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Recent developments in animal breeding programmes

K.L. MACMILLAN

MAFTech, Ruakura Agricultural Centre, Hamilton

W.W. THATCHER

Dairy Science Department, IFAS, University of Florida
Gainesville, Florida, USA

M. DROST

Department of Reproduction, College of Veterinary Medicine, University of Florida
Gainesville, Florida, USA

ABSTRACT

A single intramuscular injection of 10 g of Buserelin (Bus), a potent analogue of gonadotrophin releasing hormone (GnRH) was administered to non-lactating Holstein cows (Experiment 1) or Brangus cross animals (Experiment 2) on cycle day 12 (oestrus = cycle day 0). This treatment altered normal patterns of ovarian follicle development, causing atresia or premature luteinisation especially in follicles of 6 to 9 mm diameter. Injecting 8 or 10 g Bus every third day from cycle day 12 to cycle day 48 (Experiment 3), extended the average inter-oestrous interval to 56.2 days, maintained dioestrous concentrations of plasma progesterone (5 to 12 ng/ml), and increased corpus luteum lifespan to at least 49 days in 5 of 6 Holstein cows. This 3 day interval was also used with 18 cows and heifers which were embryo recipients when they had been in oestrus from 2.5 to 7.5 days before their contemporary embryo donors (Experiment 4). The average pregnancy rate following these asynchronous transfers was 38.9%, varying from 20% when morulas were transferred to 62.5% with blastocysts. Inserting a CIDR-B into heifers 12 h after the onset of oestrus for 3 to 15 days produced alterations in inter-oestrus intervals which indicated that progesterone treatment during metoestrus could potentiate the capacity of the uterus to synthesise and release prostaglandin $F_2\alpha$ (Experiment 5).

The results from these five experiments showed that manipulating ovarian follicle development with Bus, or altering normal follicle-uterine interactions could be utilised as forms of oestrous cycle control which may be applied after insemination or embryo transfer.

Keywords Gonadotrophin releasing hormone; Buserelin; follicle development; corpus luteum; embryo transfers; CIDR; progesterone; metoestrus

INTRODUCTION

Altering the length of the bovine oestrous cycle is most commonly achieved by varying the dioestrous period either through inducing premature luteolysis or by providing an exogenous source of a progestagen. If the objective of cycle manipulation is to synchronise oestrus, then these alternatives can be used separately or in combinations (Macmillan *et al.*, 1988). The consequent precision in synchrony will largely depend on variation in the length of the post-treatment pro-oestrus and this is primarily influenced by the stage of the ovarian follicular wave at the end of the altered period of dioestrus (Macmillan and Henderson, 1984).

In the normally cycling animal, follicular waves are also involved in the initiation of luteolysis (Pierson and Ginther, 1988; Fortune *et al.*, 1988; Savio *et al.*, 1988). Altering the pattern of follicle development, or modifying some effects of a dioestrous follicular wave may both be part of the normal process associated with the maternal recognition of an embryo and with pregnancy establishment (Thatcher *et al.*, 1989)

This paper considers a series of trials undertaken to study some of the effects of altering either the pattern of follicular development, or the relationship between a follicular wave and corpus luteum-uterine interactions on ovarian follicle activity and the lifespan of a corpus luteum.

MATERIALS AND METHODS

Experiment 1

Ten non-lactating parous cows (8 Holstein + 2 Jersey) were randomly divided into two groups, with those in the treatment group being injected intramuscularly with 2.5 ml Receptal (Hoechst-Roussel) containing 10 μ g of Buserelin (Bus), a potent analogue of gonadotrophin releasing hormone (GnRH). The ovaries of each animal were examined on cycle days 12, 13, 14 and thereafter on alternate days until oestrus using transrectal real-time linear scanning ultrasound (LS-300 UDS, Tokyo Keiki Co.) with a 7.5 mH probe. Each follicle >2 mm diameter was classified into one of three classes (Class I = 3 to 5 mm diameter; Class II = 6 to 9 mm; and Class III = >9 mm) and categorised as being "normal" or "abnormal" because of flocculence in the fluid and a follicular wall which was not sharply definable. Plasma progesterone concentrations were measured in daily samples using an enzyme immunoassay kit (Enzygnost Progesterone Test Kit; Hoechst-Roussel).

