Ann. Biol. anim. Bioch. Biophys., 1975, **15** (2), 255-262.

SYNCHRONIZATION AND INDUCTION OF ESTRUS IN HEIFERS WITH A PROGESTAGEN AND ESTROGEN

J. N. WILTBANK (1) and E. GONZALEZ-PADILLA (2)

Texas A and M University and Instituto Nacional de Investigaciones Pecuarias, Mexico

SUMMARY

Estrus has been successfully synchronized in cycling heifers and induced in pre-puberal heifers by placing of an implant containing 6 mg of 17 alpha-acetoxy-11-beta-methyl 19 nor preg 4 ene 3, 20 dione (norgestomet) in the ear for 9 days and injecting 3 mg of norgestomet and 5 mg of Estradiol Valerate (EV) at time of implantation. In trial 1 50 p. 100 of 14 non-cycling control heifers had been in heat after 45 days of breeding compared to 94 p. 100 of 16 treated heifers after 4 days of breeding. After 45 days of breeding 7 p. 100 of the control and 94 p. 100 of the treated heifers were pregnant. In the second trial 38 p. 100 of 81 non-cycling control heifers had shown heat after 48 days of breeding compared to 79 p. 100 in 79 treated heifers after 4 days of breeding. Pregnancy rates after 4, 21, and 48 days of breeding were 4, 11 and 27 p. 100 in controls and 43, 58 and 73 p. 100 in treated.

Injection of EV in conjunction with implant and injection of norgestomet in cycling heifers increased heifers in heat by 120 hours after implant removal from 74 p. 100 to 95 p. 100. Injection of 3 mg of norgestomet in conjunction with implant and EV increased the proportion of heifers in heat by 72 hours, after implant removal from 65 p. 100 to 83 p. 100 and by 120 hours after implant removal from 85 p. 100 to 94 p. 100.

In 2 field trials the proportion of heifers showing estrus by 120 hours after implant removal was over 95 p. 100 in 5 trials and 85 and 11 p. 100 in the other trials. Pregnancy rate at first service differed by 0, 0, 4, 4, 8, 21 and 25 p. 100. The proportion pregnant after 4 days in the treated animals and after 21 days in the controls differed by 0, 3, 4, 1, 11, 13 and 21 p. 100.

INTRODUCTION

A large proportion of cows and heifers do not show estrus the 1st 21 days of the breeding season in beef cattle herds (WILTBANK *et al.*, 1961 ; WILTBANK, 1971 ; WILTBANK, 1974 ; LASTER, 1974 ; REYNOLDS, 1967 ; BELLOWS, 1972). Consequently,

(1) Present address : Texas Agr. Exp. Sta., Star Route 2, Box 43C Beeville, Texas 78102 (U. S. A.).
(2) Present address : I. N. I. P., Departamento de Reproduccion animal, Apartado Postal nº 41652, Mexico 10, D. F. (Mexique)

treatments for estrous synchronization which work in the cycling animal are ineffective in a large number of cows and heifers in beef cattle herds. To be effective a treatment regime should synchronize estrus in cycling animals and induce estrus in non-cycling animals.

The length of estrous cycle was not altered in many heifers treated in the early stages of the estrous cycle when a treatment regime utilizing an ear implant containing 6 mg of norgestomet and intramuscular injection of 5 mg of estradiol valerate (EV) was used (WILTBANK *et al.*, 1975 *a*). WOODY *et al.*, (1967) ; have shown that injection of large amounts of progesterone early in the estrous cycle decreased the length of life of the corpus luteum and shortened the length of the cycle in the bovine. A treatment regime using this information was developed so corpora lutea in the early stages of the cycle would regress and therefore estrous cycle length would be altered. (WILTBANK *et al.*, 1975 *b*).

The purpose of this paper is to summarize briefly the development of a treatment regime for synchronizing estrus in heifers and present the results obtained from using this treatment in cycling and non-cycling heifers.

MATERIALS AND METHODS

Trial 1

To determine if estradiol valerate was essential in the treatment regime, eighty-one Angus and Hereford heifers were treated on day 1 (day of estrus) 2, 3, 4, 5, 6, 7 or 8 of the estrous cycle. All of the heifers were implanted for 9 days with an implant containing 6 mg of norgestomet and received an intramuscular injection of 1 mg of norgestomet. Half of the heifers received an injection of 5 mg of EV while the others served as controls.

Trial 2

The value of an intramuscular injection of norgestomet was determined by assigning beef heifers on day 1 to 5 of the cycle to 4 treatment groups. All groups received 5 mg of EV at the time of implantation. Two of the groups received one implant while the other 2 groups received two implants. One group receiving one implant and one receiving two implants received an intramuscular injection of 3 mg of norgestomet at the time of implantation.

