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Concentrations of Progesterone and the 5α-Reduced Progestins, 5α-Pregnane-3,20-Dione and 3α-Hydroxy-5α-Pregnan-20-One, in Luteal Tissue and Circulating Blood and Their Relationship to Luteal Function in the African Elephant, *Loxodonta africana*[1]

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ABSTRACT

The 5α-reduced metabolites 5α-pregnane-3,20-dione (5α-DHP) and 3α-hydroxy-5α-pregnan-20-one (5α-P-3α-OH) are the principal progestins biosynthesized by the African elephant corpus luteum. The aim of the present study was to determine luteal and circulating concentrations of these 5α-reduced progestins in relation to progesterone (P_4) and to examine whether their measurement reflects corpus luteum function. Ovarian (luteal) tissue (30 corpora lutea and 3 corpora rubra from 8 animals) and plasma samples (30 animals) were collected from pregnant and nonpregnant adult elephants shot in the Kruger National Park. Specific immunological measurement for both 5α-reduced progestins and P_4 was achieved by enzymeimmunoassay of tissue and plasma extracts following purification by HPLC. Mean (± SEM) luteal concentrations of 5α-DHP and 5α-P-3α-OH were 79,5 ± 9.4 μg/g and 196.5 ± 24.8 μg/g, respectively, approximately 2–3 orders of magnitude higher than those of P_4 (mean ± SEM, 0.16 ± 0.01 μg/g). Whereas 5α-reduced progestin concentrations tended to be lower in corpora lutea from late pregnancy compared with earlier stages and were lowest in corpora rubra, P_4 levels were similar in all tissues/stages examined. The 5α-reduced progestins also predominated over P_4 in plasma (mean 5α-DHP:P_4 and 5α-P-3α-OH:P_4 ratios 20.3 and 13.4, respectively). Similar to results for luteal tissue, plasma concentrations of 5α-reduced progestins, but not of P_4, were lower in late pregnancy than in earlier gestation stages and in nonpregnant animals. Moreover, plasma levels of both 5α-reduced metabolites were negatively correlated with gestation age, whereas those of P_4 were not. Levels of 5α-reduced metabolites (without chromatography) were also measured in weekly blood samples throughout two complete ovarian cycles in one captive female. Both measurements showed a cyclic profile (similar to that of P_4) with a luteal-phase elevation of 10- to 15-fold. The results indicate that 5α-reduced compounds are the predominant progestins contained within and secreted by the corpus luteum of the African elephant, both during the ovarian cycle and throughout pregnancy. They also provide preliminary evidence to suggest that measurements of 5α-reduced metabolites may reflect corpus luteum function more closely than those of P_4.

INTRODUCTION

The ovary of the African elephant has been the subject of considerable scientific interest and discussion for two main reasons. First, the presence of multiple corpora lutea possessing ovulation stigmata [1–3] in an apparently monovular species [4] is unusual and remains without adequate explanation [5]. Second, in terms of steroidogenic function the corpus luteum is unusual in that it appears to have a very limited secretory capacity for progesterone (P_4). Early investigators reported that in luteal extracts from both pregnant and nonpregnant animals, P_4 was either undetectable [4, 6] or present in extremely small amounts (100- to 1000-fold lower than in other mammalian species [7]), even though histologically, elephant luteal tissue appears steroidogenically active [3]. More recently, we have confirmed that quantitatively, P_4 is of minor importance and have shown that the most abundant progestins contained within and biosynthesized by the African elephant corpus luteum are the 5α-reduced metabolites, 5α-pregnane-3,20-dione (5α-DHP) and 3α-hydroxy-5α-pregnan-20-one (5α-P-3α-OH) [8]. Despite low luteal P_4 content, several studies have reported the measurement of immunoreactive P_4 in blood [5, 9–11], although concentrations are low (generally < 1 ng/ml) and not correlated with luteal volume and/or number [12]. Thus, the African elephant appears unique among mammals so far studied in that P_4 is not an abundant biosynthetic or secretory product of the corpus luteum at any stage of the reproductive cycle.

The significance of large amounts of 5α-reduced progestin metabolites with regard to ovarian physiology in this species remains unknown. In our previous study, we detected 5α-DHP and 5α-P-3α-OH as principal tissue conversion products of radiolabeled pregnenolone and as major contributors to immunoreactivity as determined by P_4 assay [8]. In the absence of specific immunological detection methods, however, quantitation of luteal content of reduced progestins was not attempted. Furthermore, the questions whether or not 5α-reduced P_4 compounds are secretory products of the corpus luteum, and whether or not they provide a useful measure of corpus luteum function in the African elephant, were not addressed.