Experiment 2

Thirty-three non-lactating cycling Brangus cross cows and heifers were slaughtered when at cycle day 17 after 17 of them had been injected with 10 μ g Bus on cycle day 12. The ovaries of each animal were examined *in vitro* using the previously described ultrasound unit.

Experiment 3

Six non-lactating cycling parous Holstein cows were injected with 8 or 10 μ g Bus at 3-day intervals from cycle day 12 to cycle day 48. There were 19 blood samplings for each animal from cycle days 12 to 48, and thereafter at daily intervals until oestrus. Follicle numbers were counted and corpus luteum size was estimated using real-time ultrasound on seven occasions.

Experiment 4

Holstein or Jersey cows and heifers were

superovulated with FSH and embryos recovered by non-surgical flushing 7 days after oestrus and insemination. Morulas (n=10) and blastocysts (n=8) of excellent morphological quality were transferred non-surgically to cow or heifer recipients which had been in oestrus from 2.5 to 7.5 days before their contemporary donor animals. Each recipient was injected with 8 μ g Bus on day 12 of its cycle, and thereafter at 3 day intervals until 12 days after embryo transfer (day 19 of embryo development). Pregnancy status was determined by ultrasound between 30 and 34 days of embryo development and confirmed by rectal palpation at around 42 days.

Experiment 5

Groups of 18 to 22 month old dairy beef heifers in which oestrus had been synchronised using an 'Eazibreed' CIDR-B containing 1.9 g progesterone (Carter Holt Harvey) and prostaglandin F₂ α (Lutalyse; Upjohn NZ) had another CIDR inserted at least 12 h after the onset of the synchronised oestrus or within 12 h of the end of oestrus. The CIDRs were removed after 3 days (n=23), 4 days (n=22), 5 days (n=16) or 15 days (n=9). The animals were checked for oestrus twice each day and responses monitored using tailpaint and an aerosol raddle (Macmillan *et al.*, 1988).

RESULTS

Experiment 1

The average number of follicles measured each day on each pair of ovaries was 4.8 Class I follicles (60.8%), 2.1 Class II follicles (26.0%) and 1.0 Class III follicles (13.1%). The effects of the Bus injection did not alter cycle length (treated *v* control = 23.2 d *v* 24.0 d), progesterone concentrations or average follicle numbers, but did produce interactions involving class, day and normality status. The treated animals had an increase in the number of abnormal follicles within 24 h of injection (Table 1). This effect was particularly obvious on cycle days 14 and 16 when at least 60% of the Class II and III follicles in the

TABLE 1 Average numbers of Class II and Class III follicles on the paired ovaries of cows examined from cycle days 12 to 20, with each follicle categorised as normal or abnormal after being injected with 10 µg Bus at cycle day 12 or left as control animals.

Cycle day	II		Follicle Class III		II + III	
	T	C	T	C	T	C
12	1.8 ^a +0 ^b	1.6+0	1.0+0.2	1.0+0.2	2.8+0.2	2.6+0.2
13	2.2+1.0	2.4+0	0.4+0.4	1.2+0	2.6+1.4	3.6+0
14	1.2+2.2	2.0+0	0.2+0.4	1.4+0.2	1.4+2.6	3.4+0.2
16	0.6+2.2	1.0+0.4	0.4+0.6	0.4+0.4	1.0+2.8	1.4+0.8
18	1.4+0.8	1.0+0.6	0.4+0.4	0.4+0.6	1.8+1.2	1.4+1.2
20	0.4+1.0	1.2+0	1.0+0.2	0+1.0	1.4+1.2	1.2+1.0

II = 6 to 9 mm diam.; III = >9 mm diam.

T = injected with 10 µg Bus at cycle day 12 (n=5); C = control animals (n=5)

^a = normal clear follicles;

^b = abnormal cloudy follicles

Treat x day x class x category $P < 0.01$

treated animals were categorised as 'abnormal' compared to less than 20% in the control animals.

Experiment 2

The average diameter of the largest follicle measured on each pair of ovaries in the 17 heifers treated with Bus was 8.8 mm compared to 10.9 mm in the 16 control heifers ($P < 0.01$). There were also significant differences in the average number of Class II and Class III follicles with only 3 of 17 treated animals having at least one Class III follicle compared to 12 of 16 of the control animals (Table 2).

