

The regulation of rabbit oviduct fluid formation

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The control of the formation of rabbit oviduct fluid and its relationship to the secretion of chloride ions has been studied using an *in vitro* vascularly perfused preparation. Fluid was produced at a rate of $43.41 \mu\text{l h}^{-1}$ in oviducts from rabbits in oestrus. The rate was increased by isoprenaline and tetraethylammonium, decreased by dibutyryl cAMP, dihydro-4,4'-diisothiocyanatostilbene-2,2'-disulfonic acid (H_2DIDS), and propranolol, while amiloride had no effect. H_2DIDS induced a small decrease and isoprenaline a small increase in vascular to lumen Cl^- flux but propranolol and dibutyryl cAMP had no effect. Oviducts from pseudopregnant animals treated with hCG three days before the experiment produced significantly less fluid than did those from rabbits in oestrus, but there was no difference in vascular to lumen Cl^- flux. The concentration of K^+ in oviduct fluid formed *in vitro* was more than three times higher than in the vascular perfusate. The ability of adrenergic agents to influence the formation of rabbit oviduct fluid could have clinical implications in the prevention or treatment of female infertility due to blockage of the Fallopian tubes and might also be useful in enhancing the secretory activity of oviduct cells maintained in co-culture with early embryos.

Introduction

Although Fallopian tube (oviduct) fluid is the medium in which fertilization and early embryo development take place, little is known about the mechanisms underlying the secretion of specific constituents or the control of these processes (Leese, 1988; Leese and Dickens, 1992). Such information is important for three main reasons. First, blockage of Fallopian tubes is a major cause of infertility, accounting for 14% of cases in infertile couples in the UK (Hull *et al.*, 1985). Tubal factors are also implicated in many cases of unexplained infertility. Information on the normal physiology of the tube is essential to understanding the onset of tubal pathology and could provide early markers of incipient damage. Second, there is a need to discover the nature of the advantage, if any, conferred by the Fallopian tube on embryo development, notably in co-culture experiments. This again requires knowledge of the physiology of tubal fluid formation. Moreover, the ability to enhance or inhibit total fluid output or the secretion of selected constituents could be used to augment any positive effect of the Fallopian tube on embryo development. Third, information on the permeability properties of the Fallopian tube wall could be valuable in the development and targeting of contraceptives designed against the gametes or early embryo.

The technique of vascular perfusion (Leese and Gray, 1985) and inhibitors of ion transport were used to show that chloride ions were involved in mediating fluid secretion in the rabbit oviduct (Gott *et al.*, 1988). Brunton and Brinster (1971), using slices of rabbit oviduct mounted in an Ussing chamber, also reported a net serosal to mucosal Cl^- flux. It is possible that changes in chloride ion flux are involved in the reduction in

volume of rabbit oviduct fluid which occurs 3 days after mating (Bishop, 1956; Gott *et al.*, 1988; Dickens *et al.*, 1992).

In other tissues, such as the small intestine (Cooke, 1987), trachea (Welsh, 1986; Leidke, 1989; Verkman *et al.*, 1992) and sweat duct gland (Pedersen, 1990), fluid secretion is stimulated by adrenergic compounds via a change in cAMP concentration. Brunton (1972) found that agents such as cAMP, adrenaline and isoprenaline increased the short circuit current and potential difference across pieces of rabbit oviduct mounted in an Ussing chamber; the response to isoprenaline was blocked by propranolol, thus signifying beta adrenergic control of short circuit current.

To study fluid formation in as physiological a manner as possible, we used an *in vitro* vascular perfusion technique (Leese and Gray, 1985) in which the ovarian artery was cannulated and the vasculature perfused with an appropriate medium. At the same time, the lumen was cannulated enabling the formation of tubal fluid to be monitored continuously. The preparation is viable for at least 3 h. We examined the effect of adrenergic agents, ion transport inhibitors and pseudo-pregnancy on fluid production and Cl^- transport in the rabbit oviduct. A preliminary account of this work has been published (Dickens *et al.*, 1992).

Materials and Methods

Materials

Rabbits were obtained from Harlan Olac Ltd, Bicester, Oxon and Froxfield Farms (UK) Ltd, Petersfield, Hampshire. Medium 199 was obtained from Flow Laboratories, High Wycombe, Bucks.; penicillin and streptomycin from Gibco Life

Technologies Ltd, Paisley; BSA from ICN Immunobiologicals, High Wycombe, Bucks.; ^{36}Cl from Amersham, Aylesbury, Buckinghamshire; Ketaset and Domitor from Vet Drug Co., Dunnington, York; all other chemicals were from Sigma Chemical Co., Poole, Dorset.

