Changes in Progesterone, Oestradiol 17 β , and Intrauterine Prostaglandin E₂ during Late Gestation in Sheep Experimentally Infected with an Ovine Abortion Strain of *Chlamydia psittaci*

By H. A. LEAVER,¹* A. HOWIE,¹ I. D. AITKEN,² B. W. APPELYARD,²
I. E. ANDERSON,² G. JONES,² L. A. HAY,² G. E. WILLIAMS² AND
D. BUXTON²

¹Department of Pharmacology, University of Edinburgh, Edinburgh EH8 9JZ, UK, and
Blood Transfusion Centre, Lauriston Place, Edinburgh EH3 9HB, UK

²Moredun Research Institute, Edinburgh EH7 7JH, UK

(Received 22 June 1988; revised 3 November 1988; accepted 16 November 1988)

The placenta is the primary site of infection of *Chlamydia psittaci* and is also intimately involved in the control of parturition. Changes in the pattern of placental hormone secretion were investigated in ewes infected with *C. psittaci* and in saline-injected controls. The concentration of progesterone in peripheral plasma of infected sheep was significantly lower than in control sheep ($P < 0.01$). A gradual decline in plasma progesterone occurred in *Chlamydia*-infected sheep, beginning on day 125 of gestation, in comparison with the sharper decline commencing on day 139 of gestation in the control population. The release of oestradiol 17 β , which was greatest on the day of parturition in control sheep, was significantly ($P < 0.02$) increased on the day before parturition in *Chlamydia*-infected sheep. The concentrations of prostaglandin E₂ in amniotic and allantoic fluids were low during late pregnancy in 12 control sheep, but were significantly raised ($P < 0.05$) in four out of 12 samples obtained from *Chlamydia*-infected sheep over the same period. The changes in progesterone and prostaglandin E₂ were temporally related to the morphological and histochemical changes characteristic of trophoblast infection. These findings suggest that *C. psittaci* infection may precipitate premature labour by altering placental steroid and prostaglandin release.

INTRODUCTION

Premature labour and abortion are major complications associated with *Chlamydia psittaci* infection of sheep (Studdert & McKercher, 1968; Novilla & Jensen, 1970). Ovine abortion strains of *C. psittaci* have been demonstrated to infect women and are associated with abortion in these subjects (Roberts *et al.*, 1967; Beer *et al.*, 1982; Johnson *et al.*, 1985). In both sheep and humans, the perinatal complications caused by *C. psittaci* appear to be more severe than those associated with *Chlamydia trachomatis*, but the pathophysiology of both infections is similar with respect to the site and timing of infectivity and the effect of initiating premature labour (Sweet *et al.*, 1987; Studdert, 1968). The widespread distribution of chlamydial infection in sheep has been known for many years (McEwen *et al.*, 1951; Studdert & McKercher, 1968). However, recent applications of monoclonal antibodies and gene probe techniques have indicated a much wider distribution of chlamydial infection than had been previously suspected in the human population (Sweet *et al.*, 1987).

The endocrinology of infectious abortion has been the subject of intermittent attention (Smith & Hughes, 1974; Roberts *et al.*, 1975; Minkoff, 1983; Lamont *et al.*, 1985; Helm *et al.*, 1987).

Abbreviations: ELD₅₀, egg LD₅₀; PGE₂, prostaglandin E₂.

However, there has been little investigation of the effects of chlamydiae on the factors which play a role in controlling parturition (Martel *et al.*, 1983; Rank *et al.*, 1982; Fredriksson *et al.*, 1988). This is surprising, since pathological studies indicate that the infectivity of chlamydiae is closely related to the stage of pregnancy, and disorders in the initiation of parturition result from this infection.

The primary focus of chlamydial infection during pregnancy is the placenta. Three placental products are important in the control of parturition, viz. progesterone, oestradiol 17 β and prostaglandin E₂ (PGE₂). Progesterone is important in the maintenance of pregnancy (Bedford *et al.*, 1972; Taylor *et al.*, 1982), and progesterone synthesis during late pregnancy in both sheep and women occurs in the placenta (Linzell & Heap, 1968). The placenta is also a major source of oestrogens in these species. The secretion and distribution of oestradiol 17 β during gestation, and its effect on prostaglandin synthesis, have been characterized (Allen, 1975; Liggins *et al.*, 1972). Plasma progesterone and oestradiol 17 β concentrations are indicators of the placental production and secretion of these steroids. The concentrations of oestradiol 17 β in the amniotic fluid and the utero-ovarian vein reflect the intrauterine production and diffusion of oestradiol 17 β .

