Effect of active immunization against oxytocin on gonadotrophin secretion and the establishment of pregnancy in the ewe

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Summary. A group of 14 ewes was actively immunized against oxytocin coupled to carrier protein, and comparisons of the reproductive status of these animals were made against ewes immunized against carrier protein only ($N = 5$) and untreated controls ($N = 6$). The last two groups were indistinguishable and were therefore combined as a single control group for analysis of the results. Oestrous cycle lengths were significantly extended in oxytocin-immunized ewes ($P < 0.005$) with 42% of cycles lasting > 18 days. Cloprostenol treatment in the mid-luteal phase resulted in apparently normal luteal regression and re-ovulation, but luteal phase FSH concentrations and follicular phase LH concentrations were elevated in the immunized ewes, although surge levels of both hormones were unaffected. Measurements of free oxytocin concentrations in the blood suggested that these were significantly raised in treated animals. Progesterone concentrations in peripheral plasma were not altered by treatment.

Mating resulted in a conception rate of 91% in control ewes compared with only 28% in oxytocin-immunized animals ($P < 0.01$). There was no evidence of any conceptus material in the uteri of non-pregnant immunized ewes 25 days after service. Some had re-ovulated, whereas the ovaries of others contained mature corpora lutea which had been maintained. Ovarian histology appeared normal.

We conclude that active immunization against oxytocin influences gonadotrophin secretion and reduces fertility. The site(s) of action for both of these effects needs to be determined.

Keywords: oxytocin; immunization; ewe; gonadotrophins; pregnancy

Introduction

The corpus luteum of the ewe is a rich source of oxytocin (Wathes & Swann, 1982). Pulses of ovarian oxytocin secreted in the late luteal phase interact with newly formed receptors on the uterine endometrium, triggering the release of prostaglandin (PG) F-2 α (McCracken *et al.*, 1981; Flint & Sheldrick, 1983; Hooper *et al.*, 1986). This interaction is thought to initiate luteolysis in the ewe as both active and passive immunization against oxytocin have been shown to prolong the oestrous cycle (Sheldrick *et al.*, 1980; Schams *et al.*, 1983). A similar effect can be achieved by continuous infusion of oxytocin in the late luteal phase, which may act by inhibiting the appearance of oxytocin receptors (Flint & Sheldrick, 1985). This role in luteolysis is only initiated from Day 14 of the cycle onwards, but ovarian oxytocin production begins in the preovulatory follicle (Wathes *et al.*, 1986), and peak concentrations of the hormone in both the corpus luteum and the circulation are reached in the first half of the luteal phase, with mean levels starting to fall some time before the onset of luteolysis (Webb *et al.*, 1981; Schams *et al.*, 1982; Sheldrick & Flint, 1983). At present no function has been attributed to the oxytocin secreted in the early and mid-luteal phases, although a

number of sites of action are possible, for example by influencing reproductive tract motility, gonadotrophin secretion or ovarian steroidogenesis (see Wathes, 1984, for review).

The purpose of this study was to see whether immunization against oxytocin affected the establishment of pregnancy in the ewe, as this would indicate a role for oxytocin other than in the process of luteolysis. As an initial experiment the endocrine changes around the time of ovulation were compared between immunized and control animals.

Materials and Methods

Animals and immunization procedure

Mature Clun Forest ewes ($N = 25$) were kept at pasture and fed hay when necessary as a winter supplement. They were divided at random into three groups for treatment: 6 remained as untreated controls, 5 were immunized against carrier protein, and 14 were immunized against oxytocin coupled to carrier protein.

Oxytocin (Cambridge Research Biochemicals Ltd, Cambridge, UK; Hoechst, Frankfurt, West Germany) was covalently bound to bovine thyroglobulin or ovalbumen (both from Sigma Chemical Co. Ltd, Poole, Dorset, UK) according to the method of Sofroniew *et al.* (1978). Studies with radiolabelled oxytocin showed that approximately 50% of the oxytocin bound to thyroglobulin and 40% to ovalbumen under these conditions. The conjugate was stored frozen at -20°C until required, at which time it was diluted as necessary with sterile saline (0.9% (w/v) NaCl) to give an aqueous solution containing either 500 μg oxytocin (first immunization) or 150 μg (subsequent immunizations) per 660 μl . This was then emulsified with Freund's incomplete adjuvant (Guildhay Antisera, Guildford, UK) (1:2 (v/v) adjuvant:conjugate) to give a total volume to dose 1 ml per sheep. A control conjugate, as above but excluding the oxytocin, was also prepared. Emulsions were either injected subcutaneously at several sites on the back or infused into the mammary gland.

