

INVESTIGATIONS ASSOCIATED WITH THE TRANS- PLANTATION OF BOVINE OVA

II. SUPEROVULATION*

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Summary. (1) Purified swine FSH and LH effectively induced superovulation in mature and immature as well as pregnant bovine females. Sixty-one of seventy-five treated cows and calves ovulated, producing a total of 1716 ovulation points which ranged from one to eighty-eight with a mean of 28.13. Twenty-eight of thirty-two cows and thirty-three of forty-three calves responded to treatment by ovulating. Calves produced an average of 12.46 more ovulations than did cows. (2) Neither the addition of prolactin to the ovulatory dosage of LH nor the administration of LH for 2 consecutive days proved superior to the single injection of LH as an ovulatory procedure for calves. (3) Calves produced 54.03 % of their total ovulations on the right ovary compared to 51.17 % for cows. (4) Nineteen cows, having undergone oestrus synchronization, produced an average of 7.89 ovulations more than were produced by nine similar individuals, superovulated without prior treatment with progesterone. (5) 60.21 % of all cows superovulated demonstrated oestrus. A significantly greater number of cows receiving progesterone as well as those undergoing enucleation of corpora lutea exhibited oestrus than did cows not receiving treatment prior to superovulation. (6) Superovulated cows exhibiting oestrus produced an average of 3.82 ovulations more than were produced by superovulated cows failing to show heat.

INTRODUCTION

An increase in the number of offspring from a given dam as compared to the number produced by the usual methods of breeding is considered one of the chief potential advantages of the technique of ovum transplantation. To attain maximum increase in number of offspring, superovulation becomes a necessity and constitutes an important facet in the overall ovum-transplantation scheme.

Superovulation may be defined as the development of and the ovulation from more than the normal number of follicles and may logically be divided into two phases. The first phase consists of the development of follicles due to the

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administration, usually subcutaneously, of follicle-stimulating hormone. Pregnant mare serum (PMS), horse pituitary extract, sheep pituitary (FSH), unfractionated sheep gonadotrophins (USG), and swine (FSH) have all been used for this purpose.

The second phase consists of the rupture of the follicles with subsequent release of ova. This may be brought about by either the intravenous administration of gonadotrophic hormone preparations which consist principally of the luteinizing hormone or by LH from the cow's own pituitary, provided timing is such that mature follicles are present at oestrus. Unfractionated sheep gonadotrophin (USG), human chorionic gonadotrophin (HCG), sheep LH and swine LH are the most commonly used materials for ovulating purposes.

Willett (1953) mentioned the importance of controlled timing of ovulation when eggs from superovulated cows are to be used for transplantation, and suggested that it could best be obtained by injecting LH intravenously rather than depending upon endogenous LH.

Superovulation in cattle was first reported by Casida, Nalbandov, McShan, Meyer & Wisnicky (1940) and Casida, Meyer, McShan & Wisnicky (1943). Since that time, it has been repeated by a number of workers using a variety of techniques. The subject has been reviewed by Dowling (1949), Hammond (1950), Chang (1949), Lamming & Rowson (1952), Donker (1952), Willett (1953), Nichols (1956) and Dziuk, Donker, Nichols & Petersen (1958).

The objectives of this study were: (1) to evaluate the efficacy of the employed superovulatory technique as a means of procuring ova from multiple ovulations; (2) to study the effect of progesterone administration prior to superovulation on incidence of heat and ovulation; and (3) to evaluate the success with which female calves might be superovulated.

METHODS AND MATERIALS

The basic superovulation procedure for cows was standardized as follows:

*Day 1	20 mg FSH and 5 mg LH subcutaneously
Day 2	10 mg FSH and 5 mg LH subcutaneously
Day 3	10 mg FSH and 5 mg LH subcutaneously
Day 4	10 mg FSH and 5 mg LH subcutaneously
Day 5	100 mg LH intravenously

This procedure was based upon the study by Nichols (1956) which indicated that a total of 50 mg of FSH would stimulate adequate development of follicles and that more consistent and desirable results as regard maturation of follicles and ovulation with a minimum of luteinization were obtained when FSH and LH were present in the ratio of 1:1 or 2:1.

