The Effect of Estradiol-17β Infusion on the Maintenance of the Induced Corpus Luteum in Cyclic Ewes

A. M. SHAREHA[1] AND R. J. FITZPATRICK[2]

ABSTRACT

Fourteen Scottish black-face ewes were injected with 100 μg of prostaglandin analogue (PGF-an) during the luteal phase (days 6–12 of the cycle). The animals were divided at random into 3 groups. Groups 1, 2 and 3 were infused for 8 hours with saline, 3 μg/h. Gonadotrophin releasing hormone (GN-RH) and 3 μg/h. E_2-17β + 3 μg/h. Gn-RH, respectively, starting 18 hr following PGF-an treatment. Blood samples for hormonal analysis were taken before and at 5, 10, 20, 30, 45, 60, 90, 120 minutes then at 2 hr for 36 hr following the start of infusion.

The mean pretreatment plasma levels of progesterone, estrogen and LH were 2.8 $\pm$ 1.5 ng/ml, 12.2 $\pm$ 1.2 pg/ml and 1.5 $\pm$ 0.25 ng/ml, respectively. Eighteen hours following PGF-an treatment, plasma progesterone concentration was significantly ($P < 0.01$) declined (0.43 $\pm$ 0.5 ng/ml). Administration of estrodiol-17β prior to Gn-RH (G-III) increased the sensitivity of the pituitary as indicated by peak of LH concentration 67.6 $\pm$ 36.5 and 134.0 $\pm$ 26.1 ng/ml for G-II and G-III, respectively.

All induced corpora lutea (CL) in animals of group 3 were maintained over a 10 day period as compared to none in animals of group 2. Thus the high level of estrogen prior to ovulation may be necessary to maintain the CL in cycling ewes.

INTRODUCTION

The administration of estradiol-17β (E_2-17β) to the ewe during the luteal phase of estrous cycle induces premature regression of the corpus luteum (CL) (3). However when it is given early in the estrous cycle (days 3 and 4) it acts in a trophic manner (3). Luteinising hormone (LH) has been suggested as a luteotrophin in the ewe and cow (6).

There is no good evidence that prolactin is luteotrophic in sheep although Kann and Denemur (8) reported that the administration of prolactin alone to the hypophysectomised hysterectomised ewes may permit some luteal activity to be maintained. LH and/or estrogen administered early or late during the cycle may not be luteotrophic unless administered as prolonged infusion or in Freund adjuvant and for a long period (1).

The induced CL following of Gonadotrophin releasing hormone (Gn-RH) infusion at different times of the year did not release progesterone (10) and was not checked visually after the first endoscopy. The objective of this work was to study factors

[1]*Department of Animal Production, Faculty of Agriculture, University of Al-Fateh, Tripoli, S.P.L.A.J.*

[2]*Department of V.C.S., Liverpool University, Leahurst, Neston, Wirral, Cheshire L64-7TE.*

affecting maintenance of induced CL following Gn-RH infusion; and compare those in sheep primed with E_2-17β infusion with those in sheep not so primed.

MATERIALS AND METHODS

Fourteen cyclical Scottish black-face ewes were run with a vasectomised ram, fitted with a marking harness, and fed twice daily during the experimental period with hay and concentrates. The animals were observed every 4 hr for estrous behaviour for at least 2 cycles. All ewes received a single intramuscular injection of 100 μg of prostaglandin analogue (PGF-an) (ICI) in 0.5 ml saline during the luteal phase (days 6–12) of the estrous cycle to induce premature regression of the cyclic corpus luteum. The animals were divided at random into 3 groups and subsequently treated as follows: Gp. 1, 4 ewes were infused in carotid artery with saline for an 8 hour period, starting 18 hours after PGF-an injection; Gp. 2, 5 ewes were infused with 3 μg/hr Gn-RH for 8 hours commencing 18 hours after PGF-an; and in Gp. 3, 5 ewes received an infusion of 3 μg/hr for 4 hours of E_2-17β also commencing 18 hours after PGF-an and followed immediately with a Gn-RH infusion as in Gp. 2 Blood samples were collected through an indwelling polyethylene catheter in the jugular vein. Samples for hormonal analysis were taken before and at 5, 10, 20, 30, 45, 60, 90 and 120 minutes, then at 2 hr for 36 hrs following the start of infusion. In addition plasma samples for progesterone determination were taken once a day for one or two estrous cycle after the start of infusion.