Vascular perfusion

The vascular perfusion apparatus was described by Leese and Gray (1985). Virgin, female Dutch rabbits (2.0–2.5 kg) were anaesthetized with i.m. injections of 45 mg Ketaset kg^{-1} and 0.5 mg Domitor kg^{-1} , maintained with 1:1 Ketaset and distilled water via the marginal ear vein. When necessary, pseudopregnancy was induced by giving 50 iu hCG i.v. 3 days before the experiment.

A glass cannula was tied into the fimbriated end of the oviduct. The ovarian artery was dissected free of fat and loosely ligatured. The artery was clamped and an incision made above the clamp into which a fine glass cannula was inserted. The vasculature was perfused at a rate of 0.7 ml min^{-1} with Medium 199 supplemented with 0.4% BSA, 4U heparin ml^{-1} , 50 μg penicillin ml^{-1} , and 50 μg streptomycin ml^{-1} gassed with 5% CO_2 in O_2 . The mean perfusion pressure was $83 \pm 2 \text{ mmHg}$ ($n = 61$), and this remained constant throughout each experiment.

The uterine end of the oviduct was tied and a calibrated tube attached to a cannula secured in the fimbriated end. The oviduct was excised and placed in a water-jacketed dish in a 37°C constant temperature cabinet. There was usually a lag period of about 1 h before fluid appeared in the lumen tube owing to the filling of the oviduct lumen. Oviduct fluid was then produced continuously and its progress along the tube could be monitored for up to 3 h.

The effect on fluid formation of the addition of dibutyryl cAMP, dihydro-4,4'-diisothiocyanatostilbene-2,2'-disulfonic acid (H_2DIDS), tetraethylammonium, amiloride, isoprenaline and propranolol to the vascular medium was investigated.

In a separate series of experiments to study unidirectional ion transport from the vascular medium into the oviduct lumen, a second cannula was tied into the lumen of the uterine end of the oviduct. Krebs Ringer bicarbonate ($118 \text{ mmol NaCl l}^{-1}$, $25 \text{ mmol NaHCO}_3 \text{ l}^{-1}$, $4.74 \text{ mmol KCl l}^{-1}$, $1.19 \text{ mmol MgSO}_4 \text{ l}^{-1}$, $1.17 \text{ mmol KH}_2\text{PO}_4 \text{ l}^{-1}$, $1.17 \text{ mmol CaCl}_2 \text{ l}^{-1}$ gassed with 5% CO_2 in air) was perfused in a single pass through the lumen at a rate of $70 \mu\text{l min}^{-1}$. After a 10 min control period $7 \text{ kBq ml}^{-1} \text{ }^{36}\text{Cl}^-$ was added to the vascular compartment and lumen wash-out samples were collected for intervals of 10 min. A 90 min control period of Cl^- flux measurement was allowed before experimental agents (dibutyryl cAMP, H_2DIDS , isoprenaline and propranolol) were added to the vascular medium. Experiments were continued for a further 90 min. Samples were added to 2 ml 'Optiphase' and counted in a liquid scintillation counter.

Analysis of oviduct fluid

Oviduct fluid formed during the vascular perfusion experiments was collected and frozen at -20°C . The ionic composition was later analysed by electron probe X-ray microanalysis. The method used was that of P. S. Hinde and

A. D. Tomos (unpublished) from the School of Biological Sciences, University College of N. Wales, Bangor. Triplicate samples were placed on a copper G100/200 mesh electron microscope grid under water-saturated paraffin oil using a constriction micropipette. An equal volume of RbFI was added to each sample. Standards for the ions to be measured were prepared in a similar manner. The completed grid was flash dried in water-saturated hexane followed by isopentane, air dried and stored over desiccant until analysis by EDAX (Energy Dispersive X-Ray Analysis).

The grids were mounted on carbon blocks and analysed using a Hitachi S520 scanning electron microscope with a LINK QX2000 EDAX detector. The grids were oriented so that the electron beam and X-ray detector were at an angle of 45° . X-ray spectra were analysed using a beryllium window.

