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PITUITARY CONTROL OF THE OVINE CORPUS LUTEUM

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Summary. The functional activity of ovine CL was assessed by their weight, DNA and RNA content, and the concentration of progesterone in ovarian venous blood.

If sheep were hysterectomized on Days 9 to 12 of the oestrous cycle, the CL were maintained in a fully functional state until at least Day 60, but their activity had begun to decline by Day 128 to 135. When hysterectomized sheep were hypophysectomized, there was a significant decline in luteal activity within 48 hr, regardless of whether or not the pituitary stalk and pars tuberalis were left intact. The CL had almost completely stopped secreting progesterone within 4 days of hypophysectomy.

Hysterectomized, hypophysectomized animals were therefore used in a series of experiments to test the luteotrophic properties of sheep pituitary gonadotrophins. Doses of up to 5 mg FSH/day were unable to prevent complete luteal regression; similarly, doses of up to 5 mg LH/day were also without effect. When mixtures of FSH and LH were given, the results were no better. However, prolactin in doses of up to 1000 i.u./day was invariably able to maintain functional CL for 12 days, although at a considerably reduced level of activity. When prolactin was combined with a small dose of LH (0·25 mg/day), the CL were maintained at a level of activity comparable to that seen in hysterectomized animals before hypophysectomy. The slight synergistic action of FSH with prolactin was probably due to LH contamination. No further beneficial effects were obtained by adding FSH to the prolactin–LH mixture.

We conclude that prolactin and LH are both necessary for the maintenance of the ovine CL, and that these two hormones together make up the 'luteotrophic complex'. But whilst prolactin on its own has some luteotrophic activity, LH by itself is completely ineffective.

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INTRODUCTION

The life of the ovine CL can be prolonged by hysterectomy (Denamur, Martinet & Short, 1968; Moor, 1968; Anderson, Bland & Melampy, 1969; Rowson, 1970), but such a CL still requires pituitary support, since it undergoes complete structural and functional regression following hypophysectomy (Denamur & Mauléon, 1963; Denamur, Martinet & Short, 1966). If, however, the pituitary is merely disconnected from the hypothalamus by severing the pituitary stalk in a hysterectomized animal, the CL can continue to secrete progesterone for 1 or 2 weeks (Denamur *et al.*, 1966). Since the disconnected pituitary is secreting small amounts of prolactin (Bryant, Greenwood, Kann, Martinet & Denamur, 1971), this suggests that prolactin may have some part to play in the maintenance of the CL following hysterectomy.

In an attempt to obtain a more precise definition of the hormonal requirements for luteal maintenance, CL morphology and secretory activity were studied in hysterectomized, hypophysectomized animals treated with prolactin, FSH or LH, alone or as mixtures. The results were then compared with the luteal changes that follow hypophysectomy in the absence of any hormonal replacement therapy. Because there is a significant amount of pars tuberalis tissue present in sheep (Courrier, Colonge, Sakiz, Guillemin & Jutisz, 1963) which may influence the life of the cyclical CL (Denamur, 1968), and which may be able to secrete a small amount of LH (Goding, Catt, Brown, Kaltenbach, Cumming & Mole, 1969; Niswender, Reichert, Midgley & Nalbandov, 1969), some sheep were hypophysectomized by a technique that also allowed removal of the pituitary stalk and most of the pars tuberalis.

Brief accounts of this work have already been published (Denamur, 1968; Denamur & Short, 1972).

MATERIALS AND METHODS

Experimental animals and hormonal replacement therapy

Experiments were carried out on 148 sheep of the Préalpes du Sud breed at 12 to 14 months of age. Hysterectomies were performed on Days 9 to 12 of the cycle (day of oestrus = Day 0), and twenty-seven animals were killed 27, 60, 128 and 135 days after hysterectomy.

Luteal development was assessed by the fresh weight of the gland, its content of DNA and RNA, expressed as μg of phosphorus in the whole gland (Gaye & Denamur, 1969), and the concentration of progesterone in ovarian vein blood.

Hypophysectomies were carried out 20 to 50 days after hysterectomy. Two techniques were used; in some animals, only the pituitary was removed and, in others, most of the pars tuberalis tissue was also destroyed. These hysterectomized, hypophysectomized animals fell into two main groups.

