OVARIAN RESPONSE TO SYNTHETIC LH-RH IN ANOESTROUS EWES

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Synthetic LH-releasing hormone (LH-RH) has been shown to induce release of both LH and FSH from the ovine pituitary *in vivo* (Jonas & co-authors, 1973; Symons, Cunningham & Saba, 1974). When administered to seasonally anoestrous Clun Forest ewes as a single intravenous injection of either 150 μg or 300 μg , the induced gonadotrophin release was sufficient to cause ovulation in 23/27 ewes (Haresign, Foster, Haynes, Crighton & Lamming, 1975). Ovulation occurred within 48 hr of injection (W. Haresign, unpublished data). However, peripheral plasma progesterone levels did not rise above basal pre-injection values in most animals after the induction of ovulation. In view of this finding the present experiment was designed to investigate whether the structures visible on the surface of the ovary after injection of LH-RH were representative of histologically normal CL. Seasonally anoestrous Scottish Blackface ewes were used because at the time of the experiment Clun Forest ewes were already showing signs of spontaneous ovulation characteristic of the onset of the breeding season. The results demonstrated a marked difference in response to LH-RH between Clun Forest and Scottish Blackface ewes in that most of the latter failed to ovulate. In consequence, the initial aims of the experiment could not be realized. Whilst macroscopic examination of ovaries has allowed preliminary observations on the follicular development induced by LH-RH in Clun Forest ewes (Haresign *et al.*, 1975), no detailed study of follicular changes resulting from LH-RH administration is apparently available for any breed of sheep. The material obtained enabled such a study to be made.

Ewes were given either 150 μg LH-RH (four ewes), 300 μg (LH-RH) (four ewes) or saline (four ewes) as a single intravenous injection during mid- to late seasonal anoestrus. All ewes were slaughtered 84 hr later and their reproductive tracts removed. The ovaries and uterus were separated, trimmed free of suspensory ligaments, weighed and stored in formol saline for subsequent histological examination.

Each ovary was cut into slices 1 mm thick and all follicles with a mean diameter >1 mm were measured and counted. Any slice containing a follicle with a maximum mean diameter >3 mm was processed and embedded in paraffin wax. Representative 6 μm sections of these follicles were stained with haematoxylin and eosin and examined microscopically for signs of atresia. The middle portion of each uterine horn was processed, embedded in wax and representative 6 μm sections stained with haematoxylin and eosin.

Ovulation was induced in only one ewe, an animal which received 300 µg LH-RH. This differed markedly from the results found previously using Clun Forest ewes, 85% of which ovulated after LH-RH injection (Haresign *et al.*, 1975).

Table 1. The effect of LH-RH on numbers of follicles and the weight of the uterus in Scottish Blackface ewes

Treatment	No. of follicles		Uterine wt (g)
	Mean diam. < 4 mm*	Mean diam. > 4 mm†	
Saline control	32.5 ± 6.1	0.50 ± 0.29 ^a	31.8 ± 1.2
150 µg LH-RH	28.0 ± 6.6	2.50 ± 0.65 ^b	29.8 ± 1.3
300 µg LH-RH	21.0 ± 2.7	1.75 ± 0.25 ^b	32.1 ± 1.7

Values are Means ± S.E.M.

* None of the values was significantly different ($P > 0.05$).

† Values with a different letter were significantly different, $P < 0.05$.

Table 1 gives the mean number of follicles that had a mean diameter < 4 mm or > 4 mm for ewes in each of the three groups. There was no significant effect of LH-RH on the mean number of follicles < 4 mm in diameter, and considerable variation occurred between individual animals. Treatment with LH-RH significantly ($P < 0.05$) increased the number of follicles > 4 mm in diameter, but there was no significant effect of dose level. Histological examination of all follicles with a mean diameter > 3 mm confirmed that none showed signs of atresia.

Uterine weight (Table 1) was not significantly altered by LH-RH; moreover the histological appearance of the uterine horns of treated ewes was similar to that of the control ewes and typical of the anoestrous condition.

These results suggest that injection of LH-RH caused release of gonadotrophins sufficient to induce some degree of follicular development, thus providing indirect evidence for the FSH-releasing properties of synthetic LH-RH in the anoestrous ewe. Since synthetic LH-RH induces concurrent release of LH and FSH (Jonas *et al.*, 1973; Symons *et al.*, 1974), it was not known whether the follicular development resulting from LH-RH was due to FSH alone or synergism between LH and FSH.

The presence of follicles > 4 mm diameter in the ovaries of the control ewes confirms the findings of Hutchinson & Robertson (1966) who found follicles of similar size in Welsh Mountain ewes during seasonal anoestrus. No follicles in the control group were > 5.5 mm in diameter. In the ewes receiving LH-RH, there were only two follicles that exceeded 5.5 mm in diameter, one in a ewe given 150 µg (9.0 mm) and the other in a ewe given 300 µg (11.0 mm). These follicles were considered to be of a size sufficient to ovulate under normal conditions. Since no follicle > 5.5 mm in diameter was found in the ovaries of the control ewes or in the experiment reported by Hutchinson & Robertson (1966), the presence of the two large follicles is likely to be a result of the LH-RH injection.

In addition to the marked difference in the incidence of induced ovulation found in previous experiments using Clun Forest ewes and the present one using Scottish Blackface ewes, there was also a difference in the degree of follicular development between breeds in the ewes that did not ovulate after LH-RH injection. All four of the Clun Forest ewes in this category had at least one large follicle >8 mm mean diameter at laparotomy 3 to 4 days after treatment (Haresign *et al.*, 1975).

Hafez (1952) postulated that the 'depth' of seasonal anoestrus in sheep was greater for 'hill breeds' than for 'lowland breeds'. The breed differences observed here substantiate this hypothesis, although it is not apparent whether they are attributable to a difference in sensitivity of the pituitary and/or ovaries.

The lack of uterine development suggests that the follicles induced to grow as a result of LH-RH administration did not secrete oestrogen. It appears, therefore, that the degree of follicular response to injected LH-RH differs markedly between Clun Forest and Scottish Blackface ewes. However, the apparent lack of oestrogen production by responsive follicles, previously alluded to in Clun Forest ewes (Haresign *et al.*, 1975), is common to both breeds, and suggests that the follicular development induced differs from that found before ovulation.

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