Experiment 3

None of the six animals was detected in oestrus during the treatment period, and average progesterone concentrations ranged from 5 to 12 ng/ml. The average interval from the last Bus injection on cycle day 48 to oestrus was 8.2 (± 1.0) days to produce an average inter-oestrus interval of 56.2 days. The original corpus luteum was still identifiable at cycle day 49 in five of the six cows, although four had at least one accessory corpus luteum. The treatment also altered the proportional relationship between follicle classes ($P > 0.01$; Table 3). Both dosages produced similar effects.

TABLE 2 Average number of follicles in each class on the paired ovaries of animals slaughtered at cycle day 17 and which had (Treated) or had not (Control) been injected with 10 µg Bus on cycle day 12.

Follicle Class	Average number follicles/animal	
	Treated	Control
I (3 to 5 mm)	14.47	14.50
II (6 to 9 mm)	2.64	1.81
III (>9 mm)	0.29	0.75

Treat x class $P < 0.01$

TABLE 3 Average number of follicles in each class categorised as normal or abnormal in six cows injected with 8 or 10 µg Bus at 3-day intervals from cycle day 12 to cycle day 48.

Cycle day	Follicle Class			I+II+III
	I	II	III	
12	6.4 ^a +0 ^b	3.0+0	1.6+0	11.0+0
18	5.0+0	3.6+1.4	0.4+1.6	9.0+3.0
22	4.4+1.0	5.0+1.6	0.6+0	10.0+2.6
27	5.4+0	3.4+0.6	0.6+0	9.4+0.6
33	4.4+1.0	3.4+1.4	0.6+0.6	8.4+3.0
42	3.0+0	2.0+0.6	2.0+0.6	7.0+1.2
49	1.4+0	2.6+0.4	0.6+2.4	4.6+2.8

I = 3 to 5 mm diam.; II = 6 to 9 mm diam.; III = >9 mm diam.

^a = normal clear follicles;

^b = abnormal cloudy follicles

class (II-III) x day x category (a or b) $P < 0.01$

TABLE 4 Pregnancy rates to asynchronous embryo transfers.

Recipient's stage of cycle (days)	Type of embryo		
	Morula (M)	Blastocyst (B)	M + B
9.5-10.5	1/1	2/2	3/3
11-12.5	1/3	2/4	3/7
13-15	0/6	1/2	1/8
Total	2/10	5/8	7/18
(%)	20%	62.5%	38.9%

Experiment 4

The average pregnancy rate for the 18 asynchronous embryo transfers was 38.9% (Table 4) with an indication that blastocyst transfers were more likely to be successful than with morulas (62.5% *v* 20%). One pregnancy diagnosed at 32 days of embryo development and involving a morula transfer was not sustained to 42 days. The interval from the last injection of Bus to oestrus among the non-pregnant recipients ranged from 17 to 20 days.

Experiment 5

The distribution of inter-oestrus intervals among heifers which had a CIDR inserted on cycle day 0 was affected by the period of progesterone supplementation, even though the shorter treatment periods varied from only 3 to 5 days (Table 5). There were no intervals of 13 or 14 days, with most intervals being short (7 to 12 d), shortened (15 to 19 d) or normal (> 19 d).

TABLE 5 Percentage distribution of inter-oestrus intervals among heifers which each had a CIDR-B inserted on cycle day 0 for 3 to 15 days.

Treatment period (days)	Inter-oestrus interval (days)					Number
	7-9	10-12	15-17	18-19	>20 ^a	
3	61	22	9	-	9	23
4	32	41	9	14	5	22
5	-	-	63	25	12	16
15	-	-	22	78	-	9

^a includes animals not detected in oestrus**DISCUSSION**

Destroying visible ovarian follicles by electrocautery followed by x-irradiation can prolong the lifespan of the corpus luteum (Villa-Godoy *et al.*, 1985) and reduce the effectiveness of injected prostaglandin F₂ α (PGF) to produce luteolysis (Hughes *et al.*, 1987). Similar effects were obtained after a single injection of 5 μ g Bus was administered during dioestrus, especially in the latter half of this phase of the cycle (Macmillan *et al.*, 1985a,b). The results from Experiments 1 and 2 indicate that the injection of 10 μ g Bus alters the normal wave-like patterns of follicle development, partly by producing premature luteinisation or atresia in medium (Class II) or large (Class III) follicles (Tables 1 and 2).

