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Prostaglandin F in the Fallopian Tube Secretion of the Ewe

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Abstract

Oviducal secretions were obtained from conscious unrestrained ewes throughout the oestrous cycle via indwelling cannulae and the content of prostaglandin F (PGF) was determined by radioimmunoassay. Levels of PGF of up to 230 ng/ml were found in oviducal fluids obtained from ewes showing regular patterns of secretion and normal cyclical ovarian function as indicated by plasma progesterone measurement. Relatively large day to day fluctuations in content were evident, but there was no consistent relationship between concentration and stage of the oestrous cycle. Concentrations of PGF in excess of 100 ng/ml were common in preparations where autopsy later revealed infection or tissue irritation, and the concentration of PGF invariably exceeded 75 ng/ml when the concentration of protein in the oviducal fluid was abnormally high.

Introduction

Infusion of prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$) into the sheep causes luteolysis (Thornburn and Nicol 1971), and $PGF_{2\alpha}$ produced by the uterus is thought to be luteolytic in the intact animal (McCraken *et al.* 1973). Prostaglandin synthesis by the ovine oviduct has not been investigated, and it is not known if prostaglandins are normal constituents of ovine oviducal fluid.

In view of the possibility of prostaglandin involvement in one or other of the multifarious roles of the oviduct, a study has been made of the content of PGF in serially collected samples of oviducal fluid from sheep.

Methods

Experimental Animals and Management

Mature Merino crossbred ewes were obtained from the University of Adelaide Experimental Farm, Mintaro, S.A., and maintained throughout the observation period in air-conditioned rooms at 25°C in a 14 h dark and 10 h light regime. Oestrus was detected by a vasectomized ram fitted with a Sire-sine harness and crayon. Oviducal cannulations were performed on the 8th day post-oestrus. Following surgery all animals were given intramuscular injections of 80 mg gentamycin sulphate (Schering Corp., U.S.A.) and 250 mg cephaloridine (Glaxo–Allenburys), and the dosage was repeated 1 week later.

Oviducal Cannulation

The procedure adopted was similar to that described by Restall (1966). Cannulae were made from silastic tubing (i.d. 1 mm, o.d. 2·2 mm; Dow Corning Medical grade) cut into 50-cm lengths with two cuffs of silicone adhesive placed 1·0 and 1·5 cm from one end of the tube to facilitate their positioning within the oviduct 1–2 cm from the infundibular opening. The uterine ends of the tubes were ligated proximal to the uterotubular junction with 2/0 braided silk, care being taken to avoid damage to blood vessels. The ends of the cannulae were exteriorized independently through

the cranial end of the midline laparotomy incision, sutured to the flank of the ewe, and the ends passed into sterile 5-ml glass collections vessels. Secretions of 24 h were collected daily at 0900 h. Strict adherence to aseptic technique at all times was found to be of paramount importance to achieve prolonged maintenance of patency of the indwelling cannulae and uncontaminated samples. The amount of fluid secreted was determined by weight and secretions were stored at −15°C until analysed.

Blood samples were taken from the jugular vein of the ewe on three occasions during each week throughout the period of study for the determination of progesterone (Obst and Seamark 1970). Where indicated, unilateral ovariectomy was carried out at the time of cannulation.

Determination of Prostaglandin

Portions of oviducal fluid, usually 20 μl, were transferred to Pyrex glass assay tubes (75 by 10 mm) and suitable reference standards containing 0–500 pg $PGF_{2\alpha}$ (tromethamine salt, Upjohn Co., Mich., U.S.A.) were prepared. To each tube was added 0·1 ml of tritiated $PGF_{1\alpha}$ (specific activity 73 Ci/mmol, New England Nuclear, Boston, U.S.A.) diluted to 50000 cpm/ml with 0·15 M phosphate buffered saline containing 0·1% (w/v) gelatin (GPBS) plus 0·1% (w/v) sodium azide. After mixing, 0·5 ml of antiserum (1 : 10000) diluted with GPBS was added and the mixture gently vortexed for 5 s. The tubes were then placed in a water bath at 4°C for at least 60 min to equilibrate.