The aim of the present study therefore was to use specific immunological measurement of 5α-reduced P_4 metabolites in conjunction with HPLC to 1) determine concentrations of 5α-DHP and 5α-P-3α-OH in relation to P_4 in individual corpora lutea and to examine whether their measurement reflects luteal functional activity and 2) establish their presence as secretory products in peripheral circulation and to compare levels and pattern of secretion to those of P_4 at various stages of the reproductive cycle.

MATERIALS AND METHODS

Animals and Sample Collection

Ovarian tissue and plasma samples were collected from adult female elephants shot for management purposes in the Kruger National Park, South Africa, during April and May

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1993. Data on luteal progestin content are presented from 2 nonpregnant animals (1 lactating, 1 nonlactating) and 6 pregnant animals (total n = 30 corpora lutea). Ovaries were removed within 30 min of death and placed on ice for transport to the field laboratory. Length and breadth of all corpora lutea were measured using calipers; to reduce processing time, weight was not determined. Tissue pieces of comparable size were removed from (to the extent possible) a similar location within the body of the corpus luteum, weighed, and placed in duplicate glass vials containing 10 ml ethanol. Macroscopically, corpora lutea appeared relatively homogeneous with respect to texture and color; care was taken to avoid collecting from external surfaces and to ensure that the sample "appeared" representative of the corpus luteum as a whole. Tissue was collected and analyzed from every corpus luteum within a pair of ovaries (range 3–6; see Fig. 1). Samples in ethanol were kept in the field at −10°C, transported to the German Primate Center, Göttingen, on dry ice, and stored at −20°C until analyzed.

Blood was collected from 22 culled animals in addition to the 8 animals mentioned above (total, n = 30). Samples were transferred to glass tubes containing EDTA as anticoagulant, and plasma was stored initially at −10°C. Sequential (weekly) blood samples were also collected for 32 wk from 1 adult female maintained in captivity in Erfurt Zoo, Erfurt, Germany. Blood (10 ml) was collected from the ear vein of the nonsedated animal into a syringe, transferred to a glass tube, and allowed to stand at 4°C for 1–2 h before being centrifuged at 500 × *g* for 20 min. Serum was collected and stored at −20°C until analysis.

In this paper, the term corpus luteum refers to pinkish-yellow to pale-brown structures formed both from follicles that have ovulated (possess ovulation stigmata) and from those that have not. The term corpora rubra is used to describe smaller structures of dark red-brown color that were removed from nonpregnant animals and assumed to represent degenerate corpora lutea from previous pregnancies [3].

Sample Extraction

Steroids were extracted from luteal tissue as described by Hodges et al. [8]. Briefly, luteal slices (~0.5 g) were hand-homogenized in 10 ml ethanol containing 50 000 cpm [^{3}H]P_4. The homogenate was decanted, together with one ethanol rinse, and centrifuged; the supernatant was decanted and reduced in volume under nitrogen to 0.5 ml. Methanol (80%, 3.5 ml) and *n*-hexane (1 ml) were added, and after vortexing and centrifugation, the methanol phase was removed. Half of the methanol phase was stored at −20°C; the other half was dried down and reconstituted in 250 μl acetonitrile (ACN):H_2O (50:50, v:v) containing [^{3}H]5α-DHP (~6000 cpm) to monitor elution positions after HPLC [12]. Irrespective of the progestin measured, efficiency of extraction (and subsequent HPLC) was monitored by the recovery of [^{3}H]P_4 added before extraction (see above). Previous tests had shown that extraction efficiencies for P_4 and 5α-DHP were similar.

Plasma samples (2 ml) from culled animals were extracted in 10 volumes diethyl ether, and recovery was monitored by the addition of 10 000 cpm [^{3}H]P_4. Sample extracts were reconstituted in ACN:H_2O for HPLC. Diethyl ether was also employed to extract steroids from serum samples (1 ml) from the captive elephant used to compile the ovarian cycle profiles, but HPLC was not performed and extractants were reconstituted in aqueous buffer ready for assay.

