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A Radioimmunoassay for Fluorogestone Acetate (FGA) and Its Application to the Measurement of Plasma FGA and Progesterone in Ewes Treated with FGA-impregnated Intravaginal Sponges

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Abstract

Simultaneous concentrations of endogenous progesterone and exogenous FGA have been measured in ewes treated with FGA-impregnated intravaginal sponges at several times relative to the expected time of release of LH.

First, a direct double antibody radioimmunoassay (RIA) for FGA, with good precision, sensitivity and reproducibility, was developed and validated. An oxime derivative was prepared and then conjugated to human serum albumen at the 3-position to produce the antigen. Antibodies raised in New Zealand White rabbits showed little cross-reactivity with related steroids. FGA was estimated in extracted and unextracted plasma; results were indistinguishable.

Second, sponges impregnated with 40 mg FGA were inserted into 20 anoestrous crossbred ewes for 12 days; 500 i.u. pregnant mare serum gonadotrophin (PMSG) was injected at withdrawal. Similar sponges were reintroduced into four ewes at each of the intervals 1, 3, 5, and 7 days later; three ewes served as controls. Plasma concentrations of progesterone and FGA were estimated by RIA daily during treatment and at intervals of 2 h for 12 h and at 18 and 24 h after withdrawal.

The plasma profiles of FGA during the two successive periods of insertion were remarkably similar. A concentration of 3·0 ng/ml (s.e.m. ±0·22) was attained on day 1, falling to 1·5 ng/ml (±0·15) by day 4. Thereafter, the concentration was maintained at 1·1 ng/ml (±0·08). Plasma progesterone concentrations were at basal levels of $<0{\cdot}2$ ng/ml during the first (acyclic) period of sponge insertion. During the second (cyclic) period there was a marked difference related to the time of sponge insertion. Insertion on day 1 (before LH release) resulted in complete inhibition of luteal activity; insertion on day 3, 5 or 7 was followed by apparently normal luteal function.

There was no evidence of any feedback mechanism of exogenous progestagen on endogenous progesterone and no interaction. It is concluded that a 12-day treatment is needed in cyclic ewes for full synchronization and that sponges impregnated with 40 mg FGA will maintain an effective plasma concentration of >1 ng/ml to the end of this period.

Introduction

Several progestagens have been used for many years for the control of reproductive phenomena in domestic animals, particularly when administered by the intravaginal route in sheep, cattle and goats. Despite this, there is a dearth of information concerning plasma concentrations of these steroids during and after treatment with intravaginal sponges and other devices. It was not until 1985 that the first such report appeared for the sheep treated with progesterone-impregnated sponges (Pearce and Robinson 1985). This was followed by a report of plasma progesterone concentrations in ovariectomized ewes treated with several types of drug-releasing devices (Hamra *et al.* 1986).

There have been no data for plasma concentrations, during and after treatment, for the progestagen most widely used by the intravaginal route for the control of the oestrous cycle of the ewe and goat doe, namely 17α-acetoxy-9α-fluoro-11β-hydroxypregn-4-ene, 3, 20-dione (SC9880; fluorogestone acetate; FGA; Cronolone; Searle), due to the absence of any published report of a radioimmunoassay (RIA). Presumptive evidence for the rate of absorption of FGA

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has been gleaned from analyses, by gas–liquid chromatography (GLC) and other means, of residual steroid in intravaginal sponges (Morgan *et al.* 1967) and in body tissues (Chien 1982).

An early study, in which progesterone was measured in ovarian vein blood by GLC (Smith and Robinson 1969), showed that exogenous FGA did not affect luteal function, with the exception of animals treated on the day of oestrus. Of 12 so treated, four failed to ovulate; the remaining eight ovulated but the resultant corpora lutea failed to develop fully and regressed early. The available technology was inadequate to measure accurately peripheral concentrations of progesterone and was quite unable to estimate FGA, so the picture was incomplete.

This paper attempts to redress this situation and deals with two aspects of the problem, namely:

(1) The development and validation of a double antibody RIA for FGA.

(2) The application of the assay to a study of the plasma concentrations of FGA during and immediately after treatment with FGA-impregnated intravaginal sponges and the interaction of the exogenous progestagen and endogenous progesterone in ewes in which the treatment commenced at predetermined stages of the oestrous cycle.

Materials and Methods

Development and Validation of the Assay

The following steps were involved. Validation was in accordance with this journal's requirements.