The free and antibody-bound prostaglandins were separated by passage through 1 by 3·5-cm columns containing 0·5 g Sephadex G25 (fine). Before used the columns were washed with 2 ml of GPBS buffer and allowed to drain. The equilibrated mixture (0·3 ml) was then loaded on to each column followed by 0·5 ml of GPBS buffer and the eluate discarded. The antibody-bound prostaglandin was then eluted from the column directly into scintillation vials with a further 1·0 ml of GPBS buffer. Three drops of acetic acid and 10 ml of toluene–Triton X-100 (2 : 1, v/v) scintillation fluid were added to each vial and, after mixing, the radioactivity in each sample was measured by liquid scintillation spectrometry. The results were analysed by logit transformation using the Wang Radioimmunoassay Data System (Riads 7/b).

The $PGF_{2\alpha}$ antiserum used in the present study was highly specific for PGF and at the dilution employed (1 : 10000) cross-reacted less than 0·2% with prostaglandins of the E, A, and B series (Fairclough *et al.* 1975). It did, however, cross-react with $PGF_{1\alpha}$ (56%) and accordingly the results are expressed at PGF equivalents.

The amount of PGF determined was proportional to the volume of fluid assayed for samples of up to 25 μl. Non-specific binding of PGF to proteins in the oviducal fluid did not appear to be a problem, and at these volumes the direct analysis was found to yield identical results to those obtained by the procedure of Caldwell *et al.* (1971) in which extracts are extensively purified by chromatography. $PGF_{2\alpha}$ (20 and 100 ng/ml) added to fluid was recovered quantitatively (87–108%). The intra-assay coefficient of variation for a sample of fluid containing PGF at 10 ng/ml was 8·5% ($n = 10$). The sensitivity of the standard curve (Ekins and Newman 1970) was 25–30 pg, thus allowing detection of about 1 ng PGF/ml of sample.

Protein was determined by the method of Lowry *et al.* (1951) with bovine serum albumin as standard. Statistical comparisons of daily secretion rates were made using a paired Student's *t*-test.

Results

Secretion of Oviducal Fluid

Thirteen intact ewes were successfully cannulated with a mean collection period of 83 days (range 29–146). Of these animals five showed regular patterns of secretion for at least five cycles and another two animals for three cycles. Typically, mean secretion rates began to increase on day −1 or day 0 of the cycle, reaching a peak of up to 1·5 ml per 24 h by about day 2 of the cycle. The rate of flow then declined to pro-oestrous levels (0·15–0·35 ml per 24 h) by about day 10. While both cannulae remained patent, the pattern of secretion between oviducts was similar, changes in one side usually being mimicked by the contralateral ovary. Two ewes had irregular secretory patterns and although there was evidence of ovarian activity at the time of cannulation, behavioural oestrus was not subsequently detected.

The remaining ewes had variable or irregular secretion rates despite apparently normal ovarian function. Excluding accidents, such as physical breaking of cannulae by the ewes (four cases), the main causes of cannula failure were infections (*Pseudomonas*, *Streptococcus* spp.) (50%) and tubal occlusion resulting from build-up of cellular debris in the cannulae. With five other ewes studied one ovary was removed at the time of cannulation. Accumulation of solid matter in cannulae was more frequent in these ewes and cannulae maintained patency for a significantly shorter period [35 days (range 19–51), $P < 0{\cdot}01$]. The secretory patterns were also less regular.