Total estrogens were measured by a radioimmunoassay (RIA) procedure similar to that of Hotchkiss, *et al.* (7). Diethyl ether was used to extract total estrogens from plasma samples (2.0–4.0 ml). The cross reactivity of the total estrogens antiserum was 80% and 10% for estriol while estradiol-17β was 100%.

Progesterone was measured by RIA similar to that described by Shareha *et al.* (11). A double-antibody technique was used to separate free from bound moieties. Plasma samples (0.05–0.5 ml) were extracted once with 2.0 ml Hexane. The cross-reactivity of the progesterone antiserum which was raised in rabbit against a conjugate of 17β-hydroxyprogesterone with bovine serum albumin was from 1.0 to 1.3% with 21-hydroxyprogesterone, 17β-hydroxyprogesterone and 11-dioxycorticosterone, and less 1% with 20α and 20β-dihydroprogesterone. The LH assay was carried out as described by Scaramuzzi, *et al.* (10). The antiserum to ovine LH was raised in rabbit, in this laboratory, and used at a dilution of 1:50,000 which bound 48% of the iodinated hormone. The limit of sensitivity of the assay was 0.7 ng/ml. The correlation coefficient between LH added and that measured was +0.999.

The first endoscopy was carried out at 45 hr after the end of Gn-RH infusion and was followed by a second endoscopy 10 days later. All induced corpora lutea were marked with Indian ink at first endoscopy.

A t-test, for comparison of treatments, and Duncan's new multiple range test, for comparison of treatment means as discussed by Snedecor and Cochran (14), were used to analyze this data.

RESULTS AND DISCUSSION

The PGF-an is luteolytic in cattle when given in low dose by intramuscular injection (4). In this study the injection of 100 μg of PGF-an resulted in regression of ovine CL as shown by the decline in progesterone concentrations in all ewes within 20 hr of treatments (Table 1 and Figs 1–3).

In all the animals ovulating, plasma progesterone concentrations started to rise again at 3–6 days following the infusion. Animals in Gp. 1 showed estrus 2–3 days after the start of infusion. In general, the duration and magnitude of hormonal pattern

Table 1 Plasma Progesterone Concentration (ng/ml) in Normal Cyclic Ewes Treated PGF-an, then Infused with Saline, Gn-RH and E_2-17β + Gn-RH. (Mean ± S.D.)

Sampling time in days	Treatment		
	Saline	Gn-RH	E_2-17β + Gn-RH
−2	2.24 ± 0.5	2.34 ± 1.03	2.65 ± 1.2
*−1	1.96 ± 0.8	2.47 ± 1.16	2.84 ± 1.5
O	0.36 ± 0.1	0.33 ± 0.12	0.46 ± 0.19
+1	0.27 ± 0.04	0.24 ± 0.09	0.24 ± 0.04
7	1.24 ± 0.5	0.95 ± 0.4	0.73 ± 0.28
8	1.34 ± 0.4	1.11 ± 0.59	0.99 ± 0.31
13	3.2 ± 0.7	3.74 ± 1.7	3.63 ± 1.9
18	1.5 ± 1.1	0.92 ± 0.42	1.4 ± 1.3
20	0.2 ± 0.1	0.36 ± 0.19	0.41 ± 0.3

* = Day of PGF-an injection (18 hrs before infusion).
O = Start of infusion of saline, Gn-RH and E_2-17β.

and estrus behaviour in animals treated with saline were comparable to those occurring during normal estrous cycle (Fig. 1, Tables 1 and 2).