The osmotic pressure of oviduct fluid samples was also measured using a modified Clifton nanolitre osmometer.

Statistical analysis

The results are expressed as mean \pm SEM. The significance of differences between means was assessed by paired or Student's *t* test as appropriate. Data for fluid formation and Cl^- flux was measured from a series of readings once a steady state had been reached.

Results

Rabbits in oestrus

Fluid formation studies. The rate of fluid production in oviducts taken from rabbits in oestrus was $43.41 \pm 4.31 \mu\text{l h}^{-1}$ ($n = 25$). The addition of $1 \text{ mmol dibutyryl cAMP l}^{-1}$ to the vascular perfusate immediately inhibited oviduct fluid formation from a mean rate of $47.7 \pm 7.08 \mu\text{l h}^{-1}$ to $6.55 \pm 4.04 \mu\text{l h}^{-1}$ ($n = 4$, $P = 0.015$; see Fig. 1). The Cl^- - HCO_3^- exchange inhibitor H_2DIDS (1 mmol l^{-1}) reduced oviduct fluid formation by 77%, from a mean rate of $45.54 \pm 6.78 \mu\text{l h}^{-1}$ to $10.26 \pm 2.80 \mu\text{l h}^{-1}$ ($n = 6$, $P = 0.004$).

Addition of $25 \text{ mmol tetraethylammonium l}^{-1}$ (TEA), a K^+ channel blocker, to the vascular medium stimulated fluid formation from 46.04 ± 13.03 to $129.01 \pm 34.73 \mu\text{l h}^{-1}$ ($n = 6$, $P = 0.044$), whereas $1 \text{ mmol amiloride l}^{-1}$, a Na^+ - H^+ exchange inhibitor, had no effect on fluid transport (control rate of $36.78 \pm 11.04 \mu\text{l h}^{-1}$ compared with $35.76 \pm 9.06 \mu\text{l h}^{-1}$, ($n = 4$)). These results are summarized (Fig. 1).

The effect of adrenergic agonists was examined. Adrenaline was an inappropriate agent to use as it raised arterial pressure owing to the stimulation of alpha receptors in the oviduct (Black, 1974). When $100 \mu\text{mol isoprenaline l}^{-1}$, a beta adrenergic stimulator, was added to the vascular medium, a large, prolonged increase (for up to 1 h) in oviduct fluid formation, from a control rate of 49.56 ± 9.42 to $120.00 \pm 37.68 \mu\text{l h}^{-1}$, a rise of 142% ($n = 9$, $P = 0.063$) was induced. Similar results were obtained with $1 \text{ mmol isoprenaline l}^{-1}$ ($n = 3$). After the addition of the beta adrenergic antagonist, propranolol ($100 \mu\text{mol l}^{-1}$), the rate fell to $27.90 \pm 10.86 \mu\text{l h}^{-1}$, a fall to 44% of the initial control value ($n = 5$, $P = 0.003$) (Fig. 2).

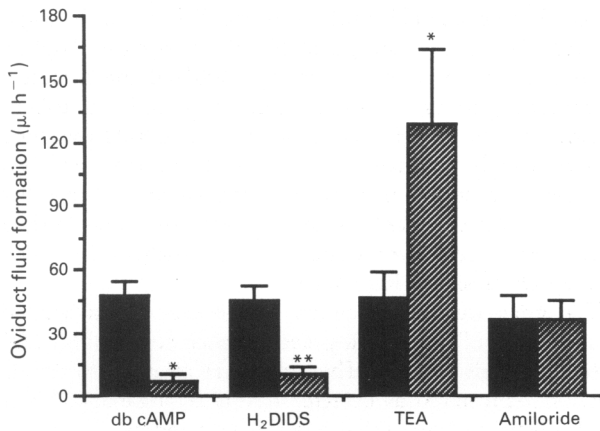


Fig. 1. The rate of fluid formation ($\mu\text{l h}^{-1}$) in oviducts taken from rabbits in oestrus. Results are paired data. (■): the control period during the first part of the experiment; (▨): the rate of fluid formation under experimental conditions. db cAMP: 1 mmol dibutyl cAMP l^{-1} ($n = 4$); H₂DIDS: 1 mmol dihydro-4,4'-diisothiocyanatostilbene-2,2'-disulfonic acid l^{-1} ($n = 6$); TEA: 25 mmol tetraethyl ammonium l^{-1} ($n = 6$) and 1 mmol amiloride l^{-1} ($n = 4$) added to the vascular medium. Values are means \pm SEM. Significantly different from control (* $P < 0.05$, ** $P < 0.01$).