Solvents and chemicals

These were of Analar grade and all solutions and buffers were prepared with double-distilled water. Human serum albumen (HSA) and bovine serum albumen (BSA) were from Sigma Chemicals (St. Louis, Missouri). 1-Ethyl-3(3-dimethylaminopropyl)-carbodiimide (EDCI), complete Freund adjuvant and goat antirabbit γ-globulin were from Calbiochem.

The steps for the development and testing of the assay were essentially as described for the development (Cornette *et al.* 1971) and testing (Hiroi *et al.* 1975) of an assay for Provera (medroxyprogesterone acetate; MPA; Upjohn).

Preparation of derivative of FGA

There were two options, namely the preparation of a monosuccinate on C_{11} or of an oxime on C_3 (Erlanger *et al.* 1957, 1959; Lindener *et al.* 1972). The first was not successful because the structure of the FGA molecule is such that the axial 11 β-hydroxyl group is sterically hindered. The other option was then chosen (Fig. 1).

FGA (3·2 g), dissolved in ethanol (30 ml) at 35–40°C, was added to aminoacetic acid hemihydrochloride (1·8 g) in water (5 ml). The pH was adjusted from 2 to 14 by the addition of KOH (4 ml, 1 M). After standing for 5 h the volume was reduced on a rotary evaporator until a precipitate separated. Water (30 ml) was added to complete the precipitation and the pH was adjusted until neutral to litmus. The precipitate was dissolved in ethyl acetate and extracted with 10% (w/v) sodium carbonate. Acidification of the aqueous layer gave a colourless precipitate after standing in the refrigerator (4°C) for 2 days. Filtration gave the *carboxymethyloxime* (FGA-3-CMO; 2·64 g), as a mixture of (E) and (Z) isomers; 65 : 35. When the reaction mixture was refluxed for 5 h before isolation, extraction with chloroform gave the ethyl ester as a mixture of (E) and (Z) isomers; 62:38.

The oxime was identified by spectroscopic methods. The infrared spectrum showed the absence of the α, β-unsaturated carbonyl group at C_3 which appeared at ν_{max} 1650 cm^{-1} in the spectrum of the original ketone (FGA). The ratio of the (E) and (Z) isomers was shown by the nuclear magnetic resonance spectrum (NMR), determined by the integral of the signals for the vinyl protons at C_4 (5·74 and 6·44, respectively). The vinyl group in the (Z) isomer is deshielded by the *syn* group. Uncorrected melting points for the oxime (196–199°C) and the ethyl ester (190–196°C) were determined on a Koffler block. Infrared spectra were recorded on a Perkin–Elmer 221 spectrophotometer as nujol mulls and NMR spectra on a Varian HA NMR spectrometer with $CDCl_3$ as solvent and $SiMe_4$ as internal standard. Analysis was carried out by the Australian Microbiological Service, Melbourne.

Preparation of steroid–protein conjugate

FGA-3-CMO was conjugated to HSA or BSA, essentially as described by Lindener *et al.* (1972). To a solution of FGA-3-CMO (50 mg in 50% v/v pyridine–water) was added 50 mg EDCI.HCl (Calbiochem), in 1 ml of 50% pyridine–water. The mixture was stirred gently for 30 min at room temperature.

HSA or BSA (100 mg in 1 ml water) was added and the mixture was stirred gently overnight. Dialysis was for 2 days, first against $NaHCO_3$ buffer (0·5 M, pH 8·0) then against several changes of distilled water. This was followed by lyophilization (Erlanger *et al.* 1957, 1959). The steroid/protein ratio was tested using an Sp 800 spectrophotometer. Results following use of HSA or BSA were indistinguishable, so HSA was used. The prepared conjugate (FGA-3CMO-HSA) was stored frozen.