The pretreatment values of LH concentrations in all groups ranged from 1.5 ± 0.2 to 1.8 ± 0.24 ng/ml. The concentrations began to increase just after the start of Gn-RH infusion reaching a mean peak value of 67.6 ± 36.5 ng/ml, and 134.0 ± 26.1 ng/ml in Group 2 and 3, respectively, 2–4 hr later (Table 2). Plasma LH

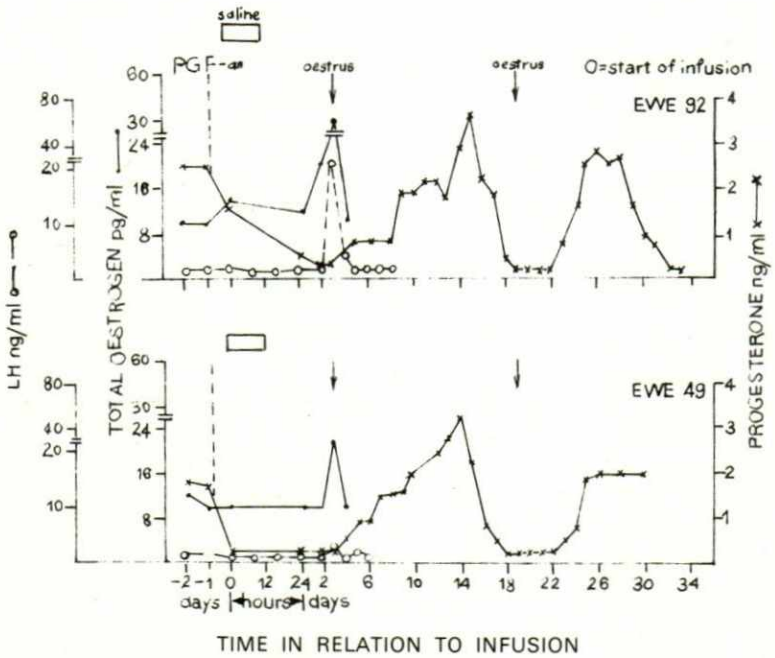

Fig. 1. Progesteron, total estrogen and LH concentration in jugular plasma of two ewes in group 1 before, during, and after PGF-an (↓) and saline (□) treatment during estrous cycle.

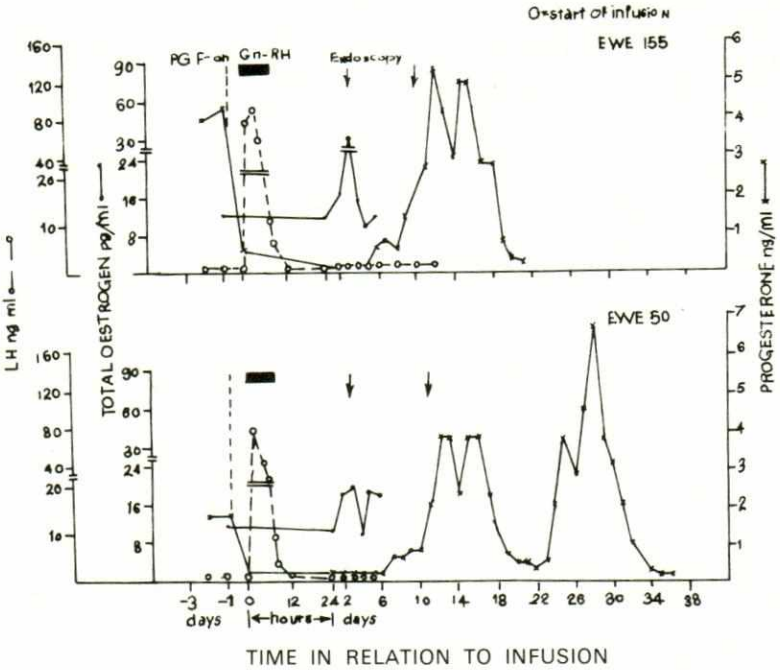

Fig. 2. The jugular plasma concentration of progesterone, total oestrogen and LH in 2 ewes of group 2 treated with PGF-an (↓) + Gn-RH (▨) during oestrous cycle.

