The effect of passive immunization against oestrogens on the onset of parturition in the ewe

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Summary. Six ewes were treated twice daily with intravenous injections of antiserum to oestrogens and 3 ewes received normal sheep serum from Day 140 of gestation until parturition. All 9 ewes experienced normal parturitions and produced healthy active lambs after similar gestation lengths. Progesterone and prostaglandin F concentrations in maternal jugular venous plasma were similar in the immunized and control ewes, but total unconjugated oestrogen concentrations were significantly higher in the immunized ewes ($P < 0.005$). It is concluded that immunization of the ewe against total unconjugated oestrogens did not affect the normal occurrence of parturition.

Introduction

The ability of oestrogen to stimulate uterine activity has long been known (see Finn & Porter, 1975) and intramuscular injections of oestrogens can induce parturition in the ewe (Delouis & Terqui, 1974). Before parturition there is a sharp increase in circulating unconjugated oestrogens in the maternal (Challis, 1971; Rawlings & Ward, 1976) and fetal (Rawlings & Ward, 1978) circulation in sheep. At term, uterine activity and circulating oestrogen concentrations are positively correlated (Rawlings & Ward, 1978) and the temporal pattern of circulating oestrogen concentrations is reflected by myometrial oestrogen concentrations (Rawlings & Ward, 1976). However, although the endocrine changes at parturition induced by the infusion of glucocorticoids and adrenocorticotrophin to the intact fetal lamb are similar to those at term (Thorburn, Challis & Robinson, 1977), it has been suggested that the pre-partum rise in oestrogen level is not essential for parturition. This premise was based on the apparent absence of an increase in maternal plasma concentrations of oestrogen when parturition was induced by adrenocorticotrophin treatment of the hypophysectomized ovine fetus (Kendall *et al.*, 1977).

The purpose of the present experiment was to investigate the effect of reducing the maternal plasma levels of unconjugated oestrogens on the onset of parturition in the ewe.

Materials and Methods

Antisera and immunization treatments

Antisera against an oestradiol-17 β hemisuccinate-bovine serum albumin conjugate were raised in 6 cyclic ewes. The ewes were injected intramuscularly with 1–4 mg of the conjugate in 1 ml of an emulsion of saline (0.9%, w/v, NaCl) and Freund's complete adjuvant (Difco, Detroit). Injections were given at monthly intervals and the ewes were bled by jugular venepuncture with a sterile procedure 10–15 days after each injection. Serum from each ewe was stored separately at -15°C until tested for titre and cross-reactions, or used for passive immunization.

From Day 140 of pregnancy until term, 6 ewes received twice daily intravenous injections of 10–20 ml of one of the antisera and 3 animals received equivalent volumes of normal serum from an anoestrous ewe. Blood samples were taken before each treatment. The volume of antiserum

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required for each injection was calculated from the ability of the antiserum to bind [2,4,6,7(n)-³H]-oestradiol-17 β *in vitro* and available estimates of daily production rates of oestradiol-17 β and oestrone in the late pregnant ewe (Bedford, Challis, Harrison & Heap, 1972). The amount of antiserum administered was estimated to be far in excess of that required to bind, at 50% saturation, the quantities of unconjugated oestrone and oestradiol-17 β present in the maternal circulation, e.g. because oestrogen production rises dramatically just before term, a greater volume of antiserum with a high titre was used from Day 143. Because the antisera cross-reacted with oestrone (68%) and oestradiol-17 α (55%), they are referred to as anti-oestrogen antisera. The comparative titres and cross-reactions of the antisera and the antibody titres created in the passively immunized sheep were estimated in the *in-vitro* liquid radioimmunoassay system referred to below. Results are expressed for 100 μ l aliquots, at a dilution of 1:10 000 or 1:1000, with 17 pg [2,4,6,7(n)-³H]oestradiol-17 β added to each assay tube.

Hormone assays

Plasma samples were analysed for progesterone, total unconjugated oestrogens and prostaglandin (PG) F concentrations by established radioimmunoassays using specific antisera after solvent extraction from plasma (Rawlings & Ward, 1978). All estimations were made in duplicate and corrected for procedural losses. Percentage cross-reactions were estimated by the method of Abraham, Odell, Edwards & Purdy (1970). Assay sensitivity was regarded as the smallest quantity of unlabelled hormone capable of significantly displacing labelled hormone from the antiserum.