Starting in November 1986, 9 ewes were immunized 4 times at 14-day intervals with oxytocin coupled to thyroglobulin, followed by a further 3 immunizations at 9–25-day intervals. To increase the titres a second series of immunizations was started in June 1987 for which thyroglobulin was replaced by ovalbumen as the conjugating protein. An additional 5 ewes were included at this point. Ewes were immunized 5 times at 14-day intervals and on 2 further occasions to boost titres before Exps 1 and 2 (see below).

From August 1987 onwards blood samples were collected twice weekly by jugular venepuncture and the plasma stored at -20°C for subsequent progesterone RIA. The ewes were run with a raddled vasectomized ram to monitor oestrous cycle lengths. Blood samples were also obtained at approximately 1-month intervals for measurement of oxytocin antibody titres.

Experiment 1

In mid-September 1987, when all 25 ewes had experienced at least one complete ovarian cycle, all animals were given an injection of cloprostenol (PGF-2a analogue, 100 μg i.m.; Coopers Animal Health Ltd, Crewe, Cheshire, UK). On the basis of (a) whether the ewes were at an appropriate stage of the cycle to show a luteolytic response to the first injection and (b) current oxytocin antibody titres, 15 animals were selected for the experiment as follows: untreated control, $N = 4$; ovalbumen-immunized, $N = 3$; and oxytocin-immunized, $N = 8$. These animals were housed in individual pens and jugular vein cannulae were inserted. Between Days 9 and 11 of the induced cycle (Day 0 = day of oestrus) all animals received a second injection of cloprostenol. Blood samples (10 or 20 ml) were collected every 2 h onto ice from 6 h before the cloprostenol injection until 96 h after. FSH and LH were measured in each sample, progesterone was measured every 2 h for the first 24 h and subsequently every 6 h, and free oxytocin concentrations were measured every 12 h. Plasma for oxytocin RIA (~ 5 ml) was acidified with 250 μl 0.1 N-HCl and frozen on solid CO_2 . Ewes were checked for behavioural oestrus every 2 h using a vasectomized ram. On Day 7 of the following cycle animals were premedicated with xylazine, and anaesthetized with halothane for laparoscopy, and the numbers of corpora lutea were counted. The ewes were then returned to the flock and twice weekly blood sampling was resumed.

Experiment 2

At 2 months after Exp. 1 (December 1987) the vasectomized ram was replaced by a single entire ram of proven fertility. Twice weekly blood sampling for progesterone RIA continued and the ewes were observed daily for raddle marks. Each ewe was slaughtered 25 days after first service and the reproductive tract was removed. The numbers of corpora lutea were counted and each ovary was then bisected and fixed in 2.5% glutaraldehyde, 4.0% paraformaldehyde in 0.1 M-sodium cacodylate buffer pH 7.4 (Wathes *et al.*, 1986). The uterus was opened, all conceptuses removed and the lengths of the embryos were recorded. Ovarian sections (7 μm) were stained with haematoxylin and eosin for histological examination.

Radioimmunoassays

Progesterone. Progesterone measurements were performed on 0.25 ml aliquants of plasma using petroleum ether extraction as described previously (Wathes *et al.*, 1986). The detection limit was 40 pg/tube, the extraction efficiency was $73 \pm 3.1\%$ and the inter- and intra-assay coefficients of variation were 15.4% and 4.5% respectively.

LH. Concentrations of LH in peripheral plasma were assayed using the specific double-antibody radioimmunoassay of Foster & Crighton (1974) as modified by McLeod *et al.* (1982). The limit of sensitivity was 0.2 ng NIH-LH-S24 equiv./ml plasma and the intra-assay coefficient of variation was <10%.

