HORMONAL MANIPULATION OF FOLLICULAR WAVES FOR IMPROVING THE SYNCHRONY OF OESTRUS INDUCED BY PROSTAGLANDIN IN DAIRY COWS

Mas'ud Hariadi[1]

Abstract

This experiment was aimed to determine whether pre-treatment with Oestradiol Benzoate (OB) on the $PGF_{2\alpha}$-induced oestrus affects the waves of follicular growth (follicular waves) and the synchrony of oestrus in Friesian dairy cows. Six cows were randomly assigned in cross-over design to either control (3 cows) or treatment (3 cows) groups. Saline (control) or treatment of OB was administered on Day 5 of the oestrous cycle (Day 0 = oestrus), and on Day 12 all cows were treated with $PGF_{2\alpha}$. Observation of ovarian follicular dynamics was carried out by means of ultrasonography. Blood samples for progesterone RIA were collected on Days 5, 7, 9, 12, 14 of the oestrous cycle and at oestrus. Oestrus was detected twice daily by means of KaMaR heat mount detector. Oestradiol benzoate administered on Day 5 of the cycle reduced the diameter and regressed ($P<0.001$) the first dominant follicle. After regression, the second follicular wave in OB-treated was emerged earlier ($P<0.001$) and with better synchrony ($P=0.03$) than in control cows. There was no correlation between the day of emergence of the second follicular wave and the day of ovulation ($r_s=0.45$, $P>0,05$). The time interval from the day of emergence of the follicular wave producing the ovulatory follicle to ovulation was longer ($P=0.013$) in OB-treated than in control cows. There was no alteration in the concentrations of plasma progesterone on Days 5, 7, 9, 12, 14 and at oestrus. The results indicates that hastening the onset of the second follicular wave can hasten the onset of oestrus, and that improved synchrony of day of emergence of the second follicular wave was not associated with improved synchrony of oestrus.

Key words: Follicular wave, cow, Follicular wave, Oestradiol benzoate, Prostaglandin, Oestrus

Introduction

In the previous studies oestradiol benzoate (OB) administered on Day 5 of the cycle shortened the interval to and improved the synchrony of oestrus (Hariadi *et al.* 1995). In that experiment, OB was given on Day 5 of the oestrous cycle (Day 0 = oestrus) and pros

[1] Faculty of Veterinary Medicine, Airlangga University, Surabaya

taglandin $F_{2\alpha}$ ($PGF_{2\alpha}$) on Day 12. However, when OB was administered on Days 3 or 7 then followed by administration of $PGF_{2\alpha}$ on Day 12 did not improve the synchrony of oestrus. This effect was probably related to the influence of OB on the development of dominant follicles.

Day 5 of the cycle was chosen for this treatment because termination of a follicular wave at this stage by atresia of a dominant follicle of the first follicular wave would permit the commencement of a second follicular wave resulting in a mature follicle at the time of $PGF_{2\alpha}$ injection on Day 12. Day 12 was chosen for $PGF_{2\alpha}$ treatment because the variability in the onset of oestrus resulting from PGF treatment in this stage is greater than at other stages of the cycle (Macmillan and Henderson, 1984; Wright and Malmo, 1992). This timing, therefore, provided a useful model for the detection of improvement of the synchrony of oestrus. The objective of this study was to gain insight into the reasons for the effect on the interval to oestrus and synchrony of $PGF_{2\alpha}$-induced oestrus of pre-treatment with OB by determining the effects of these treatments on dominant follicles and follicular waves studied using ultrasound.

Materials and Methods

Animals and Treatments

Six mature (4 to 7 years) non-lactating Friesian dairy cows, weighing 551 to 673 kilograms and with a body condition score of 4 or 5 (score 1 to 8, poor to excellent condition) (Holmes and Wilson, 1984) were used in this experiment. Cows exhibited normal oestrous cycles prior the study and had normal reproductive tracts as determined by palpation *per rectum*. Cows grazed on pasture containing mixed grasses and were supplemented with hay.

Oestrus was synchronised prior to the experiment using two doses of $PGF_{2\alpha}$ (500 μg cloprostenol, Estrumate, Pitman-Moore Australia) 12 days apart. The day of oestrus occurrence following this $PGF_{2\alpha}$ treatment was designated as Day 0. The six cows were randomly assigned according to a cross-over design, to either control (3 cows) or treatment (3 cows). Cows were treated on Day 5 of the oestrous cycle with OB (5 mg, im) control cows received saline, on Day 12 all cows were treated with $PGF_{2\alpha}$.