Total unconjugated oestrogens. The antiserum was raised against an oestradiol-17 β -protein conjugate and had a 50% cross-reaction with oestrone, 38% with oestradiol-17 α , 24% with oestriol and <0.1% with all non-oestrogenic steroids tested. The results are therefore expressed in terms of total unconjugated oestrogens. The percentage extraction ($81.52 \pm 0.68\%$, $n = 162$) of oestradiol-17 β from plasma of the 6 immunized ewes was similar to that from plasma of the 3 untreated ewes. The assay sensitivity was 5 pg/tube ($n = 30$) and the inter-assay coefficient of variation was 16.08 ± 0.86 (s.e.m.)% ($n = 92$). When 20 ($n = 27$), 50 ($n = 50$) or 100 ($n = 9$) pg oestradiol-17 β were added to plasma from cyclic ewes, the values obtained were 22 ± 1 , 49 ± 1 and 89 ± 4 pg, respectively.

Progesterone. The progesterone antiserum showed a 20% cross-reaction with 17 α -hydroxyprogesterone, 6% with 21-hydroxyprogesterone, 1% with 20 α -dihydroprogesterone, 0.5% with testosterone, 0.6% with dehydroepiandrosterone and <0.1% with other steroids tested. The sensitivity of the assay was 10 pg/tube ($n = 30$) and the interassay coefficient of variation was $17.22 \pm 2.01\%$ ($n = 37$). When progesterone was added to plasma at 50 ($n = 7$), 100 ($n = 11$) and 500 ($n = 10$) pg, the corresponding values obtained were 68 ± 11 , 119 ± 11 and 451 ± 17 pg.

PGF. The antiserum was raised against a PGF-2 α -protein conjugate and showed a 32.6% cross-reaction with PGF-1 α and <0.58% with all other PGs tested (PGE-2, 0.58%; PGE-1, 0.43%; PGA-2, 0.05%; PGA-1, 0.01%; PGB-2, 0.01%; PGB-1, 0.008%; 15-keto-PGF-2 α , 0.2%; 15-keto-PGE-2, 0.17%); the results are therefore expressed in terms of PGF (tromethamine salt). The water blank was 3 ± 2.17 pg ($n = 10$) and the assay sensitivity, regarded as the water blank value plus twice its standard deviation, was therefore 10.52 pg/tube. The interassay coefficient of variation was $15.88 \pm 7.54\%$ ($n = 10$), and estimates of 16.6 ± 3.4 , 49.4 ± 0.3 and 78.3 ± 1.4 pg PGF ($n = 3$) were obtained for added amounts of 20, 50 and 100 pg PGF-2 α .

Results

All 9 ewes had normal parturitions and produced healthy active lambs after a mean $\pm$ s.e.m. gestation length of 146.67 ± 0.56 days (6 treated ewes) and 145.00 ± 1.53 days (3 untreated ewes). These means were not significantly different. The patterns of progesterone and PGF concentrations in jugular plasma (Table 1) did not differ, but the concentrations of oestrogen were very different after treatment had started ($P < 0.005$). The percentage binding by plasma of control ewes, and preimmunization samples from treated ewes, never exceeded 4%, a figure similar to the level of non-specific binding normally observed in the liquid radioimmunoassay system employed. However, the binding capacity of plasma from the immunized ewes increased, over the period of treatment, especially following the increased dose after Day 143 of gestation.

Table 1. Concentrations of prostaglandin F, progesterone and total unconjugated oestrogens and percentage binding of [2, 4, 6, 7-(n)³H]oestradiol-17 β in the jugular plasma of 6 ewes passively immunized against oestrogens (from Days -9 to -5 until term) and 3 control ewes treated with normal sheep serum