concentrations returned to the pretreatment values by the end of infusion period (Figs 2 and 3). Infusion of E_2-17β prior to Gn-RH administration (Gp. 3) significantly ($P < 0.01$) increased the sensitivity of the pituitary as shown by the level of LH peak (Table 2). This is in agreement with a previous report (13) when reported that the administration of E_2-17β to anestrous ewes increased the sensitivity of the pituitary to Gn-RH. Moreover the first endoscopy showed that all ewes in Gp. 1, 3 of 5 in Gp. 2 and 4 of 5 in Gp. 3 ovulated in this experiment as a result of treatment in contrast to none of the anestrous ewes. This suggests the idea that seasonal anestrous is not due to a difference in the sensitivity of the hypothalamus and/or pituitary. Moreover, the response of the ovary (ovulation rate) to a given dose of Gn-RH was greater during the breeding season than the response during anestrous (11).

The 2 ewes in Gp. 2 and one ewe in Gp. 3 which did not ovulate may be due to the absence of large active follicules at the time of infusion However, the level of progesterone was low (0.31 ng/ml) prior to treatment with ether Gn-RH or E_2-17β in those ewes (Table 1).

All the induced corpora lutea in Gp. 2 regressed a few days after treatment (Fig. 4) and this is in agreement with Haresing *et al.* (5) and Shareha (11). The peripheral plasma progesterone concentrations in this experiment (Gp. 2) were not altered during a six days period following infusion with Gn-RH (Table 1). These results are in agreement with those obtained by Symons *et al.* (15) who reported no changes in the base line of progesterone concentration for 6 days after injection of Gn-RH at estrus. However, in the work of Symons *et al.* (15) endoscopy was not carried out and ovulation was assumed to have occurred based on the detection of high concentrations of LH. Despite this similarity with the normal ewes, the length of the cycle