If Bus administration was continued at 3-day intervals, at least until cycle day 48, then the lifespan of the original corpus luteum was extended in most cases (Experiment 3). The functional integrity of this corpus luteum in these Bus-treated animals could not be verified, but di-oestrous phase progesterone concentrations were maintained either by the original corpus luteum, by accessory corpora lutea or by the comparatively large number of abnormal follicles (Table 3). This form of Bus treatment also appeared to reduce the rate of follicle recruitment, as the average number of small (Class I) follicles/animal declined from 6.4 at cycle day 12 to 1.4 at cycle day 49.

A single injection of Bus can also affect the pregnancy rate of the insemination either preceding or following treatment. When 5 μ g of

Bus was injected during the metoestrus or early di-oestrus following first insemination, pregnancy rates for that insemination were reduced by 10%. If a 10 μg Bus injection was given after mid-cycle, then the pregnancy rate to the preceding first insemination was increased by 11.5%, and the effect was associated with a change in the distribution of return-to-service intervals of cows which failed to conceive (Macmillan *et al.*, 1986). Similar effects have been obtained by injecting HCG 15 days after insemination (Thatcher *et al.*, 1987).

The increase in corpus luteum lifespan or the reduced efficacy of endogenous PGF may both have contributed to the successful establishment of pregnancies in recipients which were asynchronous with the embryo donors by at least 2.5 days (Table 4). Extensive studies have previously shown that the recipient should preferably be in oestrus within 24 h of the embryo donor, and chances of conception are drastically reduced if the asynchrony exceeds 48 h (Rowson *et al.*, 1972; Wright, 1981; Putney *et al.*, 1988). Further studies are necessary to confirm the commercial potential of asynchronous transfer systems involving the use of Bus. In these trials, recipient animals were used because of their availability, and not selected because of suitability. The better results obtained when blastocysts were transferred may be related to similar observations by others (Wright, 1981; Putney *et al.*, 1988).

The intervals from the last Bus injection to oestrus among the non-pregnant recipients in Experiment 4 ranged from 17 to 20 days. This was substantially longer than the average interval of 8.2 days in Experiment 3 and of 8 to 10 days in previous trials (Macmillan *et al.*, 1985a,b). It is possible that embryo development may have continued in these non-pregnant Bus-treated asynchronous recipients to a stage where the corpus luteum continued to function beyond the time that the protective effects of the final Bus injection could have been expected to be maintained.

The results of Experiment 5 show that only 72 h (3 days) of exogenous progesterone treatment is necessary to potentiate the ability of the uterus to synthesize PGF. Once this potentiation has

occurred, then the next follicle which develops a capacity to secrete oestrogen is also capable of initiating luteolysis, especially if there is a sudden decline in progesterone concentrations. CIDR removal after 3 or 4 days would produce such a decline before the corpus luteum had acquired the capacity to secrete significant amounts of progesterone (Table 5). If the formation of the bovine

corpus luteum is similar to the ovine, then the exogenous progesterone would not have inhibited luteal formation (Ottobre *et al.*, 1980). The results obtained from animals which had CIDRs inserted for 5 days and then had inter-oestrous intervals of at least 15 days support the concept that progesterone treatment during met-oestrus potentiates the ability of the mid-cycle follicle wave, or the second wave to initiate premature luteolysis.

These results are relevant to successfully developing an alternative form of asynchronous transfer; namely, where the recipient is in oestrus after the donor. Ten day-old sheep embryos have been successfully transferred asynchronously to recipients which were in oestrus 4 days after the donors, provided that the recipients were pre-treated with progesterone during metoestrus (Lawson and Cahill, 1983). Comparable asynchronous transfers have been made in cattle (Fox *et al.*, 1988). In both studies, the recipients which failed to conceive had shortened inter-oestrous intervals. These intervals may not have been shortened if the ability of the follicle in the follicle wave after transfer to secrete oestrogen and to initiate luteolysis had been inhibited by the strategic use of Bus.

The results of this series of experiments have allowed new concepts involving ovarian follicular manipulation with Bus to be demonstrated and to be hypothesised. These concepts are particularly relevant to embryo transfer systems. Follicular manipulation may be equally applicable to donors during follicle stimulation with FSH as they are to asynchronous recipients. One important advantage involving follicle manipulation as a form of ovarian cycle control is that it can be and has been successfully applied to animals after insemination or embryo transfer. This may be

particularly relevant to reducing the major source of embryo loss at the time of maternal-embryo recognition, especially in animals with reduced fertility because of environmental stress.

