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Fertility in Ewes Treated with Luteinizing Hormone-Releasing Factor[1] (38138)

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It is well established that progestagens, estrogens and the gonadotropins will affect either the time of ovulation or estrous behavior, or both, in many mammalian species. However, subsequent fertility is often lowered in females treated in this manner. Recently it has been reported that both crude and partially purified preparations of luteinizing hormone releasing factor (LRF) from hypothalami of domestic animals will induce the release of LH in rats (1), rams (2), anestrous ewes (3), and humans (4). Administration of a synthetic decapeptide corresponding to hypothalami LRF will cause serum LH levels to increase in rats (5–7), hamsters (8), prepubertal pigs (9), ewes (10–13), cows (14, 15), and bulls (16).

There is, however, a paucity of data on subsequent fertility when time of ovulation is controlled by the administration of an LH releasing substance. This paper reports the extension of our preliminary observations (12) on the subsequent rate of fertility in ewes given synthetic LRF.

Materials and Methods. Thirty mature ewes were checked twice daily for estrus. The first day of estrus is designated Day 0. Only ewes with two consecutive 15 to 17 day estrous cycles were used in the experiment. They were mated to rams of known fertility both in the morning and afternoon of the day following the administration of synthetic LRF or 12 hr after the onset of estrus, whichever occurred first. Blood from the jugular vein was obtained immediately before and 1.5 hr after LRF administration. Serum LH levels were measured by double antibody radioimmunoassay for ovine LH (17). Samples were assayed in duplicate, with the mean value reported. Values for LH are expressed in terms of the NIH-LH-S16 ovine LH reference preparation.

By mid-ventral laparotomy 72 hr after mating, the number of corpora lutea and the cleavage stage of recovered ova were determined. Fertilized ova were redeposited into the uterine horn adjacent to the corpus luteum, and all animals were subsequently examined for fetal viability 25–35 days after ova deposition.

Trial 1. In the initial study (September), 20 ewes were given 10 mg progesterone sc daily from Day 10 through 19 of the estrous cycle. Seven of these animals were given 50 μg synthtic LRF iv in acid saline on Day 23, 4 days after the last progresterone injection. An additional 6 of these animals were given a single intramuscular injection of 25 μg estradiol benzoate on Day 22 followed by 50 μg LRF on Day 23. The remaining seven animals received only progesterone.

Trial 2. In a subsequent study (November), 10 ewes were given 3 mg progesterone daily on Day 10 through 19 of the estrous cycle. LRF (50 μg) was administered iv on Day 21, 2 days after the last progesterone injection.

Synthesis of LRF. Synthetic LRF, <Glu-His-Trp-Ser-Tyr-Gly-Leu-Arg-Pro-Gly-NH_2, was prepared by the solid phase

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TABLE 1. Effect of Luteinizing Hormone-Releasing Factor (LRF) on Fertility.

Trial	Treatment	Number of animals			Number CL	Ova recovered	
		Treated	Estrus	Ovulated		Number	Fertilized (%)
1	P[a]	7	6	6	8	6	67
	P,LRF[b]	7	4	6	9	7	57
	P,E,LRF[c]	6	5	5	7	5	60
2	P,LRF[d]	10	3	9	11	5	20

[a] Progesterone (10 mg).
[b] Progesterone (10 mg) + LRF (50 μg).
[c] Progesterone (10 mg) + estradiol-benzoate (25 μg) + LRF (50 μg).
[d] Progesterone (3 mg) + LRF (50 μg).

methodology of Merrifield as described by Stewart and Young (18). Details of the method of synthesis and chemical characterization of the LRF used in this investigation has been reported elsewhere (5). Five ng of this material elicits a nine-fold increase in serum LH levels 15 min after iv administration to ovariectomized rats pretreated with estrogen and progesterone according to the method of Ramirez and McCann (19).

Results and Discussion. Trial 1. The number of corpora lutea and ova and the percent of fertilized ova were comparable in all groups (Table I). The addition of estradiol benzoate to the regimen did not significantly affect the percent of animals showing estrus. Although mean LH levels rose from < 6 ng/ml to 40 ng/ml 90 min after LRF administration, neither ovulation nor fertility was significantly affected. Four ewes which did not show estrus after LRF did not exhibit estrus in that cycle. The latter were forcemated 24 hr after LRF and laparotomized 72 hr later. Although three ewes had ovulated, none of the ova were fertilized and no sperm were found in either the zona pellucida or in the flushing medium. This suggested that the administration of LRF in the follicular phase of the estrous cycle, but before onset of estrus, resulted in the ovulation of follicles which had not matured sufficiently (precocious ovulation) to produce the estrogen needed for initiation of estrus and sperm transport. This is consistent with a report (20) implicating estrogen in the induction of uterine contractions toward the oviduct of ovariectomized ewes.

