesterone + PG on Day 7
4. 8-day progesterone + PG on Day 7 + 1.0 mg ODB 24 hours after progesterone removal.
5. Untreated controls

At progesterone removal all animals were tail-painted and observed for heat 4 times daily for 4 days and inseminated once at 6-12 hours after the first observation of heat. On each farm and within each animal category (cows or heifers) semen from one bull was used. One inseminator was used in each herd. All animals were scanned for pregnancy at 30-40 days after insemination.

Data analysis

Heat response and pregnancy rate data was analysed using PROC CATMOD (SAS, 1988).

Results

The was no interaction (P>0.05) between the duration of the synchronisation treatment (8-day and 12-day) and ODB treatment 24 hours after progesterone removal on heat response or pregnancy rate. However, the effect of treatments on heat response and pregnancy rates was different in cows and heifers (treatment x parity interaction P<0.05). The overall heat response was similar for 12-day (151/173; 87%) and 8-day (157/173; 91%) synchronisation treatments. There was a tendency for better synchrony in heifers than cows and the 8-day progesterone + ODB given 24 hours later tended to give the best synchrony. Heat response was higher in cows following the administration of ODB (P<0.05) (Table 5). In heifers, heat response was high following all treatments and was unaffected (P>0.05) by ODB treatment after progesterone removal.

Table 5. The effect of duration of progesterone treatment with or without the administration of ODB 24hrs after progesterone removal on overall oestrous response (%) in beef cows and heifers.

Animal Category	Treatment	+ 1.0mg ODB	0mg ODB	Controls
Beef cows	12-day Progesterone	48/51 (94)^{a}	42/55 (76)^{b}	71/81 (88)
	8-day Progesterone	50/54 (93)^{a}	44/56 (78)^{b}	
Beef Heifers	12-day progesterone	32/35 (91)	29/32 (91)	30/33 (91)
	8-day progesterone	32/32 (100)	31/31 (100)	

Within row, treatments with different superscripts are significantly different (P<0.05)

Synchrony of oestrous response

The pattern of heat onset following different synchronisation treatments in both beef cows and heifers is presented in Fig 6. Duration of treatment had no effect on the % of cows observed in heat (12-Day: 75%; 8-Day: 75%) on Day 2 after progesterone removal. In heifers there was a tendency for more heifers to be in heat on Day 2 following progesterone removal following an 8-Day (86%) than a 12-Day (76%) treatment. The administration of 1.0 mg of ODB 24 hours after progesterone withdrawal improved the synchrony of oestrous response in both cows and heifers with a higher ($P<0.05$) proportion of ODB-treated animals (88%) than non-ODB treated animals (76%) observed in oestrus on Day 2 following progesterone removal.

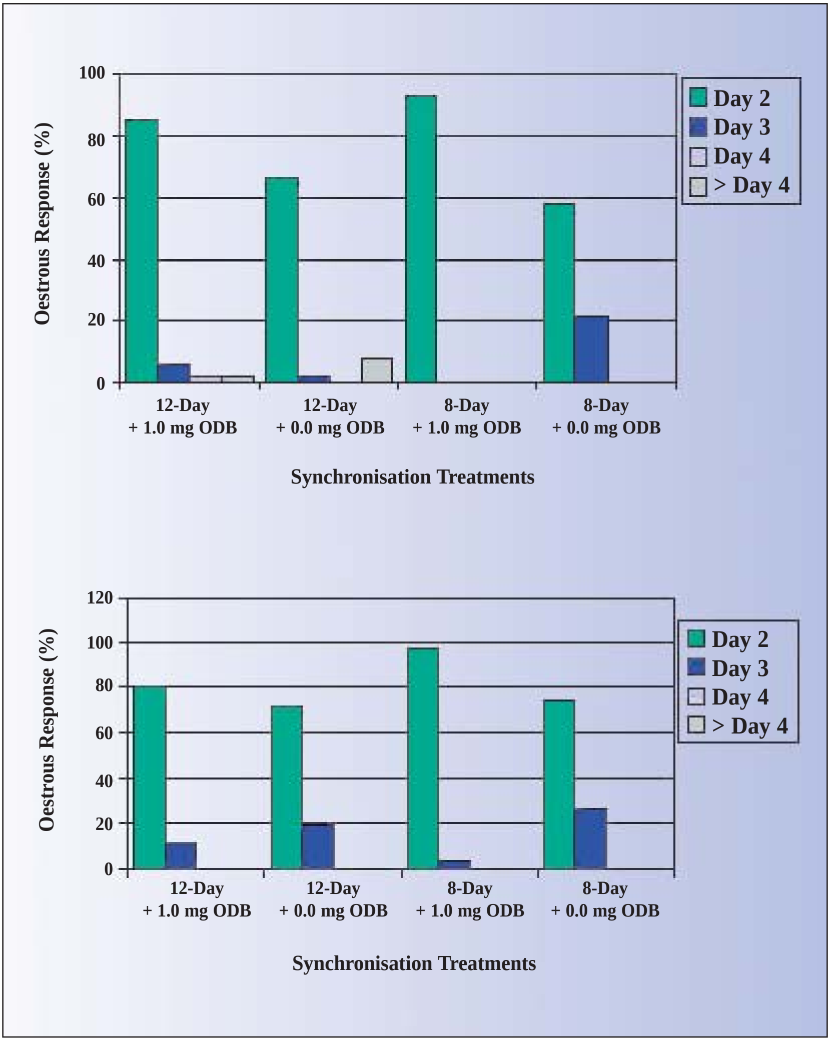


Fig 6. The effect of duration of progesterone treatment with or without the administration of 1.0 mg of ODB 24 hours after progesterone removal on oestrous response and pattern of onset of oestrus in beef cows (upper panel) and beef heifers (lower panel). Oestrous response is expressed as % of animals initially treated.