FSH. Plasma FSH concentrations were measured by a specific, homologous double-antibody radioimmunoassay as described by Cooper (1987). The immuno-reagents were developed by Dr A. F. Parlow, Pituitary Hormone and Antisera Centre, CA, USA, and were supplied by the National Hormone and Pituitary Program, Bethesda, MD, USA. The antiserum (NIAMDD-anti-oFSH-1) shows negligible cross-reaction with other pituitary hormones including ovine LH, prolactin and growth hormone. The limit of sensitivity was 0.19 ng NIAMDD/oFSH-RP-1 equiv./ml plasma and the intra-assay coefficient of variation was 4.1%. All samples were measured in the same assay.

Oxytocin. Oxytocin measurements were made after extraction of 5 ml plasma on C18 Cartridges (Jones Chromatography Ltd, Hengoed, Mid Glamorgan, Wales) as described previously (Wathes *et al.*, 1986). The limit of sensitivity was 0.5 pg/tube, the intra-assay coefficient of variation was 10.7% and all samples were measured in the same assay.

An experiment was conducted to determine the effectiveness of the C18 cartridges in separating antibody-bound from free oxytocin. An antiserum binding curve was set up with a range of antiserum dilutions from 1:2000 to 1:64 000 in 100 μ l buffer. 125 I-labelled oxytocin was added to all samples (4000 c.p.m. in 10 μ l assay buffer). Total count (label only) and non-specific binding (no antiserum) tubes were also included. All tubes were incubated overnight at 4°C. The next day all samples were diluted with 1 ml assay buffer or 1 ml sheep plasma, mixed rapidly and applied immediately to a C-18 column which had been prewetted with 10 ml 80% acetonitrile (ACN) in 0.1% (v/v) trifluoroacetic acid (TFA) followed by 20 ml 0.1% TFA to remove the antibody-bound oxytocin, which should not be retained by the column. The free 125 I-labelled oxytocin was then eluted with 3 ml 80% ACN in 0.1% TFA and the eluates were counted on a gamma counter for 2 min (see 'Results'). Longer incubations of 3 H- or 125 I-labelled oxytocin in plasma before the samples were added to the columns did not give consistent results, as the tracer was apparently degraded in the presence of plasma.

Measurement of oxytocin antibody titres and specificities

Serum samples were incubated overnight in triplicate with 5500–8000 c.p.m. 125 I-labelled oxytocin at 4°C in phosphate buffer (1.4 g $\text{Na}_2\text{HPO}_4 \cdot 2\text{H}_2\text{O}$, 0.26 g KH_2PO_4 , 8.65 g NaCl/litre) pH 7.4 containing 0.1% (w/v) bovine serum albumin and 2 mg bovine γ -globulins/ml (both from Sigma Chemical Co. Ltd) to give final dilutions of 1:100 and 1:1000 serum in a total volume of 260 μ l. Bound 125 I-labelled oxytocin was precipitated by the addition of 300 μ l 24% (w/v) polyethylene glycol, followed by immediate mixing and centrifugation (3000 g, 40 min, 4°C). The supernatant was removed by aspiration and the pellet counted. Counts precipitated in the absence of serum (5–10% of total) were subtracted from all determinations. 125 I-labelled oxytocin bound was expressed as a percentage of the total counts added.

The specificity was determined by the same method as used for the titres. Serum from each ewe was incubated at a dilution which bound 25–50% 125 I-labelled oxytocin with a range of peptides at the following dilutions: oxytocin from 2 pg/ml to 6400 pg/ml; mesotocin (Ile⁸-oxytocin), isotocin (Ser⁴, Ile⁸ oxytocin), AVP (arginine⁸ vasopressin) and AVT (arginine⁸ oxytocin) all from 20 pg/ml to 6400 pg/ml (peptides from Bachem Fine Chemicals Inc., Saffron Walden, Essex, UK).