In those instances where cows were recycled, the initial injection of gonadotrophic hormones was given on the day succeeding that of the last injection of progesterone unless otherwise specified. Gonadotrophin injections were initiated on Day 16 of the oestrous cycle for those cows receiving no progesterone. In either event, the ovulatory injection was administered at the time of heat. In most instances, this was the 5th day as indicated by the preceding

* Refers to day of injection and not to day of cycle.

schedule of injections. Some cows, however, demonstrated oestrus on the 4th day. When such occurred, the follicle-stimulating injection was omitted and the cow was given the ovulatory injection instead.

The procedure used to superovulate calves was derived by a process of trial and error. Various ratios and amounts of hormones produced a series of erratic results extending from total failure of observable response to extremely hypertrophied ovaries. The following procedure was thought to produce the most consistent and desirable results and was, accordingly, installed as the standard procedure for the development of follicles in calves.

Day 1	20 mg FSH subcutaneously
Day 2	10 mg FSH subcutaneously
Day 3	5 mg FSH subcutaneously
Day 4	No injection
Day 5	10 mg FSH subcutaneously

As no method was available whereby the stage of cycle could be discerned in calves, the day of initiation of FSH injections was unknown in the majority of cases. In those instances, where progesterone administration preceded superovulation, injections were continued for sufficient duration that the last day of the superovulation treatment would fall on or exceed what would have constituted the 21st day of the cycle had the initial injection of progesterone been administered on Day 1.

The majority of calves did not exhibit oestrus. Consequently, no indication was forthcoming regarding the most opportune time for administration of the ovulatory injection. Therefore, it became standard procedure to ovulate on Day 6. Since the ovulatory injection was to be administered at a set time, it seemed likely that giving the standard ovulatory injection for 2 consecutive days might prove advantageous.

Results obtained by most researchers have indicated that non-fractionated gonadotrophins are more effective in producing superovulation than are the more purified forms. Nichols (1956), however, using purified hFSH and hLH, obtained results comparable to those obtained by workers using the non-fractionated hormones. Although failing to establish statistical significance, he expressed the belief that more favourable results were obtained when prolactin in the order of magnitude of 650 i.u. was added to the ovulatory dosage.

For the preceding reasons, three different ovulatory procedures were used. Procedure No. 1 consisted of the intravenous administration of 100 mg of LH on Day 6 as indicated by the schedule of injections for calves. Procedure No. 2 consisted of similar injections on both Days 6 and 7. Procedure No. 3 was essentially the same as Procedure No. 1, but with the addition of 650 i.u. of prolactin. Ninety-five cows were superovulated as part of this study. Observations were made on these animals concerning the incidence of oestrus as effected by progesterone pretreatment and enucleation of the corpus luteum at the time of superovulation. Thirty-two cows were slaughtered and observations made on ovulatory response. This study also included observations made on forty-three immature female calves which were superovulated and slaughtered.

RESULTS AND DISCUSSION

Of the forty-three calves superovulated and slaughtered, three did not respond to treatment and seven developed follicles but did not ovulate. Eight of the thirty-three calves that ovulated received a single intravenous injection of LH. Twelve received LH intravenously on 2 consecutive days and thirteen received prolactin in addition to LH. Table 1 illustrates the comparative success of each