(1) Thirty-seven hysterectomized sheep received only cortisol (Roussel, 2×10 mg/day intramuscularly) following hypophysectomy, and the CL were studied 24, 48, 72 and 96 hr and 12 days after the latter operation.

(2) Eighty-four hysterectomized sheep were given cortisol daily following hypophysectomy, as in Group 1, and they also received one of the hormones

or mixtures of hormones listed below, given either by intramuscular injection or by continuous intravenous infusion for 12 days: FSH, LH, FSH+LH, prolactin+FSH, prolactin+LH, prolactin+FSH+LH. The hormones were either supplied by the National Institutes of Health (NIH-FSH-S1; NIH-LH-S8; NIH-P-S6) or were prepared by Dr Jutisz (J-FSH, 2·75×NIH-FSH-S3; J-LH, 0·67+NIH-LH-S3) or by the Byla Co (B-prolactin, 20 i.u./mg). Some batches of prolactin were treated at 100° C, pH 7·0, for 30 min in order to destroy any LH contamination (Lyons, Li & Johnson, 1958). Solutions used for infusion were freshly prepared twice daily, and stored at +4° C in a room at 25° C where the hypophysectomized animals were housed. A Technicon 1 SS pump was used with a flow rate of 6 ml/hr for infusions into the jugular vein through indwelling polyethylene catheters (PE 100, Clay-Adams). These were inserted under anaesthesia at the time of hypophysectomy. Infusions were begun only when the animals had completely recovered from the anaesthetic, no more than 2 to 4 hr after hypophysectomy, and a priming dose of the hormone was always given by intramuscular injection at the time of the operation. The route of administration (intramuscular injection or infusion), dosage and time elapsed between hypophysectomy and the commencement of treatment are summarized in the Results section.

Surgical techniques

Hysterectomy and hypophysectomy were carried out by techniques already described (Denamur *et al.*, 1966; Denamur, 1968). In some animals, after removal of the greater part of the pars tuberalis, hypophysectomy was completed as follows: the sella turcica was swabbed with 2% phenol, and then the base of the brain was detached in front of the pituitary stalk. The dura mater was incised from the pituitary stalk towards the optic chiasma, and retracted laterally. The pituitary stalk was removed and then, with the aid of a fine sucker, the opening into the third ventricle was enlarged by removing all the adjacent pars tuberalis tissue.

Histological examination of the sella turcica, estimation of nucleic acids in the corpus luteum, and progesterone determination in ovarian vein blood

These were carried out according to the procedures described by Denamur, Martinet & Short (1966, 1968, 1970). Only those animals containing no histologically detectable pituitary remnants in the sella turcica have been included in the Results.

RESULTS

Regression of corpora lutea following hypophysectomy of hysterectomized ewes

Luteal function in animals hysterectomized on Days 9 *to* 12 *of the cycle.* The fresh weight of luteal tissue, DNA and RNA content and progesterone concentration in ovarian vein plasma are shown in Table 1.

Following hysterectomy, the weight of the CL remained constant until Day 60. There was a significant decrease in weight by Days 128 and 135. The DNA and RNA content also seemed to decline with time. The progesterone

concentration in ovarian vein blood shows that the CL maintained by hysterectomy were still secreting on Day 135.

Luteal function following hypophysectomy. In animals in which the pituitary stalk was intact, the weight of the CL started to decline within 6 hr of hypophysectomy, and there was a highly significant decline within 24 hr. Involution

Table 1. Luteal function of sheep at various time intervals after hysterectomy on Days 9 to 12 of the oestrous cycle

Days after oestrus	*Luteal wt (mg)*	*Progesterone (μg/100 ml plasma)*	*Luteal content of:* *DNA (μg P)*	*RNA (μg P)*
9 (at time of hysterectomy)	564		121	174
	574		135	209
	865		187	319
	567		115	138
	562		108	223
	650		139	230
	738		130	248
	$\bar{x}$ 646±44		134±10	220±22
27		153		
		115		
		204		
	612	108	136	131
	743	221	155	202
	734		167	165
	604		122	164
	$\bar{x}$ 673±38	160±23	145±10	166±15
60	722	0·5	182	182
	498	111	122	133
	651	120	156	159
	579	82	129	140
	364	76		
	427	95		
	$\bar{x}$ 540±56	81±17	147±14	154±11
128	424	293		
	414	73		
	489	84		
	513	96		
	$\bar{x}$ 460±24	137±52		
135	451	71	111	118
	485	22	104	114
	404	44	97	104
	252	355	86	51
	$\bar{x}$ 398±51	123±78	99±5	97±16

was particularly rapid in the first 3 days after the operation, but was somewhat slower between Days 3 and 12 (Table 2).