Trial 3

The ovaries of a group of yearling heifers were examined rectally for corpora lutea. Heifers with no corpora lutea were divided by breed (*Hereford*, *Hereford-Charolais* Cross or *Angus*) to either a control group or a group receiving an implant containing 6 mg of norgestomet and an intramuscular injection of 3 mg of norgestomet and 5 mg of EV. Heifers were bred artifically for 45 days. Pregnancy diagnosis was performed at the end of the breeding season and 45 days after the end of the breeding season. More details on experimental procedure is outlined by GONZALEZ-PADILLA *et al.*, 1975.

Trial 4

A group of beef heifers in Mexico were checked for estrus for 35 days and then their ovaries were examined rectally. Heifers not showing estrus or having corpora lutea were divided into a control group and a group treated as outlined in trial 3. Heifers were bred artificially for 48 days and pregnancy was diagnosed approximately 40 days after the end of the breeding period.

Field trials

The trials were conducted on farms or ranches throughout the United States. In the first 6 trials heifers were selected by age and weight. It was hoped by this selection to have only cycling heifers on the experiment. Heifers in trial 7 had been checked for estrus for at least 30 days prior to treatment and none of the heifers placed on the trial had been noted in estrus. Heifers were divided by breed, weight and age (when available) into a control group and a group treated as outlined in trial 3. Heifers were bred artifically for 27 days and pregnancy diagnosis was performed 35 days after the end of the breeding period. Additional details are summarized by SPITZER *et al.*, (1975).

RESULTS

Trial 1

At the time of implant removal only 65 p. 100 of the corpora lutea had regressed n heifers treated on day 1 to 4 of the cycle with the implant and injection of norgestomet while 86 p. 100 of the corpora lutea had regressed in the heifers treated with the implant and injection of norgestomet and EV (table 1). Similar differences were

TABLE 1

Effect of injection of estradiol valerate on regression of corpora lutea and estrous synchronization

Stage of the cycle	Treatment	No Heifers	C. L. Regressed (3) (%)	Showing estrus by 120 hours after implant removal (%)
Day 1-4	I + IM (1)	20	65	75
	I + IM + EV (2)	22	86	91
Day 5-8	I + IM (1)	19	32	74
	I + IM + EV (2)	20	80	100

(1) 1 implant of 6 mg of norgestomet for 9 days + 1 injection of 1 mg of norgestomet.
(2) Same as 1 except 5 mg of EV at time of implantation.
(3) Regressed at time of implant removal.

noted in heifers treated on day 5-8 of the cycle. The percentage of heifers showing estrus by 120 hours after implant removal was 75 and 74 p. 100 in heifers which did not receive an injection of EV compared to 91 and 100 p. 100 in heifers receiving the EV. Therefore, EV appeared to be an important ingredient of the treatment regime.

Trial 2

The differences in the proportion of heifers showing estrus by 120 hours after implant removal were small in heifers receiving one implant versus those receiving

two implants (table 2). More heifers receiving the injection of norgestomet were in heat at 48, 72, 96 and 120 hours after implant removal than in those receiving no norgestomet.

TABLE 2

Effect of number of implants and injection of norgestomet on synchronization (1)

No Implant	IM Injection	No Heifers	Showing estrus (Hours after implant removal)				
			24	48	72	96	120
1	EV (2)	20	0	35	65	70	85
1	EV + Norgestomet (3)	18	0	61	83	94	94
2	EV	19	5	21	58	74	89
2	EV + Norgestomet	19	5	42	63	79	95

(1) All heifers day 1-5 at time of treatment.
(2) 5 mg of EV at time of implantation.
(3) Same as 2 plus 3 mg of norgestomet at time of implantation.

Trial 3

None of the control heifers had shown estrus after 21 days of breeding and only 7 had shown estrus after 45 days of breeding while 15 of 16 treated heifers had shown estrus after 4 days of breeding (table 3). Thus this treatment regime appeared to induce estrus in prepuberal heifers. The estrus appeared to be fertile as 8 out of 15 heifers bred at the induced estrus became pregnant.

TABLE 3

Induction of puberty in prepuberal heifers
(Colorado)

	Control	Treated (1)
Number	14	16
Showing estrus		
after 4 days of breeding	0	15
after 21 days of breeding	0	16
after 45 days of breeding	7	16
Pregnant		
after 4 days of breeding	0	8
after 21 days of breeding	0	12
after 45 days of breeding	1	15

(1) 6 mg of norgestomet for 9 days + IM injection of 5 mg of estradiol valerate and 3 mg of norgestomet at the time of implantation.