Nine of 13 ewes receiving LRF exhibited estrus; in 8 of these 9 ewes estrus occurred before the administration of LRF. There was no evidence of accessory ovulations at the time of laparotomy in these animals and 5 of 8 ova recovered were in the 8-cell stage of development. The embryos were replaced in the uterine horn and the pregnancy continued for at least 35 days. All fetuses were viable at this time, indicating that LRF administration did not affect fertilization and cell division even in animals which had exhibited estrus prior to its administration.

Trial 2. In the second study a smaller dose of progesterone was used to allow greater follicular development and, presumably, greater estrogen secretion. In addition, LRF was given 2 days earlier so that its action would be exerted before occurrence of a spontaneous surge of LH secretion. Nine of 10 ewes treated with LRF in this trial ovulated (Table I). Estrus was not observed in seven of the 10 ewes and all ova recovered from these 7 ewes were unfertilized. A fertilized ovum was recovered from one of three ewes which came into estrus between 12 and 24 hr after LRF. Laparotomy 30 days into the gestation period showed apparently normal fetal development. The administration of LRF resulted in a greater release of LH in this study than in Trial 1. Five of the 7 ewes not exhibiting estrus had a greater LH release after LRF than those in estrus before LRF, (Trial 1, 40 ng/ml), but a lower response than those coming into estrus after LRF (Trial 2, Table II). Elevated serum LH levels before LRF in sheep No. 9 and 17 and the exag-

TABLE II. Serum LH Levels after Injection of LRF in Trial 2.

Sheep No.	Estrus	Serum LH (ng/ml ± SE)[a] Before LRF	After LRF (1.5 hr)
37	None	< 6.0	69.4 ± 0.8
10	None	< 6.0	34.4 ± 0.2
26	None	Samples lost	
XII	None	Samples lost	
30	None	< 6.0[b]	96.2 ± 0.8
28	None	< 6.0	82.0 ± 1.3
33	None	< 6.0	60.5 ± 1.7
17	After LRF	13.3 ± 0.2	49.9 ± 0.5
9	After LRF	> 100	> 200
36	After LRF	< 6.0	> 200

[a] Expressed as NIH-LH-S16, means are of 2 analyses made on one blood sample.
[b] Determined on Day 20.

gerated response after LRF in sheep No. 36 indicate approach of spontaneous ovulation, consistent with the observation of estrus in these animals.

The present study verifies the observation that synthetic LRF can release sufficient LH to induce ovulation in the ewe. More significantly, it demonstrates that when estrus occurred close to the time of either induced or spontaneous ovulation, normal fertility was observed. However, when estrus did not occur, only unfertilized ova could be recovered. These data suggest that LRF, given at the appropriate time in the sheep estrous cycle, can cause precocious ovulation which prevents the preovulatory estrogen rise necessary for estrous behavior. Under these circumstances premature ovulation interferes with normal fertility. This finding is of obvious importance to any attempt to enhance fertility in sheep by administration of substances with gonadotropin releasing activity.

Summary. The effect of synthetic luteinizing hormone-releasing factor (LRF) on ovulation and fertility was investigated in the cyclic ewe. Twenty ewes were given 10 mg progesterone sc daily from Day 10 through Day 19 (estrus-Day 0) of the estrous cycle. Of these, 13 were also given 50 μg synthetic LRF iv on Day 23. Serum LH levels were measured by radioimmunoassay on blood samples taken on Days 22 and 23. Ewes which did not exhibit estrus were mated 24 hr after LRF; the rest were mated 12 hr after onset of estrus. Ova were examined for cleavage 72 hr after mating. In the control group, 6 of 7 ewes ovulated (86%) and 4 of 6 recovered ova (67%) were fertilized. Estrus occurred in nine of 13 ewes before or on the day of LRF administration. Eight of these ovulated (89%) with 5 of 8 ova fertilized (63%). Of the 4 ewes which did not exhibit estrus 3 ovulated (75%) but no ova were fertilized. Another 10 ewes were given 3 mg progesterone daily from Day 10 through Day 19 and 50 μg LRF on Day 21. They were mated 24 hr after LRF administration and ova were examined 72 hr later. Of 3 ewes exhibting estrus immediately after LRF, one had fertilized and 2 had unfertilized ova. Each of 7 ewes not showing estrus ovulated, but none of these ova were fertilized. All fertilized ova were replaced into the uterus and continued development to term. The data indicate that although ovulation can be induced by LRF, fertility of the released ova may be impaired if ovulation is induced prematurely.

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