Pregnancy rate.

Pregnancy rates defined as the % of cows conceiving following insemination at a synchronisation induced-oestrus as a % of animals initially inseminated are presented in Table 6. In heifers, duration of progesterone treatment (8-day versus 12-day) had no effect (P>0.05) on pregnancy rate. The use of ODB 24 hours after progesterone removal reduced (P<0.05) pregnancy rates in heifers but had no effect on pregnancy rate in cows. In beef cows overall pregnancy rate, irrespective of ODB treatment and duration of progesterone treatment, was lower (P<0.05) for treated cows (59%) than for untreated (75%) control cows while in heifers pregnancy rate was similar (P>0.05) in treated (63%) and control animals (67%).

Table 6. The effect of duration of progesterone treatment with or without the administration of ODB 24hrs after progesterone removal on pregnancy rate (%) in beef cows and heifers.

Animal Category	Treatment	+ 1.0mg ODB	0mg ODB	Controls
Beef cows	12-day Progesterone	26/48 (54)	24/42 (57)	53/71(75)
	8-day Progesterone	31/50 (62)	28/44 (64)	
Beef Heifers	12-day progesterone	12/32 (38)^{a}	20/29 (69)^{b}	20/30 (67)
	8-day progesterone	13/32 (41)^{a}	18/31(58)^{b}	

Within row, treatments with different superscripts are significantly different (P<0.05)

Conception Rate

Conception rates defined as the % of cows conceiving following insemination at a synchronisation induced-oestrus as a % of animals initially treated are presented in Table 7.

Table 7. The effect of duration of progesterone treatment with or without the administration of ODB 24hrs after progesterone removal on conception rate (%) in beef cows and heifers.

Animal Category	Treatment	+ 1.0mg ODB	0mg ODB	Controls
Beef cows	12-day Progesterone	26/51 (51)	24/55 (44)	53/71(75)
	8-day Progesterone	31/54 (57)	28/56 (50)	
Beef Heifers	12-day progesterone	12/35(34)[a]	20/32 (63)[b]	20/30 (67)
	8-day progesterone	13/32 (41)[a]	18/31(58)[b]	

Within row, treatments with different superscripts are significantly different (P<0.05)

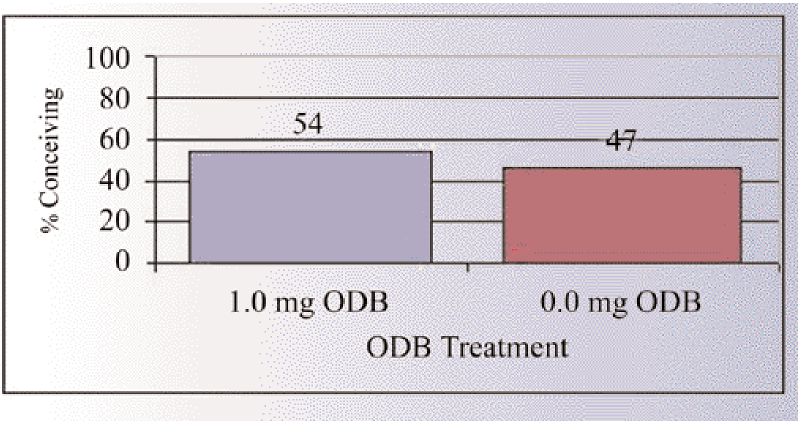


Figure 7. Effect of ODB administration 24 hours after progesterone withdrawal on conception rate in beef cows.

In cows conception rate tended to be higher following an 8-Day (54%) than a 12-Day (47%) duration treatment irrespective of ODB treatment. For beef cows the overall benefit of using 1.0 mg ODB 24 hours after progesterone removal is presented in Figure 7.

Discussion

In these farm trial studies there was no effect of progesterone duration (8-day versus 12-day) on oestrous response in either beef cows or heifers though there was some evidence that the oestrous response tended to be higher following an 8-day treatment. Similarly, duration of treatment had no effect on the synchrony of the oestrous response following withdrawal of the progesterone though there was some evidence of a more highly synchronised oestrous response following the 8-day treatment. The use of 1.0 mg oestradiol 24 hours after progesterone removal significantly increased synchronised oestrous response in beef cows, with almost all cows exhibiting oestrus on Day 2 following withdrawal of the progesterone. However, in heifers oestrous response at 91% in the non- ODB treated heifers was already high and would have been difficult to improve on. This highly synchronised oestrous response following the administration of ODB would allow one fixed-timed AI at about 54-56 hours after withdrawal of progesterone in beef cows.

