mixture with the enzyme, glucuronidase did not alter the experimental findings. 2. There was no detectable difference in the capacity of tissues of stressed or adrenalectomized animals, as compared to tissues from normal rats, to inactivate cortisone. The inactivation mechanism could not be altered by the pre-administration of progesterone, pregnenolone, testosterone, desoxycorticosterone or cortisone.

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Relation of Stage of Cycle and Source of Luteinizing Hormone to Superovulation in Dairy Cattle.* (19392)

E. L. WILLETT, W. H. MCSHAN, AND ROLAND K. MEYER.

From the American Foundation for the Study of Genetics, Madison, and the Department of Zoology, University of Wisconsin.

In research to develop practical technics for egg transplantation in dairy cattle, the first problem is that of superovulation. This problem has already been investigated by various workers(2-10). When the research reported here was initiated, some workers(2,3,10) had injected cows or heifers with gonadotrophins during the luteal phase of the cycle without expressing the corpus luteum, and the evidence indicated, although not conclusively, that cows superovulated and bred under these conditions produced infertile eggs. Rowson(8) later reported that eggs, shed by cows which had been bred and which had a functioning corpus luteum, were infertile. Various sources of gonadotrophins have been employed by different workers, including unfractionated sheep pituitary gonadotrophin (USG) and human chorionic gonadotrophin (HCG) for intravenous injections to rupture the follicles. No one has compared these two gonadotrophins quantitatively.

This paper reports the results of two series of investigations with heifers and cows to study primarily a) the effect of the stage of the cycle upon ovarian response to gonadotrophins and b) the quantitative difference in response to USG and HCG.

Experimental. Study of stage of cycle, dosages of FSH and two sources of LH. The first series consisted of a factorial experiment. It was designed to study follicular development and number and fertility of ova as affected by: a) initiation of treatment on the fourth day of the cycle (luteal) versus sixteenth day (follicular), b) 3 levels of follicle stimulating hormone (FSH), namely 20, 30 and 40 g-equivalents (g.e.) of desiccated sheep pituitaries, and c) one g.e. of USG versus 2500 I.U. of HCG (Choriogonin) for the intravenous injection. In this experiment 12

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yearling Holstein heifers were divided into two equal groups, one the "luteal" and the other the "follicular" group. Two of the animals in each group received 20, two 30 and two 40 g.e. of FSH. One of each of these pairs received USG and the other HCG for the intravenous injection. The heifers were allotted in a random manner. The treatment consisted of: a) FSH administered in equal portions subcutaneously back of the shoulders daily for 5 days, b) intravenous injection on the 6th day, and c) insemination on the 6th and 7th days. The FSH was from one batch prepared by slight modification of the method of McShan and Meyer(11). A total of 1½ g.e. given subcutaneously twice daily for 10 days to 30-day-old Sprague-Dawley hypophysectomized rats produced ovaries containing only follicles and averaging 130 mg; 3 g.e. produced similar ovaries averaging 192 mg. In another test with the method of McShan and Meyer, 1 g.e. (6.4 mg of lyophilized extract) produced ovaries averaging 54 mg in 21-day-old, intact rats. The batch of unfractionated sheep gonadotrophin was prepared by the method of McShan and Meyer (12). One g.e. of the lyophilized extract weighed 80.1 mg. One-tenth g.e. (8 mg) produced ovaries averaging 85 mg when assayed as was the FSH with intact rats. The heifers were inseminated with semen from bulls of high fertility. Immediately after slaughter the reproductive organs were removed, and the horns and oviducts were flushed with physiological saline solution. Recovered eggs were examined in hanging drop slides at a magnification of 440X. The number of ova produced, as indicated by number of ovulation points or corpora lutea, was the main criterion for measuring ovarian response. The ovaries were weighed, unruptured follicles counted, and their diameters measured with calipers. In an attempt to recover eggs from the live animal, the uteri of a few heifers were irrigated an hour or two prior to slaughter on the fifth day following the intravenous injection. No eggs were recovered, and since it appeared that the number of ova recovered was reduced, this practice was discontinued. Furthermore, after killing 2 "luteal" heifers on the 5th day, it was found that eggs could not be recovered from the uterine washings due to pyometra. The slaughtering of all heifers was subsequently done on the 3rd day at which time most of the eggs are in the oviducts.

The responses to the various treatments were extremely variable as has been reported by other workers. A summary of results is presented in Table I. Almost twice as many corpora were produced during the follicular as during the luteal phase. Folley and Malpress(5), on the other hand, observed no difference in ovarian response between the follicular and the luteal phase with the corpus luteum intact, while Rowson(8) had considerably more ovulations during the luteal phase. These discrepancies may possibly be explained by differences in treatments or criteria. None of the 34 eggs recovered in this experiment from luteal heifers showed evidence of having been fertilized, whereas 74% of the 43 from follicular heifers were fertilized. The eggs from the follicular group had blastomeres of normal uniformity in size, and spermatozoa were present in the zona pellucida. The evidence of infertility during the luteal phase is in agreement with findings of other workers (2,3,8,10). In light of all data now available, there is no doubt that bovine females, ovulated by gonadotrophins in the presence of the corpus luteum, produce infertile eggs as shown by lack of fertilization on breeding. Studies by Murphree *et al.*(13) indicate that interference with spermatozoan transport through the cervix accounts for much of the infertility in luteal phase rabbits. With the bovine, on the other hand, it appears as though other factors may be responsible. Most of the luteal heifers in the experiment described in this paper were inseminated in the uterus. Furthermore, Beschlebnov(14) and VanDemark and Moeller(15) have found that sperm reached the ovaries when cows were inseminated while not in heat.