HPLC

Sample extracts in ACN:H_2O (250 μl; [12]) were centrifuged at 5000 × *g* before 100 μl was loaded onto a Nova Pak C-18 (Millipore Corp., Bedford, MA) HPLC column (3.9 × 75 mm). Steroids were separated by reverse-phase chromatography using an ACN:H_2O mixture (38:62) as eluent at a flow rate of 1 ml/min (see [13]). Fractions (n = 60) were collected at 1-min intervals, and 400-μl portions were counted to generate a profile of radioactivity. Aliquots of the fractions associated with [^{3}H]5α-DHP tracer were pooled, dried, and reconstituted in buffer for assay. Fractions associated with P_4 tracer were also pooled and evaporated, but were then reconstituted in 250 μl chloroform: *n*-hexane (30:70) containing 7000 cpm [^{3}H]pregnenolone, ready for a second HPLC step (see below). Since 5α-P-3α-OH tracer was not available, HPLC fractions to be taken for assay were selected according to the retention times of 5α-P-3α-OH relative to those of P_4 and 5α-DHP. Based on results from test HPLC runs (n = 6), in which the elution position of 5α-P-3α-OH was identified by measurement of immunoreactivity, retention times relative to P_4 or 5α-DHP were extremely consistent, with mean (range) values of 1.4 (1.38–1.43) and 0.70 (0.68–0.72), respectively. These two relative retention values were used to predict the peak elution position of 5α-P-3α-OH, and this fraction together with three fractions on either side were pooled, dried, and reconstituted in buffer for assay. Elution positions of individual steroids separated by this procedure are in agreement with those previously reported [8].

Since P_4 co-elutes with pregnenolone in the HPLC system used, separation of the two steroids was achieved by performing a second HPLC step. Fractions associated with the P_4 tracer from the first HPLC (see above) were injected in a volume of 100 μl chloroform:*n*-hexane onto a normal-phase silica HPLC column (Waters μPorasil [Waters Assoc., Milford, MA]; 10 μm, 3.9 × 300 mm), utilizing chloroform:*n*-hexane (30:70) and a gradient of 0–3% methanol over 24 min (flow rate, 4 ml/min) for eluting steroids. Fractions (24 × 2 ml) were collected; 400 μl was evaporated and taken for counting; and fractions containing P_4 tracer were pooled, evaporated to dryness, and reconstituted in buffer for assay. With use of this system, P_4 eluted in fractions 6–8, pregnenolone in fractions 13–16.

HPLC methods were validated by demonstrating co-elution of immunoreactivity and radioactivity in individual fractions (i.e., parallel elution profiles) for P_4 and 5α-DHP, but not 5α-P-3α-OH, since tracer was not available. Measurements of all three hormones after HPLC are considered specific. Mean ± SEM recovery values for the extraction and HPLC procedures described above were as follows: extraction and HPLC1, 77.1 ± 2.1%; extraction and HPLC1 and HPLC2, 71.2 ± 2.6%.

Hormone Assays

All hormone measurements were carried out using microtiter plate enzymeimmunoassay procedures. P_4 was determined by an assay described by Heistermann et al. [13] and validated for use with African elephant corpus luteum extracts by Hodges et al. [8]. Cross-reactivities and assay sensitivity were as reported previously [8] and include 14% for 5α-DHP and 0.7% for 5α-P-3α-OH. Intra- and inter-assay coefficients of variation determined at 22% binding

were 6.9% and 8.7%, respectively. Dilutions of plasma sample extracts (with or without HPLC) demonstrated parallelism with the P_4 standard.

5α-DHP was determined by an assay system utilizing an antiserum raised against 5α-dihydroprogesterone-11-KLH-BSA [14] together with progesterone-11-glucuronide coupled to alkaline phosphatase (Ridgeway Science Ltd., Gloucestershire, UK) as label and 5α-dihydroprogesterone (5α-DHP) as standard. The antiserum showed the following cross-reactivities relative to 5α-DHP (100%): 5-pregnen-3,20-dione, 66.5%; P_4, 43%; 11α-hydroxyprogesterone, 36.2%; 5α-pregnan-3α-ol-20-one, 1.4%; 5α-pregnan-20α-ol-3-one, < 1%; and 5α-pregnan-20β-ol-3-one, < 1%. For determination of 5α-DHP in either HPLC extracts or plasma samples, diluted 50-μl aliquots were taken in duplicate to assay. Unknowns and 5α-DHP standard (50 μl, 3.9–1000 pg/well) were combined with the P_4 label (50 μl, dilution 1:6000) and 5α-DHP antiserum (50 μl, dilution 1:40 000), mixed thoroughly, sealed onto plates with plastic film, and incubated overnight at 4°C. After incubation, the plates were washed four times and blotted dry, and 150 μl of phosphatase substrate (Sigma 104; 20 mg in 15 ml substrate buffer [15]) was added to each well. The plates were incubated for a further 2 h by shaking in the dark at room temperature before absorbance was measured. Sensitivity of the assay, determined at 90% binding, was 20 pg/well. Serial dilutions of corpus luteum and plasma extracts (with or without HPLC) gave displacement curves parallel to that obtained with 5α-DHP standard. Intra- and interassay coefficients of variation determined at 37% binding were 7.0% and 8.9%, respectively.

