Evidence *in vivo* for the two-cell hypothesis of oestrogen synthesis by the sheep Graafian follicle

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After autotransplantation of different cell types to the anterior chamber of the eye in the rat, Falck (1959) proposed that granulosa and theca interna cells complemented one another in the synthesis of oestrogen by the Graafian follicle. Although numerous experiments *in vitro* support this 'two cell' hypothesis (Ryan, Petro & Kaiser, 1968; Makris & Ryan, 1975), most evidence suggests that *in vivo* oestrogen is synthesized exclusively by the theca cells (YoungLai & Short, 1970; Channing & Coudert, 1976). However, the report that FSH stimulates specifically the aromatization of androgens to oestrogens by ovaries in organ culture (Moon, Dorrington & Armstrong, 1975), as well as by granulosa cells in tissue culture (Dorrington, Moon & Armstrong, 1975), led us to re-examine the biosynthesis of oestrogens by the Graafian follicle *in vivo*. If androgens leave the thecal cell before aromatization, it should be possible to make them unavailable for the synthesis of oestrogen by anti-androgen antiserum.

Eight ewes with the left ovary autotransplanted to a carotid jugular skin loop in the neck (Goding, McCracken & Baird, 1967) were used during January and February 1976. The experiments were performed in the follicular phase of the cycle 28 h after regression of the corpus luteum had been induced by an i.m. injection of 100 µg ICI 80, 996, a potent luteolytic analogue of prostaglandin F-2 α (Baird & Scaramuzzi, 1976). Oestrus occurred 24–48 h after the experiment, i.e. approximately 48–72 h after the injection of prostaglandin. On the morning of the experiment the ovarian artery and vein were cannulated via the left carotid artery and jugular vein respectively as previously described (Collett, Land & Baird, 1973). After a rest period of 2 h, timed samples of ovarian venous blood were collected at intervals of 10 min for 180 min (Text-fig. 1). Five minutes after each ovarian venous collection a sample of blood from the right jugular vein was collected for assay of LH. The blood was centrifuged immediately at 4°C, a 5-ml sample of plasma was aspirated under sterile conditions and kept for assay at –20°C, and the remaining plasma and red cells resuspended in 0.9% (w/v) NaCl and transfused into the ewe (Baird, Swanston & Scaramuzzi, 1976).

Oestradiol and LH were measured by radioimmunoassays, LH being expressed as ng equivalents of NIH-LH-S17 (Baird *et al.*, 1976). The interassay coefficients of variation were 9.3 and 12.0% respectively.

In each experiment, antiserum was infused at the rate of 10 ml/h (about 1/70 the total ovarian blood flow) into the ovarian artery by means of a Harvard Infusion Pump. Control antiserum, obtained from ewes immunized against bovine serum albumin (BSA), was infused into 8 ewes during the 1st and 3rd hours (Text-fig. 1, Table 1). In the 2nd hour, antiserum to testosterone (4 ewes) or oestrone (4 ewes) was infused. The specificity of the antisera was determined by measuring the dilution of antiserum which bound 50% of 5–10 pg tritium-labelled steroid when incubated *in vitro*. The cross-reactions of the testosterone antiserum were 14% with androstenedione but <2% with oestrone and oestradiol. The control antiserum and antiserum to oestrone had negligible cross-reaction with androgens or oestradiol.

It has previously been reported that during the luteal phase of the oestrous cycle of the sheep, pulses of LH occur about every 2 h and are followed by a rapid increase in the secretion of oestradiol by the ovary (Baird *et al.*, 1976). In the present experiments during the follicular phase the pulses of LH and oestradiol occurred more frequently (about once per hour (Text-fig. 1)). During the 2nd hour the titre of antiserum in ovarian venous plasma, defined as the % of 5–10 pg tritium-labelled steroid

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Text-fig. 1. Ovarian secretion of oestradiol (●) and the concentration of LH (○, ng equiv. NIH-LH-S-17) in the jugular vein in two ewes with ovarian autotransplants. Antiserum to bovine serum albumin (control) and either oestrone (a) or testosterone (b) was infused at the rate of 10 ml/h via the ovarian artery.

bound at a dilution of 1/100, rose from non-specific levels (<3%) to 61.2 ± 4.2 (S.E.M.) and 82.4 ± 4.7 for oestrone and testosterone respectively. During the infusion of antibodies to testosterone the secretion of oestradiol did not increase following an LH pulse (Text-fig. 1b) although it was maintained during infusion of the anti-oestrone serum. There was no difference in concentration of LH and secretion of oestradiol during infusion of control antiserum in the 1st hour between the two groups.

Table 1. The secretion rate of oestradiol (ng/min) during the infusion of antiserum through the ovarian artery of the ewe

Antiserum	Control 0-60 min (28)	Antiserum		Control	
		60-90 min (12)	90-120 min (12)	120-150 min (12)	150-180 min (12)
Anti-oestrone	2.21 ± 0.35	2.46 ± 0.58	3.10 ± 0.56	2.94 ± 0.49	2.82 ± 0.35
Anti-testosterone	2.09 ± 0.24	1.65 ± 0.14	$**0.95 \pm 0.08$	$*1.12 \pm 0.21$	$**1.28 \pm 0.18$

Each figure is the mean $\pm$ S.E.M. of 28 or 12 (*n*) observations in 4 sheep. During the infusion of testosterone antiserum the secretion of oestradiol was significantly depressed when compared to the 1st control period ($P < 0.005$) as well as to the corresponding value in the anti-oestrone experiments:

* $P < 0.005$; ** $P < 0.001$.

However, during and after the infusion of testosterone antiserum the secretion of oestradiol was significantly lower ($P < 0.05$, Student's t test) when compared to the control hour or to the equivalent period of infusion of the oestrone antiserum (Table 1). The concentration of oestradiol in the oestrone antiserum (0.75 ng/ml) was higher than that of the testosterone antiserum (0.18 ng/ml) but the rate of infusion (0.12 ng/min) was not sufficient to account for the greater secretion in the ewes treated with oestrogen antiserum. There were no significant changes in the concentration of LH in ovarian or jugular venous plasma during the infusion although there was a tendency for the mean values to rise progressively throughout the experiment.

The sheep follicle releases both androstenedione and testosterone into the ovarian vein *in vivo* (Baird, Goding, Ichikawa & McCracken, 1968) and into the medium when incubated *in vitro* (Seamark, Moor & McIntosh, 1974). The fact that the secretion of oestradiol was depressed during the infusion of antibodies to testosterone suggests that these androgen precursors leave the ovarian cell (theca or granulosa) before aromatization *in vivo*. However, it is possible that a proportion of the oestrogen is synthesized from androgens produced locally within the cell because the secretion of oestrogen is not completely inhibited by the infusion of androgen antibodies and theca and granulosa cells can synthesize oestrogens *in vitro* (Ryan *et al.*, 1968). It is suggested that androgens synthesized by the theca interna or stroma leave the cell and traverse the extracellular space and basement membrane before being converted to oestrogens by the granulosa cells under the influence of FSH (Dorrington *et al.*, 1975). Androgen production by thecal and stromal cells has been implicated in the induction of follicular atresia (Louvét, Harman, Schreiber & Ross, 1975). Only those follicles in which the granulosa cells have been activated by FSH can aromatize androgens and thus protect themselves from atresia.

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