Changes in the concentrations of oestradiol 17 β and progesterone influence the release of PGE₂ at parturition (Thorburn & Challis, 1979; Taylor *et al.*, 1982; Olson *et al.*, 1984). The primary site of placental infection of *C. psittaci* is the chorionic membrane, which is also a major site of placental PGE₂ synthesis. PGE₂ is also released in significant quantities by leucocytes, particularly by inflammatory macrophages (Lewis, 1983). The prostaglandins exert a range of effects within the uterus at parturition. Prostaglandins act on the myometrium, where they stimulate and co-ordinate myometrial contraction (Wickland *et al.*, 1984). In the cervix, prostaglandins induce ultrastructural changes resulting in an increase in cervical patency (Keirse *et al.*, 1983). Stimulation of the chorionic membrane causes release of arachidonic acid and prostaglandin E₂ from the foetal membranes (Grieves & Liggins, 1976). A role for prostaglandin E₂ in initiating labour has been proposed (Bleasdale & Johnston, 1984). The secretion of prostaglandin E₂ and its metabolites increases during late pregnancy, and prostaglandin E₂ is synthesized in greater quantities than prostaglandin F_{2 α} during early labour (Dray & Frydman, 1976). The intrauterine concentrations of PGE₂ indicate the locally active concentrations of this metabolically labile compound close to its site of action.

In this study, the relationship between the timing of changes in placental hormone metabolism and morphological and histochemical changes in the placenta during chlamydial infection was analysed in order to establish the sequence of pathophysiological events associated with premature labour.

METHODS

Experimental infection of sheep. Twenty-two Scottish Blackface ewes were infected by subcutaneous inoculation with between 0.45×10^6 and 1.6×10^6 egg LD₅₀ (ELD₅₀) of ovine abortion strain S26/3 of *C. psittaci* between days 90 and 115 of gestation. Twenty-three pregnant ewes were used as controls. The number of foetuses was determined in all animals between days 60 and 80 of gestation using ultrasonography, and at birth. These experiments were part of a study of the pathogenesis of *C. psittaci* infection during pregnancy, as this is a serious cause of mortality in lambs and morbidity (infectious abortion) in pregnant sheep. In addition, *Chlamydia* infection has been demonstrated to be a cause of abortion in pregnant women in contact with sheep. The study was reviewed by the Ethics Committee of the Institution where the experiments were conducted before commencement.

Peripheral plasma samples for progesterone assays. Six ewes were infected on day 90 of gestation by subcutaneous injection with 5×10^5 ELD₅₀ of *C. psittaci* strain S26/3, and six control ewes were injected with sterile saline. The mean duration of gestation $\pm$ SE in control sheep was 144.7 ± 1.08 d ($n = 6$), range 144–149 d, and in *C. psittaci*-infected sheep, 137 ± 1.6 d ($n = 6$), range 132–142 d. Only sheep with single lambs were used, as twin pregnancies are associated with higher progesterone levels, which are not directly proportional to the number of lambs (Bedford *et al.*, 1972; H. A. Leaver, unpublished observations). Blood was withdrawn at 3 d intervals from the jugular vein of infected and control sheep up to day 110 of gestation and at 2 d intervals after day 110, and placed into tubes containing 2 IU preservative-free heparin ml⁻¹ (Evans Medical). Plasma was prepared by centrifugation of the heparinized sample at 2000 g for 10 min. All plasma samples were stored at -40°C .

Progesterone concentrations were expressed as the means $\pm$ SE of results from samples of plasma taken from three to six individual control or infected sheep on the same day of gestation.

Progesterone radio-immunoassay. Peripheral plasma progesterone was extracted using ethyl acetate (efficiency $71 \pm 3\%$). Progesterone was determined by a radio-immunoassay using the antiserum and technique of Scaramuzzi *et al.* (1974), and $[1,2,6,7,16,17\text{-}^3\text{H}]$ progesterone radiotracer (Amersham, batch no. 10/H/4723). Antibody-bound progesterone was precipitated using dextran charcoal. The precision of progesterone determination was 10.2% for within-assay duplication (inter-assay coefficient of variation), and 9.6% for between-assay replication (intra-assay coefficient of variation), for two plasma samples analysed six times within the same assay ($n = 6$), and two plasma samples analysed in six different assays ($n = 6$), respectively (Hunter, 1978).

Peripheral plasma samples for oestradiol 17β assays. Oestradiol 17β was analysed in peripheral plasma samples collected for progesterone analysis (see above), and in peripheral plasma samples from a second group of animals, consisting of four ewes infected on day 113–115 of gestation by subcutaneous injection with 0.5×10^6 ELD₅₀ of *C. psittaci* strain S26/3, and five control ewes injected with sterile saline. This second group were catheterized 2 d after injection, on day 115–117 of gestation, for intrauterine sampling (see below). The mean durations of gestation $\pm$ SE in this second group were 143.8 ± 1.1 d ($n = 5$), range 141–147 d, in control sheep and 139.8 ± 1.1 d ($n = 4$), range 137–141 d, in *C. psittaci*-infected sheep. Two of the control ewes had twins, and three had single lambs. All seven lambs survived. Two of the infected ewes bore twins, and two bore single lambs. Two out of six lambs of the *C. psittaci*-infected sheep were born dead: one of twin lambs was dead on delivery, and another, single lamb, died of asphyxia during delivery. In group two, blood was withdrawn from the jugular vein at 24 h intervals during the last 3 d of gestation, and placed into tubes containing 2 IU preservative-free heparin ml⁻¹. Plasma was prepared by centrifugation (see above) and stored at -40°C . Oestradiol 17β concentrations were expressed as pg ml⁻¹ $\pm$ SE in the plasma of control or infected sheep, sampled on separate days before parturition.