5α-P-3α-OH was determined using the streptavidin-biotin technique described by Meyer et al. [16]. The assay uses an antiserum raised in a rabbit against 5α-pregnan-3β-ol-20-one-3HS-BSA (supplied by E. Möstl, Institute of Biochemistry, University of Veterinary Medicine, Vienna, Austria), 5α-pregnan-3β-ol-20-one-3HS coupled to biotin as label (supplied by E. Möstl, Vienna), and 3α-hydroxy-5α-pregnan-20-one as standard. The antiserum showed the following cross-reactivities relative to 5α-P-3α-OH (100%): 5α-DHP, 66.7%; P_4, 47.3%; 5α-pregnan-20α-ol-3-one, < 1%; and 5α-pregnan-20β-ol-3-one, < 1%. For determination of 5α-P-3α-OH, 50-μl aliquots (duplicates) of diluted samples or standard (range 4.9–1250 pg/well) were combined with the label (50 μl, dilution 1:800 000) and antiserum (50 μl, dilution 1:30 000) and incubated as described for the 5α-DHP assay. After incubation, the plates were washed four times before 100 μl (20 ng) of streptavidin-peroxidase (S-5512; Sigma Chemie, Deisenhofen, Germany) in assay buffer was added to each well. The plates were incubated at room temperature in the dark for 30 min. After a second wash, substrate solution (150 μl, including 0.025% tetramethylbenzidine and 0.05% H_2O_2) was added, and the plates were incubated at room temperature in the darkness for another 45 min, after which absorbance was measured. Sensitivity of the assay determined at 90% binding was 18 pg/well. Serial dilutions of corpus luteum and plasma extracts (with and without HPLC) demonstrated parallelism with the 5α-P-3α-OH standard. Intra- and interassay coefficients of variation determined at 40% binding were 10.9% and 13.4%, respectively.

Analysis of Data

Gestation stages were calculated from fetal weights using a modification of the Huggett and Widdas equation [17] as described for the African elephant by Craig [18] and de Villiers et al. [12]. For the purposes of data shown in Figures 1 and 2, early, mid, and late stages of gestation represent the periods 150–188 days, 250–428 days, and 450–546 days of pregnancy, respectively. Pearsons Product Moment Correlation was calculated to examine the relationship between variables. Plasma hormone data were subjected to Kruskal-Wallis one-way ANOVA on ranks (data not normally distributed), using Dunn's multiple comparison method to compare hormone concentrations in samples from different reproductive states.