Follicular Dynamics Observations

Ovarian ultrasonographic examinations were performed as described by Pierson and Ginther (1984). The ultrasound machine used was a scanner 480 (Pie Medical, Philipsweg 1, the Netherlands) portable array with a 5 or 7.5 MHz intrarectal probe (transducer). Prior to the experiments oestrus was synchronised using two doses of $PGF_{2\alpha}$ 12 days apart and cows were examined and monitored daily by ultrasonography for 6 weeks to determine normal pattern of follicular waves. Oestrus was then resynchronised and the study commenced. During experiments cows were brought into the yard daily for ultrasound examination. Ovarian follicular dynamics were monitored beginning on Day 0 until ovulation following $PGF_{2\alpha}$ treatment (Day 12) at the end of each experiment.

Blood Collection and Plasma Progesterone Assay

Blood samples (10 ml) were collected from the tail vein regularly on Days 5, 7, 9, 12, 14 and at oestrus using a 10 ml heparinised vacuum – evacuated, stoppered test tube Monoject, Sherwood Medical, St. Louis, USA). The tubes were then placed on ice and transported within an hour to the laboratory. These samples were centrifuged (Clements GS200, Sydney, Australia) at 3500 r.p.m. for 15 minutes. Plasma samples were transferred to new, labelled, 5 ml plastic tubes and stored frozen (-20°C) until assayed for progesterone. Measurements of progesterone in plasma were performed by radio immunoassay using a commercially available, 125 – I labelled progesterone kit (Coat – A – Count, DPC, LA, USA). The assay sensitivity was 0.1 ng/ml. The intra-assay coefficient of variation (CV) was less than 10% over the range 0.2 - 15.8 ng/ml.

Oestrus Detection

Oestrus was detected using heat mount detector (KaMar Marketing Group Inc., Steamboat Springs, Colorado) as an aid to routine observation. These detectors were fixed firmly, using adhesive, onto the cows back parallel to hipbones in the midline. Cows were observed twice daily for an hour in the early morning and evening beginning one day after $PGF_{2\alpha}$ treatment until all cows had been observed in oestrus. The white detector turned to red after a cow stood to be mounted by others, so the red KaMar detector was regarded as indicating that standing heat had occurred.

Parameters Examined and Statistical Analysis

The time of oestrus occurrence was defined on a daily basis. Cows, which showed oestrus in the morning and evening, were considered to be in oestrus on that day. The interval to oestrus was the time from $PGF_{2\alpha}$ treatment to oestrus. Ovulation was defined as the disappearance of a large follicle that was present at a previous examination (Pierson and Ginther, 1988). The dominant follicle of a follicular wave was defined as the one that reached the largest diameter, and subordinate follicles were those that grew during periods of time when the dominant follicle was present (Ginther *et al.*, 1989). The time of emergence of a follicular wave was the day when a group of follicles ≥5 mm arose from the same pool appeared. The day of cessation of growth of the dominant follicle was the day that the dominant follicle ceased a progressive increase in diameter. The day of maximum diameter of the dominant follicle was the day on which the maximum diameter was attained. When a follicle had the same diameter for more than one day, the first day was taken as the day of maximum diameter. The initiation of regression of the dominant follicle was the first day that the follicle began a progressive decrease in diameter.

Differences between control and treatment means of those parameters were examined by two-sample t-tests appropriate to the design (Jones and Kenward, 1989). Calculations were done using the programme Super ANOVA. Differences in the interval from the emergence of follicular waves producing ovulatory follicles and ovulation induced by $PGF_{2\alpha}$ were examined by two-sample t-test (Jones and Kenward, 1989). The growth rates of the dominant follicles (mm/day) of the second follicular wave were determined by linear regression as described by others (Ginther *et al.*, 1989). This rate of growth was calculated from the emergence of the second wave to the day of $PGF_{2\alpha}$ administration. The synchrony of oestrus was the distribution of occurrence of oestrus over time following synchronisation treatment (Odde, 1990). Differences in the synchrony of oestrus were examined by comparing two correlated variances in paired observations (Snedecor and Cochran, 1980).