Utero-ovarian venous plasma, amniotic fluid and allantoic fluid samples from catheterized animals for oestradiol 17β assays. The intrauterine distribution of oestradiol 17β during chlamydial infection was analysed in the six control sheep injected with saline, and in the six sheep infected with 5×10^5 ELD₅₀ of *C. psittaci* strain S26/3 on day 113–115 of gestation, whose peripheral plasma oestradiol 17β was determined. This third group consisted of the sheep used for peripheral plasma oestradiol (see above), plus two additional infected sheep and one additional control sheep. The mean durations of gestation were 144.5 ± 1.1 d ($n = 6$), range 141–148 d, in the control group, and 141.3 ± 1.8 d ($n = 6$), range 137–141 d, in the infected group. In the control group, three of the ewes had twins and three had single lambs, and in the infected group, four of the ewes bore twins, and two bore single lambs. In the *C. psittaci*-infected group, two out of ten lambs were born dead: one of twin lambs was dead on delivery, and another, single lamb, died of asphyxia during delivery. In the control group, all nine lambs survived.

Ewes were implanted with indwelling catheters into the amniotic and allantoic cavities and into the utero-ovarian vein on day 115–117 of gestation (Mellor, 1980). Amniotic and allantoic sacs of each foetus were catheterized using Foley two-way balloon catheters (size 12 Ch, with 30–40 ml balloon; Eschmann, Sussex, UK). Small samples (0.5–2 ml) of amniotic and allantoic fluids were withdrawn using minimal suction at 24 h intervals from day 137 of gestation in control sheep and from day 133 of gestation in infected sheep. Sterility within each two-way tap was maintained by twice-daily flushing with thiomersal solution, consisting of thiomersal (BDH; 0.1% in ethanol)/acetone/ethanol (1:500:500, by vol.). Amniotic and allantoic fluids were placed immediately into 10 ml of 'analytical-reagent'-grade methanol, and stored at -40°C .

A utero-ovarian vein was also catheterized using 1.4 mm external diameter vinyl tubing (Portex Ltd). A two-way luer stopcock was attached to each catheter. Vascular catheters were sampled daily and flushed with a heparin saline solution (80 IU preservative-free heparin ml⁻¹) twice daily. Blood was placed into tubes containing 2 IU preservative-free heparin ml⁻¹; plasma was prepared by centrifugation (see above), and stored at -40°C .

Oestradiol 17β radio-immunoassay. Oestradiol 17β radio-immunoassay was carried out on samples taken from amniotic fluid, allantoic fluid, the utero-ovarian vein, and peripheral plasma, using a kit (Steranti Research; batch no. S703). The addition of up to 50 μ l of fluids did not significantly change the binding curve of the anti-oestradiol serum, unless methanol was present. The aqueous-methanol amniotic and allantoic fluid samples were taken to dryness, and resuspended in 50 μ l standard human serum containing <0.01 pg oestradiol ml⁻¹. The anti-oestradiol serum was raised in rabbits, and the secondary precipitating goat anti-rabbit IgG was coupled to a solid phase. The accuracy of the assays was controlled by using human serum with three known oestradiol concentrations, viz. 27.7 ± 3.68 , 60.2 ± 6.60 and 176 ± 13.1 pg ml⁻¹. The sensitivity of the assay was 3.1 pg oestradiol ml⁻¹ at 2.5 standard deviations from the mean, and the inter-assay and intra-assay coefficients of variation were 11.4% and 6.83%, respectively ($n = 6$).

Amniotic and allantoic fluid samples for PGE₂ assays. A fourth group of animals was used in these experiments. Twelve ewes were infected on day 90 of gestation by subcutaneous injection with 0.45×10^6 ELD₅₀ of *C. psittaci* strain S26/3; 12 control ewes were injected with saline. Four ewes (two infected animals and two controls) were killed on each of the following days of gestation: 97, 103, 109, 115, 120 and 125. Amniotic fluid, allantoic fluid, and placental tissues were removed at necropsy. Amniotic and allantoic fluids were withdrawn, using a syringe and

gauge 16 needle, during aseptic delivery of foetuses 5 min (range 3–8 min) after killing of the ewes. Care was taken during sampling to withdraw fluid as far as possible from the site of the puncture, as PGE₂ may be released during rupture of foetal membranes, or from blood or endothelial cells (Leaver *et al.*, 1988). Blood-stained or meconium-stained samples were discarded (Leaver *et al.*, 1988). Three allantoic and two amniotic samples from the control group, and one allantoic and two amniotic samples from the infected group, were rejected on this basis. All samples (3–10 ml) were placed immediately in methanol (10 ml), and stored at -40°C prior to PGE₂ radioimmunoassay. In both control and *C. psittaci*-infected ewes, five animals were carrying single lambs, and seven had twins. The local PGE₂ concentrations in amniotic and allantoic fluids of individual foetuses were analysed. The PGE₂ concentrations of individual foetal sacs were expressed as ng per ml of amniotic fluid or allantoic fluid on day 97–125 of gestation.

