Steroid metabolism in corpora lutea of the western spotted skunk (*Spilogale putorius latifrons*)*

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Summary. The present study reports steroid metabolism by corpora lutea (CL) obtained from skunks with diapausing embryos ('delay' CL) and with activated embryos (activated CL). CL from both reproductive periods were incubated with various radioactive precursors. Control incubations without any tissue or with 50 μ l of packed skunk blood cells were also conducted simultaneously. Incubation of skunk CL with [3 H]-pregnenolone for 3 h resulted in 36% of the precursor accumulating as progesterone. Metabolism of [3 H]dehydroepiandrosterone (DHEA) to androstenedione proceeded with approximately the same amount of product accumulating (34–46%) as was observed in the conversion of pregnenolone to progesterone. These results suggest that Δ^5 isomerase, 3 β -hydroxysteroid dehydrogenase, is the most prominent enzyme in skunk CL. Metabolism of [3 H]pregnenolone to 17 α -hydroxypregnenolone and [3 H]progesterone to 17 α -hydroxyprogesterone occurred at low rates (1–7%), suggesting the presence of C₂₁ steroid 17 α -hydroxylase in skunk CL. Aromatase activity, as estimated by measuring accumulation of oestradiol-17 β from [3 H]testosterone, was demonstrated in activated CL. These results suggest that skunk CL appear to metabolize steroids in a manner similar to CL of other mustelids such as the ferret and American badger.

Introduction

The western spotted skunk has an unusual reproductive cycle. It breeds in late September and early October and shortly thereafter the embryos enter the uterus, embryonic development becomes arrested and blastocyst implantation is delayed for 200–220 days (Mead, 1968, 1981). This arrest in embryonic development is accompanied by incomplete luteinization of most granulosa cells and low plasma progesterone concentrations (Sinha & Mead, 1975). Luteal development is renewed a few days before implantation and is temporally correlated with blastocyst expansion (period of embryonic activation) and increased progesterone secretion (Sinha & Mead, 1975; Mead, 1981). However, implantation cannot be induced by administering progesterone to ovariectomized skunks (Mead, Concannon & McRae, 1981) or other mustelids (Mead, 1981), although the corpora lutea (CL) appear to be the source of hormones which induce implantation in these mustelids. This theory is supported by the observation that CL are the only ovarian compartment in the spotted skunk which exhibit striking morphological and cytological changes that are temporally correlated with embryonic development and implantation (Mead & Eik-Nes, 1969; Mead, 1981). Moreover, implantation can be induced in ovariectomized mustelids, such as the ferret and mink, bearing ectopic CL but not by progesterone (Foresman & Mead, 1978; Murphy, Mead & McKibbin, 1983). It would therefore appear that the CL of these species are secreting another compound which acts in conjunction with progesterone to induce implantation. This possibility was investigated in the present study of steroid metabolism of skunk CL.

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Materials and Methods

Wild female western spotted skunks (*Spilogale putorius latifrons*), captured between 1 November and 30 January, were purchased from a trapper in Oregon. The skunks were caged individually in a room illuminated by environmental light only and supplied *ad libitum* with dried dog food and water. The CL from 22 skunks were collected between 29 January and 15 March (period of embryonic diapause), i.e. about 81–36 days before the average date of implantation (20 April). Activated CL, which were larger in size and weight than those obtained during delayed implantation, were obtained from another 25 females. Increased blastocyst diameter was also used as a criterion that blastocyst implantation was impending. All animals were anaesthetized with pentobarbitone sodium (40 mg/kg) and a blood sample was taken by cardiac puncture. The ovaries were removed, the CL dissected free of any adhering follicles or interstitial tissue and placed in medium TC-199 at 4°C. Only CL from skunks possessing unimplanted blastocysts, which were flushed from the uterus and measured with a calibrated ocular micrometer, were used. The CL were then quartered, pooled and weighed into 7.5 mg samples.

The steroids used were [7-³H]pregnenolone (sp. act. 10 Ci/mmol: Amersham International, Bucks, U.K.), [1,2-³H]progesterone (sp. act. 57 Ci/mmol: New England Nuclear, MA, U.S.A.), [1,2,6,7-³H]dehydroepiandrosterone (DHEA) (sp. act. 64 Ci/mmol: Amersham) and [1,2,6,7,16,17-³H]testosterone (sp. act. 135 Ci/mmol: New England Nuclear). All tritiated precursors were purified 1 day before use as described by Kintner & Mead (1983). The samples of luteal tissue were incubated with 5 µCi of one of the above radiolabelled precursors for 10, 30, 60 or 180 min at 37°C, in 3 ml of Krebs–Ringer–bicarbonate (KRB) buffer pH 7.1 in an atmosphere of 95% air : 5% CO₂. Control incubations with the same amount of radiolabelled steroid were conducted in flasks containing KRB buffer without tissue. Another control consisted of 50 µl packed skunk blood cells which were also incubated in KRB buffer. The incubations were stopped by the addition of 5 ml ethyl acetate.

