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THE INFLUENCE OF STEROID HORMONES ON THE UTERINE CERVIX DURING PREGNANCY

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Summary—This paper reviews the evidence concerning the actions of steroid hormones on the connective tissues of the pelvis. Most available data concern the effects of steroids on the cervix. The time course of cervical softening in rats, sheep and humans suggests the possibility that the changes in connective tissue biochemistry that underlie the physiological phenomenon of cervical softening are under hormonal control. Both oestrogens and progestogens have been implicated in the control of cervical softening. However, recent experiments using inhibitors of 3β-hydroxysteroid dehydrogenase suggest that cervical softening can be produced in both sheep and humans by progesterone withdrawal in the absence of high circulating concentrations of oestradiol-17β.

INTRODUCTION

The physiological changes which prepare for birth are complex. The uterus, which must remain quiescent throughout pregnancy becomes capable of producing co-ordinated contractions of sufficient force to expel the fetus. However, in addition, the birth canal must become distensible to permit the fetus to pass through into the world without excessive trauma. To facilitate this, changes in the structure of the connective tissues which form the canal must take place prior to delivery. These changes are most obviously seen in the uterine cervix and it is this tissue which has figured most prominently in scientific studies of connective tissue re-modelling and parturition. However, it is important to point out that biochemical changes of equal significance must also take place in other structures such as the uterus, amnion and pelvic ligaments as shown years ago by Harkness and Harkness[1].

Although the anatomy of the cervix varies considerably between species, all mammals have a seal at the entrance to the uterus which serves to retain the fetus during pregnancy. At term, the circumference of this structure increases rapidly and concurrently with the increase in uterine activity. However, cervical dilatation is merely the final, if most obvious stage of a remodelling process which takes place throughout late pregnancy. This is most apparent in the human, where the consistency of the cervix has been shown to alter progressively during the last trimester [2] and in the rat, where cervical softening occurs throughout the last quarter of pregnancy [3]. Events may be more rapid in the sheep [4, 5], but it is clear that in each species there are pre-labour changes in the structure of the cervix and pelvic ligaments which permit delivery to take place. This paper examines the evidence for the involvement of steroid hormones in the control of these changes.

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OESTROGENS

There is a close temporal relationship between the preterm rise in the circulating concentration of oestrogens seen in rats [6, 7], and sheep [8, 9] and the changes in cervical mechanical properties recorded in these species [3, 5, 10]. However, attempts to produce increases in cervical extensibility experimentally by treatment with oestrogens have produced conflicting results. Treatment with oestradiol benzoate does not increase the extensibility of the cervix of the late pregnant rat [3], and although oestrogens will increase the weight of the cervix of the non-pregnant ovariectomised rat, this is not associated with any increase in the internal diameter of the cervix [11–15] or in the rate of creep, a measure of extensibility [14]. Such studies suggest that oestrogens may be involved in the growth of the cervix in late pregnancy, but not in cervical softening in the rat.

Contrasting evidence has been obtained in the sheep. In this species, s.c. injection of diethylstilboestrol during late pregnancy was shown to produce a transient increase in cervical compliance as measured *in vivo* using a water-filled balloon lying in the cervical lumen [16]. The ovine cervix can also be softened by i.v. infusion of oestradiol-17β [17].

Intravenous oestradiol-17β has also been shown to produce a degree of cervical ripening in pregnant women at term [18] and extra-amniotic administration of a gel containing oestradiol valerate also caused cervical ripening when assessment was made

18 h later [19]. However, although these authors found that treatment with oestradiol via the extra-amniotic route caused little increase in uterine activity, a later study of this technique [20] found that extra-amniotic administration of oestradiol valerate only altered the cervical score in those patients in whom labour became established. Intra-amniotic injection of oestriol valerate has also been shown to be ineffective as an agent for cervical ripening, although the assessment of cervical score was made only 4 h after administration [21]. Comparison of oestrogens with prostaglandins in clinical studies on cervical ripening at term have shown both agents to be of approximately equal efficiency [22, 23]. However, oestradiol given vaginally has no effect on the cervix earlier in pregnancy [24].