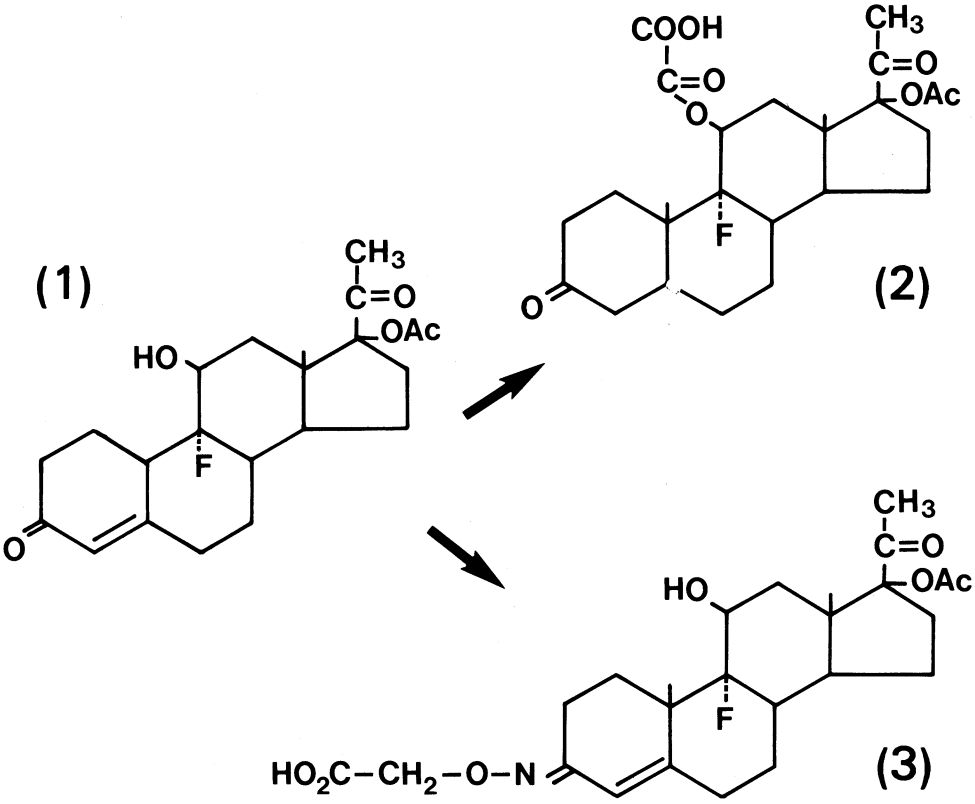

Fig. 1. Structural formulae for (1) fluorogestone acetate (FGA) and two potential derivatives, namely (2) FGA-11-hemisuccinate and (3) FGA-3-carboxymethyloxime of which the latter was chosen for conjugation to human serum albumin.

Production of antibody to FGA

FGA-3-CMO-HSA was injected into young male New Zealand White rabbits (2–4 mg/ml in 0·9% (w/v) saline-complete Freund adjuvant emulsion) at intervals of 2 weeks. Bleeding commenced after 4 weeks. Aliquots of all serum samples were stored frozen. Boosters of 4–6 mg/ml were given once a month.

Labelled FGA

This was prepared as a customer service by Amersham (England) at a specific activity of 11–22 GBq/mmole and a purity of 95%: 1·85 MBq was prepared and aliquots of 92·5 kBq placed in sealed ampoules and stored at −15°C. Unlabelled FGA (purity 100%) was provided by G.D. Searle (France).

Preparation and storage of standards and controls

All standards were made up in plasma from a wether which always gave a reading less than the limit of detection of the assay ('zero wether plasma'). The standards and controls were prepared from a stock of FGA in ethanol (200 ng/ml). The appropriate amount was taken into a clean vial and dried at 37°C in a stream of dry nitrogen. The standard was then made to volume with zero wether plasma. All standards and controls were stored cold overnight, aliquots taken and frozen. They were stable up to 6 months, as were stock solutions of FGA in ethanol stored at 4°C. Antiserum was stored as neat aliquots or in 1 : 50 dilution. Stability exceeded 2 years, as did that of labelled FGA.

Extraction

Because of the possibility of interference of water-soluble metabolites, a comparison was made of FGA concentration in 20 samples of extracted and unextracted plasma of FGA-treated ewes. For extraction, 0·4 ml of each sample and standard was mixed gently with 2 ml benzene–isooctane mixture (2:1 v:v) and equilibrated for 30 min at room temperature. The solvent layer was then transferred to RP-10D glass tubes and evaporated at 4°C under a stream of dry nitrogen. In order to conform with the matrix of standards which were in similar plasma and for valid comparison with unextracted plasma, the dried extract was reconstituted with 0·4 ml zero wether plasma and vortexed. Three aliquots of 0·1 ml then were transferred to RIA tubes and processed as described below. Extraction efficiency was determined by the addition of a tracer (1000 c.p.m. of [1, 2-^{3}H]FGA) and its recovery after the extraction procedure. Values of 90–95% were obtained, so obviating the need for a correction factor. There was excellent agreement between the results for extracted and unextracted plasma. The regression equation had the form $Y = 1{\cdot}04X - 0{\cdot}18$, where X and Y were the values for FGA in extracted or unextracted plasma, and the correlation coefficient (r) was 0·95. Hence test assays were conducted on unextracted plasma.