Tissue homogenization, extraction, addition of authentic labelled steroids to monitor recovery, paper chromatography to separate the various steroid complexes (oestradiol and 17α-hydroxypregnenolone, oestrone and androstenediol, 17α-hydroxyprogesterone and testosterone, 20α-dihydroprogesterone, DHEA and androstenedione, and 5α-dihydroprogesterone and progesterone), thin-layer chromatography (t.l.c.) to isolate individual steroids, and quantitation were identical to those described by Kintner & Mead (1983) with the following exceptions.

Oestradiol and 17α-hydroxypregnenolone

After eluting this steroid complex from paper chromatograms, an appropriate amount of [¹⁴C]-oestradiol was added to monitor recovery. Due to the lack of a labelled 17α-hydroxypregnenolone standard, recovery of this steroid was estimated on the basis of the average loss expected for each step involved in the isolation process. Initial separation of these steroids was achieved by t.l.c. (benzene : diethyl ether, 3 : 1 v/v). The 17α-hydroxypregnenolone was acetylated and the products were extracted, chromatographed, and quantitated as described by Kintner & Mead (1983). Oestradiol was methylated (Brown, 1955) and the methyl ether of oestradiol was isolated by t.l.c. (benzene : methanol, 95 : 5 v/v) and quantitated by liquid scintillation spectrometry.

Oestrone and androstenediol (5-androsten-3β,17β-diol)

These were separated by t.l.c. (chloroform : ether, 4 : 1 v/v). Recovery of androstenediol was monitored by processing additional tubes containing authentic tritiated and unlabelled androstenediol whereas [¹⁴C]oestrone was added directly to each sample after elution from the paper to monitor recovery of oestrone. Oestrone was methylated (Brown, 1955), the methyl ether was isolated by t.l.c. (benzene : methanol, 95 : 5 v/v) and quantitated by liquid scintillation

spectrometry. Androstenediol was acetylated, isolated and quantitated as described by Kintner & Mead (1983).

Aliquants of each major radioactive steroid product obtained from luteal samples were pooled and stored at 4°C until further use. Then 100 000–250 000 c.p.m. of the ^3H -products were mixed with 25–35 mg of the appropriate authentic steroid and recrystallized up to 5 times. Dried samples of each set of crystals were weighed on a Cahn electrobalance and counted in a liquid scintillation spectrometer. The steroid was considered radiochemically pure if three successive sets of crystals had specific activities within a 5% deviation about the mean.

All values are reported as the percentage of initial ^3H -labelled precursor metabolized to each steroid. Results of all incubations were first statistically analysed by analysis of variance. Significant differences between means were subsequently determined using least squares means. Values were considered significantly different if $P < 0.05$.

Results

Blastocysts from skunks exhibiting embryonic diapause averaged 0.9 mm in diameter (0.7–1.1 mm) and the mean weight of each CL was 2.7 mg; whereas activated blastocysts from skunks averaged 1.4 mm (1.2–2.0 mm) and the mean weight of each CL was 5.6 mg.

Activated CL and those in delay metabolized [^3H]pregnenolone to other steroids to approximately the same extent (Table 1). Depending upon the techniques used for separation, the average recoveries for the different steroids ranged from 46 to 76%. About 36% of the total radioactivity added accumulated as progesterone after 180 min of incubation ($P < 0.05$) during both stages of pregnancy. Only small (<3%) but statistically significant ($P < 0.05$) amounts of 17 α -hydroxypregnenolone and androstenedione accumulated during the course of 3-h incubations of both types of CL (Table 1). Small amounts of label were likewise incorporated into testosterone by 'delay' CL. However, the amounts of 17 α -hydroxypregesterone and DHEA which accumulated during incubations for 10–180 min were not statistically different from control incubations containing plain buffer or packed blood cells (data not shown). Progesterone, which was isolated as 20 β -dihydroprogesterone, and androstenedione were demonstrated to be radiochemically pure by recrystallization to constant specific activity (Table 2). However, attempts to recrystallize 17 α -

Table 1. Percentage of [^3H]pregnenolone metabolized ($\pm$ s.d., no. of observations in parentheses) to product by skunk CL