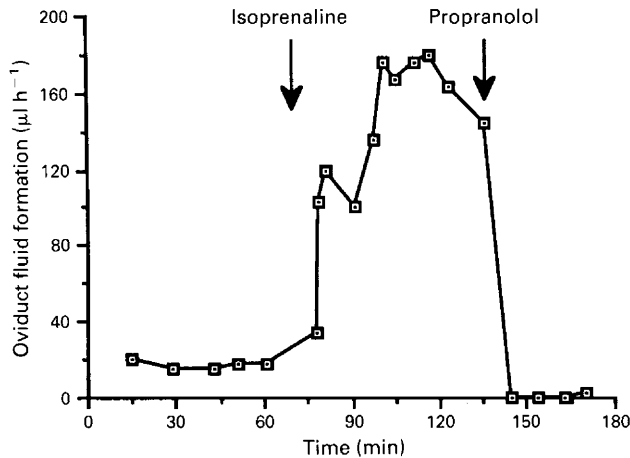


Fig. 2. Rate of formation of rabbit oviduct fluid ($\mu\text{l h}^{-1}$) when, after a control period of 70 min, 100 μmol isoprenaline l^{-1} (final concentration) was added to the vascular medium. After a further 65 min, 100 μmol propranolol l^{-1} (final concentration) was also added to the vascular medium.

The different effects exerted by dibutyl cAMP and isoprenaline on fluid transport were unexpected. Perfusions were therefore carried out in which isoprenaline was added to the vascular medium followed later by dibutyl cAMP in the same experiment. The control rate of fluid formation was $35.64 \pm 8.40 \mu\text{l h}^{-1}$ ($n = 5$). After the addition of isoprenaline it increased to $63.90 \pm 20.82 \mu\text{l h}^{-1}$ before falling to a mean of $23.70 \pm 9.66 \mu\text{l h}^{-1}$ with the addition of dibutyl cAMP (that is, below the control rate).

Ion transport studies. The mean rate of Cl^{-} transport from the vascular compartment into the lumen was $15.7 \pm 1.85 \mu\text{mol}$

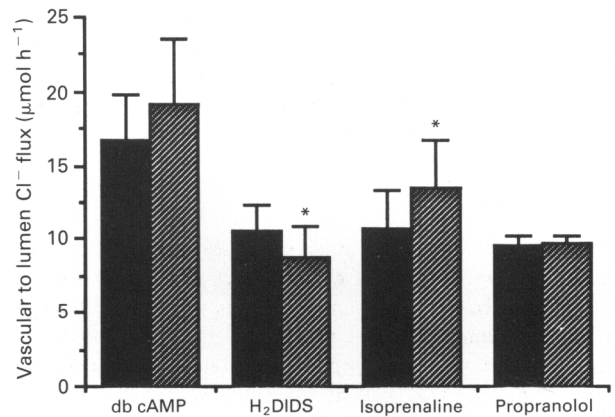


Fig. 3. Vascular to lumen Cl^{-} flux ($\mu\text{mol h}^{-1}$) across rabbit oviducts. db cAMP: 1 mmol dibutyl cAMP l^{-1} ($n = 5$); H₂DIDS: 1 mmol dihydro-4,4'-diisothiocyanatostilbene-2,2'-disulfonic acid l^{-1} ($n = 5$), 100 μmol isoprenaline l^{-1} ($n = 7$) and 100 μmol propranolol l^{-1} ($n = 4$) added to the vascular medium of the preparation after a steady control flux had been established. (■) Control flux during the first part of the experiment; (▨) the flux under experimental conditions. Values are means \pm SEM. *Significantly different from control ($P < 0.05$).

h^{-1} for rabbits in oestrus ($n = 26$). Addition of 1 mmol dibutyl cAMP l^{-1} to the vascular compartment had no significant effect on Cl^{-} transport into the oviduct lumen, with a rate of $16.67 \pm 3.06 \mu\text{mol h}^{-1}$ before and $19.21 \pm 4.28 \mu\text{mol h}^{-1}$ after addition of cAMP ($n = 5$, $P = 0.4$, see Fig. 3). Chloride ion transport was also monitored after the addition of H₂DIDS to the vascular medium. Over a 30 min period, 1 h after the addition of 1 mmol H₂DIDS l^{-1} , there was a small but significant fall in Cl^{-} flux compared with control values before the addition of H₂DIDS, from $10.53 \pm 1.78 \mu\text{mol h}^{-1}$ to $8.81 \pm 2.09 \mu\text{mol h}^{-1}$ ($n = 5$, $P = 0.028$).