Statistical analysis

The distribution of oestrous cycle lengths was analysed by using the Kruskal–Wallis test. Comparisons of hormone concentrations over time were made by ANOVA, taking account of repeated measures from the same animal. Concentrations of different hormones within the same ewe were compared by linear correlation analysis. Conception rates were compared by χ^2 analysis. All other comparisons were made using Student's *t* test.

Results

Oxytocin antibody titres and specificities

Oxytocin antibody titres rose steadily during the first immunization period but did not attain very high levels. All ewes responded to the second immunization period using ovalbumen as carrier protein by a further increase in titres (Fig. 1). In the ewes receiving only the second series of

Fig. 1. Development of oxytocin antibody titres in immunized ewes. Nine animals were given a series of immunizations against oxytocin coupled to thyroglobulin beginning in November 1986. These animals were given a second series of immunizations against oxytocin coupled to ovalbumen beginning in June 1987. A further 5 animals received only the second series of injections. The mean $\pm$ s.e.m antibody titres (expressed as the % binding of ^{125}I -labelled oxytocin to antibody at a dilution of 1:100) for each group are shown, together with the values for the 2 individual animals showing the highest (Ewe 73 $\square$ --- $\square$) and lowest (Ewe 8 $\circ$... $\circ$) titres at the conclusion of the experiment. The arrows indicate the timing of immunizations. The bars show the timing of Exp. 1 and Exp. 2 (see text for further details).

Fig. 2. The effect of oxytocin antiserum concentration on the measurement of oxytocin in samples extracted on a C-18 column. The amount of ^{125}I -labelled oxytocin retained by the columns is expressed as a percentage of the counts measured with no antiserum present (non-specific binding). Before extraction, 1 ml buffer ($\triangle$ --- $\triangle$) or sheep plasma ($\blacktriangle$ — $\blacktriangle$) was added to the samples.

Fig. 3. Histogram showing the distribution of oestrous cycle lengths in control ewes ($N = 6$), ovalbumen-immunized ewes ($N = 5$) and oxytocin-immunized ewes ($N = 14$) during the autumn of 1987.

Table 1. Comparison of gonadotrophin concentrations in control and oxytocin-immunized ewes before and after an injection of cloprostenol (PG) in the mid-luteal phase

	Control ($N = 7$)	Oxytocin-immunized ($N = 8$)	<i>P</i>
LH			
Peak height (ng/ml)	24.1 ± 4.17	28.3 ± 4.34	NS
Time from PG to start of surge (h)	70.3 ± 2.49	69.7 ± 2.55	NS
Mean luteal-phase conc.* (ng/ml)	0.3 ± 0.07	0.6 ± 0.12	0.059
Mean follicular-phase conc.† (ng/ml)	0.5 ± 0.03	0.7 ± 0.04	0.021
FSH			
Peak height (ng/ml)	4.6 ± 0.51	4.9 ± 0.51	NS
Mean luteal-phase conc.* (ng/ml)	1.5 ± 0.09	2.4 ± 0.19	0.028
Mean follicular-phase conc.† (ng/ml)	1.1 ± 0.03	1.7 ± 0.06	0.054
Ovulation rate	1.7 ± 0.33	2.0 ± 0.19	NS
Time from PG to onset oestrus (h)	59.7 ± 3.51	58.6 ± 2.84	NS

*Calculated from 3 samples per ewe taken before cloprostenol treatment.

†Includes all samples from that after cloprostenol treatment until the start of the LH surge.

Fig. 4. Changes in the mean $\pm$ s.e.m. concentrations of progesterone and free oxytocin in jugular venous plasma of control (●—●) and oxytocin-immunized (○---○) ewes after cloprostenol treatment (arrow) in the mid-luteal phase. Progesterone values did not differ between the groups, and most of the error bars have been omitted for clarity. Free oxytocin concentrations were significantly elevated in immunized ewes ($P < 0.01$).

immunizations, oxytocin antibody reached titres comparable to those of ewes receiving both series before the start of Exp. 2.

None of the antisera cross-reacted significantly with arginine vasopressin or isotocin. The serum from only 1 out of 14 ewes cross-reacted with vasotocin (11.25%) and the sera from all but 1 ewe cross-reacted with mesotocin (range 0.4–32%).