In contrast to these rapid weight changes, the DNA content was not significantly altered 24 hr after hypophysectomy. However, it fell rapidly between 24 and 72 hr so that on the 3rd day there was half the preoperative amount of DNA.

There was a significant decline in RNA content by 24 hr after the operation. After 72 hr, the CL had only a third as much RNA as the hysterectomized controls. Thus, the decline in RNA was greater and more rapid than that of DNA, which resulted in an ever decreasing RNA/DNA ratio. The progesterone

Table 2. Luteal function of hysterectomized sheep at various time intervals after hypophysectomy

Time after hypophysectomy (hr)	*Luteal wt (mg)*	*Progesterone (μg/100 ml plasma)*	*Luteal content of:* *DNA (μg P)*	*RNA (μg P)*
6	579		171	172
	529		131	117
	590		95	146
	509		164	135
	$\bar{x}$ 552±19		140±17	143±12
24	507	33	139	118
	488	78	149	99
	593	92	133	131
	462	52		
	$\bar{x}$ 519±28	64±13	140± 5	116± 9
48	448	17	123	92
	292	13	83	51
	315	13	81	66
	416	109	123	95
	363	21	131	92
	$\bar{x}$ 367±29	35±19	108±11	79±9
72	277	0·3	90	62
	259	11	82	49
	229	0·6	71	45
	217	21	66	45
	$\bar{x}$ 246±14	8·23 ±4·93	77± 6	50± 4
96	266	1·1	72	52
	244	0·8	70	48
	265	0·5	78	50
	205	1·5	68	34
	$\bar{x}$ 245±14	0·97 ±0·21	72± 2	46± 4
12 days	72			
	59			
	46			
	72			
	$\bar{x}$ 62± 6			

Hysterectomy was performed on Days 9 to 12 of the cycle, hypophysectomy 20 to 50 days later. The pituitary stalk and pars tuberalis were not removed.

concentration in ovarian vein blood declined significantly within 24 hr. By 72 hr, the values had fallen to 5 to 7% of the controls.

In sheep in which the pituitary stalk and pars tuberalis tissue were also removed, the decline in luteal weight, RNA and DNA content and progesterone

concentration was slightly more rapid than after simple hypophysectomy, although the difference was not significant (Table 3).

Endocrine control of corpora lutea maintained by hysterectomy

Since the CL had regressed completely 12 days after hypophysectomy of ewes which had previously been hysterectomized, this time interval seemed appropriate for studying the possible luteotrophic effects of FSH, LH and prolactin.

Influence of FSH. The following experimental design was used:

A. Intramuscular injection of 5 mg NIH-FSH/day/12 days, divided into four injections, into hysterectomized, hypophysectomized animals with the

Table 3. Luteal function of hysterectomized sheep at various time intervals after hypophysectomy

Time after hypophysectomy (hr)	*Luteal wt (mg)*	*Progesterone (μg/100 ml plasma)*	*Luteal content of:*	
			DNA (μg P)	*RNA (μg P)*
6	440			
	700		134	135
	472		135	145
	491		89	95
	$\bar{x}$ 526±59		119	125
24	379		116	99
	434		116	112
	406		121	96
	508		151	117
	504		128	99
	$\bar{x}$ 446±26		126± 7	105± 4
48	344	22	119	89
	314	47	108	84
	305	15	109	99
	313	39	129	68
	$\bar{x}$ 319± 9	31± 7	116± 5	85± 6
96	159	2	51	20
	255	1	96	38
	179	2		22
	243	3	93	62
	$\bar{x}$ 209±24	2±0·4	80±15	36±10

Hysterectomy was performed on Days 9 to 12 of the cycle, and hypophysectomy and removal of the pituitary stalk and pars tuberalis 20 to 50 days later.

pituitary stalk present; and 1 mg J-FSH/day/12 days, divided into four injections, into hysterectomized, hypophysectomized animals in which the pituitary stalk and pars tuberalis had been removed. The first injection was given at the time of hypophysectomy.

