A COMPARISON OF PMSG AND LHRH AGONIST TREATMENT IN THE INDUCTION OF OVULATION IN THE ANOESTROUS EWE

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ABSTRACT

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The aim of this experiment was to compare the use of pregnant mares' serum gonadotrophin (PMSG) with that of a luteinizing hormone releasing hormone (LHRH) agonist in the induction of ovulation in anoestrous sheep. Anoestrous ewes were treated with progestagen-impregnated sponges for 12 days. They were given either PMSG at the time of sponge withdrawal or the LHRH agonist D-Ser(But)^{6}desGlyNH$_2$ ^{10}LHRH ethylamide 20 h after sponge withdrawal. This protocol was followed over 2 consecutive years. Plasma concentrations of oestradiol and LH were measured, and in the first year a comparison was made of the ovulation rate, conception rate and luteal function of the two groups after artificial insemination. During the first year, all of the PMSG-treated group but none of the agonist-treated group exhibited oestrus. Five of the eight PMSG-treated ewes had embryos in utero at slaughter whilst none was present in the agonist-treated ewes. The secretion of progesterone was greatest in the PMSG-treated ewes ($P < 0.001$). During the second year, a more frequent blood-sampling regime was employed. Increased plasma concentrations of LH occurred within 3 h of agonist administration. Plasma oestradiol concentrations peaked at 20 h and 45 h after sponge withdrawal in both groups. Both peaks were larger in the agonist-treated group. It is concluded that a single dose of the highly potent LHRH agonist is unable to produce normal luteal function or conception using the present protocol.

INTRODUCTION

Previous work has demonstrated that a single intravenous injection of D-Ser(But)^{6}desGlyNH$_2$ ^{10}LHRH ethylamide (LHRH agonist) early in the anoestrous season could stimulate ovulation and luteal function in the ewe (Swift and Crighton, 1980). This is in contrast to injection of synthetic LHRH which causes ovulation but not luteal function (Haresign et al., 1975).

The most widely used method of ovulation induction during anoestrum

is by PMSG injection of progestagen-primed ewes (Robinson, 1951, 1961, 1971). The present study was undertaken to compare the efficacy of LHRH agonist treatment with that of PMSG treatment of progestagen-primed ewes in terms of ovulation induction and luteal function.

MATERIALS AND METHODS

Experimental protocol (Experiment 1)

During the anoestrous season of 1979 (day 165) 16 ewes from the University of Leeds flock which had lambed earlier in the year were implanted with medroxy progesterone acetate (60 mg) impregnated tampons (Veramix, Upjohn, Crawley, U.K.). Twelve days later (day 177), the tampons were removed and eight ewes were injected intramusculary with PMSG (Folligon, Intervet, Cambridgeshire, U.K.; 600 i.u) at 13.00 h. One day later (day 178) at 09.00 h, the remaining untreated ewes were injected via the jugular vein with the LHRH agonist (50 μg) and blood sampling (10 ml) was commenced. A sample was taken every 2 h until 19.00 h. Further blood samples were taken at 05.00 h on day 179 and every 2 h until 13.00 h and then every 2 days for the remainder of the luteal phase. On day 179, all of the ewes were artificially inseminated with 400×10^6 sperm from a Suffolk ram 48 and 60 h after sponge withdrawal. On days 198 and 199 all of the ewes were slaughtered and their ovaries and uteri examined for ovulation points and embryos.

Experimental protocol (Experiment 2)

During the anoestrous season of 1980 (day 122), ten ewes were implanted with progestagen-impregnated tampons. Twelve days later (day 134) these were removed at 13.00 h and five ewes were injected intramuscularly with PMSG (600 i.u.). At 09.30 h the following day, the remaining five ewes were injected with 50 μg of the LHRH agonist via the jugular vein.

Blood samples (10 ml) were taken half-hourly commencing at 09.00 h on day 135 for 4 h. Samples were then taken hourly until 18.00 h and then 2 hourly until 06.00 on day 136. Hourly samples were taken until 18.00 h on day 136.

Treatment of blood samples

Blood samples were placed in heparinized tubes in ice and centrifuged within 20 min of collection. The plasma samples were collected after centrifugation at 3000 g for 15 min and then stored on ice until transported back to the laboratory where they were stored at $-20°C$ until required for assay. All plasmas were assayed for LH and oestradiol and selected plasmas for progesterone.

