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THE TIME OF OVULATION IN THE EWE FOLLOWING TREATMENT WITH HUMAN CHORIONIC GONADOTROPHIN

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The object of the experiments described below was to estimate the time at which ovulation occurs in sheep treated with gonadotrophin and to provide, if possible, sheep oocytes of known age in connection with experiments on the hybridization of sheep and goats.

Ortavant, Thibault & Wintenberger (1949) found that ovulation occurred about 24 hr after the intravenous injection, given at the beginning of oestrus, of a mixture of pregnant mares' serum (PMSG) and human chorionic gonadotrophin (HCG). Braden, Lamond & Radford (1960) found that ovulation occurred between 20 and 28 hr after injection of HCG in ewes previously treated with progesterone and PMSG. In ewes previously treated with 6-methyl-17-acetoxyprogesterone, ovulation was found to occur 25 hr after injection of HCG (Dziuk, Hinds, Mansfield & Baker, 1964). Moor, Rowson, Hay & Caldwell (1969), without giving details of the source of their information, say that ovulation occurs approximately 24 hr after administration of HCG.

The experiments reported here were designed to examine the time of ovulation in ewes treated with HCG alone, as well as in ewes previously treated with PMSG to induce superovulation. Some observations were also made on the effect of the dose and route of administration of HCG.

Welsh Mountain ewes, 4 to 5 years old, were run with crayoned, vasectomized rams and were examined for evidence of the onset of oestrus twice daily, at 09.00 hours and 17.00 hours. Ewes which had been marked were tested for oestrus with a second vasectomized ram. The day of onset of oestrus is referred to as Day 0.

The animals were allocated arbitrarily into five treatment groups. The type of treatment and the distribution of animals between the groups are shown in Table 1. Ewes receiving PMSG were injected subcutaneously with 1000 i.u. 'Gestyl' (Organon Laboratories Ltd) at approximately 17.00 hours on Day 13. HCG-treated ewes received 250 i.u. or 1000 i.u. Lutormone (Burroughs Wellcome & Co.) intramuscularly or intravenously on Day 15. The time of administration of HCG was varied for practical considerations but most injections were made between 09.00 and 11.00 hours. Only ewes which were not in oestrus on the morning of Day 15 were included in the experiments. This selection was made in order to reduce the possibility that ovulation might occur spontaneously within the experimental period.

The animals were subjected to laparotomy on Day 16 at various times after HCG injection. In the case of the PMSG-treated ewes which did not receive HCG (Group 3), laparotomy was performed during the afternoon of Day 16. The anaesthetic and surgical procedures used were the same as those described by Hancock & Hovell (1961). The ovaries were examined and the number of corpora lutea present was recorded. The Fallopian tubes of those ewes which had ovulated were flushed with Hanks' solution and a record made of the number of recovered eggs.

All animals were again tested for oestrus on the evening of Day 15 and on the morning of operation, on Day 16. Observations were also made on the length of the oestrous cycle in the sixth group of ewes which received no hormone treatment. Because ewes could not be tested after laparotomy, the comparison of findings between treated (operated) and untreated ewes is restricted to the proportion of ewes showing heat up to the time of operation of the treated ewes.

The findings regarding the time and occurrence of ovulation are summarized in Table 1.

TABLE 1

THE OCCURRENCE OF OVULATION (EWES OVULATED/EWES EXAMINED) IN FIVE GROUPS OF SHEEP FOLLOWING TREATMENT WITH GONADOTROPHIN

Group	*Gonadotrophin*		*Interval from injection of HCG to ovulation (hr)*							
	PMSG (i.u.)	*HCG (i.u.)*	*18 to 22*	*23*	*24*	*25*	*26*	*27*	*28*	*29*
1	–	250	–	–	0/4	0/2	–	3/4	1/2	–
2	1000	250	0/3	–	3/3	2/2	0/1	–	3/3	3/4
3	1000	–	–	–	–	–	–	–	0/4†	–
4	–	1000	–	–	–	–	–	2/4	4/4	–
5	–	1000*	–	–	–	–	–	1/2	3/4	1/2

* HCG given by intravenous injection. Other groups received HCG intramuscularly.
† Animals examined 70 hr after injection of PMSG, i.e. at a time comparable with that for HCG-treated ewes of other groups.