Measurement of plasma oxytocin concentrations

The results of the experiment to determine whether C-18 cartridges separate antibody-bound from free oxytocin are shown in Fig. 2. In the presence of high antibody concentrations very little ^{125}I -labelled oxytocin was retained by the columns, but as the antibody concentration was decreased the measured concentration of ^{125}I -labelled oxytocin increased. Separation was slightly improved by the presence of plasma in the samples. These results suggest that, if this method is used

Fig. 5. Changes in the mean $\pm$ s.e.m. concentrations of LH and FSH in control (●—●) and oxytocin-immunized (○---○) ewes after cloprostenol treatment in the mid-luteal phase. Values are shown in relation to the start of the LH surge. Note difference in scale for surge levels of LH. A statistical analysis of these data is given in Table 1.

to extract the blood of immunized ewes (in which the oxytocin antiserum is undiluted), only the free (as opposed to antibody-bound) oxytocin concentrations will be measured.

Oestrous cycle lengths

The lengths of the oestrous cycles for the 1987/8 breeding season were estimated based both on the raddle marks and the progesterone profiles which were in good agreement. The first cycle of the breeding season and those following prostaglandin treatment were excluded from the analysis. The mean cycle lengths for the three groups were: control 16.7 ± 0.17 days (32 cycles from 6 ewes); ovalbumen-immunized 16.8 ± 0.25 days (27 cycles from 5 ewes) and oxytocin-immunized 19.6 ± 0.75 days (60 cycles from 14 ewes). The distribution of cycle lengths (Fig. 3) shows that in oxytocin-immunized ewes 42% of the cycles were extended (> 18 days) with a significant difference in the distribution of the cycle lengths between the oxytocin-immunized and the other two groups ($P < 0.005$). All but one of the ewes had at least one extended cycle, and the one exception (Ewe 8,

Fig. 6. Values of LH (■—■) and FSH (□---□) in 3 individual ewes after cloprostenol treatment (arrow) in the mid-luteal phase: (a) ovalbumen-immunized control ewe; (b) oxytocin-immunized ewe with elevated FSH concentrations; (c) oxytocin-immunized ewe (No. 73) with abbreviated LH surge and no FSH surge.

see Fig. 1) had the lowest antibody titres. However, in the majority of animals extended cycles were interspersed with those of normal length in an irregular fashion.

Experiment 1

None of the hormone measurements (progesterone, oxytocin, LH or FSH) differed between untreated and ovalbumen-immunized groups, and so data from these animals were combined in a single control group for further analysis. All 15 ewes responded to the mid-cycle cloprostenol

Table 2. Pregnancy statistics for the control compared with the oxytocin-immunized ewes

	Control	Oxytocin-immunized	<i>P</i>
No. of ewes	11	14	
No. pregnant* (%)	10 (91%)	4 (28%)	<0.01
No. of CL/ewe	2.4 ± 0.20	2.3 ± 0.12	NS
No. of embryos/ pregnant ewe	2.4 ± 0.20†	2.2 ± 0.25	NS
Ewe wt (kg)	64.6 ± 3.10	68.3 ± 2.43	NS

*Determined at slaughter, 25 days after mating.

†In each of 3 control ewes one of the embryos was degenerating, and so the number of viable embryos/ewe was 2.1 ± 0.23.

treatment by a fall in both progesterone and free oxytocin concentrations (Fig. 4). There was no difference in either the progesterone concentrations or the rate of fall of progesterone between the control and oxytocin-immunized groups. However, free oxytocin concentrations were significantly higher in the oxytocin-immunized ewes ($P < 0.01$) and although oxytocin values fell after treatment they only reached basal values in 2 out of the 8 treated animals.