Radioimmunoassay

Standard curves were constructed initially covering the range of 8–1000 pg but, for routine use, 0·1 ml of each standard was dispensed in triplicate to P8 tubes to construct a seven-point standard curve from 16 to 1000 pg. Zeros were run in quadruplicate. Non-specific binding tubes were included in each assay, the first antibody being replaced by assay buffer (0·1 M Tris; pH 7·4): 0·1 ml of samples and controls were also dispensed. This was followed by the addition of 100 μl of 1 : 6000 – 1 : 8000 to give a final dilution of 1 : 18 000–1 : 24 000, according to the sensitivity desired. Tubes were then vortexed: 100 μl of 6000–8000 cpm of [^{3}H]FGA was added and the tubes were vortexed again and equilibrated overnight

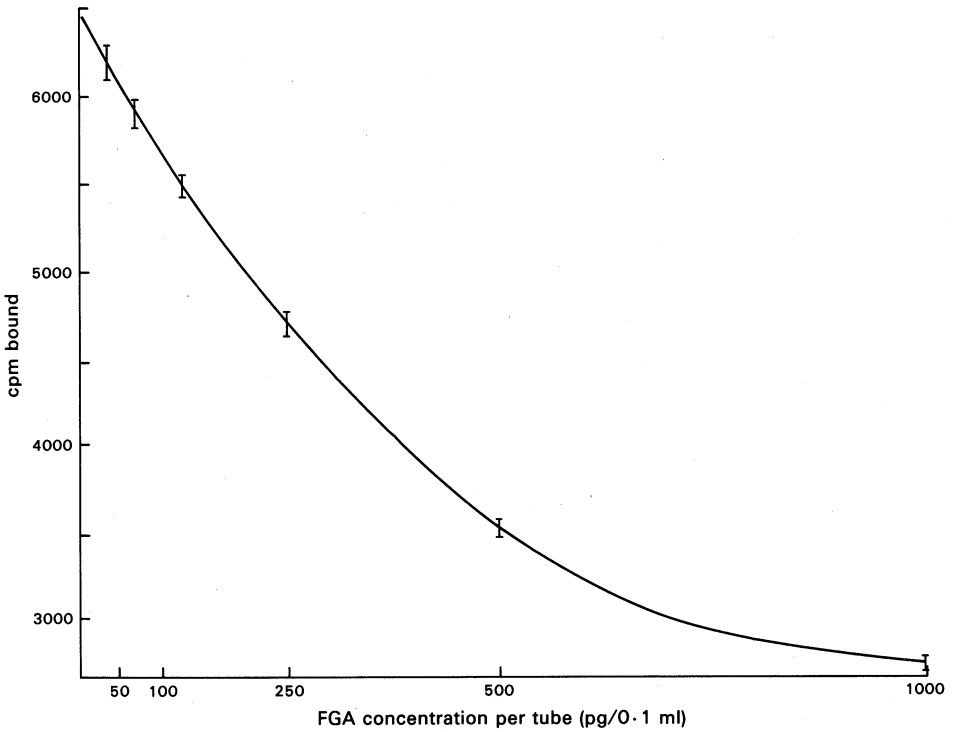

Fig. 2. Dose response curve for FGA antiserum, with associated standard errors.

at 4°C. The second antibody (100 μl goat antirabbit γ-globulin) was added, together with 100 μl normal rabbit serum (2–3%) to produce a heavier precipitate. After incubation overnight, the antigen–antibody complex was centrifuged at 2000 r.p.m. for 20 min. An aliquot of 400 μl was taken into counting vials:

10 ml toluene scintillant was added and vials were equilibrated overnight. They were counted in a LKB liquid scintillation counter. The data were processed using a weighted logit–log model on a RIA software program run on a Hewlett–Packard 85 computer.

Specificity

The assay was highly specific, as shown by the dose response curve for FGA in Fig. 2 and the following cross-reactions of several related steroids, as tested at the 50% inhibition points: progesterone, 8·5%; testosterone, 0·7%; oestrone, 2·0%; cortisone, 0·7%; 17β-oestradiol, 2·6%; 11β-hydroxyprogesterone, 0·1%; 17α-hydroxyprogesterone, 0·8%; 20α-hydroxyprogesterone, 0·0%.