Trial 4

Thirty-eight percent of the control heifers had shown estrus and 27 p. 100 had become pregnant after 48 days of breeding (table 4), while 79 p. 100 of the treated heifers had shown estrus after 4 days of breeding, and 43, 58 and 73 p. 100 became pregnant after 4, 21 and 45 days of breeding, respectively. The ability of the treatment to induce estrus appeared to be related to weight of the heifers at the time of treatment (GONZALEZ-PADILLA *et al.*, 1974).

TABLE 4

Induction of puberty in prepuberal heifers
(Mexico)

	Control	Treated (1)
Number	81	77
Showing estrus (%)		
after 4 days of breeding	6	79
after 21 days of breeding	28	84
after 48 days of breeding	38	84
Pregnant (%)		
after 4 days of breeding	4	43
after 21 days of breeding	11	58
after 48 days of breeding	27	73
Anestrous after 1st observed estrus (%)	13	6

(1) 6 mg of norgestomet for 9 days + IM injection of 5 mg of estradiol valerate and 3 mg of norgestomet at the time of implantation.

Field trials

The ability of the treatment regime to control the estrous cycle and induce estrus can be seen by comparing the proportion of treated heifers showing estrus by 5 days after implant removal to the proportion of controls showing estrus by 27 days after implant removal. In all 7 trials the proportion of treated heifers either exceeded or was equal to the proportion of controls (table 5). Thus the treatment regime appeared capable of controlling the estrous cycle and inducing estrus in some but not all of the prepuberal heifers.

Fertility at the synchronized estrus was not different from that noted in the controls at 1st service in 4 of the 7 trials (table 6). However, in 3 trials (1, 4 and 7) fertility was markedly lower. In trial 1, one of the 2 technicians involved had not previously bred heifers and consequently had a difficult time breeding heifers during the synchronized period. The proportion of heifers conceiving at 1st service in the

TABLE 5

Heifers showing estrus in seven field trials

Trial	Number		In estrus by			
			5 days after implant removal (%)		27 days after implant removal (%)	
	Control	Treated (1)	Control	Treated (1)	Control	Treated (1)
1	77	78	27	100	100	100
2	95	98	27	98	92	92
3	53	56	28	98	96	100
4	39	39	10	85	77	98
5	99	99	26	96	93	100
6	99	99	26	96	92	99
7	89	97	16	77	46	80

(1) Implant containing 6 mg of norgestomet for 9 days and an IM injection of 6 mg of estradiol valerate 3 mg of norgestomet.

TABLE 6

Heifers pregnant in seven field trials

Trial	Pregnant							
	After 1st Service (%)		After 4 days of breeding (%)		After 21 days of breeding (%)		After 27 days of breeding (%)	
	Control	Treated (1)	Control	Treated (1)	Control	Treated (1)	Control	Treated (1)
1	54	33	14	33	54	53	60	60
2	63	63	10	63	70	70	74	80
3	60	56	15	55	58	64	64	71
4	63	38	5	31	44	38	51	51
5	45	49	15	43	47	50	50	56
6	45	45	15	43	43	48	50	56
7	52	40	4	30	19	34	26	38

(1) Implant containing 6 mg of norgestomet for 9 days and an IM injection of 6 mg of estradiol valerate and 3 mg of norgestomet.

treated and control animals differed markedly for this technician while little difference between treated and controls were noted for the other technician. Similar results were experienced for 2 technicans in trial 7 were one had been recently trained and had bred only a limited number of animals prior to the trial. In trial 4 the technican had not done any breeding since last breeding season and became tired during the synchronized period. Therefore, it is thought that the poor results in the treated animals noted for trial 1, 4 and 7 could be the result of technician efficiency rather than treatment regime.

To be useful a treatment regime should provide a method so that the proportion of treated heifers pregnant after 4 days of breeding would be equal to or exceed that noted in the controls after 21 days of breeding. In two trials (1 and 4) the difference in the proportion pregnant after 21 days in the control animals exceeded the proportion pregnant after 4 days by 21 and 13 p. 100 ($P < .05$). Non-significant differences of 7, 3, 4 in favor of control animals were noted in trial 2, 3 and 5, respectively ($P < .05$). No difference was noted in trial 6 and a difference of 11 p. 100 in favor of the treated animals was seen in trial 7 ($P < .05$).

The results indicate the ability of the treatment regime to synchronize estrus in cycling animals without a marked decrease in fertility. The treatment also had ability to induce estrus in some prepuberal heifers without a marked reduction of fertility. However, the limitations of this treatment in the prepuberal heifers need to be identified more accurately.

Colloque : Control of sexual cycles in domestic animals
October 27-30, 1974, Nouzilly.