All the heifers in the luteal group had pyometra. Since this observation was originally reported(1), a comparable condition has been reported in swine(16) and rabbits(13). It is improbable that substances in the semen diluter (egg yolk-citrate) used for breeding the heifers were responsible, for one heifer was inseminated in the cervix with undiluted se-

TABLE I. Summarization of Comparisons Between Various Treatments in Factorial Experiment. Figures are heifer averages.

Treatment	Total No. heifers	Wt of ovaries, g	No. Unruptured follicles >4 mm diam.	No. corpora lutea	No. heifers yielding eggs	Eggs		
						No. ovulated	No. recovered	% cleaved
Luteal	6	36	20	12.7	5	14.4	6.8	0
Follicular	6	53	28.2	21.8	5	23.4	8.6	74
20 g.e. FSH	4	34	19	11.8	2	14.5	8	6
30 " "	4	50	29.8	22.2	4	22.2	11.2	62
40 " "	4	48	23.5	17.8	4	17.8	4	19
USG	6	42	30.5	23	6	23	9.7	6
HCG	6	47	17.7	11.5	4	12.8	4.8	26

men because of inability to penetrate the cervix with the insemination tube. All other heifers were inseminated in the uterus with semen in yolk-citrate diluent. Whether changes during the cycle in regard to uterine sensitivity to foreign substances or to defenses against bacteria introduced at insemination or whether other factors produced this pyometra merit investigation. An answer to this problem might shed light on the cause of spontaneously occurring pyometra in cows(17).

The lowest dose of FSH gave the lowest response; there was not much difference between the two higher dosages. USG injected intravenously produced twice as many corpora as HCG. The data from this experiment were tested by analysis of variance. Due to the small number of heifers and the great variability, there were no significant differences between any of the treatment means. Neither were there any significant interactions between any two of the treatments.

Further study of two sources of LH. The data from the above experiment were considered adequate in regard to stage of cycle and dosage levels of FSH. It was concluded, however, that more information was needed as to the relative number of corpora produced with USG and HCG. Consequently, a second series of animals was used to obtain more data for this comparison. Heifers or cows were superovulated by giving a single subcutaneous injection of gonadotrophin from pregnant mare serum (PMS) on the 15th or 16th day of the cycle and an intravenous injection of USG or HCG five days later. Dosages given were 2,000, 3,000 or 5,000 I.U. of PMS, 2,500 or 5,000 I.U. of HCG and 1 g.e. of USG. Animals were paired as to size, breed and parity. The 2 animals in each pair received the same dose of the same batch of PMS on the same day of the cycle, but one received USG and the other HCG for the intravenous injection. There were 6 pairs of Holsteins and one of Guernseys. The 5 pairs of heifers were slaughtered, and the ovaries studied in the laboratory. Animals in 2 of the pairs were mature cows, and the number of corpora lutea in their ovaries was counted *in vivo* by making an incision through the dorsal wall of the vagina and the peritoneum of the recto-vaginal pouch, drawing the ovaries into the vagina, and examining them with a speculum.

The data for individual heifers are presented in Table II. The data from this series were combined with those from series I, for the animals in both series had been paired similarly.

A summary of the data from the 2 series and of the combined data is given in Table III. Results from the 2 series were similar in that a greater number of corpora was obtained with USG than with HCG. When the combined data were analyzed by analysis of variance, it was found that the over-all means were almost highly significantly different (probability slightly greater than 0.01). There was no significant interaction between the subcutaneous and intravenous treatments. The evidence that a larger number of corpora can be obtained with USG than with HCG is,

TABLE II. Ovarian Response and Infertile and Fertile Eggs Recovered from Individual Heifers and Cows in Series II Receiving PMS and Two Sources of LH.