Similar experiments were carried out in which 100 μmol isoprenaline l^{-1} was added to the vascular compartment after an initial 90 min control period. During the first 30 min after addition of isoprenaline, there was a significant increase in Cl^{-} flux, from 10.71 ± 2.54 to $13.44 \pm 3.36 \mu\text{mol h}^{-1}$ ($n = 7$, $P = 0.025$). In a separate series of experiments, the addition of 100 μmol propranolol l^{-1} to the vascular compartment had no effect on Cl^{-} transport ($n = 4$) (Fig. 3).

hCG-treated rabbits

The rate of fluid formation was monitored in oviducts taken from day 3 pseudopregnant rabbits. In five experiments, the mean rate of fluid formation was $21.42 \pm 3.19 \mu\text{l h}^{-1}$, significantly lower than the rate of $43.41 \pm 7.08 \mu\text{l h}^{-1}$ ($n = 25$) obtained for oviducts obtained from rabbits in oestrus ($P = 0.0001$; Fig. 4a). Chloride ion transport from the vascular medium to the oviduct lumen in hCG treated animals ($13.56 \pm 3.74 \mu\text{mol h}^{-1}$, $n = 4$) was not significantly different from that found in animals in oestrus ($15.71 \pm 1.85 \mu\text{mol h}^{-1}$) ($n = 26$, Fig. 4b).

Composition of oviduct fluid

The concentrations of Na^{+} , K^{+} and Cl^{-} were analysed in fluid collected during the control period of perfusion of

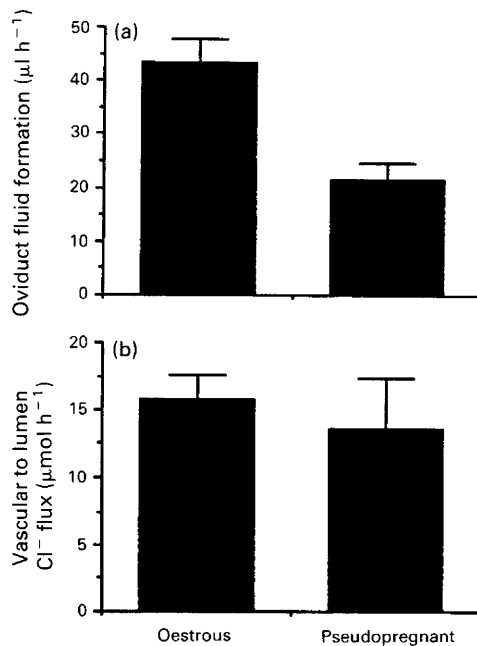


Fig. 4. (a) Fluid production ($\mu\text{l h}^{-1}$) in oviducts from rabbits in oestrus (oestrous; $n = 25$) and from rabbits treated 3 days previously with hCG (pseudopregnant; $n = 5$). (b) Vascular to lumen Cl^{-} transport ($\mu\text{mol h}^{-1}$) in oestrous ($n = 26$) and pseudopregnant rabbits ($n = 4$). Bars represent means \pm SEM.

oviducts from rabbits in oestrus (Table 1). The concentration of K^{+} in oviduct fluid was more than three times higher than in the vascular perfusate, whereas Na^{+} and Cl^{-} concentrations were similar to those in the perfusate. The osmolality of fluid in the oviduct was similar to that of Medium 199 and of rabbit plasma.

Discussion

The technique of vascular perfusion enables the formation of rabbit oviduct fluid to be monitored continuously for at least 3 h. Once a steady rate is established, potential modulators may be added to the vascular perfusate and their effect on fluid production compared with the initial control rate. By perfusing the lumen, the unidirectional flux of ions from the vascular compartment may also be studied. Attempts were made to

measure the absorptive Cl^{-} flux, that is, in the lumen to vascular direction, but the results proved unreliable.