The gonadotrophin concentrations before and after treatment are shown in Figs 5 and 6 and the results are summarized in Table 1. There was no difference in the peak height of the LH or the concurrent FSH surge or in the timing of the surges relative to the start of treatment in the control or oxytocin-immunized groups. The time of onset of oestrus was also similar in both groups. The three luteal-phase samples collected before cloprostenol treatment contained significantly elevated concentrations of FSH in oxytocin-immunized ewes. Values of both gonadotrophins were also elevated throughout the follicular phase in these animals with P values of 0.021 for LH and 0.054 for FSH. Correlation analysis was performed within the treated ewes between both the free oxytocin concentrations and oxytocin antibody titres and the mean luteal- and follicular-phase FSH and LH concentrations for each ewe. The only significant correlation was between free oxytocin and luteal-phase FSH concentrations ($r = 0.705$, 7 df, $P < 0.05$). It may be of significance that the ewe with the highest oxytocin antibody titre by a considerable margin (Ewe 73, see Fig. 1) was the only one with an abnormally abbreviated LH surge and no detectable FSH surge (Fig. 5c), although the progesterone response was normal and the ovaries contained 2 presumed corpora lutea with no visible abnormalities at laparoscopy.

Experiment 2

There was again no difference between untreated and ovalbumen-immunized ewes, and so these two groups are considered together (Table 2). All but one of the control ewes conceived at a single oestrous period, giving a conception rate of 91%. The one exception was in poor body condition and the ovaries contained 2 luteinized cysts at slaughter. In contrast, only 4 of the oxytocin-immunized ewes conceived (28%, $P < 0.01$), although all received raddle marks at an appropriate stage of the cycle as judged from the progesterone profiles. There were no differences in ovulation rate, number of embryos per pregnant ewe or ewe weight between the control and immunized ewes. There was a tendency for ewes which conceived to have lower oxytocin-antibody titres than those which did not (ranked 4th, 9th, 10th and 13th out of 14), but this was not significant. The progesterone profiles (obtained from twice-weekly sampling) from 6 days before service until slaughter were compared between control ewes, and immunized ewes which did or did not conceive. No differences were apparent until 15–18 days after service when luteolysis began in some of the non-pregnant animals (data not shown). At slaughter (25 days) gross examination and ovarian histology showed that 6 of the non-pregnant ewes had re-ovulated, the ovaries of 3 contained

mature corpora lutea which were undergoing luteolytic changes in one animal, and one ewe had luteinized cysts. Although detailed examination was not performed, there were no obvious histological abnormalities in the ovaries of the oxytocin-immunized ewes.

Discussion

The results of this work confirm previous reports by showing that active immunization against oxytocin increases oestrous cycle lengths. In addition we show that gonadotrophin concentrations tend to be raised in immunized ewes and that immunization results in a significant reduction in the conception rate.

Our results for cycle lengths are similar to those of both Sheldrick *et al.* (1980) and Schams *et al.* (1983) in that about 40% of the cycles were extended to over 18 days. In all three studies there has been some correlation between the oxytocin antibody titres and the degree of extension. These results are consistent with the hypothesis that oxytocin is involved in the initiation of luteolysis in the ewe, and that alteration of oxytocin concentrations can delay this process, presumably by interfering with the feedback loop involving endometrial secretion of prostaglandin F-2 α (McCracken *et al.*, 1981; Flint & Sheldrick, 1983). However, when cloprostenol was given to immunized ewes, there was no difference in either the rate of fall of progesterone or in the timing of the LH surge or oestrus between control and immunized animals, suggesting that, once luteolysis has begun, the normal oxytocin secretion pattern may not be essential for its completion.