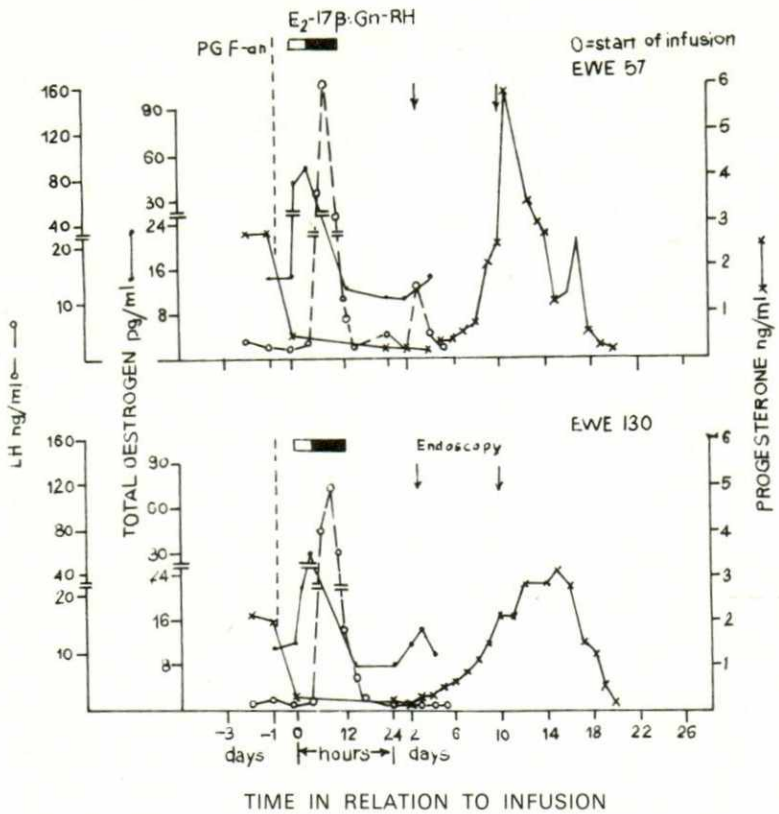

Fig. 3. The jugular plasma concentration of progesterone, total estrogen and LH in 2 ewes of group 3 treated with PGF-an (↓) + $E_2 17\beta$ + Gn-RH (□■) during breeding season.

after treatment in ewes in groups 2 and 3 was longer ($P < 0.2$) than the normal cycle period for the same ewes.

When ewes were treated with E_2-17β prior to treatment with Gn-RH, the induced corpora lutea were maintained in all the animals, even when ovulation occurred again within 10 days (Fig. 5). There is no significant difference in the magnitude or duration of the rise in progesterone concentration in those animals with multiple corpora lutea as compared to the control animals or to the next cycle in the same animal. These results are in agreement with those obtained by Lamond *et al.* (9) and Shareha (11) who reported that progesterone concentration is similar whether 1 or 2 corpora lutea were present. These results in Gp. 3 when compared with those of Gp. 2 indicate that the administration of E_2-17β prior to Gn-RH maintains the induced corpora lutea for 10 days or more.

With these experiments it was difficult to decide whether the induced or the new corpora lutea or both were secreting progesterone. However, one ewe in Gp. 3 had only the induced CL as a result of the treatment and nevertheless its progesterone concentrations were similar to those in the control animals and remained high for the normal length of the luteal phase. Perhaps the high concentration of E_2-17β and LH prior to ovulation, are necessary for the maintenance of the CL. Thus the action of estradiol on the CL appears to be mainly through the hypothalmic pituitary system in

Table 2. Hormone Concentrations in Jugular Plasma of Normal Cyclic Ewes Treated with PGF-an Then Infused with Saline, Gn-RH and E_2-17β + Gn-RH (Mean ± S.D.).

Sampling Time in Days	Treatment		
	Saline	Gn-RH	E_2-17β + Gn-RH
	Total estrogen (Pg/nl)		
- 2	11.6 ± 0.8	13.2 ± 1.3	13.5 ± 1.2
*- 1	10.3 ± 0.2	16.1 ± 2.6	13.8 ± 1.9
0	11.7 ± 1.9	12.3 ± 0.8	12.6 ± 1.5
+2			40.4 ± 10.8
+4 hrs			45.5 ± 9.4
+6			14.2 ± 3.8
1	11.4 ± 0.9	16.7 ± 4.7	13.9 ± 4.8
2	15.1 ± 0.9	14.3 ± 3.1	19.0 ± 9.2
3	17.9 ± 8.9	20.3 ± 8.1	16.5 ± 6.2
4	13.8 ± 7.1	16.3 ± 3.6	11.5 ± 3.1
	LH Concentration (ng/ml)		
- 2	1.5 ± 0.2	1.5 ± 0.3	2.4 ± 2.1
*- 1	1.3 ± 0.3	1.6 ± 0.1	1.8 ± 0.2
0	1.5 ± 0.3	1.4 ± 0.2	1.4 ± 0.3
+2			1.7 ± 0.3
+4			1.5 ± 0.2
6 hrs	1.5 ± 0.2	67.6 ± 36.5	68.4 ± 30.2
8	1.5 ± 0.3	28.5 ± 42.3	134.0 ± 26.1
10	1.5 ± 0.3	28.5 ± 19.6	46.6 ± 18.3
12	1.5 ± 0.2	8.2 ± 3.5	11.4 ± 4.8
14	1.5 ± 0.1	4.1 ± 2.7	5.7 ± 3.2
+ 1	1.6 ± 0.1	2.1 ± 1.1	2.3 ± 0.9
+ 2	2.9 ± 3.4	1.4 ± 0.2	1.7 ± 0.5
+ 3	1.9 ± 0.6	1.5 ± 0.2	1.5 ± 0.4
+ 4	5.6 ± 6.6	1.5 ± 0.2	3.9 ± 5.1
+ 5	1.6 ± 0.4	1.4 ± 0.3	2.2 ± 1.2
+ 6	1.5 ± 0.25	1.4 ± 0.1	1.7 ± 0.4