Intravenous injection of dehydroepiandrosterone sulphate (DHAS) also produces changes in the state of the cervix in late pregnant women. This treatment has been associated with increased levels of oestradiol-17β in biopsy specimens of human cervix taken during late pregnancy as well as with an increased production of collagenase by cervical explants [25–27] and release of oestrogens from cervical biopsy material collected during labour [28]. When used as an agent for cervical softening, the interval between the beginning of the DHAS treatment and delivery was again shortened, particularly in the primiparous group in which the greatest effect on the cervix was seen. Thus DHAS may also be acting indirectly on the cervix by way of an increase in myometrial contractility.

Given the seemingly unavoidable problem of the induction of uterine contractions as well as cervical softening by treatment with oestrogens in late pregnancy, it is necessary to consider evidence drawn from other sources in order to determine the part played by oestrogens in the control of cervical softening. Low circulating concentrations of oestradiol-17β have been associated with the failure of intra-vaginal prostaglandin E_2 (PGE_2) treatment for cervical softening prior to induction of labour [29]. There was no similar relationship seen with progesterone or prolactin. Thus oestrogens might be involved in modulating the response of the cervix to ripening agents. This suggestion is supported by the finding that some pregnancies complicated by placental sulphatase deficiency and low oestriol excretion may result in a failure of the cervix to ripen at term [30–32]. However, delivery by caesarean section was usually only necessary in primigravidae and subsequent studies have identified certain patients with placental sulphatase deficiency who were able to deliver vaginally after labour of spontaneous onset [33] indicating the absence of any significant degree of cervical dystocia.

The contradictions posed by the data presented above might be resolved by studies of the receptor populations of the cervix during pregnancy. The non-pregnant human cervix has been shown to contain specific oestrogen-binding proteins, the numbers of which may increase in response to oral contraceptive regimes, although they do not vary during the menstrual cycle [34–36]. The identification of receptors for oestrogens within tissue taken from the cervix during pregnancy would suggest that this group of steroid hormones might have some direct action on the cervix during labour.

PROGESTAGENS

The theory that progesterone withdrawal is the key event in the initiation of parturition has been the subject of considerable interest since it was first proposed by Csapo in 1956 [37]. Although most studies have concentrated on the relationship between progesterone concentrations and myometrial contractility the possible involvement of progesterone in the control of cervical softening has also been examined.

Results of early studies were mostly negative. In the rat, the increase in cervical weight in response to treatment with oestrogen or relaxin was not reduced by concurrent treatment with progesterone [13–15]. Neither administration of progesterone to late pregnant rats nor withdrawal of progesterone from the hormone replacement regime used to maintain pregnancy following ovariectomy caused any increase in cervical extensibility [3]. The increase in cervical compliance seen in sheep after induction of labour could not be inhibited by treatment with progesterone [5] although uterine activity was effectively abolished by the progesterone treatment. Although relatively high circulating concentrations of progesterone have been implicated in the lack of success of infusions of prostaglandins for softening the ovine cervix [38], we have shown that it is possible to alter cervical extensibility in late pregnant sheep in the presence of high concentrations of progesterone in peripheral plasma [39]. In this study, cervical softening was induced in chronically catheterised late pregnant sheep by direct infusion of prostaglandin E_2 (PGE_2) into a cervical artery. Assessment *in vitro* showed a significant increase in cervical extensibility after treatment with PGE_2 in comparison with saline infusion (Fig. 1). Measurements of the concentrations of progesterone in jugular venous plasma showed that these levels remained within the normal range for late pregnancy in the sheep (Fig. 2).

The effects of progesterone on the human cervix are poorly understood. Progesterone treatment during early and mid-pregnancy has been shown to be ineffective in preventing abortion and premature labour [40–42], suggesting that neither myometrial contractility nor cervical ripening can be inhibited by progesterone at this time. However, progesterone

Effect of PGE_2 on the Cervix of the Pregnant Ewe

Results

	Infusion	
	PGE	Saline
	(Mean ± SEM)	
Initial circumference (mm)	41.2 ± 3.6*	27.9 ± 2.7
Gradient (mm min^{-1})	0.32 ± 0.05**	0.08 ± 0.01
Extensibility	8.6 ± 1.8*	2.84 ± 0.3
Wet weight (g)	30.6 ± 3.5	24.8 ± 1.4
% Water	82.5 ± 0.9	80.7 ± 1.7

