

Fate of the Two Largest Follicles in the Ewe after Injection of Gonadotrophins at Two Stages of the Estrus Cycle (39682)

J. BHERER¹, P. MATTON¹, AND J. J. DUFOUR²

¹Department of Biology, University of Sherbrooke, Sherbrooke, Quebec, ²Research Station, Agriculture Canada, Lennoxville, Quebec, Canada

During the estrus cycle ovulation can be induced in the ewe by either removing the corpus luteum (CL) (1, 2) or by injecting exogenous gonadotrophins (3-5). In these experiments it has not been determined however, whether the follicles that ovulated were the largest follicles at the time of treatment or that ovulation resulted from the rapid growth of smaller follicles.

In a previous experiment, Bherer *et al.*, (6) reported that of the two largest follicles identified at Day 10 or Day 14 of the cycle in the ewe, only the largest follicle observed at Day 14 ovulated spontaneously at the next estrus. It was also observed that the removal of the CL when identifying the largest follicle at Day 10 of the cycle, did not at the ensuing estrus 2 days later, result in ovulation of the largest marked follicle. These findings were interpreted that the largest follicles present at mid-cycle were not responsive to the ovulating stimulus, or that this ovulating stimulus came too late or was not appropriate in bringing about ovulation of the largest follicle. It was also observed in these experiments that while the largest follicle marked on Day 14 lead to one ovulation, in cases of double ovulation, the second ovulation resulted from a smaller unmarked follicle.

The present experiment was designed to determine the ovulating capacity of the two largest follicles present at Day 10 or 14 of the estrus cycle with and without injection of exogenous gonadotrophins.

Materials and methods. Twenty crossbred ewes, 3 to 4 years of age, were checked daily for estrus (Day 0 of the cycle and of the experiment) with vasectomized rams and distributed at random to a 2 × 2 factorial experiment: the factors were (a) time of identification of the two largest follicles (Day 10 or day 14), and (b) type of injections (saline or saline and gonadotrophins).

For surgery, a general anesthetic (pento-

barbital sodium) was used. The ovaries were exposed through a mid-ventral laparotomy. The largest and the second largest (F₁ and F₂, respectively) follicles were marked by injecting "dots" of India ink with a small syringe around the periphery of the follicle, just beneath the ovarian tunic and locations and diameters of the two largest follicles were recorded. One hour after recovery from anesthesia, the ewes were injected intravenously with 1 ml of Vetrophin or with 1 ml of saline. Vetrophin (Abbot Laboratories) is a sheep anterior pituitary extract; according to the manufacturer, one ml has an hormonal activity equivalent to 1 ml LH (NIH-LH-S1) + 1 mg FSH (NIH-FSH-S1).

On Day 19 of the experiment (i.e., 2 to 3 days after the postoperative estrus) the animals were autopsied. The ovaries were examined and a marked follicle was considered to have ovulated if the luteal structure was completely outlined by dots of India ink. Data were analyzed by the χ^2 method.

Results and discussion. The results obtained in the present experiment confirm previous observations (6) that marking of the follicles in the peripheral stroma does not interfere with ovulation of the marked follicles. Similar observation has also been done in cow (7) and Hunter and Baker (8) have shown that scratching and marking of the porcine follicle without piercing the follicle itself had no effect on ovulation.

The results presented in Table I show that in the five ewes laparotomized at Day 10 of the cycle, none of the marked largest follicles (F₁ and F₂) ovulated (Group I), although the follicles appeared similar to those found on Day 14 of the cycle and had the same diameter (7.0 ± 0.2 mm vs 7.3 + 0.3 mm). However, when gonadotrophins were injected immediately after marking at Day 10, a significant number of ewes (4/5) ovulated their largest follicle (Group II).

Among the three F_2 follicles which ovulated after gonadotrophin injection, two were found in ewes in which the F_1 follicles had also ovulated.