B. Continuous intravenous infusion of 1 mg J-FSH/day/12 days into hysterectomized, hypophysectomized animals with the pituitary stalk removed. In this experiment, the animals also received 0·5 mg FSH intramuscularly at the time of the hypophysectomy.

The results are shown in Table 4, and demonstrate quite clearly that, regardless of the method of administration or the dosage, the CL had regressed completely 12 days after hypophysectomy. Because of the involuted state of the CL, it was not possible to estimate the nucleic acid content.

Influence of LH. This hormone was administered for 12 days to hysterectomized, hypophysectomized animals, either intramuscularly, 5 mg NIH-LH/day, divided into four injections, or by continuous intravenous infusion (0·25, 1·0 or 2·5 mg J-LH/day). The sheep in which the pituitary stalk and the pars tuberalis were also removed during hypophysectomy were given 0·5 mg J-LH/day by infusion. An intramuscular injection of LH equivalent to half the daily infused dose was given at the time of hypophysectomy to all the animals on the infusion.

Table 4. Luteal weights of hysterectomized, hypophysectomized sheep treated with FSH for 12 days

Treatment	*Luteal wt (mg)*
5 mg NIH-FSH/day i.m., pituitary stalk intact	97, regressed, 49
1 mg J-FSH/day i.m., pituitary stalk removed	30, 50
1 mg J-FSH/day i.v. infusion, pituitary stalk removed	42, 52

i.m. = intramuscularly, i.v. = intravenous.

Table 5. Luteal weights of hysterectomized, hypophysectomized sheep treated with LH for 12 days

Treatment	*Luteal wt (mg)*
5 mg NIH-LH/day i.m., pituitary stalk intact	39, 43, regressed, 42
0·25 mg J-LH/day i.v. infusion, pituitary stalk intact	51, 74, 126, 49
1 mg J-LH/day i.v. infusion, pituitary stalk intact	45, 74
2·5 mg J-LH/day i.v. infusion, pituitary stalk intact	46, 84, 78, 64, 154
0·5 mg J-LH/day i.v. infusion, pituitary stalk removed	51, 43, 75

i.m. = intramuscularly, i.v. = intravenous.

The results in Table 5 show that by 12 days after the operation the CL had regressed. Progesterone concentrations were always negligible, ranging from 0·3 to 3·0 μg/100 ml plasma. Because of the regression, it was not possible to estimate luteal nucleic acid content.

Influence of FSH+LH. The FSH-LH mixture was given by intravenous infusion at a dosage rate of 0·5 mg J-FSH+0·25 mg J-LH/day/12 days. Half the daily dose was given by intramuscular injection at the time of hypophy-

sectomy, and the pituitary stalk and pars tuberalis tissue were present in all the animals. The results (see Table 6) of injecting this gonadotrophin mixture were no better than those obtained by injecting the two hormones separately, and the CL were regressed 12 days after hypophysectomy.

The same gonadotrophin mixture injected intramuscularly four times a day at twice the dosage rate into hypophysectomized ewes in which both the pituitary stalk and pars tuberalis tissue were removed, did not allow maintenance of the CL.

Influence of prolactin on hysterectomized, hypophysectomized animals with the pituitary stalk and pars tuberalis intact. The effect of administration of prolactin (500 i.u. and 1000 i.u./day/12 days), divided between four daily intramuscular injections, on the fate of the CL was studied (see Table 7). In addition, two animals received an infusion into the jugular vein for 12 days of a prolactin solution at a dosage rate of 500 i.u./day.

Table 6. Luteal weights of hysterectomized, hypophysectomized sheep treated with FSH + LH for 12 days

Treatment	*Luteal wt (mg)*
1 mg J-FSH/day + 0·5 mg J-LH/day i.m., pituitary stalk removed	66, 78, regressed, regressed
0·5 mg J-FSH/day + 0·25 mg J-LH/day i.v. infusion, pituitary stalk intact	48, 92, 97, 97

i.m. = intramuscularly, i.v. = intravenous.