Prostaglandin E₂ radio-immunoassay. Prostaglandin E₂ radio-immunoassay was carried out on samples of amniotic and allantoic fluids, either taken to dryness and resuspended in buffer, or extracted using ethyl acetate. There was no significant difference in the PGE₂ concentration in ethyl acetate-extracted and in methanol-treated samples, after correcting for the efficiency of extraction. Addition of amniotic or allantoic fluid to PGE₂ standards did not influence the binding curve of the anti-PGE₂ antiserum. Radio-immunoassay of PGE₂ was carried out using anti-PGE₂ antiserum (Institut Pasteur, Paris; batch no. D7), and [5,6,8,11,12,14,15(n)-³H] PGE₂ radiotracer (Amersham; batch no. 60) under previously described conditions (Leaver *et al.*, 1987). Antiserum was precipitated using donkey anti-rabbit IgG donated by the Scottish Antibody Production Unit, Carlisle, Lanarkshire, UK. The precision of PGE₂ determination was 12.6% for within-assay duplication, and 9.6% for between-assay replication ($n = 6$).

Pathology of placentae. Placentae were removed from 12 infected and 12 control ewes (fourth group of animals used for PGE₂ assays). Two infected and two control sheep were killed on each of the following days of gestation: 97, 103, 109, 115, 120, 125. Six cotyledons from each uterine horn bearing a foetus were examined in paraffin section, stained by haematoxylin and eosin. *C. psittaci* inclusions were identified using an immunoperoxidase method (Finlayson *et al.*, 1985).

Statistics. Results were expressed as mean $\pm$ standard error (SE) of the mean for n determinations. The normality of distribution of each group being tested was analysed using the standard score (z test), and the statistical significance of differences between paired and unpaired data was analysed using the paired and unpaired Student's t -tests, respectively (Moroney, 1951). The unpaired t -test incorporated the Behrens Fisher statistic and, therefore, did not assume that the variances of the population groups being compared were the same.

RESULTS

Plasma progesterone in sheep infected with C. psittaci

The mean peripheral plasma progesterone concentrations of six control sheep and of six sheep experimentally infected with *C. psittaci* on day 90 of gestation are shown in Fig. 1. Parturition

Fig. 1. Progesterone in peripheral plasma of control and *C. psittaci*-infected sheep. Ewes were infected by subcutaneous injection on day 90 of gestation with 5×10^5 ELD₅₀ of *C. psittaci* strain S26/3. The filled arrow indicates the mean day of parturition in infected, and the open arrow, in control ewes (the mean duration of gestation in control sheep was 144.7 ± 1.08 , $n = 6$, and in *C. psittaci*-infected sheep, 137 ± 1.6 , $n = 6$). All animals had a single lamb. Progesterone concentrations are the means of three to six determinations of plasma progesterone from individual control (○) or infected (●) sheep on separate days of gestation.

Table 1. Oestradiol 17 β in peripheral plasma, utero-ovarian venous plasma and amniotic fluid of *C. psittaci*-infected and control sheep

Sample	Sheep	Oestradiol 17 β concentrations (mean $\pm$ SE pg ml ⁻¹) on indicated days before parturition†		
		-2	-1	0
Peripheral plasma	Control (n = 5)	9.15 $\pm$ 2.60	21.7 $\pm$ 5.70*	63.1 $\pm$ 10.3
	Infected (n = 4)	16.6 $\pm$ 0.80	56.3 $\pm$ 7.05*	26.4 $\pm$ 15.9
Utero-ovarian venous plasma	Control (n = 6)	10.1 $\pm$ 0.58	18.3 $\pm$ 1.82	29.5 $\pm$ 7.36
	Infected (n = 6)	6.37 $\pm$ 0.17	12.4 $\pm$ 4.90	32.0 $\pm$ 5.31
Amniotic fluid	Control (n = 6)	5.86 $\pm$ 0.86*	5.67 $\pm$ 0.87*	9.31 $\pm$ 0.96
	Infected (n = 6)	17.6 $\pm$ 5.28*	12.9 $\pm$ 3.07*	24.3 $\pm$ 17.3

* Indicates significant differences between control and infected groups ($P < 0.02$), using the Student's unpaired *t*-test.

† The concentrations of oestradiol 17 β in sheep infected subcutaneously with *C. psittaci* (strain S26/3, 5×10^5 ELD₅₀), and of control ewes injected with sterile saline.

was significantly ($P < 0.05$) earlier in the infected group. The profiles of progesterone release in the two populations showed the following differences. There was a greater variation in the concentration of plasma progesterone in infected animals. The mean plasma progesterone concentration in infected animals, compared on eight days between days 100 and 130 of gestation, was $80 \pm 5.3\%$ of the mean plasma progesterone concentration in control animals. The concentration of progesterone in the plasma of control sheep dropped sharply during the 8 d before parturition, while that of infected sheep showed a more gradual decline, commencing 13 d before parturition, on day 125 of gestation. The difference between the mean progesterone concentrations, paired between day 100 and day 144 of gestation and analysed using the paired Student's *t*-test, was highly significant ($P < 0.01$, no. of paired concentrations = 22). The difference between the curves was significant well before delivery: when plasma progesterone levels from individual infected sheep were paired randomly with progesterone concentrations in controls on the same day of gestation, and were compared between days 125 to 135 of gestation, using the paired *t*-test, a significant difference was observed ($P < 0.05$, no. of paired concentrations = 9). The progesterone concentrations in infected and control sheep were also analysed in relation to the day of parturition, using the paired *t*-test for samples collected between 0 and 8 d before parturition, and between 9 and 20 d before parturition. There was a significant ($P < 0.05$) difference in plasma progesterone concentrations between 16 paired samples from infected and control sheep in the earlier (day -9 to -20) period. However, there was no significant difference in the plasma progesterone concentration of 16 paired samples from infected and control sheep during the period of progesterone decline immediately before parturition (day -8 to 0).