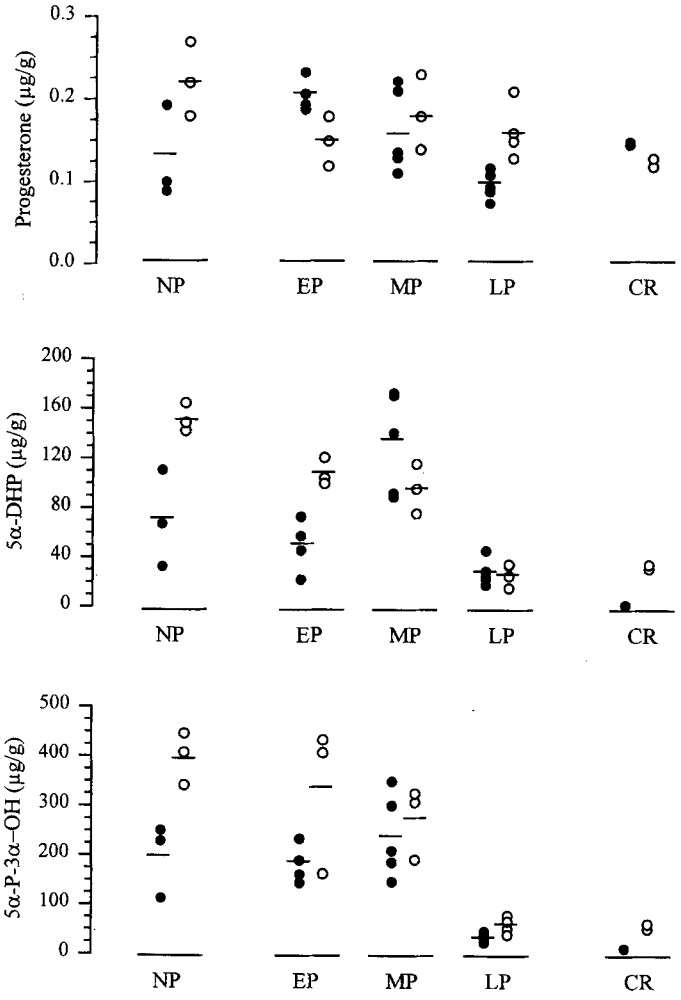

FIG. 1. Concentrations of P_4, 5α-DHP, and 5α-P-3α-OH in individual corpora lutea (n = 30) from nonpregnant (NP, n = 2), early (EP, n = 2), mid (MP, n = 2), and late (LP, n = 2) pregnant African elephants (see *Materials and Methods* for explanation of staging pregnancy). All corpora lutea present in each animal were analyzed. Hormone levels in 3 corpora rubra (CR) from the two nonpregnant animals are shown for comparison. Horizontal bars represent the mean for each animal.

RESULTS

Figure 1 shows concentrations of immunoreactive progestins in individual corpora lutea, grouped according to reproductive stage. Results indicate that P_4, 5α-DHP, and 5α-P-3α-OH were detectable in all corpora lutea measured, although there were marked differences in the absolute levels of the three hormones. Concentrations of the

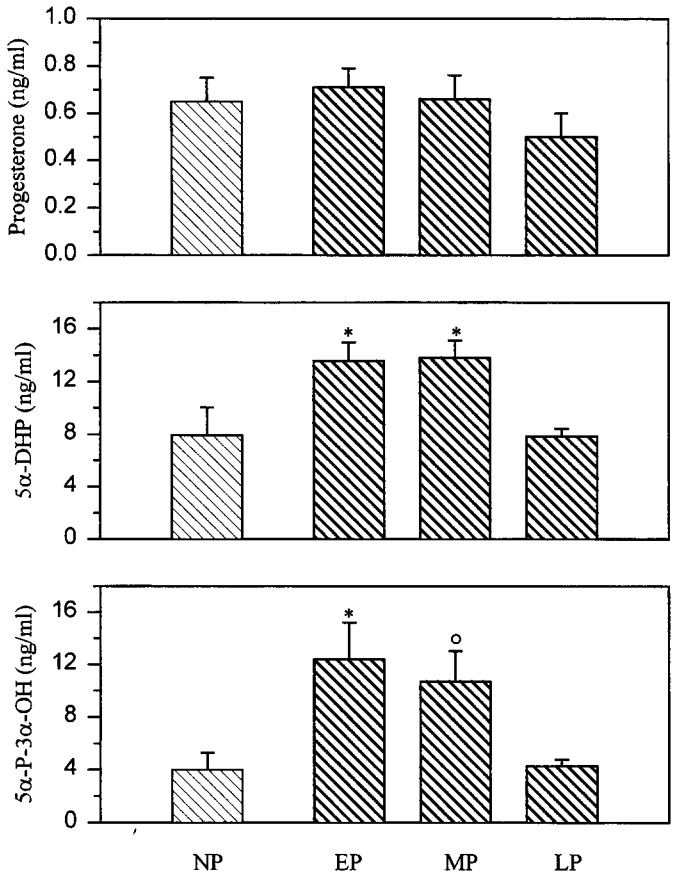

FIG. 2. Circulating concentrations of P_4, 5α-DHP, and 5α-P-3α-OH in pregnant and nonpregnant African elephants. Values are mean ± SEM; n = 8, 8, 7, and 7 for nonpregnant (NP) and early (EP), mid (MP), and late (LP) pregnant animals, respectively. * $p < 0.05$ vs. NP and LP; ° $p < 0.05$ vs. NP.

two 5α-reduced P_4 metabolites were much higher than those of P_4, with mean (± SEM) levels for the 30 corpora lutea measured being 79.5 ± 9.4 and 196.5 ± 24.8 μg/g for 5α-DHP and 5α-P-3α-OH, respectively, in comparison to 0.16 ± 0.01 μg/g for P_4. Although statistical comparison among reproductive categories was not possible, a divergent trend between the data for P_4 and the 5α-reduced progestins can be seen. Thus, whereas luteal P_4 content appeared similar in all reproductive categories, 5α-reduced progestin concentrations tended to be lower and less variable in late pregnancy. Differences are more apparent for 5α-P-3α-OH, levels of which were on average 4- to 6-fold lower in late pregnancy than in any of the other stages.