* $p = < 0.02$

** $p = < 0.002$

Fig. 1. Properties of the cervix of late pregnant ewes after infusion of PGE_2 (5 mg/24 h) or saline into a cervical artery for 48 h.

treatment has been found to diminish collagen breakdown in cervical explants from non-pregnant women [43] and also in parturient rat uterus [44] and guinea-pig pubic symphysis [45]. Specific binding of progesterone has been measured in specimens of non-pregnant human cervix [35], indicating that the cervix may be a target organ for this hormone. However, there are no reports of progesterone receptors being identified in cervical tissue from pregnant women.

PROGESTERONE WITHDRAWAL BY INHIBITION OF 3β-HYDROXYSTEROID DEHYDROGENASE

Until recently it has not been possible to study the effect of progesterone withdrawal *in vivo* without initiating a concomitant rise in the circulating concentration of oestrogens. The development of inhibitors of 3-hydroxysteroid dehydrogenase (3β-HSD) has allowed these two endocrine events to be separated. Treatment with such inhibitors has been shown to cause a precipitate fall in the concentration of progesterone in peripheral plasma both in sheep [46, 47] and humans [48]. Progesterone withdrawal in sheep was followed by labour, with delivery occurring approximately 32 h after injection of the drug [46]. Circulating concentrations of a metabolite of prostaglanding $F_2\alpha$ ($PGF_2\alpha$) increased during labour but concentrations of oestradiol-17β were at most only slightly above the normal low values seen in late pregnancy in sheep.

Concentration of Progesterone (ng/ml) in Maternal Jugular Venous Plasma Immediately Before and After Infusion of PGE_2

Ewe	Time (h)	
	0	48
1	27.5	25.3
2	26.2	23.7
3	20.3	18.7
4	11.5	6.7
5	5.6	11.3
6	2.7	5.5

Fig. 2. Concentrations of progesterone in jugular venous plasma collected immediately before and immediately after infusion of PGE_2 or saline.

These results suggested that increases in prostaglandin synthesis could occur without an increase in circulating concentrations of oestradiol-17β, supporting the "progesterone block" hypothesis. We have recently used an inhibitor of 3β HSD, Epostane (Sterling Winthrop Ltd), to investigate the effects of progesterone withdrawal on cervical softening in the pregnant ewe [49]. Catheters were surgically implanted in 8 ewes approximately 30 days before term. This allowed longitudinal sampling of blood from the maternal peripheral and utero-ovarian veins. A catheter lying within the uterine cavity was used to record changes in intra-uterine pressure during the experiments. After a recovery period each ewe received an i.v. injection of Epostane (100 mg). One-half of the animals were also given a continuous infusion of mefenamic acid (62.5 mg/h) (Warner Lambert, Parke Davis) for the duration of the experiment. Measurements made on plasma collected from the utero-ovarian vein showed that injection of Epostane produced a rapid but transient fall in the concentration of progesterone (Fig. 3). This was not accompanied by any increase in the concentration of oestradiol 17-β. Measurements of 11-deoxy-13, 14-dihydro-15-oxo-11β, 16ε-cyclo PGE_2 (bicyclo-PGEM) and 13,14-dihydro-15-oxo-$PGF_2\alpha$ (PGFM), stable circulating metabolites of PGE_2 and $PGF_2\alpha$ in plasma [50–52] showed that