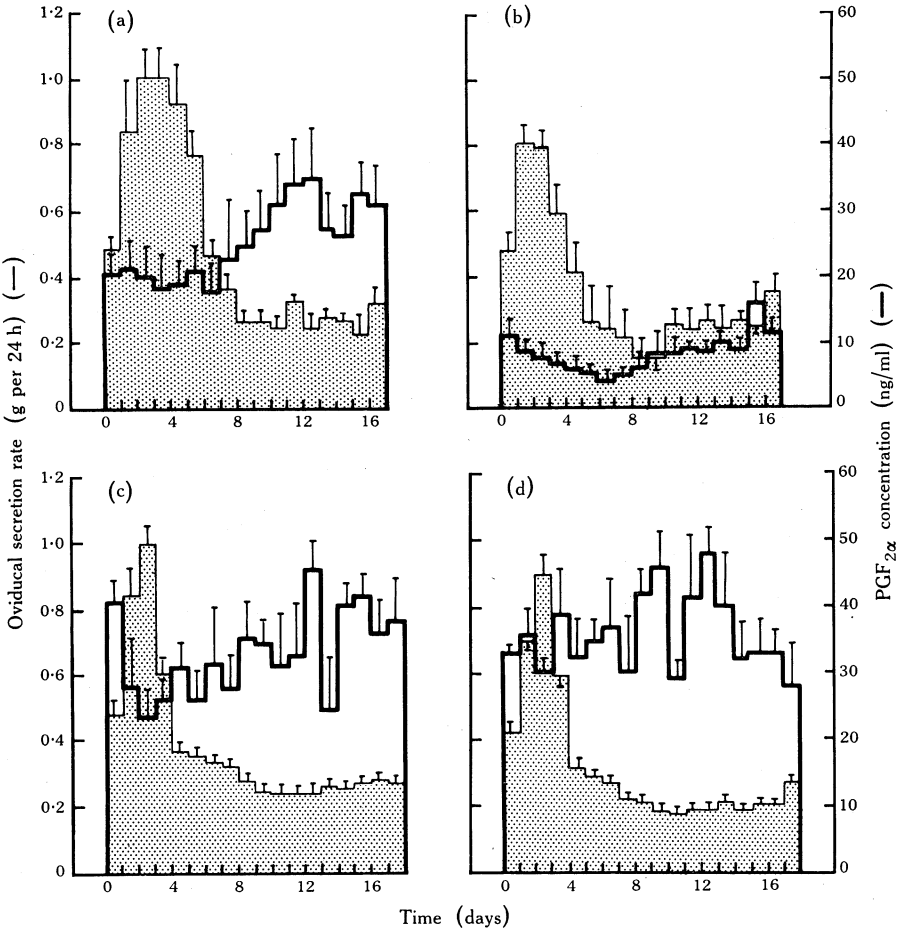

Fig. 1. Secretion of oviducal fluid throughout the oestrous cycle and the PGF content for (*a*) the left and (*b*) the right oviduct of ewe 272, and (*c*) the left and (*d*) the right oviduct of ewe 214. The results are the mean ± s.e.m. for four and five complete oestrous cycles in ewes 272 and 214 respectively. Behavioural oestrus was first detected on day 0.

Interestingly, higher rates of oviducal secretion were observed in the oviduct contralateral to the remaining ovary in each of the five ewes (mean differences 0·09–0·28 ml/24 h, $P < 0{\cdot}05$), indicating that local ovarian events can modulate the secretion of oviducal fluid from the adjacent oviduct (see Fig. 2).

PGF Content of Oviducal Fluid

Four ewes were selected for prostaglandin studies—three were entire (ewes 214, 272 and 166) and one (ewe 160) was hemicastrate—on the basis of collections being made from both oviducts for at least four cycles without accident or signs of infection.