Oestradiol 17 β in *C. psittaci*-infected and control sheep

The oestradiol 17 β concentrations in the peripheral plasma of the sheep whose progesterone secretion is shown in Fig. 1 were assayed at 48 h intervals. In the *C. psittaci*-infected group, a pre-partum increase in plasma oestradiol 17 β was detected in the one sheep sampled at 24 h before parturition (24 pg ml⁻¹, compared with 9.2 pg ml⁻¹ and 12.9 pg ml⁻¹ in two control sheep). The five *C. psittaci*-infected sheep sampled on the day of parturition had a mean plasma oestradiol concentration of 7.2 ± 4.8 pg ml⁻¹, which was significantly ($P < 0.05$) lower than that of control sheep (45 ± 7.3 pg ml⁻¹, $n = 4$).

Peripheral plasma oestradiol 17 β was analysed daily in a second group of ewes, consisting of four infected and five control sheep during the 2 d prior to parturition (Table 1). The duration of gestation in the infected group (139.8 ± 1.1 d, $n = 4$) was not significantly different from that of the control group (143.8 ± 1.1 d, $n = 5$). Abnormalities in the timing of oestradiol 17 β release

Fig. 2. Prostaglandin E_2 in amniotic and allantoic fluid in (a) control and (b) *C. psittaci*-infected sheep. Twelve ewes were infected by subcutaneous injection on day 90 of gestation with 4.5×10^5 ELD₅₀ of *C. psittaci* strain S26/3, and 12 controls were injected with sterile saline. Four ewes (two infected and two control) were killed on each of the following days of gestation: 97, 103, 109, 115, 120 and 125. Amniotic and allantoic fluids were withdrawn during aseptic delivery of foetuses, 5 min post mortem. In both the control and *C. psittaci*-infected groups of ewes, five animals were carrying single lambs, and seven had twins. Five and three samples, respectively, were rejected from the control and infected groups because they were stained with blood or meconium. The PGE₂ concentrations in amniotic fluid (open symbols) and allantoic fluid (filled symbols) of individual foetuses were analysed. Amniotic fluid and allantoic fluid from the same animal are shown as like pairs of open and filled symbols.

were observed in the sheep infected with *C. psittaci* compared with controls. The characteristic rise in plasma oestradiol 17β , which was observed on the day of parturition in control animals, was detected 24 h earlier in *C. psittaci*-infected sheep. In contrast with the control group, the mean peripheral plasma oestradiol 17β concentration in the *C. psittaci*-infected group 24 h before parturition was higher than the mean concentration of oestradiol 17β on the day of parturition.

As uterine prostaglandin release is controlled by oestradiol 17β (Liggins *et al.*, 1972; Olson *et al.*, 1984), the effect of chlamydial infection on the concentration of oestradiol 17β within the uterus of these sheep was analysed by cannulation of the utero-ovarian vein and amniotic sacs. The oestradiol 17β concentrations in the utero-ovarian plasma and amniotic fluid were determined (Table 1). The oestradiol 17β concentrations in utero-ovarian venous plasma of control sheep were similar to previously reported values (Bedford *et al.*, 1972). There was no significant difference in the oestradiol 17β concentrations released into the utero-ovarian vein by infected, compared with control uteri. However, significantly higher local intrauterine concentrations of oestradiol 17β were observed in the amniotic fluid of *C. psittaci*-infected animals compared to controls (Table 1), indicating that chlamydial infection may have compromised local intrauterine diffusion barriers.

*Intrauterine prostaglandin E_2 in *C. psittaci*-infected and control sheep*

The concentrations of prostaglandin E_2 in the amniotic fluid and allantoic fluid of 24 ewes, killed between day 97 and day 125 of gestation, were determined, in order to investigate whether

premature release of prostaglandins occurred during late gestation (see Fig. 2). The concentrations of prostaglandin E_2 in amniotic and allantoic fluids were low (0.89 ± 0.12 ng ml^{-1} , $n = 12$), between days 97 and 125 in the 12 control sheep, but were significantly raised in four out of 12 samples obtained from *C. psittaci*-infected sheep over the same period (6.46 ± 1.49 ng ml^{-1} , $P < 0.05$). The PGE_2 concentrations in the amniotic and allantoic fluids of the same animals showed a highly significant correlation ($P < 0.01$) in the infected group ($n = 12$), but not in the control sheep ($n = 12$). The distribution of intrauterine PGE_2 concentrations was wider in infected sheep than the distribution of PGE_2 in the corresponding control population (Fig. 2).