Results

Before the start of the experiments during which ovarian follicular dynamics were observed daily, all cows had three waves of follicular growth. Oestrus was designated as Day 0. Emergence of the first wave was identified at oestrus, ovulation or the day after ovulation (Day 0.9±0.35), of the second follicular wave was identified between Days 7 and 11 of the oestrous cycle (Day 8.7±1.21), and of the third follicular wave between Days 14 and 17 (Day 15.6±1.0). The interovulatory interval was 21.3±0.8 days.

Oestradiol benzoate administered on Day 5 of the cycle reduced ($P=0.008$) the diameter of the first dominant follicle and caused it to regress sooner ($P=0.001$) than in control cows (Table 1, Figure 1). The day of the emergence of the second follicular wave in OB-treated was earlier ($P<0.001$) and with better synchrony ($P=0.03$) than in control cows but the interval from $PGF_{2\alpha}$ treatment to oestrus and the synchrony of oestrus and ovulation were similar ($P>0.05$) to that in control cows. The day of emergence of the second follicular wave and the day of ovulation in control but not in OB-treated cows were correlated ($r_s=0.87$, $P<0,05$). The time interval from the day of emergence of the follicular wave producing the ovulatory follicle to ovulation was longer ($P=0.013$) in OB-treated than in control cows. The daily growth rate of the dominant follicle of the second follicular wave was similar ($P=1.0$) for control and OB-treated cows. The plasma P4 concentrations on Days 5, 7, 9, 12, 14 and at oestrus were similar ($P>0.05$, Figure 2) for cows treated on Day 5 of the oestrous cycle with OB or with saline (control).

Table 1.Characteristics of dominant follicles of the follicular waves and oestrus occurrence in cows after treatment with saline (control), oestradiol benzoate on Day 5 of the oestrous cycle and prostaglandin on Day 12

Characteristics	Experiment	
	Saline (Control)	Oestradiol Benzoate
Number of cows (n)	6	6
First dominant follicle:		
Day of emergence	0.8±0.17	0.5±0.22
Cessation of growth (Day)	5.7±0.21	5.0±0.0
Maximum diameter (mm)	14.0±0.58[a]	10.0±0.36[b]
Initiation of regression (Day)	12.0±0.45[a]	9.2±0.40[b]
Second dominant follicle:		
Day of emergence	9.8±0.40[a]	8.5±0.22[b]
Diameter on Day 12 (mm)	9.7±0.76[a]	11.3±0.33[b]
Growth rate (mm/day)	1.8±0.14	1.8±0.08
Diameter at oestrus (mm)	15.7±0.31	14.8±0.31
Third dominant follicle:		
Day of emergence	-	-
Diameter at oestrus (mm)	-	-
Day of oestrus occurrence	15.2±0.31	14.8±0.17
Interval to oestrus (days)	3.2±0.31	2.8±0.17
Day of ovulation	16.2±0.31	15.8±0.17
Interval wave emergence to ovulation (days)	6.3±0.21[a]	7.3±0.21[b]
r_s(wave emergence & ovulation)	0.87*	0.45

[a,b] Mean values between column within the experiment are different ($P<0.05$) and those without superscripts are not different ($P>0.05$)

r_s Spearman's rank correlation coefficient, $P=0.05$ for $r_s=0.83$ from a sample size of 6; NC values are indentical, could not be calculated.

Discussion

This study demonstrated clear effects of the administration of OB on Day 5 of the oestrous cycle on the growth of ovarian follicles and follicular waves. These observations offered explanations for the effects of treatments on the synchrony of PG-induced oestrus.

