Androstenedione, dehydroepiandrosterone and testosterone in ovarian vein plasma and androstenedione in peripheral arterial plasma during the bovine oestrous cycle

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Summary. Catheters were placed in the carotid artery via a facial artery ($n = 12$) and in the ovarian vein ($n = 12$), and, in conjunction, electromagnetic flow meters were placed around the ovarian artery ($n = 6$) in cyclic beef cows. Androstenedione was quantitatively the highest and dehydroepiandrosterone the lowest of the ovarian androgens measured. Ovarian androgens were correlated positively with each other ($P < 0.05$) but not with ovarian blood flow or day of the cycle. There was a trend for spikes of androgen release (ovarian vein concentration $\times$ ovarian blood flow) from the ovary to be greatest during the period of decreasing progesterone and CL regression. However, only with testosterone were spikes of release different (Days -13 to $-9 < \text{Days } -8$ to -4 ; $P < 0.05$; Day 0 = oestrus). The dynamic changes in ovarian androgens noted in this study were compatible with the concept of continuous follicular development and atresia throughout the oestrous cycle.

Introduction

Androgens appear to be involved in the regulation of follicular growth (Louvet, Harman, Schreiber & Ross, 1975) and in the regulation of the hypothalamic–pituitary axis (Martensz, Baird, Scaramuzzi & Van Look, 1976). Short (1962) isolated androgens from the bovine ovary and Shemesh & Hansel (1974), Kanchev, Dobson, Ward & Fitzpatrick (1976) and Kanchev & Dobson (1976) noted increases in peripheral testosterone concentrations before oestrus and corpus luteum (CL) regression, but no relationships were found with androstenedione during the oestrous cycle of the cow. Peterson, Fairclough & Smith (1978) found no change in testosterone values before CL regression or at oestrus in the cow. Relationships of dehydroepiandrosterone are totally unknown in the cow although dehydroepiandrosterone may be important in bovine ovarian steroidogenic pathways (Lacroix, Eechaute & Leusen, 1974). We have therefore examined the secretion of androstenedione, testosterone and dehydroepiandrosterone from the ovary of beef cows.

Materials and Methods

Animals

The 12 Hereford and Angus cows used were confirmed as having normal oestrous cycles. A cannula was placed into the ovarian vein on the side ipsilateral to the CL and into the carotid artery

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(via the facial artery; Wise, Caton, Thatcher, Barron & Fields, 1982). Blood samples were then collected during the oestrous cycle. The ovarian vasculature was closely observed for the location of the ovarian-uterine vein anastomosis so that the ovarian cannula was not inserted into or beyond this area. Patency of the ovarian vein cannula was generally limited to 5–6 days. It was not possible to study an individual animal throughout the entire period but the records for the different cows do overlap.

Ovarian blood flow was monitored in 6 animals as described by Wise *et al.* (1982). This, in conjunction with the sampling of ovarian vein and peripheral arterial blood, permitted the calculation of total androgen release from the ovary [(ovarian vein concentration – arterial concentration) $\times$ (ovarian blood flow)].

The blood samples (10 ml) were collected four times daily (06:00, 12:00, 18:00 and 24:00 h) into slightly greased, heparinized syringes. The blood was centrifuged and the plasma stored at -20°C until analysed for steroids.

Extraction and chromatography

To each 1 ml ovarian vein plasma [1,2,6,7- ^3H]androstenedione (sp. act. 114 Ci/mmol), [1,2- ^3H]dehydroepiandrosterone (sp. act. 58.6 Ci/mmol) and [1,2,6,7- ^3H]testosterone (sp. act. 98.8 Ci/mmol) were added (3500 d.p.m. of each) to determine procedural losses. All radioactive steroids were obtained from New England Nuclear, Boston, Massachusetts. Each sample was extracted twice with 10 ml freshly distilled diethyl ether. Aqueous phases were frozen in liquid nitrogen, permitting the ether to be decanted and subsequently dried under nitrogen at 40°C . The residue was applied to a 1×25 cm LH-20 column with cyclohexane–benzene–methanol (90:25:5 by vol.), and eluates were collected in 2-ml aliquants (Wise *et al.*, 1982).