Placental pathology of chlamydial infection

The histopathology of placentae during chlamydial infection was investigated, in order to study the histological distribution of *C. psittaci* inclusions, the associated necrosis and the extent of leucocyte infiltration into the placenta of the 24 sheep used for PGE_2 determinations (see above). Placental lesions consistent with those seen in cases of ovine chlamydial abortion (Stamp *et al.*, 1950; Studdert, 1968; Novilla & Jensen, 1970) were observed in the cotyledons of all animals in the infected group killed on days 125 and 120 of gestation and in one of the two infected animals examined on day 115 of gestation. The placental cotyledons of these sheep showed foci of infection, which consisted of *C. psittaci* inclusions in the cytoplasm of trophoblast cells, identified by immunoperoxidase staining, associated with cellular necrosis and phagocyte infiltration. The severity of the lesions increased during the period (days 115–125 of gestation) when infection was detected. No lesions were detected in the placentae from infected ewes killed between days 97 and 110 of gestation or in any of the placentae of control sheep examined on days 97–125 of gestation.

DISCUSSION

A characteristic feature of infection with both *C. psittaci* and *C. trachomatis* during pregnancy is a period of latency, followed by the onset of infection of the foetal membranes and placenta during the last quarter of gestation (Stamp *et al.*, 1950; Studdert, 1968; Novilla & Jensen, 1970; Johnson *et al.*, 1985; Aitken, 1986; Sweet *et al.*, 1987). The severity of infection and the perinatal mortality associated with *C. psittaci* is greater than for *C. trachomatis* in both women and sheep (Sweet *et al.*, 1987; Studdert, 1968). However, both chlamydial species are associated with the initiation of premature labour.

The chorionic membrane of the placenta is the primary focus of intrauterine *C. psittaci* infection, which then spreads to the caruncular regions of the placenta (McEwen *et al.*, 1951; Studdert, 1968). We observed a disruption of placental steroidogenesis, and a stimulation of foetal membrane prostaglandin synthesis during the early stages of chlamydial infiltration into the uterus.

In addition to evidence of changes in steroid hormone and prostaglandin synthesis by *C. psittaci*-infected tissues, we detected changes in the intrauterine distribution of oestradiol 17β and PGE_2 . Similar concentrations of oestradiol 17β and PGE_2 were detected in the amniotic and allantoic fluid of infected, but not of control sheep, and this may reflect changes in foetal membrane integrity during chlamydial infection.

The pathogenesis which we describe may be relevant to other mammalian species, because the placenta and foetal membranes are major sources of steroid hormones and prostaglandins in most species, including humans, during late pregnancy. There are considerable inter-species differences in the pattern of hormone secretion, and in the relative importance of the stimuli which initiate labour (Allen, 1975). However, in all mammalian species, pregnancy is dependent on maintained progesterone secretion, and oestradiol and prostaglandins stimulate uterine responses at the time of parturition (Thorburn & Challis, 1979; Bedford *et al.*, 1972). Premature progesterone withdrawal can initiate labour and increase oestradiol synthesis in the sheep (Mitchell *et al.*, 1983). The abortifacient agent *Actinobacillus seminis* has been reported to cause a decline in plasma progesterone in pregnant ewes (Smith & Hughes, 1974), although this occurred earlier in pregnancy than the inhibition which we observed in *Chlamydia*-infected

sheep (Fig. 1). Premature increases in oestradiol 17β and prostaglandin $F_{2\alpha}$ synthesis have been observed during infectious abortion due to intrauterine surgery (Bedford *et al.*, 1972; Silver *et al.*, 1986) or endotoxin infusion (Roberts *et al.*, 1975) respectively. There has been a recent report of decreased plasma progesterone and oestradiol 17β concentrations and elevated peripheral $PGF_{2\alpha}$ metabolite concentrations in four sheep infected with *C. psittaci* (Fredriksson *et al.*, 1988). There has only been one report on PGE_2 , which is thought to play a role in the initiation of labour (Bleasdale & Johnston, 1984) in infectious abortion (Romero *et al.*, 1988). Our report is the first description of the effect of *C. psittaci* infection on the intrauterine distribution of oestradiol 17β and prostaglandin E_2 during pregnancy.

The effect of the hormonal environment on chlamydial infectivity during pregnancy has recently been investigated *in vivo* and *in vitro*. It was found that oestradiol 17β enhanced the growth of *C. trachomatis* in a guinea-pig model (Rank *et al.*, 1982), and in cultured human lymphoma cells (Bose & Goswami, 1986; Sugarman & Agbor, 1986). The early increases in plasma oestradiol 17β concentrations, and the high intrauterine concentrations of this steroid which we detected, may induce the metabolic and vascular changes which enhance chlamydial growth at a specific stage of late gestation.

In conclusion, experimental infection of ewes with *C. psittaci* was associated with changes in placental steroid and prostaglandin synthesis. It is likely that the premature decline in progesterone, and the premature rise in oestradiol 17β and prostaglandin E_2 concentrations which we report, contribute to the initiation of premature labour in *C. psittaci*-infected sheep.