*- Day of PGF-an injection (18 hrs before infusion)

0 = start of infusion of E_2-17β for 4 hr. ☐

or/and Gn-RH for 8 hrs. ▨

its trophic action and through the uterus in its lytic action as reported by Bolt and Hawk (2).

It was not possible however, to deduce from these experiments in intact sheep whether the apparent induced luteotropic effect brought about by estradiol was due to direct action of this hormone on luteal tissue or an indirect action involving other hormonal secretions.

Table 3. Ovarian Changes in Ewes Infused with Saline (G.I), Gn-RH (GI-II) and E_2-17β + Gn-RH (G -III) 18 hrs after PGF-an Injection.

Group	Treatment	No. of ewes	*No. ovulated at first endoscopy	No. of C.L. at 2nd endoscopy XX		
				Fresh C.L.	marked and regressed C.L.	marked and maintained C.L.
GP I	Saline	4	4	-	-	4
GP II	Gn-RH	5	3	6**	3	-
GP III	E_2-17β + Gn-RH	5	4	4***	-	4+

* - All C.L. in the first endoscopy (48 hrs after the end of imfusion) were marked with Indian ink.

** - One ewe had 2 C.L.

*** - All ewes in this group except one ovulated again after the first endoscopy.

\+ - All induced C.L. as a result of treatment in this group were maintained.

XX (10 days after the first endoscopy)

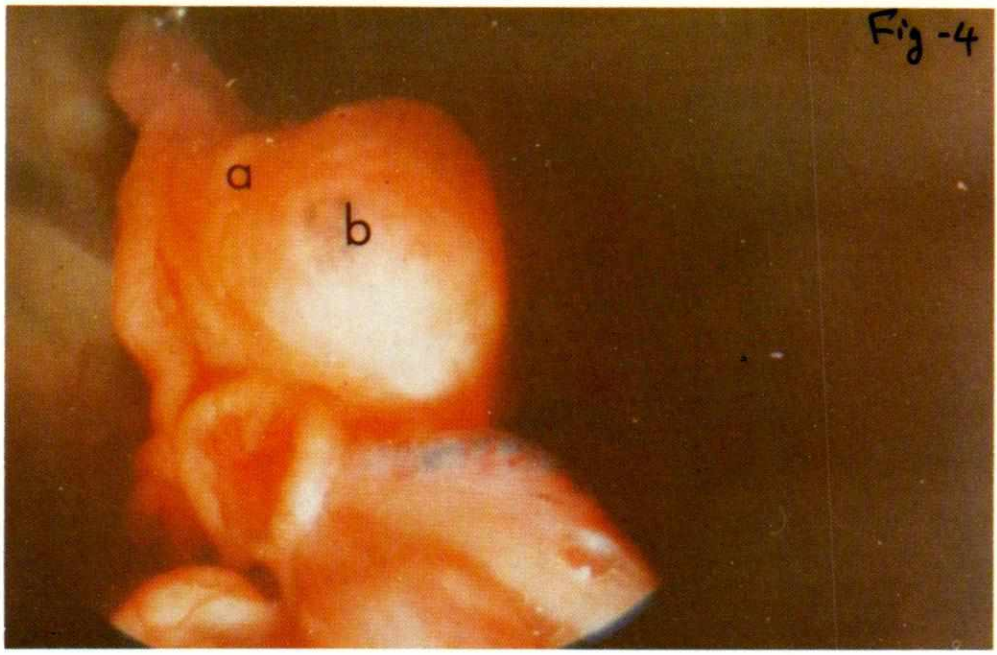

Fig. 4. At the second endoscopy the ovary shows CL (a) and the persistence of the Indian ink after induced CL had regressed (b).