Expressed in relative terms, the mean (± SEM) ratio of 5α-DHP:P_4 values within individual corpora lutea (n = 30) was 490.9 ± 49.7, the corresponding value for 5α-P-3α-OH:P_4 being 1193.9 ± 140.2. With the exception of three corpora lutea from late pregnancy, luteal concentrations of 5α-P-3α-OH were higher than corresponding values for 5α-DPH, with a mean ± SEM ratio in all samples (n = 30) of 2.5 ± 0.2. Luteal progestin concentrations in the order 5α-P-3α-OH > 5α-DPH >>> P_4 were found for 27 of the 30 corpora lutea measured. Ratios of the 5α-reduced progestins to P_4 tended to be lower during late pregnancy than in other stages, whereas levels relative to each other were unchanged. Accordingly, concentrations of the two 5α-reduced progestins were more closely correlated with each other ($r = 0.87$, $p < 0.001$) than they were to those of P_4 ($r = 0.53$ and 0.57 for 5α-DPH and 5α-P-3α-OH, respectively, $p < 0.01$).

TABLE 1. Associations among plasma concentrations of P_4, 5α-DHP, and 5α-P-3α-OH during pregnancy and between hormone concentrations and gestation age in the African elephant.

	Correlation coefficient		
Parameter	P_4	5α-DHP	Gestation stage
P_4	—	0.25	−0.29
5α-DHP	—	—	−0.52*
5α-P-3α-OH	0.26	0.56**	−0.51*

* $p < 0.05$, ** $p < 0.01$.

In addition to measurements of corpora lutea, hormone analysis of three corpora rubra from the two nonpregnant animals was also carried out (Fig. 1). Despite the limited sample size, it can be seen that levels of 5α-reduced progestins were low relative to those in corpora lutea in all stages except late pregnancy, whereas P_4 values in corpora rubra were comparable to those of corpora lutea irrespective of reproductive stage. Moreover, of the three structures analyzed, the smallest (< 1-cm diameter) and darkest contained markedly reduced levels of 5α-reduced metabolites (1.4 and 10.9 μg/g 5α-DHP and 5α-P-3α-OH, respectively), whereas P_4 content (0.15 μg/g) was close to the overall mean value (0.16 μg/g) for luteal tissue.

Hormone concentrations in the tissue pieces sampled were correlated to overall corpus luteum size (determined as [length plus width]/2) for each of the three progestins (correlation coefficients had values ranging from 0.45 to 0.62; the p value for two was < 0.05 and for one, < 0.01). Of the 30 corpora lutea examined, ovulation points were clearly visible on 12. Distribution of ovulation points within animals ranged from 0 (one late-pregnant animal) to 3, while distribution of corpora lutea ranged from 3 to 6. Although ovulation points occurred on the largest corpus luteum in 7 of the 8 animals examined, corpora lutea with ovulation points (n = 12) were not consistently larger than those without (n = 18).

Plasma concentrations of P_4, 5α-DHP, and 5α-P-3α-OH in pregnant and nonpregnant animals are shown in Figure 2. Individual P_4 concentrations varied between 0.26 to 1.13 ng/ml, but values did not differ according to reproductive stage. In contrast, levels of 5α-reduced progestins were much higher (range 0.8–26.9 ng/ml) and were significantly elevated during early and mid-gestation compared with values from nonpregnant and late-pregnant animals. Furthermore, both 5α-DHP and 5α-P-3α-OH, but not P_4, were negatively correlated with gestational stage (Table 1).

Overall, mean ratios of 5α-DHP:P_4 and 5α-P-3α-OH:P_4 were 20.3 ± 3.4 and 13.4 ± 1.9, respectively; with few exceptions, 5α-P-3α-OH values were lower than those of 5α-DHP (mean ratio, 0.74 ± 0.1). Concentrations of 5α-DHP and 5α-P-3α-OH in all samples (n = 30) were significantly correlated with each other, but not with those of P_4, both during pregnancy (n = 21, Table 1) and as a whole (all samples, n = 30).