TABLE 1

OVULATION AMONG CALVES AS INFLUENCED BY NATURE OF OVULATORY DOSAGE

Treatment		Observations	Ovulations		
No.	Nature	No.	No.	Range	Mean
1	100 mg LH; single injections	8	305	7 to 88	38·12
2	100 mg LH; 2 consecutive days	12	444	1 to 77	37·00
3	100 mg LH and 650 i.u. prolactin; single injection	13	367	5 to 74	28·23

treatment. The eight calves on Treatment No. 1 produced an average of 38·12 ovulation points. Twelve calves on Treatment No. 2, and thirteen calves on Treatment No. 3 produced averages of 37·00 and 28·23 points respectively. Although based on a limited number of observations, these data indicate that no advantage is to be derived by administering LH for 2 consecutive days or by the addition of prolactin to the ovulatory injection. Calves ranged in age from 65 to 173 days. Ovulatory response of these young individuals to administered gonadotrophins varied from a single point of ovulation to eighty-eight. The regression of total ovulation on age revealed that regression did not account for a significant portion of this variation ($F = 0·082$, $C.R. > 4·17$) and suggested that, within the limits 65 to 173 days, no difference existed in ovulatory response attributable to age. Calves failing to ovulate fell into all three groups in approximately equal proportions. Pl. 1 demonstrates the ovulatory response.

TABLE 2

OVULATION OF COWS VERSUS CALVES AND RIGHT VERSUS LEFT OVARY IN RESPONSE TO SUPEROVULATION

Animals				Ovulation points								
Observed		Ovulated		Total			Right ovary			Left ovary		
Type	No.	No.	%	No.	Range	Mean	No.	Mean	% of total	No.	Mean	% of total
Cows	32	28	87·50	598	4 to 55	21·36	306	10·93	51·17	292	10·43	48·8
Calves	43	33	76·64	1116	1 to 88	33·82	604	18·30	54·03	512	15·6	45·97
Total	75	61	81·33	1716	1 to 88	28·13	910	—	53·03	806	—	46·97

In the group of thirty-two cows superovulated and slaughtered, four failed to respond to hormone treatment of which three failed to ovulate and the fourth did not develop follicles. Table 2 contains data which provide a basis for

comparing the relative ovulatory response of cows and calves respectively to superovulation. Observations are based upon only those animals slaughtered subsequent to superovulation. Seventy-five observations, thirty-two pertaining to cows and forty-three to calves, were made. Twenty-eight of thirty-two cows or 87.50 % responded to treatment by ovulating, compared to thirty-three of forty-three or 76.64 % of the calves. The difference was not found to be statistically significant (χ^2 , 1.44). Ovulation points for twenty-eight cows ranged in number from four to fifty-five with a total of 598 and a mean of 21.36. Similar enumeration of the thirty-three calves revealed a total of 1116 ovulation points with a mean of 33.82 and a range of one to eighty-eight. The calves, therefore, produced an average of 12.46 more ovulations than did cows, a difference found to be highly significant (χ^2 , 83.92). Table 2 also provides a breakdown of ovulation by right and left ovaries. The right ovary contained 53.03 % of all ovulations, compared to 46.97 % for the left. This compared to 57 % for the right and 43 % for the left, based upon 10,260 observations of cows having single ovulations as reported by Neilsen (1949). The calves in this study were observed to have a greater percentage of ovulation on the right ovary than were cows.

Table 3 contains data that compares the effect of progesterone administration

TABLE 3

INFLUENCE OF PROGESTERONE PRETREATMENT ON OVULATION OF SUPEROVATED FEMALES

Basis of comparison	No. observations			No. ovulations					
	Cows	Calves	Total	Total			Average		
				Cows	Calves	Combined	Cows	Calves	Combined
Progesterone	19	7	26	454	247	701	23.89	35.28	26.96
No progesterone	9	26	35	144	869	1013	16.00	33.42	28.94

TABLE 4

INCIDENCE OF OESTRUS AMONG SUPEROVULATED COWS AS INFLUENCED BY PRETREATMENT

Nature of treatment	No. observations	Cows manifesting oestrus	
		No.	% of total
Progesterone	56	37	69.64
No progesterone	14	4	28.57
Enucleation of corpus luteum	25	19	76.00
Total	95	59	60.21

prior to superovulation on ovulation numbers. Nineteen cows receiving progesterone were observed to produce an average of 7.89 ovulations more than were produced by nine similar individuals, superovulated without prior treatment with progesterone. This represents a highly significant difference (χ^2 , 17.67). Of the thirty-three calves observed, the seven receiving progesterone produced an average of 1.86 ovulations more than did twenty-six not receiving progesterone. The difference was not significant (χ^2 , 0.53).