Radioimmunoassay of LH

Radioimmunoassay of LH was conducted as described by Foster and Crighton (1974). The sensitivity of the assay was 2.6 ng NIH-LH-S18. The inter- and intra-assay coefficients of variation were 11.2% and 4.9% respectively. (n = 5 and 10 respectively)

Radioimmunoassay of oestradiol

Radioimmunoassay of oestradiol was conducted using an antiserum raised in sheep against the conjugate 6-oximeoestradiol BSA donated by Dr. G. Pope of the National Institute for Research in Dairying, Reading. Plasma samples (1 ml) in duplicate were rendered alkaline with 1 M NaOH (50 μl) and the oestradiol extracted with diethyl ether (5 ml). The aqueous phase was frozen in solid CO_2/ethanol freezing mixture and the organic phase decanted into tubes suitable for radioimmunoassay. Standards (Steraloids Ltd) in ethanol (0.05 ml) ranging from 0 to 552 fmol were added to the tubes containing 5 ml of diethyl ether which had been saturated by shaking 0.05 M NaOH (1 ml).

The assay tubes containing the plasma extracts were balanced with an appropriate volume of ethanol. All tubes were dried under a stream of nitrogen at 37°C. When dry, tritiated oestradiol (2,3,6,7(n)-^{3}H oestradiol, 3600 cpm in 0.5 M phosphate buffer saline, 0.1 ml, specific activity 85—91 μCi/mmol, Radiochemical Centre, Amersham) was added to each tube and 0.1 ml antiserum (final dilution 1:40 000 diluted in phosphate buffered saline). The tubes were then incubated at 4°C for a minimum of 2 h. Bound and free fractions were separated by the addition of 0.5 ml of dextran charcoal (0.5% charcoal; 0.05% dextran in saline diluent [0.9% NaCl, 0.01% gelatin, 0.001% thiomersal]) suspension. After centrifugation, the supernatant was decanted into scintillation vials (Scintillation cocktail: 3 g PPO, 0.2 g dimethyl POPOP; 250 ml Triton X; 750 ml xylene; 10 ml), cooled and counted. The efficiency of this extraction was assessed by the addition of tritiated oestradiol (4000 cpm) to five plasma samples (1 ml) which were extracted as outlined above. The organic phase was decanted into scintillation vials to which an excess of unlabelled oestradiol had been added and then dried under a stream of nitrogen. Scintillation fluid was then added and these were then cooled and counted. The mean extraction was found to be 82 ± 1.28% (mean ± S.D.; n = 75).

The precision profile of a typical assay is shown in Fig. 1. The precision potential was calculated as the coefficient of variation expressed as a percentage of the concentration estimated from duplicate counts from an assay containing 200 points covering a range from <5 pmol/l to 1000 pmol/l. The useable range of the assay, defined as that part of the standard curve having a percentage coefficient of variation of < 10%, was 14 pmol/l to 380 pmol/l (Ekins, 1983). The sensitivity of the assay as defined as the

quantity of unlabelled hormone required to inhibit the binding of the tracer by an amount equal to 2 S.D. compared with inhibition in the absence of unlabelled hormone was 21 pmol/l. The intra-assay coefficient of variation, as determined by repeated analysis of a plasma sample (estimated concentration 40 pmol/l) every five 'unknown' samples, was 6.0% ($n = 5$) and the inter-assay coefficient of variation, determined by taking the mean of the repeating pool for each assay, was 8.4% ($n = 15$).
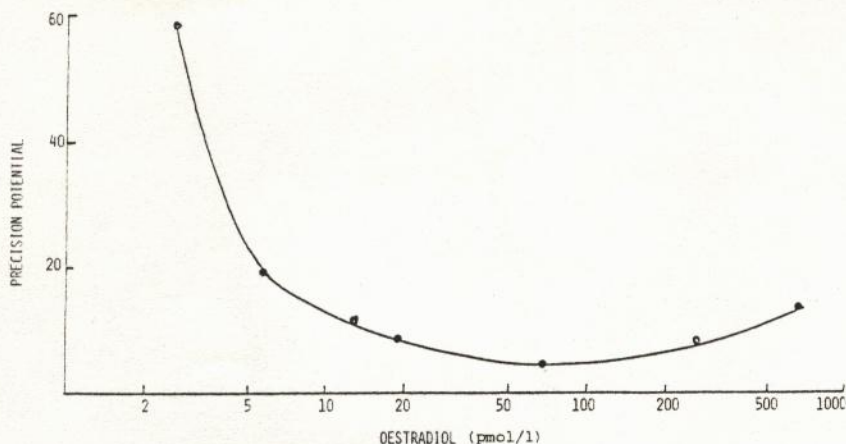

Fig. 1. Precision profile of the oestradiol assay.