Although the CL were maintained, luteal weight at both doses of prolactin was definitely less than in the hysterectomized controls. The DNA content of the CL was also less than that in the hysterectomized controls, even after the administration of 1000 i.u. prolactin. The RNA content was also lower, whatever the dose of prolactin.

The luteal weight in the two animals infused with 500 i.u./day did not differ from that in the animals receiving injections (350, 352 mg).

The progesterone concentrations in ovarian vein blood of animals given 500 i.u. prolactin/day were lower than in the hysterectomized controls.

Influence of prolactin on hysterectomized, hypophysectomized animals with the pituitary stalk and pars tuberalis removed. The weight of the CL in animals treated with 500 i.u. prolactin/day/12 days was a little less than in the hypophysectomized animals with the pituitary stalk and pars tuberalis left intact, which were nevertheless receiving the same dose of hormone (Table 7). It was about 40% of the weight of the hypophysectomized controls, regardless of whether the prolactin was used before or after heat treatment. The quantities of DNA and RNA were diminished, and the RNA/DNA ratio was less than 1.

Doses of 250 i.u. prolactin/day divided into four injections also maintained the CL, but a dosage of 50 i.u./day was insufficient.

Effect of prolactin+FSH. Prolactin was given, as in the previous group, in

Table 7. Luteal function of hysterectomized, hypophysectomized sheep treated with prolactin

Treatment	*Luteal wt (mg)*	*Progesterone (μg/100 ml)*	*Luteal content of:* *DNA (μg P)*	*RNA (μg P)*
1000 i.u. B-prolactin/day, pituitary stalk intact	377		104	95
	206		63	59
	528		129	137
	470		130	135
	$\bar{x}$ 395 ± 70		107 ± 16	107 ± 19
500 i.u. NIH-prolactin/day, pituitary stalk intact	436	41		79
	373	125		110
	341	25		108
	$\bar{x}$ 383	64		99
500 i.u. B-prolactin/day, pituitary stalk removed	184	67	70	53
	295	370	97	94
	368	638	112	102
	318	70	107	88
	289	66	82	72
	419		149	138
	355		165	122
	320		122	120
	$\bar{x}$ 318 ± 24	242 ± 115	113 ± 11	99 ± 10
500 i.u. heated B-prolactin/day, pituitary stalk removed	337	99	105	67
	303	26	96	80
	232	66	74	59
	$\bar{x}$ 291	64	92	69
250 i.u. B-prolactin/day, pituitary stalk removed	360			
	475			
	464			
	281			
	$\bar{x}$ 395 ± 46			
250 i.u. B-prolactin/day, heated, pituitary stalk removed	327			
	232			
	298			
	$\bar{x}$ 286			
50 i.u. B-prolactin/day, heated, pituitary stalk removed	183			
	101			
	122			
	$\bar{x}$ 135			

Prolactin given as four intramuscular injections/day for 12 days.

the presence of the pituitary stalk and pars tuberalis, in a dose of 500 i.u./day/12 days divided between four intramuscular injections. The J-FSH was given by intravenous infusion (0·5 mg/day/12 days).

The weights of the CL were slightly increased by the addition of FSH to the prolactin (Table 8), and the same was also true of the RNA content. The progesterone concentrations in the ovarian vein were similar to the values in the previous group.

Table 8. Luteal function of hysterectomized, hypophysectomized sheep treated with prolactin+FSH

Treatment	*Luteal wt (mg)*	*Progesterone (μg/100 ml plasma)*	*Luteal content of:*	
			DNA (μg P)	*RNA (μg P)*
500 i.u. B-prolactin/day +0·5 mg J-FSH/day, pituitary stalk intact	349	218	85	72
	548		117	176
	411	119	106	132
	537	76	133	174
	$\bar{x}$ 461±49	138	110±10	139±24

Prolactin was given as four intramuscular injections/day with an intravenous infusion of FSH for 12 days.