Sensitivity

The least detectable dose was defined as the apparent concentration (±2 s.d.) of zero point, namely that of maximum binding. The ratio of antiserum and [^{3}H]FGA was varied in order to achieve the desired sensitivity. The lower limit was 3·1 pg (mean sensitivity = 1·2 pg; s.d. = 0·98; n = 10). Scatchard plots showed the antibody to be heterogenous, with two populations: high affinity – low capacity and low affinity – high capacity. The antibody had a high avidity for the FGA molecule, as shown by the variation in affinity according to the antibody dilution for the required sensitivity. The K_a value at 1:6000 initial dilution (1: 18 000 final dilution), and sensitivity of 5 pg, was $3{\cdot}7 \times 10^{10}$ mol/l.

Parallelism

Diluted standards in zero wether plasma and diluted plasma from FGA-treated ewes exhibited parallelism (Table 1).

Table 1. Test for parallelism of the radioimmunoassay

Sample	Dilution	FGA (pg/100 μl) Observed	Expected	Recovery (%)[A]
	(*a*) Serial dilution with 'zero wether plasma'			
Plasma of FGA-treated ewes	Undiluted	270	—	—
	1/2	160	135	118
	1/4	69	68	101
	1/8	35	34	103
	(*b*) Direct dilution with 'zero wether plasma'			
Plasma of FGA-treated ewes	Undiluted	337	—	—
	1 : 1	162	169	96
	1 : 3	94	84	112
	1 : 4	66	67	99
	(*c*) Serial dilution with 'zero wether plasma'			
Zero wether plasma +FGA (500 pg/100 μl)	Undiluted	507	500	101
	1/2	257	250	103
	1/4	134	125	107
	1/8	60	63	96

[A]Ratio of observed to expected values.

Accuracy

Table 2 presents the results for tests for accuracy, using a spiking recovery method. Recoveries ranged from 84 to 98%.

Precision

The mean intra-assay coefficient of variation, (s.d./mean) × 100, estimated from 10 replicates each of three FGA concentrations, was 7·6%. The mean interassay coefficient was 10·7%, as determined from the following data: 800 pg, n = 20, mean = 734 pg, s.d. = 29, c.v. = 4%; 250 pg, n = 18, mean = 247 pg, s.d. = 29, c.v. = 11·8%; 50 pg, n = 18, mean = 50·7, s.d. = 8·3, c.v. = 16·4%.

Plasma FGA in treated ewes

Two tests were conducted, the first with six acyclic ewes treated with intravaginal sponges containing 40 mg FGA for 12 days, and the second with 10 acyclic ewes, five of which were treated with 40 mg

and five with 80 mg sponges, all for 10 days. Ewes were bled daily during the period of insertion and at intervals of 2 h after withdrawal until 12 h, and again at 18 and 24 h.

Table 2. Test for accuracy of the radioimmunoassay

Two pooled wether plasma samples, with detectable basal levels of 7 and 65 pg/100ml, were assayed in triplicate following 'spiking' with 250, 500 or 1000 pg FGA

Basal level (pg/100ml)	Spiking solution (pg)	FGA (pg/100ml) Observed	Expected	Recovery (%)[A]
7	250	215	257	84
	500	487	507	96
	1000	920	1007	91
65	250	295	315	94
	500	556	565	98
	1000	1000	1065	94

[A]Ratio of observed to expected values.

Interaction between Exogenous Progestagen and Endogenous Progesterone

Treatments and bleeding schedules

Sponges impregnated with 40 mg FGA were inserted into 20 mature Border Leicester × Merino ewes in early September — deep anoestrus in Australia. Ewes were bled daily by jugular venipuncture using 10 ml Vacutainers. Following centrifugation the plasma was withdrawn and frozen pending assay for progesterone and FGA. On the twelfth day, sponges were withdrawn and 500 i.u. PMSG (Intervet) was injected. Bleeding continued at intervals of 2 h until 12 h, and again at 18 and 24 h.

Fresh sponges were re-inserted into four ewes at each of the intervals 1, 3, 5, and 7 days after sponge withdrawal–PMSG injection (i.e. −1, 1, 4, and 5 days after oestrus) and remained in place for a further 12 days. Blank sponges were inserted into the remaining four ewes as controls. Daily bleedings continued throughout. Following sponge withdrawal, bleeding continued on the same schedule as following the first withdrawal.