Androstenedione was measured in 4-ml samples of carotid arterial plasma following addition of 1800 d.p.m. [1,2,6,7- ^3H]androstenedione and double extraction with 15 ml ether. A 0.5×7 cm LH-20 column with a cyclohexane–benzene–methanol (90:5:5 by vol.) solvent system was used to effect separation of the androstenedione (Wise *et al.*, 1982).

Hormone assays

Androgen concentrations were measured by radioimmunoassay as described by Buster & Abraham (1972) for androstenedione (antiserum S-1557 No. 2), Coyotupa, Parlow & Abraham (1972) for dehydroepiandrosterone (antiserum S-1502 No. 7) and Abraham & Chakmakjian (1973) for testosterone (antiserum S-741 No. 2). The antisera originated in sheep and were raised against androstenedione-3-oxime–human serum albumin, dehydroepiandrosterone- 3β -mono-hemisuccinate–human serum albumin and testosterone-3-oxime–human serum albumin antigen. The cross-reaction values (percentage binding at 50% displacement) were 25% for the dehydroepiandrosterone and 3% for testosterone with the androstenedione antiserum, 12% for androstenedione and $<0.01\%$ for testosterone with the dehydroepiandrosterone antiserum and $<0.1\%$ for androstenedione and $<0.06\%$ for dehydroepiandrosterone with the testosterone antiserum. Cross-reactivity with other steroids was $<1\%$.

The average ($\pm$ CV) recovery of each ^3H -labelled steroid added to plasma as an internal standard was $86\% \pm 0.23$ for androstenedione, $85\% \pm 0.01$ for dehydroepiandrosterone and $68\% \pm 0.23$ for testosterone.

Known amounts of steroids were added to duplicate plasma samples (1 ml each) from an ovariectomized cow, and the steroids were extracted, chromatographed and measured by radioimmunoassay so that the accuracy and reproducibility of the method could be determined. The pooled intra-assay coefficient of variation when 100, 200, 500 and 1000 pg androgen were added were 10.9% ($n = 39$) for androstenedione, 8.8% ($n = 27$) for dehydroepiandrosterone and 8.9% ($n = 28$) for testosterone. The pooled interassay coefficients of variation were 11.5 ($n = 4$), 9.8 ($n = 3$)

and 7.5% ($n = 3$) respectively. The sensitivity of the assays, defined as the smallest amount detectable from 0, was 15 pg/ml. Steroid concentrations were determined by a linear regression calculated from the log-logit transformation of known standards.

Statistical analysis

Data were analysed by split-plot least-squares regression as described by Barr & Goodnight (1976). An analysis of spike release of hormones as an index of ovarian secretory activity was conducted by χ^2 tests. Hormonal levels exceeding the overall mean plus two standard deviations calculated from all experimental animals was considered a spike of steroid released from the ovary by that animal (method of Brandt as described by Batson, 1956).

Changes in ovarian and peripheral androgens were analysed by least-squares to detect possible differences between groups of animals with or without blood flow transducers among the four periods (06:00, 12:00, 18:00 and 24:00 h) monitored within day, and among days before and after functional luteolysis. No differences in patterns of androgen concentrations in the ovarian vein or ovarian release were detected between animals with or without blood flow transducers.

To overcome differences in oestrous cycle lengths, all responses were adjusted to Day - 4, Day 0 being the day of expected oestrus. Day - 4 was identified when two successive peripheral blood samples contained <1 ng progesterone/ml (Wise *et al.*, 1982), indicating functional luteal regression. No attempts were made to detect oestrus for fear of damage to the ovarian vein catheters and blood flow transducers; however, any cyclic phenomena observed were supported by (1) determination of ovarian structural changes by palpation *per rectum*, (2) observed behavioural oestrus when the cow was placed with other animals after removal of the blood flow transducer and (3) a cyclic decline and increase in ovarian blood flow and progesterone levels associated with ovulation and CL renewal (Wise *et al.*, 1982).