Table 9. Luteal function of hysterectomized, hypophysectomized sheep treated with prolactin+LH

Treatment	*Luteal wt (mg)*	*Progesterone (μg/100 ml plasma)*	*Luteal content of:*	
			DNA (μg P)	*RNA (μg P)*
(1) 500 i.u. B-prolactin/day +0·25 mg J-LH/day, pituitary stalk intact	536	155	127	156
	940	188	225	281
	548		122	139
	846	246	182	233
	$\bar{x}$ 717±103	196	178±28	224±36
(2) 500 i.u. B-prolactin/day +0·5 mg J-LH/day, pituary stalk removed	774	540		
	754	836		
	725	539		
	$\bar{x}$ 751	638		

Prolactin was given as four injections/day with an intravenous infusion of LH for 12 days.

In another experiment, two animals were injected with 2·5 mg NIH-FSH in four daily intramuscular injections, together with 500 i.u. NIH-prolactin, and although luteal weight and DNA and RNA content were identical to the values obtained after infusing 0·5 mg FSH, the progesterone concentrations were high (245 and 269 μg%), possibly due to LH contamination of the FSH (see next Section below).

Effect of prolactin+LH on hysterectomized, hypophysectomized animals. A dose of

500 i.u. prolactin/day divided between four intramuscular injections was given at the same time as an intravenous infusion of 0·25 mg J-LH (see Table 9). These gonadotrophin mixtures, given for 12 days, maintained the weights of the CL (717±103 mg) and the content of DNA (178±28 μg) at values similar to those in the control hysterectomized group. They increased the luteal content of RNA (224±36 μg), and there were high concentrations of progesterone (196 μg) in ovarian vein blood (Table 9).

In another experiment in which four daily intramuscular injections of B-prolactin and NIH-LH were given (four animals), in a total dose of 500 i.u. and 0·25 mg/day, respectively, comparable results were obtained (luteal weight 665±31 mg).

Effect of prolactin and LH on hysterectomized, hypophysectomized ewes with pituitary stalk and pars tuberalis removed. B-prolactin (500 i.u./day) was given as four daily intramuscular injections, and LH (0·5 mg/day) was given by intravenous infusion for 12 days following hypophysectomy. The weight of the CL was maintained at 751 mg, and the concentration of progesterone remained very high.

Table 10. Luteal function of hysterectomized, hypophysectomized sheep treated with prolactin+FSH and LH

Treatment	*Luteal wt (mg)*	*Progesterone (μg/100 ml plasma)*	*Luteal content of:* *DNA (μg P)*	*RNA (μg P)*
500 i.u. B-prolactin/day	934	305	176	269
+0·5 mg J-FSH/day	576	271		143
+0·25 mg J-LH/day,	638	303	137	213
pituitary stalk intact	389	45	112	118
	x̄ 634±113	231±63	142	186±34

Prolactin was given as four injections/day with an intravenous infusion of FSH and LH for 12 days.

Effect of prolactin+FSH+LH. B-prolactin was injected four times a day in total doses of 500 i.u./day; J-LH (0·25 mg/day) and J-FSH (0·5 mg/day) were given by intravenous infusion to hysterectomized, hypophysectomized animals with the pituitary stalk and pars tuberalis intact.

The weight of the CL, the content of DNA and RNA, the RNA/DNA ratio and the progesterone concentrations were comparable to the values in the hysterectomized controls, and there did not seem to be any beneficial effects from adding FSH to the prolactin-LH mixtures (Table 10).

DISCUSSION

The change in weight of the CL in the 130 days following hysterectomy was similar to that reported previously by Kiracofe & Spies (1966). The luteal content of RNA gradually declined with increasing time intervals after hysterectomy, and so did the progesterone concentration in the ovarian vein. The effects of hypophysectomy on CL maintained by hysterectomy were

extremely rapid; within 24 hr, luteal weight, RNA content and progesterone concentration declined significantly, but the DNA content was still unaffected. Thus, functional regression occurred rapidly and was soon complete; structural regression also consisted of an extremely rapid phase during the first 3 days following hypophysectomy, and was followed by a second, slower stage from the 3rd to the 12th postoperative day. These results, therefore, confirm and extend earlier observations on the histology of the CL (Denamur & Mauléon, 1963) and the progesterone concentration in the ovarian vein (Denamur *et al.*, 1966).

Of the three hormones investigated, FSH administered by either route alone never showed any luteotrophic effect, even in the presence of pars tuberalis tissue. Doses of 0·5 and 1 mg FSH did not produce any follicular development.