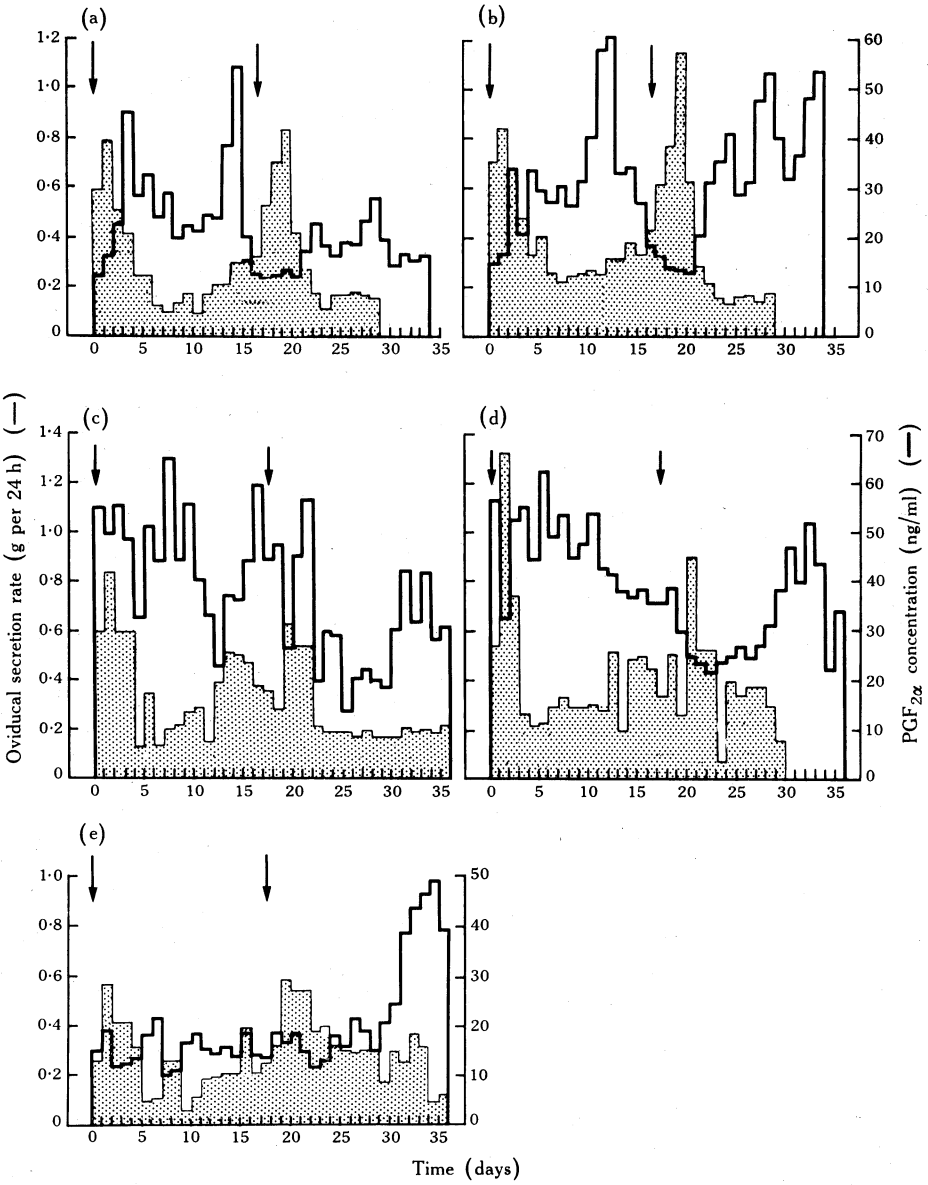

Fig. 2. $PGF_{2\alpha}$ content of oviducal fluid obtained from (*a*) the left and (*b*) the right oviduct of hemicastrate ewe 160, and (*c*) the left and (*d*) the right oviduct of entire ewe 166. (*e*) The effect of aspirin on the secretion of the left oviduct of ewe 166. The right ovary of ewe 160 was removed at the time of cannulation. After two completed cycles with patent canulae ewe 166 was given an oral dose (900 mg) of aspirin each day for two consecutive cycles. Behavioural oestrus is indicated by arrows (i.e. starting on day 0, Fig. 2*e*).

In the entire ewes regular patterns of oviducal secretion were found. In ewes 214 and 272 (Fig. 1) secretion rates were significantly greater (7 cycles, $P < 0{\cdot}01$) in the left than the right oviduct, but there were no significant differences in the other ewe studied (ewe 166, 6 cycles, Figs 2*c* and 2*d*). The pattern of prostaglandin secretion found in these ewes is shown in Figs 1, 2*c* and 2*d*.

No constant relationship was found between the content of F-prostaglandins and the cyclical changes in fluid secretion rate or the endocrine status of the ewe, as assessed by plasma progestin determination. However, if the data are expressed in terms of PGF secretion rate (concentration × flow), a rise (two- to fourfold) in PGF production was seen around oestrus in ewes 272 and 214 (Fig. 1) but not in ewe 166 (Figs 2*c* and 2*d*).

Fluids from the hemicastrate ewe (ewe 160, Figs 2*a* and 2*b*) were examined in detail for PGF content. In this ewe significantly ($P < 0{\cdot}05$) higher levels of PGF were found in secretions collected from the tract contralateral to the remaining ovary, but similar differences were also found in the intact ewes examined.

Affect of Aspirin

After two completed cycles of collection, ewe 166 was given an oral dose of 900 mg of aspirin (acetylsalicyclic acid, Ca salt) each day for the following two cycles (Fig. 2*e*). The concentration of PGF was significantly reduced during the first cycle of treatment, but rose during the subsequent cycle. Aspirin did not appear to affect the rate of fluid secretion, the length of the cycle, or the level of circulating progesterone (data not shown).