Of six ewes examined 24 to 25 hr after HCG injection alone (Group 1), none had ovulated but four of six ewes in this group were found to have ovulated when examined 27 to 28 hr after injection. There was evidence that pre-treatment with PMSG reduced the interval between injection and ovulation since ovulation had occurred within 24 to 26 hr of injection in five of six ewes (Group 2) previously treated with PMSG; ovulation had occurred in none of three PMSG-treated ewes injected with HCG 18 to 22 hr before examination.

The earlier ovulation of PMSG-treated ewes which received HCG was apparently independent of endogenous LH since four ewes which received PMSG alone (Group 3) had not ovulated when examined 70 hr after injection of PMSG although ovulation had occurred by this time in six of seven PMSG-treated ewes (Group 2) which had received HCG 28 to 29 hr previously. The shorter interval between injection of HCG and ovulation may be evidence of greater maturity, at the time of HCG treatment, of the follicles of PMSG-treated sheep; it could be due to a synergistic action of the two hormones.

In all treatment groups, some ewes had failed to ovulate when examined as

late as 28 to 29 hr after injection of HCG. Attempts to ensure the occurrence of ovulation by varying the dosage and route of administration of HCG were unsuccessful. Thus, two of eight ewes receiving 1000 i.u. HCG intramuscularly (Group 4) had not ovulated when examined 27 to 28 hr after injection and ovulations were recorded in only five of eight ewes which had received the same dose intravenously 27 to 29 hr previously (Group 5).

The findings on the occurrence of oestrus in ewes in the separate treatment groups are summarized in Table 2. There is evidence that treatment with HCG,

TABLE 2

THE OCCURRENCE OF OESTRUS IN GONADOTROPHIN-TREATED SHEEP AND THE NUMBER OF EGGS SHED AND RECOVERED

Group	*Treatment*		*Ewes*		*Eggs*	
	PMSG (i.u.)	*HCG (i.u.)*	*Oestrous*†	*Total*	*Recovered*	*Shed*
1	–	250	3	12	2	4
2	1000	250	6	16	8	23
3	1000	–	2	4	–	0
4	–	1000	0	8	3	7
5	–	1000*	1	8	2	8
6	–	–	7	13	–	–

* HCG injected intravenously.
† Excludes ewes showing heat on the afternoon of Day 16 or later (see text).

at least at the higher dosage level, prevented (or perhaps delayed) the occurrence of oestrus. Thus, of sixteen ewes which received 1000 i.u. HCG (Groups 4 and 5), only one showed heat by the morning of Day 16; seven of thirteen untreated ewes (Group 6) showed oestrus by this time. Inhibition of oestrus in ewes treated with HCG was also recorded by Braden *et al.* (1960).

There was no significant effect of treatment with PMSG on the number of eggs shed. Twenty-three corpora lutea were counted in the ovaries of eleven PMSG-treated ewes; nineteen corpora lutea were found in fifteen ewes which had not received PMSG. We have no explanation for the relative failure of PMSG to induce superovulation.

Of forty-two eggs shed, only fifteen were recovered. The distribution of recovered eggs between the different experimental groups is shown in Table 2. Any suggestion that the rate of egg recovery was low because the operations were performed so near to the time of ovulation is not supported by available evidence. An apparent difference in recovery rate between ewes of Group 2 examined 24 to 26 hr after injection of HCG (two eggs recovered of eleven eggs shed) and of those examined 27 to 29 hr after the injection of HCG (six eggs recovered of twelve eggs shed) is not statistically significant ($\chi^2_{(1)}$ 2·00; $0{\cdot}2 > P > 0{\cdot}1$).

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