We are very grateful to Vicky Sweeting and John Seth of the Edinburgh Radioimmunoassay Team for advice on oestradiol 17β radioimmunoassay, and to Professor David Mellor for advice on surgical techniques and scientific guidance. This work was supported by a project grant from the Agricultural and Food Research Council.

REFERENCES

- AITKEN, I. D. (1986). Chlamydial abortion in sheep. *In Practice* **8**, 236–237.
- ALLEN, W. R. (1975). Endocrine functions of the placenta. In *Comparative Placentation*, pp. 214–261. Edited by D. H. Steven. London: Academic Press.
- BEDFORD, C. A., CHALLIS, J. R. G., HARRISON, F. A. & HEAP, R. B. (1972). The role of oestrogens and progesterone in the onset of parturition in various species. *Journal of Reproduction and Fertility*, Suppl. **16**, 1–23.
- BEER, R. J. S., BRADFORD, W. P. & HART, R. J. C. (1982). Pregnancy complicated by psittacosis acquired from sheep. *British Medical Journal* **284**, 1156–1157.
- BLEASDALE, J. E. & JOHNSTON, J. M. (1984). Prostaglandins and human parturition: regulation of arachidonic acid metabolism. *Reviews of Perinatal Medicine* **5**, 151–191.
- BOSE, S. K. & GOSWAMI, P. C. (1986). Enhancement of adherence and growth of *Chlamydia trachomatis* by estrogen treatment of HeLa cells. *Infection and Immunity* **53**, 646–650.
- DRAY, F. & FRYDMAN, R. (1976). Primary prostaglandins in amniotic fluid in pregnancy and spontaneous labour. *American Journal of Obstetrics and Gynecology* **126**, 13–20.
- FINLAYSON, J., BUXTON, D., ANDERSON, I. E. & DONALD, K. M. (1985). Direct immunoperoxidase method for demonstrating *Chlamydia psittaci* in tissue sections. *Journal of Clinical Pathology* **38**, 712–714.
- FREDRIKSSON, G., BUXTON, D., CARLSSON, U., EDQUIST, L. E., ELVANDER, M., KINDHAHL, H. & UGGLA, A. (1988). Effect of bovine virus diarrhoea virus, *Toxoplasma gondii* and *Chlamydia psittaci* on pregnancy in the ewe monitored by ultrasound scanning and levels of 15-keto-13,14-dihydro- $PGF_{2\alpha}$ progesterone and estrone sulphate. *Proceedings of the XI International Congress of Animal Reproduction* **4**, 515–517.
- GRIEVES, S. A. & LIGGINS, G. C. (1976). Phospholipase A activity in human and ovine uterine tissues. *Prostaglandins* **12**, 229–242.
- HELM, C. W., SMART, G. E., GRAY, J. A., CUMMING, A. D., LAMBIE, A. T., SMITH, I. W. & ALLAN, N. C. (1987). Exposure to *Chlamydia psittaci* in pregnancy. *Lancet* **i**, 1144–1145.
- HUNTER, W. M. (1978). Radioimmunoassay. In *Handbook of Experimental Immunology*, pp. 17.1–17.36. Edited by D. M. Weir. Oxford: Blackwell.
- JOHNSON, F. W. A., MATHESON, B. A., WILLIAMS, H., LAING, A. G., JANDIAL, V., DAVIDSON-LAMB, R., HALLIDAY, G. J., HOBSON, D., WONG, S. Y., HADLEY, K. M., MOFFAT, M. A. J. & POSTLETHWAITE, R. (1985). Abortion due to infection with *Chlamydia psittaci* in a sheep farmer's wife. *British Medical Journal* **290**, 592–594.
- KEIRSE, J. N. C., THIERY, M., PARENHIJK, W. & MITCHELL, M. D. (1983). Chronic stimulation of uterine oestradiol synthesis during cervical ripening. *Prostaglandins* **25**, 671–681.