Plasma progesterone

Plasma progesterone was estimated by RIA, as described by Pearce and Robinson (1985), based on the method of Thorneycroft and Stone (1972). Progesterone antiserum was prepared in our laboratory and was used at a final concentration of 1 : 12 000. Cross-reactions were: 17α-hydroxyprogesterone, 3·4%; 20α-dihydroxyprogesterone, 1%; other steroids, <1%. Progesterone (4-pregnene-3, 20-dione, Cat. No. P-0130, lot 25c-0106, Sigma, MO, U.S.A.) was used to prepare standards in buffer. The extraction efficiency of the assay was tested on six occasions and had a mean (± s.e.m.) of 81% (±3%). The extracted water blank was always <45 pg progesterone per tube and non-specific binding was <4%. Control samples of progesterone of concentrations 0·6 and 5·3 ng/ml had interassay coefficients of variation of 14·8 and 11·5% and intra-assay coefficients of variation of 11·3 and 9·1, respectively. The limit of assay sensitivity, expressed as 2 s.d. below maximum (zero progesterone standard) binding, was 0·27 ng/ml for 100 μl plasma assayed.

Statistical analyses

In view of their uniformity, data for plasma profiles of FGA were pooled to provide overall means (± s.e.m.) for each time, during and after treatment, and 95% fiducial limits were calculated using standard *t*-tests. The data for plasma progesterone concentrations following sponge insertion at various times were subjected to split-plot analyses of variance.

Results

Development and Validation of the Assay

The ultimate value of the assay lay in its simplicity, in that direct assay could be made on unextracted plasma, and in its application to plasma samples of ewes treated with different doses of FGA. Results from the two tests were remarkably similar. Further, the variation between animals was small. Following a rapid rise to a plasma concentration of 4·2 (±0·6), ng/ml

in test 1 (40 mg) and 4·4 (±1·1) and 6·3 (±0·6) ng/ml in test 2 (40 and 80 mg), FGA fell steadily over the next 4 or 5 days and levelled out at 1·1–1·5 (±0·3) ng/ml (40 mg) or 2–2·5 (±0·3) ng/ml (80 mg) until withdrawal 1 week later.

Following withdrawal, plasma concentration remained constant for 2 h and then fell rapidly, attaining a basal level of 0·2–0·3 (±0·08) ng/ml by 10 h.

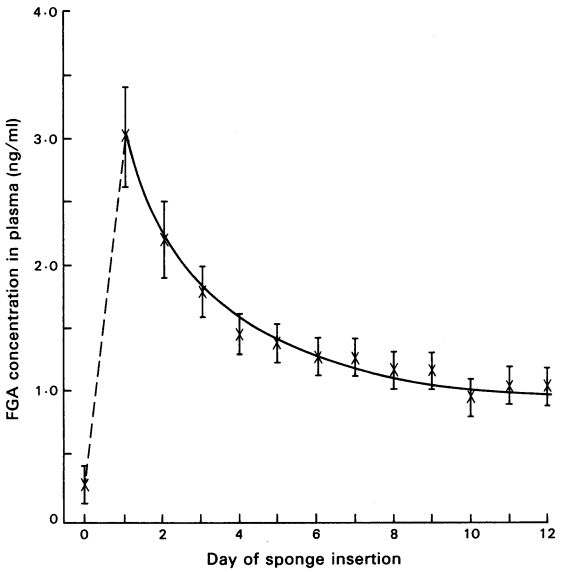

Fig. 3. Mean plasma concentrations of FGA, with 95% fiducial limits, in anoestrous crossbred ewes during insertion of intravaginal sponges impregnated with 40 mg FGA. Each point is the mean of 38 observations, expressed as ng/ml.

Interaction between Exogenous Progestagen and Endogenous Progesterone

Data for one control ewe were lost. Those for the remaining 19 ewes are presented in Figs 3–5.

The FGA plasma profiles were indistinguishable during the two successive periods of insertion. There was a rise to a concentration of 3 ng/ml plasma by 24 h, followed by a fall to a level of 1·4 ng/ml by the 4th day. A concentration of 1·3–1·1 ng/ml was then maintained until the 12th day, when the sponges were withdrawn (Fig. 3). This concentration was held until 2 h after withdrawal, and then fell away to a basal level of 0·25 ng/ml by 12 h (Fig. 4).