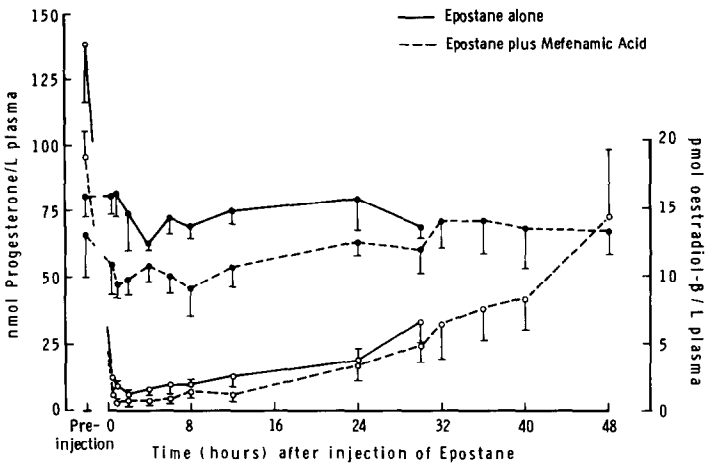

Fig. 3. Concentrations of progesterone and oestradiol-17 β in utero-ovarian venous plasma immediately before and at intervals after injection of Epostane (100 mg) alone (solid lines, $n = 4$) or in combination with continuous infusion of mefenamic acid (broken lines, $n = 4$). Values are mean ± SEM. *$2P < 0.02$ compared with mefenamic acid values measured at the same time (Mann–Whitney rank sum test).

progesterone withdrawal was followed by significant increases in the concentrations of both metabolites in utero-ovarian vein plasma (Fig. 4). Treatment with mefenamic acid abolished much of these increases. Mefenamic acid also inhibited the marked increases in uterine activity seen after Epostane treatment. Measurements made *in vitro* at the end of each experiment showed that withdrawal of progesterone by inhibition of 3β-HSD caused substantial increases in cervical extensibility (Fig. 5). These were not seen in the mefenamic-acid-treated group. Thus it would appear that cervical softening can be produced in sheep by progesterone withdrawal in the absence of high circulating concentrations of oestradiol-17β.

Clinical studies using inhibitors of 3β HSD have shown that progesterone withdrawal in early pregnancy will produce abortion, possibly by increasing myometrial sensitivity to prostaglandins [53, 54]. Although no measurements of cervical ripening were made it seems likely that in this situation the human cervix became softened and dilated in the absence of increases in plasma oestrogens. Studies with inhibitors of 3β-HSD therefore suggest that progesterone withdrawal may be a key event in the initiation of cervical softening. However, it may be that oestrogens facilitate the recovery of the cervix from the suppressive action of progesterone, rendering it susceptible to the effects of the prostaglandins and relaxin.

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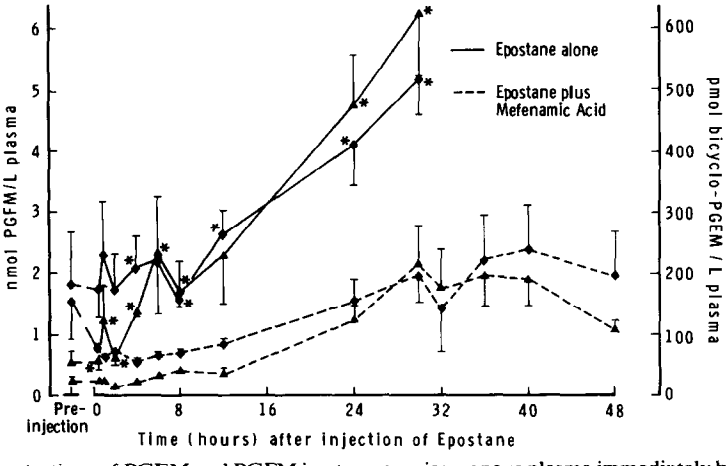

Fig. 4. Concentrations of PGEM and PGFM in utero-ovarian venous plasma immediately before and at intervals after injection of Epostane (100 mg) alone (solid lines, $n = 4$) or in combination with mefenamic acid (broken lines, $n = 4$). Values are mean ± SEM. *$2P < 0.02$ compared with mefenamic acid values measured at the same time. (Mann–Whitney rank sum test).

Cervical extensibility following treatment with epostane (E) alone, or with mefenamic acid (E + MF)

Animal	Treatment	Duration of experiment (h)	Cervical extensibility (min^{-1}) at sacrifice
1	E	32	16.0
2	E	33	14.5
3	E	35	13.6
4	E	32	10.8
5	E + MF	42	6.9
6	E + MF	48	6.6
7	E + MF	48	3.5
8	E + MF	48	4.0

Fig. 5. Experimental data on ewes treated with Epostane (Group A) or Epostane and mefenamic acid (Group B).

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