By itself, LH had no luteotrophic effects in hysterectomized, hypophysectomized ewes, regardless of the route of administration. In every case, an intramuscular injection of LH was given before complete removal of the pituitary.

Certain doses of LH used in these experiments approximate closely to the daily secretion of the hormone during the oestrous cycle and pregnancy. The concentration of LH in the peripheral blood of sheep has been measured by radioimmunoassay (Geschwind & Dewey, 1968; Niswender, Roche, Foster & Midgley, 1968; Pelletier, Kann, Dolais & Rosselin, 1968; Goding *et al.*, 1969; Wheatley & Radford, 1969; Reeves, Arimura & Schally, 1970; Scaramuzzi, Caldwell & Moor, 1970; Kann, 1971). It is extremely low, except for a pronounced preovulatory peak, and corresponds to a daily secretion during the cycle and pregnancy of about 0·4 to 0·5 mg LH (NIH-S1) (Geschwind & Dewey, 1968). The absence of any luteotrophic effects observed in this study with 0·25, 0·5 and even 1 mg LH/day, shows that this hormone cannot be held solely responsible for the maintenance of the CL following hysterectomy. Even larger quantities (2·5 mg/day) are still without effect. Kaltenbach, Graber, Niswender & Nalbandov (1968) and Hixon & Clegg (1969) have claimed that LH is capable of maintaining progesterone secretion by the CL in sheep hypophysectomized during the cycle or in pregnancy. However, the dosages of LH that they used were unphysiological (2·5 to 50 mg/day) and in the work of Kaltenbach *et al.* (1968), 'pure' NIH-LH was ineffective, and only 'crude' preparations worked.

In striking contrast to FSH or LH, prolactin injected on its own (250 to 1000 i.u./day) maintained the CL for 12 days after hypophysectomy, even though the weight was only about 40% of the control values, and the RNA content and progesterone concentration were similarly diminished. There were no differences between intravenous infusion or intramuscular injection of prolactin (500 i.u./day), if injections were given four times a day. It was immaterial whether or not the pars tuberalis was present.

Large doses of prolactin (250 to 500 i.u./day) were still luteotrophic after boiling, a procedure which would destroy any contaminating LH or growth hormone. However, 50 i.u. prolactin/day was unable to maintain the CL, and yet this amount will give a physiological blood level of prolactin at least for a short time when injected into hypophysectomized sheep (G. Kann & R. Denamur, unpublished data).

It is interesting to note the comparable effects of prolactin therapy and pituitary stalk section. The latter operation did not allow the CL to survive in hysterectomized animals for as long as 24 days (Denamur *et al.*, 1966), probably because the amount of pituitary tissue surviving after stalk section is so small that prolactin secretion is diminished to the point where it is no longer adequate to maintain the CL (Bryant *et al.*, 1971). The fact that, in large quantities, prolactin is partially luteotrophic in hysterectomized, hypophysectomized sheep, but is ineffective if the uterus is present (Denamur & Mauléon, 1963; Karsch, Cook, Ellicott, Foster, Jackson & Nalbandov, 1971), shows that prolactin can only exhibit its luteotrophic effects if the uterus is no longer capable of producing a luteolytic stimulus.

Any gonadotrophic combination which included prolactin was luteotrophic. The addition of only 0·25 to 0·5 mg LH/day produced CL which were twice as heavy as those maintained by prolactin alone, and their RNA content and the progesterone concentrations in ovarian vein blood were comparable to those of control animals. The addition of FSH to prolactin, or to a prolactin+LH mixture, did not produce any obvious changes in the CL.

Prolactin and LH must, therefore, be looked upon as incomplete luteotrophic hormones, and the ovine CL is probably controlled by a luteotrophic complex of prolactin and LH. A low, tonic secretion of LH is probably important for luteal maintenance, since the administration of an LH antiserum to sheep brings about a decline in progesterone secretion (Dermody & Foote, 1969; Fuller & Hansel, 1970; McCracken, Baird & Goding, 1971), and physiological doses of prolactin alone (50 i.u./day) cannot maintain the CL in hypophysectomized animals. But, whilst some degree of luteal function is preserved by treating hysterectomized, hypophysectomized sheep with large doses of prolactin alone, no such luteotrophic effects can be produced by LH alone.

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