PLATE 1

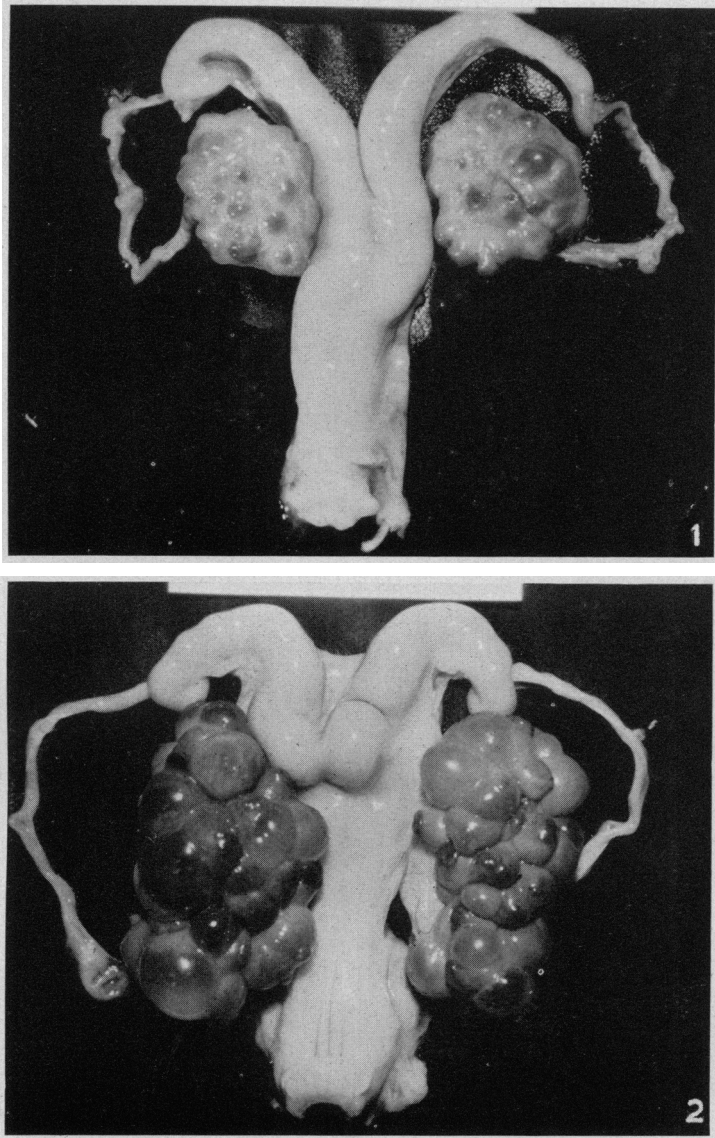


FIG. 1. Reproductive tract of Holstein calf 127 days of age. Ovaries contained thirty-seven ovulation points.

FIG. 2. Reproductive tract of Holstein calf 131 days of age. Ovaries contained fifty-seven ovulation points. Ten ova were recovered from horns and uterus.

[facing p. 216]

Table 4 contains data relating to the incidence of oestrus among super-ovulated cows. Fifty-six animals received progesterone prior to being super-ovulated. Thirty-seven or 69.64% exhibited oestrus as compared to only four or 28.57% of those not receiving treatment prior to superovulation. An additional twenty-five cows were superovulated following enucleation of the corpus luteum. Nineteen or 76% exhibited oestrus. A significantly greater number of cows receiving progesterone as well as those undergoing enucleation of corpora lutea exhibited oestrus than did cows not receiving progesterone treatment prior to superovulation (χ^2 , 5.85 and 7.34 respectively). Differences between the progesterone treated and the animals in which corpora lutea were enucleated were not significant. Also animals superovulated which exhibited standing heat produced on an average 3.83 more ovulations than did cows that were superovulated, but failed to show standing oestrus.

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