During the first period of FGA sponge insertion, plasma progesterone concentrations were consistently <0·5 ng/ml (mean <0·2 ng/ml). During the second period, a distinct progesterone profile was evident in the three control ewes and in all 12 into which sponges were inserted 3 or more days after sponge withdrawal–PMSG injection, but not in the four ewes into which sponges were inserted at 24 h (Fig. 5).

There was an apparent reduction in plasma progesterone concentrations in ewes treated at 3 days, but this was not significant. There was a highly significant difference between days ($P < 0{\cdot}001$) and an interaction with time of insertion ($P < 0{\cdot}001$). The curve of progesterone concentration shifted, relative to the day of sponge insertion, peak levels occurring 10 days after sponge withdrawal–PMSG injection, regardless of the time of the subsequent sponge insertion. A similar pattern, quantitatively and temporally, was exhibited by the control ewes.

Basal progesterone levels of <0·2 ng/ml plasma were attained by the day of sponge withdrawal in ewes treated on days 5 and 7 but not in ewes treated on day 3, in which the mean was 0·7 ng/ml, falling to basal levels by the next day.

Discussion

The development of 3-*O*-carboxymethyloxime as a suitable haptene for the production of the immunogen, and hence of anti-FGA antibodies, has permitted the development of a highly

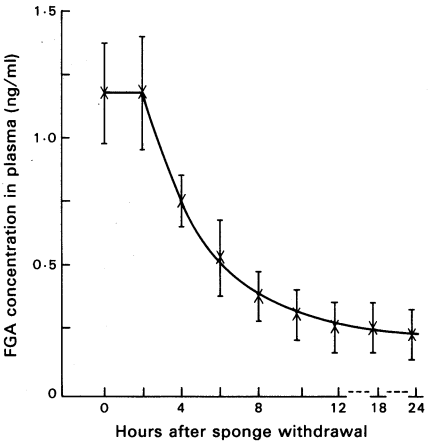

Fig. 4. Mean plasma concentrations of FGA, with 95% fiducial limits, in anoestrous crossbred ewes following withdrawal of intravaginal sponges impregnated with 40 mg FGA. Each point is the mean of 38 observations, expressed as ng/ml.

sensitive, accurate and specific assay system for FGA. The antibody showed low cross-reactivity with other structurally related steroids, resulting in good specificity.

A base level for FGA of 0·2–0·3 ng/ml was a constant feature of ewe plasma, for which few zero values were obtained. It probably represents the sum of the cross-reactions to which the antibody is susceptible. Also, as comparison between extracted and unextracted plasma showed such excellent agreement, a direct assay was developed. However, the extraction does not account for the metabolites soluble in the organic phase; only those soluble in water are eliminated.

Four points of particular interest emerge from the data for plasma FGA and progesterone in treated ewes. First of all, the shape of the profile for plasma concentrations of FGA in acyclic ewes treated with impregnated sponges was remarkably similar to that of residual FGA in the sponges, as had been estimated by GLC by Morgan *et al.* (1967).

The second point is the remarkable uniformity of the plasma profile of FGA during and after treatment with impregnated intravaginal sponges, as shown by the repeatability in two successive periods of insertion and the narrow fiducial limits associated with the estimates. This explains the precision of subsequent events, as defined by Robinson *et al.* (1987).

The third point concerns the quantitative and temporal pattern of the plasma profiles of FGA during and after treatment. The plasma concentrations of FGA during treatment (3 ng/ml, dropping to a relatively uniform level of 1·2 ng/ml) were unexpectedly high, bearing in mind the potency of this progestagen relative to that of progesterone. When Shelton (1965) first identified this steroid as a substitute for progesterone in priming the ewe to respond to oestrogen, he reported an activity 25 times that of progesterone, but with an equally short biological activity. This has been confirmed for other functions of the compound, notably the suppression and subsequent accurate release of ovulation (see Robinson 1967). Despite this, 40 mg FGA in intravaginal sponges has resulted in a plasma profile of FGA not much lower than that of progesterone observed by Pearce and Robinson (1985) during treatment of cyclic ewes with 400 mg progesterone and similar to that observed by Hamra *et al.* (1986) in progesterone-treated

ovariectomized ewes. This may be associated with a slightly longer half life; the decline in the plasma profile of FGA to a more or less constant level during the period of sponge insertion, and the drop to a basal level following withdrawal, were less steep than that reported for progesterone by Pearce and Robinson (1985). The respective times of this decline for FGA and progesterone were 10 h and 5 h, both markedly quicker than normal luteolysis.