RÉSUMÉ

SYNCHRONISATION ET INDUCTION DE L'ŒSTRUS CHEZ LES GÉNISSES A L'AIDE D'UN PROGESTAGÈNE ET D'UN ŒSTROGÈNE

L'œstrus a été synchronisé avec succès chez des génisses cycliques et induit chez des génisses prépubères en plaçant un implant contenant 6 mg de norgestomet dans l'oreille pendant 9 jours et en injectant 3 mg de norgestomet et 5 mg de valérate d'œstradiol au moment de la pose de l'implant.

Dans l'essai 1, 50 p. 100 des 14 génisses témoins non cycliques ont été en chaleur après 45 jours de mise à la reproduction contre 94 p. 100 des 16 génisses traitées dans les 4 jours qui suivent le retrait de l'implant. Après 45 jours de mise à la reproduction, 7 p. 100 des génisses témoins étaient gestantes alors que ce taux était égal à 94 p. 100 chez les génisses traitées.

Dans l'essai 2, 38 p. 100 des 81 génisses témoins non cycliques ont été détectées en chaleur dans les 48 jours qui suivent la mise à la reproduction ; ceci est à comparer aux 79 p. 100 des 79 génisses traitées dans les 4 premiers jours. Les taux de gestation après 4, 21 et 48 jours de mise à la reproduction ont été de 4, 11 et 27 p. 100 chez les génisses témoins et 43, 58 et 73 p. 100 chez les génisses traitées.

L'injection de valérate d'œstradiol, en plus de la pose d'un implant et de l'injection de norgestomet chez des génisses cycliques augmente la proportion de génisses en chaleur dans les 120 heures qui suivent la fin du traitement de 74 p. 100 à 95 p. 100. L'injection de 3 mg de norgestomet en plus de la pose d'un implant et de l'injection de valérate d'œstradiol augmente la proportion de génisses en chaleur dans les 72 heures qui suivent la fin du traitement de 65 p. 100 à 83 p. 100 et dans les 120 heures de 85 p. 100 à 94 p. 100.

Dans les 5 essais sur le terrain, la proportion de génisses en chaleur dans les 120 heures qui suivent le retrait de l'implant était supérieure à 95 p. 100 dans 5 essais et à 85 et 11 p. 100 dans l'autre. Les taux de gestation au premier service différaient de 0, 0, 4, 4, 8, 21 et 25 p. 100 par rapport aux

témoins dans chacun des essais. Les proportions de génisses gestantes après 4 jours chez les animaux traités et après 21 jours chez les témoins différaient de 0, 3, 4, 1, 11, 13 et 21 p. 100 en faveur des animaux traités.

REFERENCES

BELLOWS R. A., 1972. Factors affecting losses at calving. In improving reproductive efficiency in Beef cattle. *Proceed. 21st and 22nd Texas Beef Cattle Short Course*, 165.

GONZALEZ-PADILLA E., RUIZ R., LEFEVER D., DENHAM A., WILTBANK J. N., 1975. Puberty in Beef heifers. III. Induction of fertile estrus. (To be published).

LASTER D. B., 1974. Sources and causes of conception losses in the cow. *Proceed. 8th Conf. on Artificial Insemination of Beef Cattle.*

REYNOLDS W. L., 1972. Breeds and reproduction. In *Factors affecting Calf crop*. Univ. Fla. Press, 244.

SPITZER J. C., JONES D. L., MIKSCH E. D., WILTBANK J. N., 1975. Synchronization of estrus in Beef cattle. V. Field trials in heifers. (To be published).

WILTBANK J. N., 1971. How to increase Beef A. I. in the 1970's. *N. A. A. B., 22th Convention*, 206.

WILTBANK J. N., 1974. Management programs to increase reproductive efficiency of Beef herds. *J. Anim. Sci.*, **38**, Suppl. 1, 58.

WILTBANK J. N., BURRELL C., DEYOUNG J., LEFEVER D. G., 1975 *a*. Estrous synchronization in Beef cattle. I. Use of an ear implant containing Norgestomet and injection of estradiol valerate in heifers. (To be published).

WILTBANK J. N., SLADE B., MORRIS L. E., LEFEVER D. G., 1975 *b*. Estrous synchronization in Beef cattle. III. Modification of ear implant and estradiol valerate treatment by injection of a progesten in heifers. (To be published).

WILTBANK J. N., WARWICK E. J., VERNON E. H., PRIODE B. M., 1961. Factors affecting net Calf crop in Beef cattle. *J. Anim. Sci.*, **20**, 409.

WOODY C. O., FIRST W. L., POPE A. L., 1967. Effect of exogenous progesterone on estrous cycle length. *J. Anim. Sci.*, **26**, 139-141.