Protein Content of Oviducal Fluids

The protein content of oviducal fluids was usually in the range from 4 to 20 mg/ml, and although relatively large day to day variations were not uncommon (possibly due to contamination by cellular debris), the concentration of protein was found to be significantly ($P < 0{\cdot}05$) higher (mean value 13·1 mg/ml) during the pro-oestrous and oestrous phases of the cycle than in the luteal phase (mean value 6·3 mg/ml). In those animals which had patent cannulae in both oviducts there was no overall difference in protein content between left and right oviducts. Higher protein concentrations (>20 mg/ml) were often detected in other ewes studied during periods of irregular secretion or when infections were subsequently shown to be present within the cannulated oviduct. In such samples PGF levels were invariably elevated (>75 ng/ml) relative to normal values.

Discussion

Previous investigations of PGF in the reproductive tract of the ewe have mostly been concerned with the uterus, culminating in the identification of $PGF_{2\alpha}$ as the uterine luteolytic hormone (McCracken *et al.* 1972). These studies clearly indicated the great potential of uterine tissues to form prostaglandins (Harrison *et al.* 1972), and the present data indicate that the tubule tissues have similar capacities. Prior studies on prostaglandins in fallopian tubes have largely been concerned with their occurrence in the human (Zetler and Wiechell 1969; Ogra *et al.* 1974; Vastik-Fernandez *et al.* 1975) although there is one report of PGF in the oviducal tissues

of the rabbit (Saksena and Harper 1975). Of the studies in the human, only one concerns PGF in oviducal secretions; this is by Ogra *et al.* (1974) who found levels of 7·1 and 5·0 ng/ml in two samples of human oviducal fluids obtained during a pre- and post-ovulatory stage, respectively, of the menstrual cycle. These values are much lower than those presently obtained with the studies on sheep, and the question arises as to whether this is due to species differences or is an artifact of the collection technique employed.

It is well known that tissue distortion or irritation can lead to the release of prostaglandins (Piper and Vane 1971). The possibility remains that the cannulae acted as a chronic irritant although great care was taken to select ewes which remained free of infection and which showed regular cyclical activity and repeatable patterns of secretion for prolonged periods indicative of minimally disturbed function.

There are other problems also which can arise from this approach towards collecting tubal secretion, including the possibility that PGF may have been metabolized or even generated while the fluids were within the collection tube, as the volume of the cannulae employed (0·39 ml) was large in relation to the overall flow rates (0·15–1·5 ml per 24 h).

Although it is accepted that prostaglandins are a normal constituent of tubal secretions, it is of interest to speculate on their possible physiological significance.

No consistent data were obtained to suggest that tubular PGF secretion increased at the same time as the rise in uterine secretory activity associated with luteolysis (McCracken *et al.* 1973). However, there was a tendency for prostaglandin secretion to increase early in the cycle with the overall increase in secretory function. Although these changes were nowhere near as marked as those found, for example, in the uterine secretion of the monkey where there is a 20-fold increase in the PGF content at oestrus (Demers *et al.* 1974), they may indicate that the secretion of fluid and PGF are causally related. Alternatively, the increase may relate to a more direct role of PGF in the reproductive process, possibly in the transport and maintenance of the gametes or in the fertilization process itself.

That prostaglandins have an active role in the functioning of the fallopian tubes has now been suggested by many authors (see Flint and Hillier 1975). Prostaglandins are known to be potential stimulators of tubule motility (see Elder *et al.* 1977) and compelling data have been presented to suggest that they may play an active part in ovum transport (Wakeling and Spilman 1974; Saksena and Harper 1975; Vastik-Fernandez *et al.* 1975; Wechsunge and Houvenaghel 1976; Maia *et al.* 1977). Their possible effects on the gametes (Cohen *et al.* 1977) and development of the conceptus (Kirkpatrick 1974; Spilman 1974), however, remains conjectural. Alternatively, PGF of tubule origin could act to promote ovarian contraction especially at the time of peak secretion just after oestrus. However, if this happens it does not appear to affect ovulation (Chang *et al.* 1974; Richman *et al.* 1974).

Reduction of prostaglandin synthesis using one of the armoury of known prostaglandin synthetase inhibitors offers one possible experimental approach towards investigating the possible actions of prostaglandins in fallopian tube functions.

The preliminary experiment which indicated that the PGF content of oviducal fluid can be partially reduced by administration of aspirin requires confirmation. The incomplete nature of the block could have resulted from the oral route of administration, and the experiment requires repetition with more effective antagonists.

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