We have shown that the rate of formation of oviduct fluid is inhibited by H_2DIDS , and stimulated by TEA, with amiloride having no effect. Although H_2DIDS is not solely specific for the inhibition of $\text{Cl}^{-}-\text{HCO}_3^{-}$ exchange, the data did suggest a role for chloride ions in the production of oviduct fluid. However, there was little correlation between the effect of the various agents on fluid formation and on vascular to lumen chloride flux, indicating that other ions or metabolites must be involved in fluid secretion. A likely candidate is potassium, the concentration of which is about three times greater in oviduct fluid than in plasma (this study and Brackett and Mastroianni (1974)). This contention is supported by the effect of TEA on fluid formation. TEA is considered to block the K^{+} channels involved in recycling K^{+} across the basolateral membrane, concomitant with the action of the $\text{Na}^{+}-\text{K}^{+}$ ATPase. This could lead to a rise in intracellular K^{+} and force its exit into the lumen via apical K^{+} channels (James and Okada, 1990), thereby drawing water into the lumen and leading to the observed increase in oviduct fluid production.

The concentration of sodium ions is also slightly higher in oviduct fluid than in plasma (this study), although the lack of effect of amiloride would seem to preclude its involvement in fluid production. The observation that the osmolality of the fluid formed was similar to that of the vascular perfusate indicates that water transport is isotonic in this tissue.

A reduction in the rate of fluid production by pseudopregnant animals has been reported by Bishop (1956), Gott *et al.* (1988) and Leese (1988), and may be associated with the passage of the embryo from the oviduct into the uterus. The fall in fluid production was not associated with a change in Cl^{-} transport, and other mechanisms are therefore likely to be involved as discussed above. For example, there is some evidence for a change in the concentrations of H^{+} (Mass *et al.*, 1979), Na^{+} and Ca^{2+} (Holmdahl and Mastroianni, 1965) in rabbit oviduct fluid collected after ovulation.

The results reported here represent the first direct evidence for the regulation of oviduct fluid formation by adrenergic agents. However, such agents could act at a variety of sites as is the case in the anatomically similar small intestine (Cooke, 1987). For example, alpha- and beta-receptors have been found in the musculature and vasculature of rabbit oviducts (Black, 1974) and in oviduct epithelial cells in rats (Tolszczuk and Pelletier, 1988) and rabbits (Dickens *et al.*, 1993). The receptors in the musculature are thought to be under ovarian steroid

Table 1. Ion concentrations and osmolality of oviductal fluid from rabbits in oestrus, Medium 199 and doe plasma

Source	Na^{+} (mmol l^{-1})	Cl^{-} (mmol l^{-1})	K^{+} (mmol l^{-1})	Osmolality (mosmol l^{-1})
Rabbit oviductal fluid ^a	183 \pm 7	132 \pm 8	18 \pm 2	307 \pm 12
Medium 199 ^b	170	127	5.4	306
Doe plasma ^c	142 \pm 1	104 \pm 3	3.7 \pm 0.2	293 \pm 2

^aMean \pm SEM from samples of fluid from five experiments.

^bCalculated from composition data supplied by the manufacturer.

^cData from Brackett and Mastroianni (1974).

control; alpha-receptors are influenced by oestrogen and beta-receptors by progesterone (Black, 1974). However, with regard to their effect on fluid transport, we consider that the epithelial cells lining the oviduct lumen are the most likely targets for adrenergic agents as these agents have a dramatic effect on primary cultures of polarized monolayers of rabbit oviduct epithelial cells (Dickens *et al.*, 1993).

Our results lead to a paradox in that it is well established that beta adrenergic agonists, such as isoprenaline exert their effect via an increase in the intracellular cAMP concentration (Brunton, 1972; Pedersen, 1990; Verkman *et al.*, 1992), whereas our data clearly show that cAMP and isoprenaline have opposite effects on the rate of oviduct fluid formation. To our knowledge this apparent anomaly has not been reported in other tissues. There are two possible explanations. First, cAMP may not be the second messenger for isoprenaline in the rabbit oviduct. Second, isoprenaline added exogenously may give rise to a different response in terms of intracellular cAMP concentration to that induced by external cAMP, which could be acting at other levels in the tissue, such as the vasculature, the stromal cells or the musculature, as discussed above.

Adrenergic agents, of which a great many have been tested clinically, might, by their action on oviduct fluid formation be useful in treating or preventing blockage of Fallopian tubes and also in enhancing, or inhibiting, the secretory activity of oviduct cells maintained with early embryos in co-culture.

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