The surge release of gonadotrophins was unaffected in 13 out of 14 animals, but the luteal-phase FSH concentrations and the follicular-phase LH concentrations were significantly raised in oxytocin-immunized ewes. Oxytocin is released into the hypophyseal portal vasculature (Gibbs, 1984) and oxytocin has been shown to play a potential role in the regulation of both ACTH (Beny & Baertsghe, 1980; Antoni *et al.*, 1984) and prolactin (Samson *et al.*, 1986) secretion. Several previous studies have examined the possibility that short-term treatment with oxytocin could influence gonadotrophin secretion in rats, cattle and humans, but a substantial majority reported no effect (reviewed by Wathes, 1984) and evidence for a direct action on pituitary gonadotrophin secretion is therefore sparse. An alternative explanation could be that oxytocin is altering some aspect of ovarian function and thus modulating the feedback of another hormone involved in regulating gonadotrophin output. As both LH and FSH were affected an influence on inhibin production seems unlikely and there was also no evidence for an alteration in progesterone production by the corpus luteum in immunized ewes. Another possibility might be oestradiol-17 β , as the work of Adashi & Hsueh (1981) and Adashi *et al.* (1984) in the male rat has indicated that treatment of testicular cells *in vitro* with oxytocin can reduce androgen production, probably by inhibiting the enzymes 17 β -hydroxylase and 17-20-desmolase which convert progesterone to androstenedione. A similar action in the female could limit the amount of substrate available for follicular conversion to oestradiol-17 β and thus reduce circulating oestrogen concentrations. Further data are obviously needed to resolve this point. The differences in gonadotrophin concentrations observed are unlikely to be attributable to differences in cycle length between the groups as the follicular phases compared were induced prematurely by PGF-2 α treatment.

It is well established that active immunization against many hormones increases the total amount of the hormone in the circulation by both increasing the production rate and decreasing the metabolic clearance rate. Accurate measurements of free, and therefore biologically active, hormone concentrations in the circulation of immunized animals are more difficult and are often omitted. Whereas active immunization against testosterone causes atrophy of the accessory reproductive organs, animals immunized against oestrogen generally continue to show some oestrogenic stimulation of the reproductive tract, indicating that the antibodies do not neutralize all the circulating steroid (see Nieschlag & Wickings, 1978, for review of this subject). There has also been a previous suggestion that free steroid levels were raised in both male and female rats actively

immunized against oestradiol-17 β (Hillier *et al.*, 1975). The total oxytocin concentrations in ewes (Sheldrick *et al.*, 1980); Schams *et al.*, 1983) and goats (Cooke & Homeida, 1985) actively immunized against oxytocin were significantly elevated. The results of the present experiment indicate that free oxytocin concentrations were also raised, although this result needs to be interpreted with a degree of caution. Whilst the C-18 cartridge method of separating antibody-bound and free hormone can be shown to work (Schams *et al.*, 1983; present paper) it remains a possibility that, in actively immunized ewes with very high circulating oxytocin values, some dissociation of hormone from antibody could occur between the time of blood collection and application of the plasma to the cartridge. It is, however, likely that the high binding capacity for oxytocin in the blood of the treated ewes would alter the concentration of hormone reaching any target organ at different times by damping any pulses. The normal pattern of major peaks and troughs which occur during luteolysis (Flint & Sheldrick, 1983; Hooper *et al.*, 1986) could therefore be converted into a more constant intermediate level of biologically active hormone. This possibility is supported by the observation that both immunization against oxytocin (Sheldrick *et al.*, 1980; Schams *et al.*, 1983) and constant infusion of oxytocin (Flint & Sheldrick 1985) have the same biological effect, namely prolongation of the cycle.

The final part of the experiment showed that the conception rate in oxytocin-immunized ewes was significantly reduced from 91% to 28%. At present the only established role for ovarian oxytocin is in the control of luteolysis. The treated animals had normal progesterone concentrations and several had prolonged cycles, but in no cases were degenerating conceptuses found at slaughter. This suggests that the loss occurred earlier in the cycle and is unlikely to be related to luteolysis. Although gonadotrophin concentrations were slightly raised, the ovulation rate was unaffected and the ovarian histology indicated that the process of ovulation and luteinization proceeded normally in immunized animals. Another possibility may be that the altered oxytocin-release pattern during the oestrous period affected the motility of the reproductive tract as both the myometrium (Sheldrick & Flint, 1985) and the oviduct (Ayad *et al.*, 1988) of the ewe have high concentrations of oxytocin receptors at this time. The reduction in fertility could therefore have been mediated via interference with gamete or embryo transport. Further experiments are in progress to try to define the critical stage of the cycle at which the anti-fertility effect occurs in order to establish the most likely site of action. The results of this study suggest that oxytocin analogues might be used to prevent conception, and also raise the possibility that inappropriate secretion of oxytocin might be one cause of infertility.

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