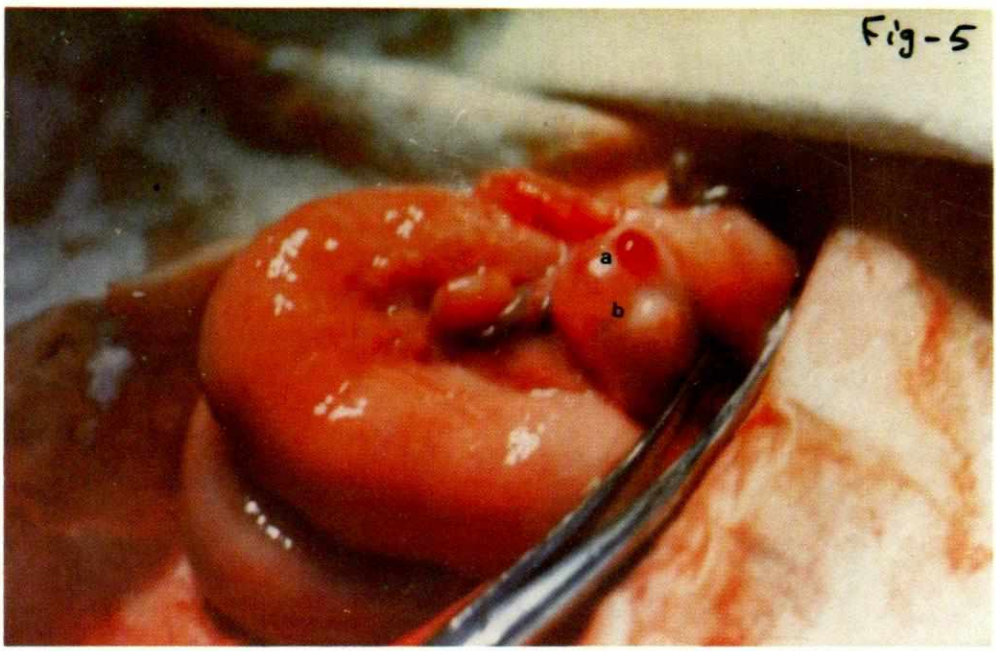

Fig. 5. Group 3 second endoscopy at 10th day after infusion (b) C.L. which was present at first endoscopy examination at 45 hrs and which has persisted (marked with Indian ink which was observed at the second endoscopy, but which does not show up in the photograph). (a) New CL which arose from an ovulation after the first endoscopy.

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تأثير الحقن المستمر لهرمون الاستروجين (E_2 - 17 B) على حياة الاجسام الصفراء المستحدثه فى موسم التلقيح عند الاغنام

د. عاشور مسعود شريحه ور.ج فيتزباترك

مستخلص

١ - الحقن المستمر لهرمون الاستروجين (E_2 - 17 B) قبل الحقن المستمر للهرمون المسبب (Gn-RH) قد سبب زيادة فى انتاج هرمون المبيض (LH) وان جميع الاجسام الصفراء الناتجه عن هذا الحقن قد استمرت باقيه ولمدة اكثر من عشرة ايام فى جميع حيوانات المجاميع التجريبية .

٢ - الحقن المستمر للهرمون المسبب (Gn-RH) قد اسفر عنه زيادة فى انتاج هرمون المبيض (LH) وان جميع الاجسام الناتجه عن هذا الحقن قد ضمرت قبل اوانها فى نفس حيوانات المجاميع التجريبية .

٣ - لذا نستخلص من هذه التجربة ان هرمون الاستروجين (E_2 - 17 B) قد يكون مهما فى حياة الاجسام الصفراء فى الاغنام .