Profiles of immunoreactive P_4, 5α-DHP, and 5α-P-3α-OH as determined without chromatography, over a 32-wk period encompassing two complete ovarian cycles, are shown in Figure 3. Specificity of measurement was as-

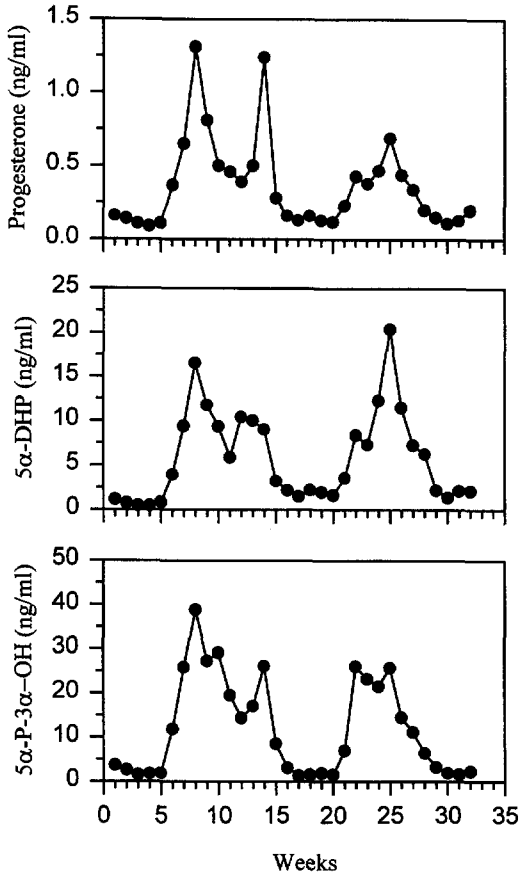

FIG. 3. Concentrations of immunoreactive P_4, 5α-DHP, and 5α-P-3α-OH in blood samples collected once weekly over a 32-wk period in an African elephant maintained in captivity. The period spans two ovarian cycles, each comprising an approximately 5-wk preovulatory (low progestin) and a 9- to 10-wk postovulatory (high progestin) phase. Unlike measurements for data presented in Figures 1 and 2, measurements were made without chromatography.

sessed separately by HPLC co-chromatography of selected midluteal-phase samples. Several peaks of immunoreactivity could be detected in the 5α-P-3α-OH assay (predominantly 5α-P-3α-OH and 5α-DHP), and there was substantial contamination (up to 50%) of P_4 measurements with 5α-DHP. In contrast, a single peak of immunoreactivity was obtained in the 5α-DHP assay, implying relative specificity. All three measurements yielded similar profiles, in which a 4- to 5-wk preovulatory (low progestin concentrations) and a 9- to 10-wk postovulatory (high progestin concentrations) period could be clearly distinguished. Luteal-phase concentrations of 5α-reduced progestins were approximately 10- to 15-fold elevated over follicular-phase values. Measurements of the three hormones were significantly correlated ($r = \geq 0.79$, $p < 0.0001$).

DISCUSSION

This paper reports the concentrations of 5α-DHP and 5α-P-3α-OH in individual corpora lutea and in peripheral blood at various stages of the reproductive cycle in the African elephant. The results confirm and extend our previous observations [8] by demonstrating the quantitative importance of 5α-reduced metabolites as biosynthetic and secretory products of the corpus luteum both during the ovarian cycle and during pregnancy.

Whereas we have previously detected 5α-reduced metabolites as cross-reacting substances demonstrating P_4 immunoreactivity, the present report provides quantitative data based on measurement by hormone-specific assays. Luteal concentrations of 5α-P-3α-OH generally exceeded those of 5α-DHP, although both progestins were present in the 100–250 μg/g tissue range, which is, on average, 500- to 1000-fold higher than corresponding values for P_4. That the P_4 content in the African elephant luteal tissue is unusually low was initially demonstrated by Short and colleagues [6, 7], who reported a difference of 2–3 orders of magnitude between P_4 content in a pregnant elephant corpus luteum (0.18 μg/g) and that in luteal tissue from numerous other mammalian species (approximate range 10–200 μg/g). Interestingly, the concentrations of 5α-reduced metabolites described here for the elephant (mean values for 5α-DHP and 5α-P-3α-OH were 80 and 200 μg/g, respectively) are in the upper range of those reported for P_4 in luteal tissue of these other mammals [7]. Thus, while the elephant corpus luteum clearly has limited secretory capacity for P_4, it is not necessarily less steroidogenically active (with respect to progestin secretion) than in other species but favors biosynthesis and secretion of 5α-reduced P_4 compounds rather than P_4 itself. In this respect, the corpus luteum of the African elephant appears to be unique among mammals studied so far.