Results

Ovarian vein androgen concentrations

As shown in Text-fig. 1 and Table 1, androgens tended to increase as the CL regressed although statistical regression analysis of androgen changes over days detected no significant changes. There

Table 1. Concentrations (arithmetic mean and range in parentheses) of androgens in the ovarian vein blood of cyclic cows (Day 0 = day of expected oestrus)

Day	No. of samples	Androstenedione (ng/ml)	Dehydroepiandrosterone (ng/ml)	Testosterone (ng/ml)
-17	4	2.5 (3.7-7.8)	0.35 (0.16-0.48)	0.7 (0.3-1.6)
-16	9	2.6 (0.3-8.1)	0.26 (0.01-0.91)	0.5 (0.0-1.5)
-15	9	4.6 (0.4-20.0)	0.46 (0.05-2.00)	0.6 (0.1-1.9)
-14	8	3.6 (0.3-11.0)	0.50 (0.05-1.10)	0.9 (0.0-2.2)
-13	9	6.9 (0.4-13.0)	3.20 (0.06-24.00)	1.7 (0.1-8.5)
-12	14	3.3 (0.2-8.5)	0.63 (0.03-1.90)	1.5 (0.2-4.3)
-11	13	5.4 (0.4-26.7)	0.29 (0.04-0.76)	0.9 (0.2-1.7)
-10	9	7.7 (1.6-26.6)	0.56 (0.18-1.30)	1.5 (0.3-3.4)
-9	9	7.5 (0.4-24.5)	0.98 (0.21-2.20)	2.1 (0.6-9.4)
-8	18	10.4 (1.4-29.5)	0.84 (0.12-2.20)	3.6 (0.5-17.3)
-7	17	8.4 (0.8-18.4)	0.78 (0.15-2.80)	4.1 (0.2-38.5)
-6	18	12.1 (1.0-39.1)	1.80 (0.48-9.20)	3.2 (0.3-7.9)
-5	13	9.9 (0.9-38.4)	2.10 (0.13-6.50)	4.7 (0.1-10.6)
-4	11	9.7 (0.8-37.6)	1.20 (0.01-10.50)	2.8 (0.8-6.6)
-3	1	3.5	0.13	0.4

Text-fig. 1. (a) Concentrations of androgens in the ovarian vein of cows and (b) the release rate of androgens from the ovary (see text) during the oestrous cycle. See Table 1 for numbers of samples and ranges of values for (a). For (b), there were 2–11 observations per day.

were no differences in androgen levels between time periods within days. Within-animal correlations between androstenedione and dehydroepiandrosterone (0.26), androstenedione and testosterone (0.24) and testosterone and dehydroepiandrosterone (0.14) were detected ($P < 0.05$); but there were no relationships of androgens with ovarian blood flow or day ($P > 0.10$). Decreases in ovarian blood flow were associated with CL regression (from 25 to < 8 ml/min), and limited blood sampling and androgen analysis from the ovarian vein after regression of the CL.

Ovarian androgens displayed wide fluctuations within day (Text-fig. 1a). The sampling period was divided into four intervals (Days -17 to -14 , -13 to -9 , -8 to -4 and -3 to $+1$ for χ^2 analysis of peaks of secretion. Although the majority of androgen peaks were found during Days -8 to -4 (1–4 days before CL regression), χ^2 analysis with orthogonal comparisons failed to detect significant differences among any of the periods.

Peripheral androstenedione

Androstenedione concentrations tended to increase 1–4 days before luteal regression (Table 2) and on Day 0. However, there were no significant day trends when androstenedione concentrations

Table 2. Concentrations (arithmetic mean and range in parentheses) of androstenedione in peripheral arterial blood of cyclic cows (Day 0 = day of expected oestrus).

