INVESTIGATIONS ASSOCIATED WITH THE TRANS-PLANTATION OF BOVINE OVA

I. SYNCHRONIZATION OF OESTRUS*

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Summary. Synchronization of bovine oestrous cycles was studied with respect to methods of accomplishment. A total of 328 cows were recycled by: (a) daily subcutaneous injections of progesterone, (b) larger subcutaneous injections every 3rd day and (c) corpus luteum expression. Standing oestrous was exhibited by 262 cows. Twelve did not exhibit oestrus and fifty-four exhibited symptoms but refused mounting. Time between treatment and oestrus was 4.97 ± 1.82 , 6.52 ± 1.25 and 2.84 ± 0.68 days, respectively, for the three methods employed. Length of time between injection and oestrus did not differ significantly as influenced by day of initiation, the duration of injections or by superovulation. Differences between fifty-nine recycled and fifty-three control cows, with respect to rate of conception, were not significant nor were differences between the first and second services after recycling.

INTRODUCTION

Synchronization of the oestrus cycles of donor and recipient individuals is believed to be essential to the ultimate survival of transferred ova. The uterus is subject to continual changes in response to fluctuating hormonal levels. The transferred ovum, if it is to survive, must be placed in an environment that as nearly as possible approximates the one from which it was removed. Chang (1950) investigated the fate of transferred rabbit ova in relation to ovulation time of recipients. He concluded that the reception and nourishment of ova in the uterus requires the participation of secretions of corpora lutea at definite stages, and that little opportunity exists for transferred ova to develop into embryos when placed in tracts more than 1 or 2 days prior to or following the corresponding luteal stage.

It has been demonstrated that progesterone, endogenous or of exogenous origin, is effective in preventing oestrus for such time as it is present and for a reasonably predictable period of time following its withdrawal. Reports pertaining to certain aspects of the problem such as effective dosage levels and

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length of time between injection and oestrus are fairly consistent; however, a decided disunity of opinion exists concerning alteration of the oestrous cycle upon rate of conception. It seemed advisable, therefore, that an attempt be made to ascertain whether progesterone synchronization would be satisfactory for ovum-transfer work or whether other means should be sought.

The objectives of this study were threefold: (1) to establish the consistency with which oestrous cycles can be readjusted and predictability of the time of first heat period subsequent to such readjustment, (2) to determine whether a recycled animal is capable of subsequent normal ovulation, fertilization and complete gestation and (3) to study treatment effects on incidence of heat among superovulated cows.

METHODS AND MATERIALS

Synchronization of oestrus in the cow was studied by three methods:

(1) SYNCHRONIZATION BY DAILY INJECTIONS OF PROGESTERONE

Conception rate of synchronized animals

Progesterone was administered at the rate of 50 mg daily. This dosage was based upon the findings of Christian & Casida (1948) and Ulberg, Christian & Casida (1951). The progesterone used in this study was purchased in crystalline form and dissolved in corn oil prior to use. Injections were initiated on Day 14 of the oestrous cycle, counting day of heat as Day 1, and continued for such duration as to exceed the normal cycle. The recycling procedure was initiated subsequent to the first heat period occurring after 40 days following parturition. Only first-service animals having had at least one calf were used. Animals coming into heat the 1st, 3rd, 5th and 7th 10-day periods received subcutaneous injections of progesterone daily. Those coming into heat the 2nd, 4th, 6th and 8th 10-day periods were designated as controls.

Animals recycled as potential donors and recipients of ova

Animals in this group were handled in a manner similar to the aforementioned, as to method of synchronization. With the exception of those individuals selected as donors of ova, the animals were observed for oestrus but were not bred.

(2) SYNCHRONIZATION BY INCREASED DOSAGES OF PROGESTERONE ADMINISTERED EVERY 3RD DAY

Cows in this group were recycled by the administration of greater amounts of progesterone administered less frequently. Injections consisted of 150 mg and were administered every 3rd day. Some individuals received two injections or a total of 300 mg and others three injections or 450 mg of progesterone.

(3) SYNCHRONIZATION BY EXPRESSION OF CORPUS LUTEUM

Animals in this group were recycled by the expression of the corpus luteum. The technique of rectal palpation was utilized to remove luteal tissue by manually exerting pressure upon the ovaries. Corpora lutea were expressed on

Days 12, 13 and 14 of the oestrous cycle, day of oestrus being considered as Day 1. No distinction was made between days. Some of the cows in this group were superovulated.

RESULTS AND DISCUSSION

(1) SYNCHRONIZATION BY DAILY INJECTIONS OF PROGESTERONE

Conception rate of synchronized animals

One hundred and twelve cows were included in this group. Fifty-nine were designated as experimental animals and fifty-three as controls. Table 1 compares rates of conception between the two groups. Conception is based upon

TABLE 1
INFLUENCE OF RECYCLING AND OF NORMAL OESTRUS ON FERTILITY IN COWS

	Experimental				χ^2 Diff.	Control			
	No. obs.	Conceived		Services per conception		No. obs.	Conceived		Services per conception
		No.	%				No.	%	
*First service	59	20	33.9	2.95	0.163	53	17	32.2	3.12
Second service	39	17	43.6	2.29	0.902	36	12	33.3	3.00
First and second	59	37	62.7	2.65	0.592	53	29	54.7	3.07
Total	59	50	84.7	2.90	0.25	53	45	84.9	3.33

* χ^2 between first and second services = 0.880

actual calving. Conception to first, second and total services is compared. Differences between groups were not statistically significant for either first or second service nor were differences noted between services within groups as tested by chi-square analysis. Percentage conception for first and second services combined were found to be 62.7 and 54.7 respectively for experimental and control groups. Total cows conceiving, regardless of the number of services involved, were observed to be 50 of 59 or 84.7 % for the progesterone synchronized group and 45 of 53 or 84.9 % for the control group. The progesterone group required an average of 2.90 services per conception, as compared to 3.33 for the controls. Though overall conception was low, the conclusion is drawn that, under the conditions of this experiment, synchronization of oestrus by the daily injection of progesterone did not effect fertility.