Although the data presented in Figure 1 are limited in that they are derived from only two animals per reproductive stage, they nevertheless suggest the existence of qualitative as well as quantitative differences between luteal P_4 concentrations and those of the 5α-reduced metabolites. Thus, whereas P_4 concentrations (range of individual values and animal means) were similar at all stages, luteal content of both 5α-DHP and 5α-P-3α-OH tended to be lower and less variable in samples from late pregnancy compared with the other stages. The smaller size [12] and much darker appearance of luteal bodies during later stages of pregnancy ([3]; present study) suggest a decrease in functional activity toward the end of gestation and indeed, a reduction in steroid secretory capacity of African elephant corpora lutea during the latter part of pregnancy has been suggested on the basis of histological appearance [3]. The present finding of similar P_4 content of corpora lutea from all reproductive stages, including three corpora rubra (old, degenerate corpora lutea) almost certainly from previous pregnancies, suggests that P_4 content does not accurately reflect the functional status of luteal tissue. On the other hand, 5α-reduced metabolites, which are quantitatively more important throughout gestation but present in relatively low concentrations toward the end of pregnancy, may provide a more reliable indication of luteal steroidogenic activity.

The present study also demonstrates that 5α-reduced metabolites are important secretory products of the corpus luteum and are the predominant progestins in the circulation at all stages of the reproductive cycle. Moreover, in showing an elevation during early and mid-pregnancy, followed by a significant decline during late gestation, the pattern of circulating levels of 5α-reduced metabolites during pregnancy contrasts with that of P_4, for which stage-specific differences were not found. A reduction in circulating levels during late pregnancy supports the tendency toward reduced luteal content at this stage (Fig. 1) and substantiates the notion that synthesis and secretion

of 5α-reduced metabolites decline during the latter part of gestation.

Our findings of a lack of association between circulating P_4 concentrations and gestation stage are not in agreement with the data of de Villiers et al. [12] that describe a significant negative correlation between the two parameters. Part of the explanation for this may be that statistical significance was not reached in our study because of the relatively low numbers of samples analyzed (21 during pregnancy compared with 46 in the study of de Villiers et al. [12]). Since, however, the antibody used by de Villiers was reported to demonstrate substantial cross-reactivity with 5α-DHP (21.7%), a further possibility is that the measurements reported by de Villiers were not specific for P_4 and that the negative trend is at least in part a reflection of contamination with (and co-measurement of) 5α-DHP. Since the levels of P_4 reported by us appear, in general, to be lower than those described by de Villiers et al., the possibility of some contamination with 5α-DHP in the latter study seems likely. Similarly, it is also probable that the midpregnancy elevation in P_4 values (up to 3 ng/ml) reported by McNeilly et al. [10] reflects co-measurement of 5α-DHP in the P_4 assay (cross-reactivity of the antiserum with 5α-DHP was not tested) and that the values reported overestimate authentic P_4. Furthermore, the cyclic pattern of 5α-reduced metabolite secretion and predominance over P_4 in the nonpregnant animal (Fig. 3) indicate a similar risk of overestimation of P_4 measurements during the ovarian cycle. The present results therefore suggest a general need for caution in reporting absolute values for P_4 concentrations in the African elephant, since in the absence of evidence to exclude cross-reactivity with 5α-reduced compounds, results would be more accurately reported as P_4 immunoreactivity.

The physiological significance of the abundance of 5α-reduced metabolites in the elephant is unclear, although certain parallels exist with the situation during pregnancy in the mare, in which 5α-reduced metabolites predominate in circulation and P_4 levels (especially in the later stages) are extremely low [19, 20]. In the mare, 5α-DHP is derived primarily from endometrial metabolism of pregnenolone or P_4 [14], whereas in the elephant, much appears to be derived from ovarian (luteal) secretion. Although a contribution from sources other than the ovary has not been examined in the elephant, the negative association between 5α-reduced metabolite output and gestation stage argues against substantial placental and/or fetal production. A possible endometrial contribution, however, cannot be excluded.

With regard to biological activity, 5α-reduced P_4 metabolites are known to exert potent receptor-mediated effects on the adult rat brain, where among other actions they modulate GnRH and gonadotropin secretion [21–23]. Although 5α-DHP has been shown to bind weakly to human uterine microsomal P_4 receptors [24], significant P_4-like effects on uterine progestational parameters (endometrial secretion, myometrial stability) have not yet been described. Whether 5α-reduced compounds contribute to such effects in the African elephant depends (among other things) on the extent to which they demonstrate receptor binding in elephant uterine tissue. Given the abundance of the 5α-reduced metabolites in circulation in relation to P_4, even a relatively low binding affinity (vs. P_4) may be sufficient to be of biological relevance. In this respect, preliminary results demonstrating significant binding of 5α-DHP to the cytosolic P_4 receptor prepared from elephant endometrium [25] suggest that a progestational role for this hormone in the elephant remains an interesting possibility.

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