No. of pair	No. of heifer	Dosages: PMS, I.U. × 1000	Dosages: USG, g.c.	Dosages: HCG, I.U. × 1000	Interval intrav. to autopsy, days	Wt of ovaries, g	No. unruptured follicles: 5-25 mm	No. unruptured follicles: >25 mm	No. corpora lutea	Eggs: No. recovered	Eggs: Cleaved No.	Eggs: Cleaved %
I	22	5	1		5	61	14	0	27	16	14	87
II	28	3	1		7	205	16	5	33	4	1	25
III	33	2	1		5	25	11	0	10	5	4	80
IV	54	3	1		—	—	—	—	39	—	—	—
V	57	2	1		—	—	—	—	6	—	—	—
VI	67	2	1		8	18	1	0	5	1	0	0
VII	68	2	1		6	46	4	0	8	5	5	100
Avg						71	9.2	1	18.3	6.2	4.8	77
I	21	5		2.5	5	33	6	0	14	8	5	62
II	27	3		2.5	4	29	7	0	4	1	1	100
III	32	2		5	5	52	4	0	12	6	6	100
IV	55	3		5	—	—	—	—	2	—	—	—
V	56	2		5	—	—	—	—	2	—	—	—
VI	65	2		5	8	15	5	0	1	0	0	—
VII	60	2		2.5	4	12	5	0	1	0	0	—
Avg						28	5.4	0	5.1	3	2.4	80

therefore, quite conclusive. Umbaugh(9) also presented data indicating that such differences might occur, although other variables were involved in the comparison. It would appear, on the basis of the limited data for individual heifers (Table II), that the dosage of HCG was not a factor causing this difference in our experiments, for the relationship of the response with HCG to that with USG treatment was apparently the same whether 2,500 or 5,000 I.U. of HCG was given. Probably USG produces more corpora when given intravenously than HCG because it contains considerably more FSH. In this connection, it is of interest that, in both experiments, USG also produced more unruptured follicles than did HCG. This difference in number of follicles was not significant, however.

A comparison of the data from series I with series II gives some indication of the relative merits of sheep FSH and PMS. Injections with PMS-USG have frequently resulted in large ovaries with follicles greater than 25 mm in diameter, which might be classed as cystic. One heifer in series II, No. 28, had such follicles. In cases where the animals were not slaughtered, these large follicles were later resorbed. Dowling(4), and Folley and Malpress(5) have observed such abnormal responses when injecting PMS and the latter have also observed resorption. There is a possibility that PMS-USG overstimulates the ovaries of some animals causing a rapid thickening of the follicle wall and thus preventing ovulation(18). The presence of some LH in PMS may aggravate this condition.

The animals injected with PMS and HCG had fewer corpora than those receiving PMS and USG but did not have extremely large ovaries or follicles. Of 15 eggs recovered from this group, 80% had apparently cleaved normally. One animal in this group received the highest dose of PMS (5,000 I.U.) and also 2,500 I.U. HCG. She produced 14 corpora. Eight eggs were recovered and 5 had cleaved. This combination of PMS and HCG merits additional investigation in reference to its possibilities of producing superovulation.

The 5 pairs of animals in series II which were slaughtered and whose eggs were studied

TABLE III. Number of Unruptured Follicles and Corpora Lutea in Ovaries of Heifers and Cows in Series I and II Receiving USG or HCG in the Intravenous Injection. Figures are avg.

Series	Subcut. inj.	No. animal pairs' follicles studied	No. unruptured follicles >4 mm diam. USG	HCG	No. animal pairs' corpora studied	No. Corpora lutea USG	HCG
I	Sheep FSH	6	30.5	17.7	6	23	11.5
II	PMS	5	10.2	5.4	7	18.3	5.1
	Totals and avg	11	21.3	12.1	13	20.5	8.1

were killed on the 5th to 8th day following the intravenous injection. All had been inseminated at the time of the intravenous injection and the day following. Of the 31 eggs recovered from the PMS-USG treated animals, 77% had apparently cleaved normally. The one animal in this series (No. 28) which had extremely large ovaries had a total of 33 corpora. She was killed on the 7th day following the intravenous injection. Three of 4 eggs recovered had not cleaved. The fourth apparently had, but the blastomeres showed evidence of degeneration. Dowling(4) has found that, when animals were injected during the follicular stage of the cycle with PMS and when abnormal ovaries were produced, a small percentage of the ova were fertilized but abnormal in appearance.

The question has frequently been raised as to whether fertilized superovulated bovine eggs are capable of normal development. The American Foundation for the Study of Genetics has data indicating that at least some of them are. As of this writing, one living calf and 2 pregnancies (114 days and 150 days) confirmed by palpation have been produced from 5 transplantations. These transplantations have been performed with methods similar to those described in the report of the birth of the calf(19), including superovulation of the donor with sheep FSH and USG during the follicular stage of the cycle.

Summary. 1. Two series of studies involving a total of 26 heifers and cows are reported. 2. Five heifers superovulated during the luteal phase of the estrual cycle produced 34 eggs none of which were fertilized. Five heifers treated during the follicular phase produced 43 eggs of which 74% had cleaved. All 6 heifers treated during the luteal phase, but none of the 6 follicular heifers, had pyometra. 3. Thirty and 40 g.e. of sheep FSH gave responses about equal but considerably greater than a 20 g.e. dose. 4. USG produced approximately twice as many corpora as HCG in the intravenous injection when they were used in combination with either sheep pituitary FSH or PMS. 5. A combination of PMS and HCG may produce satisfactory superovulation and should be studied further.

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