Animals recycled as potential donors and recipients of ova

A total of 262 cows received daily injections of 50 mg of progesterone. Two hundred and four stood for mounting by other cows. Forty-two refused to stand but manifested other symptoms of oestrus. Twelve failed to indicate oestrus and four manifested heat while receiving progesterone. Thirty-seven of the 204 'oestrous' cows were superovulated. They received treatment an average of 10.38 days and returned to oestrus 4.97 ± 1.36 days later. One hundred and sixty-five of the 167 non-superovulated cows were injected an average of 10.68 days and stood to be mounted 4.97 ± 1.97 days later. The

remaining two were treated 44 and 60 days and both demonstrated oestrus 5 days subsequent to the last injection. No difference was found to exist between the superovulated and the non-superovulated groups, in terms of time required to return to oestrus. The frequency with which the 204 'oestrous' cows returned to oestrus is depicted by the frequency histogram (Text-fig. 1). Length of

TEXT-FIG. 1. Frequency of return to oestrus after cessation of progesterone administration to superovulated and non-superovulated cows. Black, superovulated; white, non-superovulated.

treatment varied considerably among cows. Table 2 lists the 204 'oestrous' cows and mean time of return to oestrus by number of days treated. Two hundred and two were treated for 3 through 24 days. The remaining two were treated for 44 and 50 days. Length of treatment had no significant effect on time required to exhibit oestrus following cessation of treatment.

TABLE 2
MEAN TIME OF RETURN TO OESTRUS AS INFLUENCED BY DURATION OF PROGESTERONE TREATMENT

<i>No. observations</i>	<i>No. injections</i>	<i>Mean day of return to oestrus</i>
72	3 to 8	5.44
103	9 to 16	6.14
27	17 to 24	5.18
2	44 to 50	5.00

Table 3 lists the 'oestrous' cows and mean time of return to oestrus by day of the oestrous cycle on which treatment was initiated. In the majority of cases (143), injections were begun on Day 14. This was done as a matter of expediency since it facilitated recycling with a minimum of injections. Treatment initiated on Day 14, resulted in the occurrence of oestrus an average of 4.89 days following the last injection. Initiation of treatment at other stages of the oestrus cycle did not differ significantly.

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(2) SYNCHRONIZATION BY INCREASED DOSAGES OF PROGESTERONE
ADMINISTERED EVERY 3RD DAY

This group consisted of thirty-eight cows in which the oestrous cycle was controlled by injections of 150 mg of progesterone at 3-day intervals. One-half of this number was given two injections or a total of 300 mg of progesterone. The remaining one-half received three injections or 450 mg total dosage. Fifteen of the '300-mg' group and eighteen of the '450-mg' group expressed standing heat. Those cows receiving 300 mg of progesterone demonstrated oestrus an average of 6.80 ± 1.42 days after the last injection while the 450-mg group returned to heat in an average time of 6.28 ± 1.07 days. Combining of the two groups resulted in a mean time of return of 6.52 ± 1.25 days.

TABLE 3

MEAN TIME OF RETURN TO OESTRUS AS INFLUENCED BY TIME OF PROGESTERONE TREATMENT INITIATION

<i>No. observations</i>	<i>Day of treatment initiation</i>	<i>Mean day of return to oestrus</i>
18	1 to 8	4.83
19	8 to 14	5.31
143	14	4.89
9	15 to 18	4.77
15	Random	5.53

TABLE 4

EFFECT OF TREATMENT UPON TIME OF SUBSEQUENT OESTRUS IN COWS SHOWING STANDING HEAT

<i>Treatment</i>	<i>No. observations</i>	<i>Day of return to oestrus</i>		
		<i>Mode</i>	<i>Mean</i>	<i>S.D.</i>
Progesterone daily (50 mg/injection)	204	5	4.97	1.82
Progesterone at 3-day intervals (150 mg/injection)	33	6	6.52	1.25
Expression of corpus luteum	25	3	2.84	0.68

(3) SYNCHRONIZATION BY EXPRESSION OF THE CORPUS LUTEUM

Thirty-two cows were synchronized as to oestrus by corpora lutea removal. Twenty-five expressed standing heat. Nineteen of the cows expressing standing heat were superovulated and six were not. The superovulated group showed heat an average of 2.74 ± 0.65 days following corpus luteum expression. The non-superovulated group showed heat 3.17 ± 0.75 days. The pooled average was 2.84 ± 0.68 days. Table 4 summarized the total number of cows recycled that expressed standing oestrus by each of the three methods utilized in this study.

Differences between the different levels of progesterone administration with respect to the time of subsequent oestrus were found to be highly significant ($F=611$, C.R. >6.63). Cows that received 150 mg of progesterone in the last injection required an average of 1.55 days more to return to oestrus than did cows that received only 50 mg. The possibility is thereby suggested that for every milligramme increase of progesterone in the last injection, a resulting increase of approximately 0.016 days might result in time required to return to oestrus. This would be equivalent to an increase of 0.8 of a day for an additional 50 mg of progesterone. Time-of-oestrus differences between daily injections and corpus luteum removal were also observed to be highly significant ($F=31.06$, C.R. >6.63).

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