- LAMONT, R. F., ROSE, M. & ELDER, M. G. (1985). Effect of bacterial products on prostaglandin E production by amnion cells. *Lancet* **II**, 1331–1333.
- LEAVER, H. A., MACPHERSON, H. D. & HUTCHON, D. J. R. (1987). Amniotic fluid prostaglandin $F_{2\alpha}$ measured at artificial rupture of the membranes, predicts the subsequent progress of labour. *Prostaglandins, Leucotrienes and Medicine* **28**, 237–241.
- LEAVER, H. A., RICHMOND, D. H., MACPHERSON, H. D. & HUTCHON, D. J. R. (1988). Prostaglandin E_2 in induction of labour: a pharmacokinetic study of dose and treatment protocols. *Prostaglandins, Leucotrienes and Essential Fatty Acids* **31**, 1–13.
- LEWIS, G. P. (1983). Immunoregulatory activity of metabolites of arachidonic acid and their role in inflammation. *British Medical Bulletin* **39**, 243–248.
- LIGGINS, G. C., GRIEVES, S. A., KENDALL, J. Z. & KNOX, B. S. (1972). The physiological role of progesterone, oestradiol 17β , and prostaglandin $F_{2\alpha}$ in the control of parturition. *Journal of Reproduction and Fertility*, Suppl. 16, 85–103.
- LINZELL, J. L. & HEAP, R. B. (1968). Progesterone metabolism in the sheep and goat: production and uptake by target organs. *Journal of Endocrinology* **73**, 433–438.
- MARTEL, J. L., PERRIN, M., RODAKIS, A., RUSSO, P., DESCHAND, J. P. & GARNIER, F. (1983). Infection experimentale de la vache gestante par *Chlamydia psittaci*. *Annales de recherches vétérinaires* **14**, 117–120.
- MC EWEN, A. D., STAMP, J. T. & LITTLEJOHN A. I. (1951). Enzootic abortion in ewes: immunisation and infection. *Veterinary Record* **63**, 197–201.
- MELLOR, D. J. (1980). Investigations of fluid spaces of the sheep conceptus. In *Animal Models in Fetal Medicine*, pp. 61–106. Edited by P. W. Nathanielz. Amsterdam: Elsevier.
- MINKOFF, H. (1983). Prematurity: infection as an etiologic factor. *Obstetrics and Gynecology* **62**, 137–144.
- MITCHELL, M. D., BRENNKE, S. P., KRAEMER, D. L. & WEBB, R. (1983). Progesterone withdrawal without parturition. *European Journal of Obstetrics and Gynaecology* **15**, 25–30.
- MORONEY, M. J. (1951). *Facts from Figures*. Harmondsworth: Penguin Books.
- NOVILLA, M. N. & JENSEN, R. (1970). Placental pathology of experimentally induced enzootic abortion in ewes. *American Journal of Veterinary Research* **31**, 1983–2000.
- OLSON, D. M., LYE, S. L., SKINNER, K. & CHALLIS, J. R. G. (1984). Early changes in PG concentration in dispersed cells from ovine uterine tissues. *Journal of Reproduction and Fertility* **71**, 45–55.
- RANK, R. G., WHITE, H. J., HOUGH, A. J., PASLEY, J. N. & BOURON, A. L. (1982). Effect of estradiol on chlamydial genital infection of female guinea pigs. *Infection and Immunity* **38**, 699–705.
- ROBERTS, J. S., BARCIKOWSKI, B., WILSON, L., SKARNES, R. C. & MCCracken, J. A. (1975). Hormonal and related factors affecting the release of prostaglandin $F_{2\alpha}$ from the uterus. *Journal of Steroid Biochemistry* **6**, 1091–1097.
- ROBERTS, W., GRIST, N. R. & GIROUD, P. (1967). Human abortion associated with infection by ovine abortion agent. *British Medical Journal* **iv**, 37–41.
- ROMERO, R., HOBBS, J. C. & MITCHELL, M. D. (1988). Endotoxin stimulates prostaglandin E_2 production by human amnion. *Obstetrics and Gynecology* **157**, 815–820.
- SCARAMUZZI, R. J., CORKER, C. S., YOUNG, G. & BAIRD, D. T. (1974). Production of antisera to steroid hormones in sheep. In *Steroid Immunoassay: Proceedings of Fifth Tenovus Workshop*, pp 111–122. Edited by E. H. D. Cameron. Cardiff: Alpha Omega Publishing.
- SILVER, R. K., GIBBS, R. S. & CASTILLO, M. (1986). Effect of amniotic fluid bacteria on the course of labour in nulliparous women at term. *Obstetrics and Gynecology* **68**, 587–592.
- SMITH, I. D. & HUGHES, K. L. (1974). Progesterone concentrations in the peripheral plasma of pregnant ewes following infection with an abortifacient organism. *Research in Veterinary Science* **16**, 116–118.
- STAMP, J. T., MC EWEN, A. D., WATT, J. A. A. & NISBET, D. I. (1950). Enzootic abortion in ewes: transmission of the diseases. *Veterinary Record* **62**, 251–254.
- STUDDERT, M. J. (1968). *Bedsonia* abortion of sheep: pathology and pathogenesis. *Research in Veterinary Science* **9**, 57–64.
- STUDDERT, M. J. & MCKERCHER, D. G. (1968). *Bedsonia* abortion of sheep: aetiological studies. *Research in Veterinary Science* **9**, 48–56.
- SUGARMAN, B. & AGBOR, P. (1986). Estrogens and *Chlamydia trachomatis*. *Proceedings of the Society for Experimental Biology and Medicine* **183**, 125–131.
- SWEET, R. L., LANDERS, D. V., WALKER, C. & SCHACHTER, J. (1987). *Chlamydia trachomatis* infection and pregnancy outcome. *American Journal of Obstetrics and Gynecology* **156**, 824–833.
- TAYLOR, M. J., WEBB, R., MITCHELL, M. D. & ROBINSON, J. S. (1982). Effect of progesterone withdrawal in sheep during late pregnancy. *Journal of Endocrinology* **92**, 85–93.
- THORBURN, G. & CHALLIS, J. R. (1979). Endocrine control of parturition. *Physiological Reviews* **59**, 863–918.
- WICKLAND, M., LINDBLOOM, B. & WIKQUIST, N. (1984). Myometrial response to PG during labour. *Gynaecological and Obstetrical Investigation* **17**, 131–138.