Time before parturition	Prostaglandin F (ng/ml)		Progesterone (ng/ml)		Total unconjugated oestrogens (pg/ml)		% binding	
	Immunized ewes	Control ewes	Immunized ewes	Control ewes	Immunized ewes	Control ewes	Immunized ewes	Control ewes
Days								
-15	0.28	—	16 $\pm$ 2.1 (3)	—	74 $\pm$ 13 (3)	—	<4 (6)	<4 (3)
-13	—	—	61	—	66	—	<4	<4
-11	—	—	14, 25	28	38, 49	52	<4	<4
-10	0.11	0.20	23	—	81	—	<4	<4
-9	0.12	0.11	28	12	96	50	<4	<4
-8	0.13	—	11, 45	11	49, 83	46	4	<4
-7	—	—	15, 42	12	64, 989	53	14, 18	<4
-6	0.29	0.13	20 $\pm$ 3.5 (5)	10, 16	682 $\pm$ 340 (5)	55, 57	—	<4
-5	0.12, 0.06	—	16 $\pm$ 3.3 (5)	16	1360 $\pm$ 470 (5)	48	19	<4
-4	0.19, 0.08	0.38	20 $\pm$ 3.5 (5)	12	1350 $\pm$ 180 (5)	28	13, 14	<4
-3	0.20, 0.09	—	15 $\pm$ 3.2 (5)	10, 13	1730 $\pm$ 550 (5)	30, 64	14, 29	<4
Hours								
-72	0.46	0.25	18 $\pm$ 5.5 (5)	13 $\pm$ 4 (3)	3670 $\pm$ 2100 (5)	47 $\pm$ 17 (3)	—	<4
-60	0.24 $\pm$ 0.04 (3)	0.13, 0.24	12 $\pm$ 3.0 (5)	9.9 $\pm$ 2.5 (3)	4470 $\pm$ 2130 (5)	53 $\pm$ 23 (3)	—	<4
-48	0.25 $\pm$ 0.05 (5)	0.28 $\pm$ 0.07 (3)	9.5 $\pm$ 1.8 (5)	10 $\pm$ 4.0 (3)	12224 $\pm$ 5000 (5)	45 $\pm$ 16 (3)	26, 33	<4
-36	0.35 $\pm$ 0.06 (5)	0.22 $\pm$ 0.03 (3)	8.3 $\pm$ 1.8 (5)	7.3 $\pm$ 1.6 (3)	16030 $\pm$ 3000 (5)	47 $\pm$ 19 (3)	—	<4
-24	0.40 $\pm$ 0.05 (6)	0.41 $\pm$ 0.11 (3)	6.3 $\pm$ 1.5 (5)	8.5 $\pm$ 3.6 (3)	20640 $\pm$ 4710 (6)	218 $\pm$ 148 (3)	23 $\pm$ 1.3 (6)	<4
-12	0.46 $\pm$ 0.08 (6)	0.52 $\pm$ 0.19 (3)	4.9 $\pm$ 1.1 (6)	6.9 $\pm$ 0.6 (3)	31110 $\pm$ 3770 (6)	410 $\pm$ 178 (3)	—	<4
0	1.35 $\pm$ 0.49 (3)	—	4.1 $\pm$ 1.6 (3)	2.4	35550 $\pm$ 11270 (3)	540	27 $\pm$ 1.7 (6)	<4

Values are mean $\pm$ s.e.m., no. of samples in parentheses.

Discussion

Ferin, Raziano, Tempone & Vandewiele (1970) have demonstrated that passive immunization of rats with an antiserum raised against oestradiol-17 β will effectively neutralize the biological activity of endogenous and exogenous oestrogens. Caldwell, Scaramuzzi, Tillson & Thorneycroft (1970) were able to block cyclic activity in ewes by actively immunizing them against oestradiol-17 β . In the present study, immunization of the preparturient ewe against unconjugated oestrogens had no effect on parturition, although there was a massive rise in total unconjugated oestrogen concentrations in the plasma of the treated ewes. It is presumed that the anti-oestrogen sera bound circulating unconjugated oestrogens with such high affinity as to prevent hepatic removal, metabolism and conjugation.

Although plasma ether-extractable oestrogen concentrations rose in parallel with antisera titre, the calculated dose of antisera was sufficient to neutralize unconjugated oestrogens far in excess of their calculated production rates: 100 μ l aliquots of 1:1000 dilutions of plasma from passively immunized ewes bound 27% of 17 pg labelled oestradiol-17 β at term, indicating spare antibody capacity even at this time.

Because immunization against oestrogens is usually able to block known oestrogen-dependent events and because there was a surfeit of antibodies in the plasma of the passively immunized ewes in the present study, it is suggested that unconjugated oestrogens in the maternal blood compartment are not involved in the initiation of parturition in the ewe and that any effect of placental unconjugated oestrogen on the myometrium probably involves a local tissue transfer. Oestrogen is produced primarily by the placenta during pregnancy in the ewe, and conjugated as well as unconjugated oestrogens are secreted (Pierrepont, Anderson, Harvey, Turnbull & Griffiths, 1971). Oestradiol-17 β (Challis, Harrison & Heap, 1973) and oestrone (Findlay & Seamark, 1973) are rapidly and extensively conjugated after intravenous injection into the pregnant ewe (67–74% and 76% respectively). Oestrogen sulphates may have oestrogenic effects on the uterus (Pierrepont *et al.*, 1971), but in the ovariectomized ewe oestradiol benzoate appears to be 20 times more effective in stimulating uterine motility than oestrone sulphate (unpublished results of J. Prud'Homme, cited by Bosc, Delouis & Terqui, 1977). The present experiment investigated only the involvement of unconjugated oestrogens in the maternal circulation and the antisera used would probably not have bound any oestrogen conjugates (Boilert, Edqvist, Johannsson, Lindberg & Martinsson, 1973).

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