In cows there was a tendency for the pregnancy rate to be higher following an 8- than a 12-day progesterone treatment. The lower pregnancy rate following the longer duration treatment may be the result of the ovulation of follicles with an excessive long period of dominance caused by the 12-day treatment. The administration of ODB had no effect on pregnancy rate in beef cows but significantly reduced pregnancy rate in heifers. It would appear that the dosage used in heifers was excessive and that a lower dose would be more appropriate. However, the high and synchronous oestrous response recorded in heifers would obviate the need to use ODB after progesterone removal in heifers.

In beef cows the overall benefit of using ODB is evident by the higher conception rate (+7 percentage points), defined as the proportion of animals initially treated that conceived at the synchronised oestrus. This results from a small increase in both the oestrous response and pregnancy rate in ODB-treated animals.

An 8-day progesterone treatment, with 0.75 mg of ODB at start, prostaglandin (PG) administered 24 hours before and 1.0 mg ODB administered 24 hours after progesterone removal will give a highly synchronous heat response in beef cows facilitating fixed-times AI at 54-56 hours after progesterone removal. Average conception rates of 55-60% are achievable.

CONCLUSIONS

- The administration of exogenous oestradiol by injection, in the form of oestradiol benzoate (ODB), resulted in dose-dependent increase in peak plasma oestradiol and a dose-dependent transient decrease in follicle stimulating hormone (FSH) in ovariectomised heifers.

- Administration of higher doses (5mg and 10mg) of oestradiol by capsule attached to a progesterone releasing intravaginal devise generated much lower plasma concentrations of oestradiol compared with the lowest dose (0.75mg) of oestradiol administered intramuscularly. Both the maximum % decline in FSH and interval to FSH nadir were related to peak concentrations of oestradiol.

- Plasma concentrations of FSH began to increase 1-2 days after ODB administration while concentration of oestradiol were declining but still high.

- The administration of ODB, caused a dose-dependent increased in plasma concentrations of oestradiol and a dose-dependent decrease in plasma FSH in intact heifers, and, in a high proportion of animals caused atresia of the follicle wave present at treatment initiation followed by the emergence of new follicle wave 3 to 6 days later.

- The administration of oestradiol, by a capsule attached to the progesterone releasing device, was the least effective way of administering oestradiol in terms of its ability to generate plasma concentrations of 5-15 pg/ml which, would appear to be required to terminate the current follicle wave and cause new wave emergence. The low plasma concentrations of 3-5 pg/ml generated by the gelatine capsule attached to the progesterone releasing device were insufficient to consistently ensure new follicle wave emergence but were sufficient to shorten the lifespan of the corpus luteum.

- The use of oestradiol at the beginning of a progesterone synchronisation treatment with animals at the beginning of their oestrous cycles, even when the duration of the treatment is extended to 12 days, did not ensure that corpus luteum regression was complete in all animals at the time of progesterone withdrawal, thus allowing animals to exhibit a synchronous heat. Consequently, there is a requirement to administer a luteolytic agent like prostaglandin F_{2a} at or before progesterone withdrawal to ensure complete corpus luteum regression.

- The administration of oestradiol by injection was superior to administration by a capsule attached the progesterone releasing device. There was no evidence of any beneficial effects of increasing the dose of oestradiol from 0.75/animal to 5mg/animal in terms of controlling follicle wave dynamics, shortening the lifespan of the corpus luteum, or in terms of synchrony of heat or overall heat response.

- There was no effect of progesterone duration (8-day versus 12-day) on oestrous response in either beef cows or heifers though in the latter oestrous response tended to be higher following an 8-day treatment. Similarly, duration of treatment had no effect on the synchrony of the oestrous response following withdrawal of the progesterone though there was some evidence of a more highly synchronised oestrous response following the 8-day treatment.

- The use of 1.0-mg oestradiol, 24 hours after progesterone removal, significantly increased synchronised oestrous response in beef cows. However, in beef heifers it had no beneficial effect, though in this group oestrous response at 91% in the non- ODB treated heifers was already high and would have been difficult to improve on.

- The use of ODB improved the synchrony of oestrous response with almost all cows exhibiting oestrus on Day 2 following withdrawal of the progesterone. This highly synchronised oestrous response following the administration of ODB would allow one fixed-timed AI at about 54-56 hours after withdrawal of the progesterone. In cows there was a tendency for the pregnancy rate to be higher following an 8-day than a 12-day duration progesterone treatment.

- The administration of ODB had no effect on pregnancy rate in beef cows but significantly reduced pregnancy rate in heifers. It would appear that the dosage used in heifers was excessive and that a lower dose would be more. However, the high and synchronous oestrous recorded in heifers would obviate the need to use ODB after progesterone removal in heifers.

- The overall benefit of using ODB is evident by the higher conception rate (+7 percentage points at the synchronised oestrus. This results from a small increase in both the oestrous response and pregnancy rate in ODB-treated cows.

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