Determination of oestradiol concentration in tubes where plasma had been substituted by buffer gave a concentration not significantly different from the zero point. Determination of oestradiol concentration in dextran-charcoal treated plasma samples from which the removal of tritiated oestradiol was 99.1% yielded estimated values of between 5 and 10 pmol/l (below the formal limits of detection for each individual assay). Samples from one ewe were run in one single assay to minimise inter-assay variation. Random samples from the previous assay were reassayed in the current assay to ensure the inter-assay stability.

Cross-reaction, as determined by the method of Abraham (1974) was found to be 10% with oestrone, 1% with oestriol and less than 1% with all other steroids tested.

Radioimmunoassay of progesterone

Progesterone concentrations were determined using antiserum also donated by Dr. G. Pope of the N.I.R.D., Reading, U.K. The assay was conducted as described elsewhere (Senior et al., 1978) after the appropriate validation for sheep plasma. The sensitivity of the assay was 30 fmol. Inter- and intra-assay coefficients of variation were both 8% ($n = 5$ and 8 respectively).

Statistical analyses

Results were analysed by either Student's *t* test or analysis of variance. All figures show means and standard errors of the mean (except where these were less than the diameter of the point).
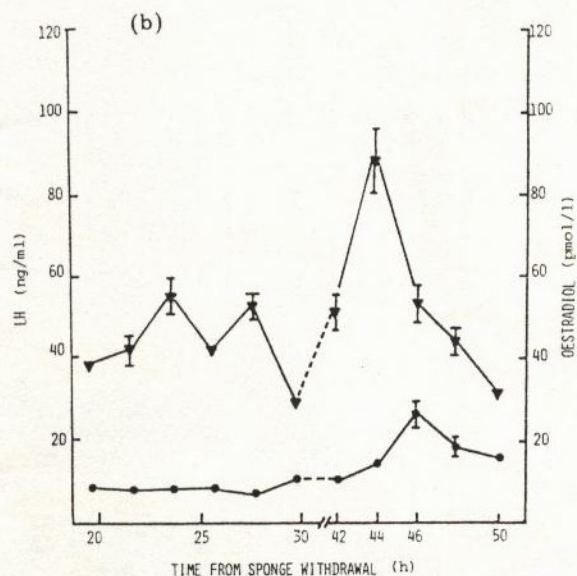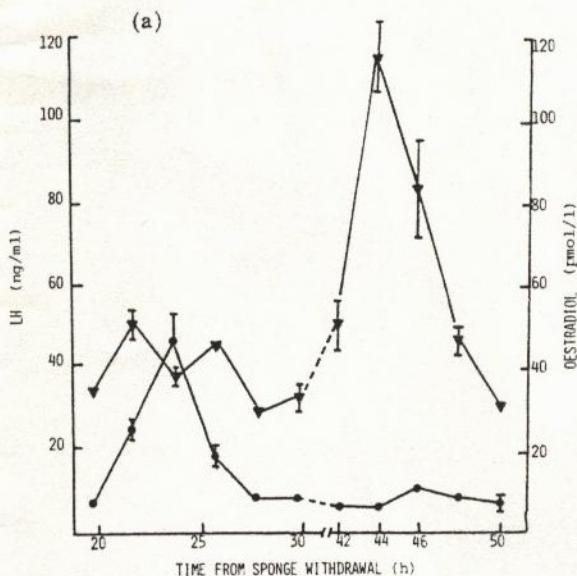

Fig. 2. Plasma concentrations of oestradiol (triangles) and of LH (circles) after (a) LHRH agonist treatment and (b) PMSG treatment in experiment 1.

RESULTS

Experiment 1

LHRH agonist-treated animals showed a peak of plasma LH within 3 h of injection (Fig. 2). This was followed by a large oestradiol peak approximately 45 h after sponge withdrawal. The PMSG-treated animals had a similar oestradiol peak again about 45 h after sponge withdrawal and preceding an LH peak by a few hours. All the PMSG-treated animals exhibited oestrus as detected by a vasectomised ram, but none of the LHRH agonist-treated ewes did so.