When marked on the 14th day of the cycle, the largest follicle (F_1) ovulated in four out of five ewes which agrees with our previous findings (6). When double ovulation occurred, the second ovulation never resulted from the F_2 follicle. Injection of gonadotrophins on Day 14 (Group IV) did not change the proportion of F_1 ovulating (3/4) but allowed 50% of the F_2 to ovulate. These were found in ewes where the F_1 follicle had also ovulated. Ovulation of the F_2 follicle in Group IV appear to result from the effect of exogenous gonadotrophin since the difference in ovulation of F_2 follicle between Group III and IV is significant at the 0.07 level and because spontaneous ovulation of the F_2 follicle identified at day 14 was never encountered either in the present or previous experiment.

According to Hay and Moor (9), follicles are growing continuously and replacing each other during the cycle. The data of the present experiment thus suggest that the period of time during which the F_2 grew from a mean diameter of 4.9 mm to become the largest follicle (with a mean diameter of 7.3 mm) is shorter than 4 days. It seems also that the largest follicle seen at Day 14 can keep on growing slowly for at least 2 days before becoming a much larger preovulatory follicle on the day of estrus; Brand and de Jong (10) and Hutchinson and Robert-

son (11) have shown that there was very little change in the diameter of the largest follicle during the last days of the cycle.

While in another study (6) the largest follicles (F_1 and F_2) present at the 10th day of the cycle could not be ovulated by CL removal, the present experiment shows that these follicles can be ovulated by exogenous gonadotrophins. These two studies do suggest that the largest follicle (F_1) present at mid-cycle is not as responsive as the largest follicle present at the end of the cycle, but that it can be ovulated if properly stimulated and that exogenous gonadotrophins are a more appropriate stimulus than CL removal in making the largest follicle of day 10 ovulate.

The present results also agree with previous observations that F_2 follicles of the 14th day of the normal cycle do not ovulate spontaneously (Group III) and that the second ovulation in ewes having a spontaneous double ovulation came from smaller unmarked follicles.

This raises the question as to why F_2 follicles present on Day 14 do not ovulate spontaneously at the next estrus. The F_2 follicles at Day 14, as well as at Day 10, probably represent a mixed population: actively growing follicles on one hand and on the other hand former F_1 follicles which are undergoing atresia and are regressing. If some of the F_2 follicles are regressing, it is not surprising that they do not ovulate at the next estrus. However it is possible, as suggested by Hay and Moor (9), that in some of these follicles, atresia could be stopped and reversed by the gonadotrophin injection so that some of them would ovulate. However this does not explain why among the F_2 follicles present at day 14, those which are actively growing do not ovulate spontaneously and are replaced by smaller follicles. We have already advanced the hypothesis (6) that the F_1 follicle could exert an inhibiting influence on the F_2 follicle. Although the present experiment was not devised to prove this hypothesis, it seems to support the idea that the F_2 follicles of day 14 do not normally ovulate and that they require an extra stimulus to do so.

Summary. The response of the two largest follicles, identified at Day 10 or Day 14 of the estrous cycle in ewe, was determined by

TABLE I. FATE OF THE TWO LARGEST FOLLICLES IN EWES AFTER VETROPHINE INJECTIONS ON DAY 10 OR 14 OF THE ESTROUS CYCLE.

Group	Vetrophin (1 ml) ^a	Time of marking and of hormone injection	Ovulated follicle ^b		
			F_1	F_2	F_u
I (5) ^c	-	10	0/5	0/5	6
II (5)	+	10	4/5 ^d	3/5 ^d	4
III (5)	-	14	4/5 ^d	0/5 ^e	3
IV (4)	+	14	3/4	2/4	4

^a One milliliter was equivalent to 1 mg LH (NIH-LH-S1) + 1 mg FSH (NIH-FSH-S1).

^b F_1 , largest follicle; F_2 , second largest follicle; F_u , unmarked follicle.

^c Number of ewes per group.

^d Significantly different ($P < 0.05$) from corresponding value of Group I.

^e Significantly different ($P < 0.05$) from F_1 value.

injecting exogenous gonadotrophins. The largest follicle marked on the 10th day of the estrus cycle ovulated only when the ewes were given gonadotrophins (control 0/5; treated 4/5), while when marked on the 14th day, it did not require gonadotrophin injection to ovulate (control 4/5; treated 3/4). The second largest follicle marked on the 10th or 14th day ovulated in 60% and 50% of the treated ewes respectively, while nontreated ewes did not ovulate any of the second largest follicles at the ensuing estrus.

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