Day	No. of samples	Androstenedione (pg/ml)	Day	No. of samples	Androstenedione (pg/ml)
-17	4	9.0 (7.0-13)	-7	27	160.5 (4.0-169)
-16	9	29.0 (2.0-152)	-6	30	109.0 (3.0-660)
-15	9	35.0 (4.2-91)	-5	31	56.0 (5.0-315)
-14	9	26.7 (2.1-108)	-4	31	126.0 (4.0-940)
-13	10	20.8 (6.0-67)	-3	15	34.0 (0.4-100)
-12	13	21.6 (5.0-70)	-2	14	51.5 (2.0-175)
-11	13	48.0 (2.0-352)	-1	12	28.8 (2.0-55)
-10	14	48.7 (0.3-300)	0	10	153.0 (7.0-1270)
-9	16	41.7 (10.0-119)	+1	9	24.7 (5.0-57)
-8	25	79.0 (0.3-382)	+2	10	31.0 (4.0-97)

were analysed by statistical regression analysis or for spike releases. Androstenedione concentrations in peripheral plasma were positively correlated with those in ovarian vein blood plasma ($r = 0.17$; $P < 0.05$), but no relationships were detected for time within day or ovarian blood flow.

Ovarian release of androgens

Androgen release rates from the ovary were monitored by the establishment of ovarian venous catheters and ovarian arterial blood flow transducers ($n = 6$), i.e. [(ovarian vein concentration - peripheral concentrations) $\times$ ovarian blood flow (Text-fig. 1b)] for androstenedione and (ovarian vein concentration $\times$ ovarian blood flow) for dehydroepiandrosterone and testosterone. There were no within-time or among-day trends for release of any of the hormones. Most peaks of release were during Days -8 to -4, but only the value for testosterone differed among periods (Days -12 to -9 $<$ Days -9 to -4; $P < 0.05$) in number of spikes.

Discussion

Of the androgens measured in this analysis, androstenedione was quantitatively the highest and dehydroepiandrosterone the lowest. Since Lacroix *et al.* (1974) showed that the predominant in-vitro pathway of follicular oestrogen synthesis in the cow may be via the Δ^5 pathway (pregnenolone through dehydroepiandrosterone to oestrogens) the low levels of dehydroepiandrosterone noted in this study may be due to an active metabolism of this substrate to oestrogens and a pooling of other androgens.

Shemesh & Hansel (1974) and Kanchev & Dobson (1976) reported patterns of peripheral testosterone in the cow that were similar to patterns of oestradiol. Since androgens are precursors of oestrogens, changes in ovarian androgen concentrations may reflect periods of follicular development during the bovine oestrous cycle. The rise and fall of ovarian androgens among days of the oestrous cycle may be indicative of 'waves' of follicular growth (Thibault, 1977); however, statistical regression analysis for day trends revealed no predictable changes among days in the present study. The rapid fluctuations (Text-fig. 1) in ovarian androgens suggested surges of release into the ovarian vein over a short duration similar to the peripheral androstenedione patterns noted by Kanchev & Dobson (1976) and Peterson *et al.* (1978). The dynamic changes in ovarian androgens during the oestrous cycle noted in this study may be more compatible with the data of Choudary, Gier & Marion (1968) who stressed that follicular development was continuous and independent of specific days of the oestrous cycle.

The highest levels of testosterone in the cyclic cow and ewe are during the luteal phase and just before CL regression (Kanchev *et al.*, 1976; Hoffman & Rattenberger, 1977; Herriman, Harwood, Mallinson & Heitzman, 1979). We found differences for the release of testosterone on Days -8 to -4 and Days -13 to -9: the significant increases in spikes of testosterone release before CL regression concur with the increases noted by Kanchev *et al.* (1976) in peripheral testosterone levels during the oestrous cycle.

The origin of bovine ovarian androgens is unknown. Shemesh, Hansel & Concannon (1975) reported that the bovine CL at Day 12 of the cycle contained appreciable amounts of testosterone (2.6 ng/g tissue) and that prostaglandin F-2 α added to in-vitro incubation systems stimulated testosterone synthesis. Increases in ovarian testosterone within the 4-day period before CL regression may be of luteal origin, indicating a change in CL steroid metabolism, possibly induced by prostaglandin F-2 α at the time of CL regression. The possibility of uterine prostaglandin F-2 α co-ordinating follicle growth and atresia with the fate of the CL through luteal androgens requires further elucidation.

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