All sheep in the PMSG group and seven of the eight in the agonist-treated group showed elevated plasma progesterone levels during the 2 weeks following sponge withdrawal. However, PMSG-treated sheep had significantly ($P < 0.001$) higher levels than did the LHRH agonist-treated animals (Fig. 3).

At slaughter five of the eight PMSG-treated animals were pregnant, with an average of 1.8 embryos and 2.8 corpora lutea per pregnant animal. The ovaries of the other three contained corpora albicantia. None of the LHRH agonist-treated animals was pregnant and no corpora lutea were seen, although four animals had corpora albicantia and three had follicles greater than 5 mm in diameter.
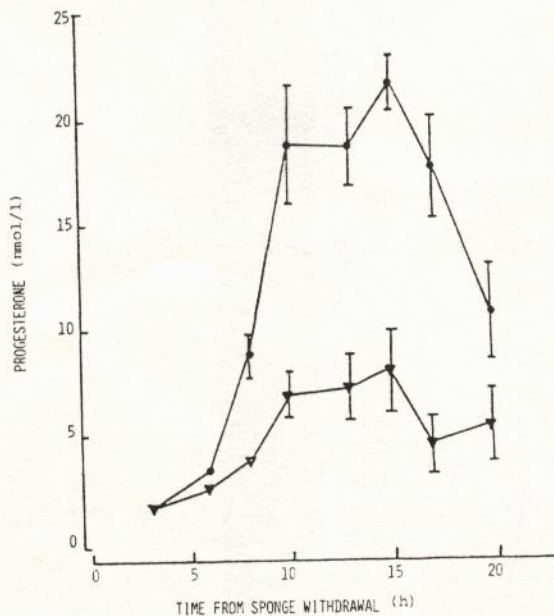

Fig. 3. Plasma progesterone concentrations after LHRH (triangles) or PMSG (circles) treatment in experiment 1.

Experiment 2

This was essentially a repeat of the first experiment except that more frequent blood samples were taken and the animals were not inseminated or slaughtered. Fig. 4 shows that essentially similar hormonal profiles were obtained, although in the LHRH agonist-treated group a secondary release of
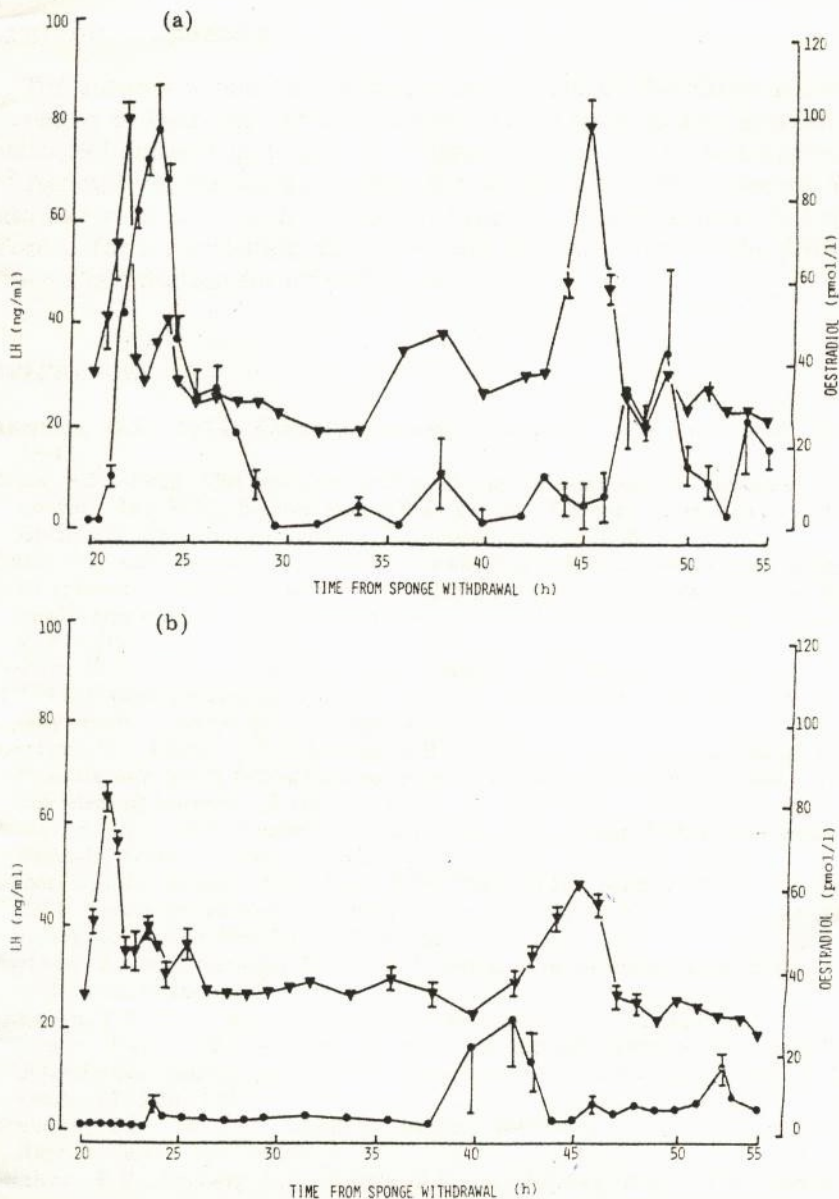