Findings that administration of OB on Day 5 of the oestrous cycle caused early regression of the dominant follicle of the first follicular wave and led to the early emergence of the second follicular wave explain the observation in the previous study (Hariadi *et al.* 1995) that OB pre-treatment on Day 5 improved the synchrony and hastened the onset of PG-induced oestrus. There is no report of the effect short-acting oestradiol administered alone to cows on Day 4 or 5 of the oestrous cycle, when the corpus luteum is functional, on the wave of follicular growth. The reduction in size and early regression of the dominant follicle of the first follicular wave and the early emergence of the second follicular wave were similar to the report of Bo *et al.* (1994). Oestradiol (E-17β) administered on Day 3 to 5 (Day 0 = ovulation, equivalent to Day 4 to 6 of the cycle in this study) to progestogen-treated cows suppressed the growth and caused early regression of the first dominant follicle compared with control (progestogen-treated) cows. These treatments hastened and made more synchronous the day of emergence of the second follicular wave. Although these workers had no untreated control cows it was unlikely that progestogen treatment alone would have influenced the follicular wave. High plasma P4 concentrations during early cycle suppressed the growth of the first dominant follicle but did not have an effect on the emergence of the second follicular wave (Burke *et al.*, 1994). Long-acting oestradiol (oestradiol valerate, OV) alone suppressed the growth of the first dominant follicle when administered to heifers on Day 3 (Bo *et al.*, 1993). This OV treatment, however, delayed the emergence of the second follicular wave. This delayed emergence of the second follicular wave was probably due to the long duration of action of OV resulting in prolonged gonadotropin suppression (Bo *et al.*, 1993). The lack of significant improvement of the synchrony of oestrus despite the improved synchrony of emergence of the second follicular wave may have reflected factors additional to follicular wave synchrony and an adequate number of animals for statistical significance.

An overview of the results indicates that hastening the onset of the second follicular wave can hasten the onset of oestrus, and that improved synchrony of day of emergence of the second follicular wave was not associated with improved synchrony of oestrus. Failure to detect relationship between day of emergence of the second follicular wave and day of oestrus in this experiment may reflect small numbers of cows per group and factors additional to day of emergence of the second follicular wave affecting the variability in the day of oestrus. Our data indicates the growth rate of the follicles to be such a factor.

Acknowledgements

We wish to thanks Dr. P.J. Wright and Prof. D.B. Galloway for supervising the project and reading the manuscript. The project was funded by The Six Universities Development Rehabilitation Project – Asian Development Bank (SUDR – ADB), Loan No. 1013 – INO.

References

Bo, G,A., Adams, G.P., Nasser, L.F. Pierson, R.A. and Mapletoft, R.J. 1993. Effect of estradiol valerate on ovarian follicles, emergence of follicular waves and circulating gonadotropins in heifers. Theriogenology 40:225-239.

Bo, G.A., Adams, G.P., Pierson, R.A., Tribulo, H.E., Caccia. M. and Mapletoft, R.J. 1994. Follicular wave dynamics after estradiol-17β treatment of heifers with or without a progestogen implant. Theriogenology 41:1555-1569.

Burke, C.R., Mihm, M., Macmillan, K.L., Roche, J.F. 1994. Some effect of prematurely elevated concentrations of progesterone on luteal and follicular characteristics during the estrous cycle in heifers. Anim. Reprod. Sci. 35:27-39.

Ginther, O.J., Kastelic, J.P. and Knopf, L. 1989. Intraovarian relationships among dominant and subordinate follicles and the *corpus luteum* in heifers. Theriogenology 32:787-795.

Hariadi, M. and Wright, P.J. 1995. Administration of oestradiol benzoate improves the degree of synchrony of prostaglandin – induced oestrus in cows. Proc. 17thAnnu. Reprod. Biol. 27:88.

Holmes, C.W., Wilson, G.F. 1984. Milk Production from Pasture. Butterworths Agricultural Books. Wellington. N.Z. pp. 29-31.

Jones, B. and Kenward, M.G. 1989. Design and Analysis of Cross-over Trials. Chapman and Hall, London. pp. 25-27.

Macmillan, K.L. and Henderson, H.V. 1984. Analyses of the variation in the interval from an injection of prostaglandin $F_{2\alpha}$ to estrus as a method of studying patterns of follicle development during dioestrus in dairy cows. Anim. Reprod. Sci. 6:245-254.

Odde, K.G. 1990. A review of synchronization of estrus in postpartum cattle. J. Anim. Sci. 68:817-830.

Pierson, R.A. and Ginther, O.J. 1984. Ultrasonography of the bovine ovary. Theriogenology 21:495-504.

Pierson,R.A. and Ginther, O.J. 1988. Follicular populations during the estrous cycle in heifers III. Time of selection of the ovulatory follicle. Anim. Reprod. Sci. 16:81-95.

Snedecor, G.W. and Cochran, W.G. 1980. Statistical Methods. 7th ed. Iowa State University Press, Ames, Iowa, U.S.A.

Wright, P.J. and Malmo, J. 1992. Applied pharmacology therapeutics II: Pharmacologic manipulation of fertility. Vet. Clin. North Am.: Food Anim. Pract 8:57-89.