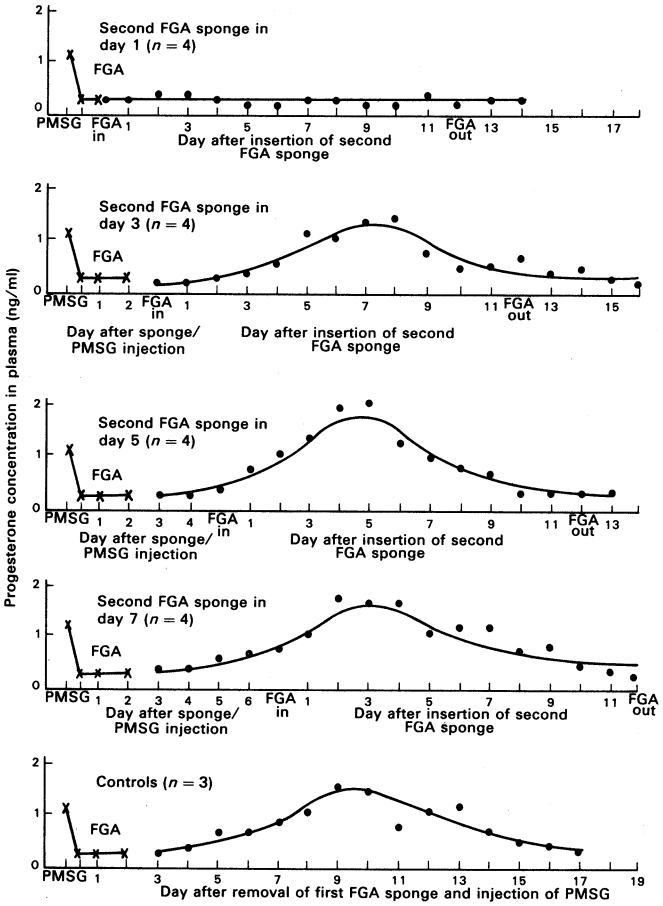

Fig. 5. Mean plasma concentrations of progesterone (ng/ml) in anoestrous crossbred ewes with a cycle induced by FGA/PMSG and then treated with intravaginal sponges impregnated with 40 mg FGA on days, 1, 3, 5 or 7, together with data for untreated controls.

The fourth point concerns the relationship between exogenous progestagen and endogenous luteal function. Early studies of Pincus and Chang (1953), Inskeep *et al.* (1963, 1964), and others had led to the conclusion that the suppression of ovulation by an exogenous progestagen is probably due to an inhibition of LH release (see Smith and Robinson 1969). Robertson and Rahka (1965, 1966) showed the release of LH to take place in the first 6 h of oestrus in the cyclic ewe. A similar time relationship applies also to the anoestrous ewe treated with PMSG following withdrawal of FGA-impregnated sponges. The time of onset of oestrus is highly predictable, with a normal distribution centred around 33–36 h, with a range of 24–48 h. This normal distribution is accompanied by a similar distribution of time of LH release, with a time lag of some 6 h (Robinson *et al.* 1987). Hence, in this experiment, ewes into which sponges were inserted at 24 h after FGA withdrawal–PMSG injection would be expected to

release LH between 6 and 30 h after sponge insertion. Administration of FGA at this time resulted in complete inhibition of luteal activity. Ewes into which sponges were inserted at 72 h would have released LH between 18 and 36 h before sponge insertion. Administration of FGA at this time had no significant effect on subsequent luteal development and function.

These data conform to earlier data from this laboratory (Smith and Robinson 1969) from which it was concluded that there was no inhibitory action of exogenous progestagen on luteal function if treatment starts on or after the 4th day of the oestrous cycle. They add to those data in that they show the independence of levels of exogenous and endogenous progestagen and highlight the vexed question of the absence of any feedback mechanism between circulating levels of progestagen and luteal activity, once the initial neural stimulus and LH release have been initiated (see Robertson 1977). They show, also, that a period of 12 days FGA sponge treatment is advisable in cyclic ewes if all ewes are to be synchronized. A plasma concentration of the order of 1–1·2 ng/ml is maintained to the end of this treatment period.

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