Fig. 4. Plasma concentrations of oestradiol (triangles) and of LH (circles) after (a) LHRH agonist treatment and (b) PMSG treatment in experiment 2.

LH occurred about 24 h after the initial peak. Again, in both groups, large peaks of oestradiol occurred about 45 h after sponge withdrawal. That of the LHRH agonist-treated group was significantly ($P < 0.05$) larger than that of the PMSG group. In this experiment, however, both groups also had peaks of oestradiol at about 20 h after sponge withdrawal. The main LH peak in the PMSG-treated animals occurred about 24 h after sponge withdrawal and appeared to precede the oestradiol peak by several hours.

DISCUSSION

The hormonal profiles obtained were essentially similar in both years. However, in experiment 2, peaks of oestradiol occurred about 20 h after sponge removal in both treatment groups. These were not observed the previous year although as they occurred very shortly after the start of sampling, they could have been missed. As they occurred coincidently in both treatments they were presumably due to the sponge withdrawal rather than to the LHRH or PMSG treatments. The timing of the LH peak in the PMSG-treated group in experiment 2 is difficult to reconcile with current opinion that the LH surge occurs as a result of the preceding oestradiol surge. The LH peak here occurred several hours before the oestradiol peak but could possibly have been triggered by the oestradiol peak which occurred some 20 hours previously. In the first experiment the peaks occur in their expected order.

The conception rate achieved with the PMSG regime (63%) was within the expected range, and the individual progesterone profiles (not shown) indicate that all the ewes ovulated in the PMSG group in Experiment 1. In the LHRH agonist-treated group no conceptions occurred, presumably due to the inadequate luteal function. In a similar study using another LHRH agonist, although without progestagen sponge treatment, Kinder et al. (1976) were also unable to establish pregnancy, but in this work luteal function was not monitored. Segerson et al. (1974) have suggested that oestrogen secretion may be inadequate for normal sperm transport in ewes treated with GnRH during the anoestrous season. However, in the present work oestrogen secretion was higher in the agonist-treated animals than in the PMSG-treated group. Egg transport in the oviduct is also susceptible to altered oestrogen: progesterone ratios (see Hunter, 1980), and this is therefore another possible cause of failure of conception.

Normal luteal function after LHRH agonist treatment during the anoestrous season appears to have been obtained in only one study (Frandle et al., 1977), although Swift and Crighton (1980) obtained an almost normal pattern of progesterone secretion using the same agonist as was used in the present study. This varying response to LHRH certainly depends on the timing of the treatment during the anoestrum (Shareha et al., 1976; Swift and Crighton, 1980) and possibly on the breed of sheep. It appears from recent work using either multiple LHRH injections or slow infusions (MacLeod

and Haresign, 1982) that a longer period of elevated LH is required to stimulate more natural development of pre-ovulatory follicles which are presumably then able to form functionally adequate corpora lutea and therefore to maintain the early stages of pregnancy. There appears to be little chance that a single injection of LHRH or its agonist will prove routinely successful in establishing normal luteal function in the anoestrous ewe.

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