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REFERENCES

- Fortune J.E.; Sirois J.; Quirk S.M. 1988. The growth and differentiation of ovarian follicles during the bovine estrous cycle. *Theriogenology* 29:95-110.
- Fox T.C.; Geisert R.D.; Morgan G.L.; Wells M.E.; Zavy M.T. 1988. Survival of bovine embryos transferred asynchronously to progesterone treated recipients. *Journal of animal science* 66:(Suppl. 1) 414.
- Hughes T.L.; Villa-Godoy A.; Kesner J.S. Fogwell R.L. 1987. Destruction of bovine ovarian follicles: Effects on the pulsatile release of luteinising hormone and prostaglandin $F_{2\alpha}$ -induced luteal regression. *Biology of reproduction* 36:523-529.
- Lawson R.A.S.; Cahill L.P. 1983. Modification of the embryo-maternal relationship in ewes by progesterone treatment early in the oestrous cycle. *Journal of reproduction and fertility* 67:473-475.
- Macmillan K.L.; A.M.; Taufa V.K.; Gibb M.; Pearce M.G. 1985a. Effects of an agonist of gonadotrophin releasing hormone in cattle I. Hormone concentrations and oestrous cycle length. *Animal reproduction science* 8:203-212.
- Macmillan K.L.; Day A.M.; Taufa V.K.; Gibb M.; Pearce M.G. 1985b. Effects of an agonist of gonadotrophin releasing hormone in cattle II. Interactions with injected prostaglandin $F_{2\alpha}$ and unilateral ovariectomy. *Animal reproduction science* 8:213-223.
- Macmillan K.L.; Henderson H.V. 1984. Analyses of the variation in the interval from an injection of prostaglandin $F_{2\alpha}$ to oestrus as a method of studying patterns of follicle development during dioestrus in dairy cows. *Animal reproduction science* 6:245-254.
- Macmillan K.L.; Taufa V.K.; Barnes D.R.; Day A.M.; Henry R. 1988. Detecting oestrus in synchronised heifers using tailpaint and an aerosol raddle. *Theriogenology* 30:1099-1114
- Macmillan K.L.; Taufa V.K.; Day A.M. 1986. Effects of an agonist of gonadotrophin releasing hormone (Busarelin) in cattle III. Pregnancy rates after a post-insemination injection during metoestrus or dioestrus. *Animal reproduction science* 11:1-10.
- Ottobre J.S.; Lewis G.S.; Thayne W.V.; Inskoop E.K. 1980. Mechanism by which progesterone shortens the estrous cycle of the ewe. *Biology of reproduction* 23:1046-1053.
- Pierson R.A.; Ginther O.J. 1988. Follicle populations during the estrous cycle in heifers I. Influence of day. *Animal reproduction science* 14:165-176.
- Putney D.J.; Drost M.; Thatcher W.W. 1989 Influence of summer heat stress on pregnancy rates of lactating dairy cattle following embryo transfer or artificial insemination. *Theriogenology* 31:765-778.
- Rowson L.E.A.; Lawson R.A.S.; Moor R.M.; Baker A.A. 1972. Egg transfer in the cow: Synchronisation requirements. *Journal of reproduction and fertility* 28:427-431.
- Savio J.D.; Keenan L.; Boland M.P.; Roche J.F. 1988. Pattern of growth of dominant follicles during the oestrous cycle of heifers. *Journal of reproduction and fertility* 83:663-671.
- Thatcher W.W.; Larson L.E. Jr.; Drost M.; Putney D.J. 1987. HCG-induced alterations in pregnancy rate of lactating cows during summer months in South Florida. *Journal of dairy science* 70:(Suppl. 1) 206.
- Thatcher W.W.; Macmillan K.L.; Hansen P.J.; Drost M. 1989. Concepts for regulation of corpus luteum function by the conceptus and ovarian follicles to improve fertility. *Theriogenology* 31:149-164.
- Villa-Godoy A.; Ireland J.J.; Wortman J.A.; Ames N.K.; Hughes T.L.; Fogwell R.L. 1985: Effect of ovarian follicles on luteal regression in heifers. *Journal of animal science* 60:517-527.
- Wright J.M. 1981. Non-surgical embryo transfer in cattle. Embryo-recipient interactions. *Theriogenology* 15:43-56.