Product	Incubation time (min)	Blank	Blood cells	'Delay' CL	Activated CL
Progesterone (62%)*	10	$^{a}0.1 \pm 0.1$ (2)	$^{a}0.1 \pm 0.1$ (3)	$^{b}3.7 \pm 0.5$ (2)	$^{a}2.6 \pm 2.2$ (2)
	30	$^{a}0.1 \pm 0.2$ (5)	$^{a}0.0$ (4)	$^{b}7.2 \pm 2.8$ (3)	$^{b}7.5 \pm 2.8$ (4)
	60	$^{a}0.0$ (4)	$^{a}0.1 \pm 0.1$ (5)	$^{b}22.9 \pm 14.3$ (2)	$^{b}12.9 \pm 2.2$ (3)
	180	$^{a}0.1 \pm 0.1$ (7)	$^{a}0.1 \pm 0.1$ (7)	$^{b}36.5 \pm 5.3$ (3)	$^{b}36.7 \pm 12.4$ (5)
Androstenedione (46%)*	10	$^{a}0.2 \pm 0.1$ (2)	$^{a}0.1 \pm 0.2$ (2)	$^{a}0.0$ (2)	$^{a}0.2 \pm 0.1$ (2)
	30	$^{a}0.1 \pm 0.1$ (4)	$^{a}0.1 \pm 0.1$ (4)	$^{a}0.1 \pm 0.0$ (2)	$^{b}0.6 \pm 0.5$ (4)
	60	$^{a}0.2 \pm 0.2$ (5)	$^{a}0.0$ (5)	$^{b}0.4 \pm 0.2$ (3)	$^{b}0.4 \pm 0.3$ (3)
	180	$^{a}0.3 \pm 0.3$ (7)	$^{a}0.0$ (6)	$^{b}0.9 \pm 0.3$ (2)	$^{b}0.5 \pm 0.3$ (5)
Testosterone (63%)*	60	$^{a}0.2 \pm 0.1$ (3)	$^{a}0.0$ (2)	$^{b}0.3 \pm 0.2$ (3)	
	180	$^{a}0.2 \pm 0.2$ (3)	$^{a}0.0 \pm 0.1$ (3)	$^{b}0.6 \pm 0.2$ (3)	
17 α -OH pregnenolone (76%)*	10	$^{a}0.3 \pm 0.2$ (3)	$^{a}0.2 \pm 0.1$ (2)	$^{a}0.1 \pm 0$ (2)	$^{b}0.8 \pm 0.7$ (2)
	30	$^{a}0.3 \pm 0.2$ (3)	$^{a}0.3 \pm 0.1$ (4)	$^{a}0.2 \pm 0$ (3)	$^{b}1.0 \pm 0.4$ (4)
	60	$^{a}0.1 \pm 0.1$ (5)	$^{a}0.1 \pm 0.1$ (4)	$^{b}1.0 \pm 0.2$ (3)	$^{b}0.5 \pm 0.1$ (3)
	180	$^{a}0.2 \pm 0.1$ (7)	$^{a}0.2 \pm 0.1$ (7)	$^{b}2.2 \pm 0.5$ (3)	$^{b}1.3 \pm 0.5$ (5)

Different superscripts indicate significant differences ($P < 0.05$) across rows.

* Average recovery of product after chromatography and derivative formation.

Table 2. Recrystallization of radiolabelled products isolated after incubation of skunk CL with [^3H]pregnenolone

Recrystallization number	20 β -Dihydroprogesterone acetate (d.p.m./ μg)	Androstenedione (d.p.m./ μg)
1	14.7 (pentane : benzene v/v)	5.5 (pentane : acetone)
2	14.8 (petroleum ether : benzene v/v)	3.4 (benzene : acetone)
3	15.5 (MeOH : H_2O)	3.6 (pentane : acetone)
4	15.2 (MeOH : H_2O)	3.5 (benzene : acetone)
5	14.9 (acetone : MeOH)	

Table 3. Percentage of [^3H]progesterone metabolized ($\pm$ s.d., no. of observations in parentheses) to product by skunk CL

Product	Incubation time (min)	Blank	Blood cells	'Delay' CL	Activated CL
20 α -Dihydroprogesterone (56%)*	30	^a 0.0 (7)	^a 0.0 (8)	^a 0.0 (3)	^a 0.0 (5)
	60	^a 0.0 (4)	^a 0.0 (3)	^a 0.0 (2)	^a 0.0 (4)
	180	^a 0.0 (8)	^a 0.0 (9)	^b 0.2 $\pm$ 0.1 (2)	^a 0.0 (4)
17 α -Hydroxyprogesterone (48%)*	30	^a 0.4 $\pm$ 1.0 (7)	^a 0.0 $\pm$ 0.1 (8)	^a 0.7 $\pm$ 0.2 (3)	^a 0.6 $\pm$ 0.7 (5)
	60	^a 0.1 $\pm$ 0.2 (4)	^a 0.0 (4)	^b 3.0 $\pm$ 0.5 (2)	^a 0.5 $\pm$ 0.1 (4)
	180	^a 0.1 $\pm$ 0.1 (6)	^a 0.1 $\pm$ 0.1 (8)	^c 7.5 $\pm$ 0.7 (2)	^b 1.3 $\pm$ 2.2 (4)

Different superscripts indicate significant differences ($P < 0.05$) across rows.

* Average recovery of product after chromatography and derivative formation.

hydroxypregnenolone and testosterone to constant specific activity were not successful due to lack of sufficient ^3H -labelled product.

Small amounts of 17 α -hydroxyprogesterone ($P < 0.05$) accumulated after incubating 'delay' and activated CL with [^3H]progesterone (Table 3). The amount of 20 α -dihydroprogesterone accumulated after incubating 'delay', but not activated CL, with [^3H]progesterone was very low but statistically significant ($P < 0.05$). Metabolism of [^3H]progesterone to 5 α -dihydroprogesterone, androstenedione and testosterone did not occur in significant quantities ($P > 0.05$) (data not presented). Recoveries of various steroid products which accumulated after incubation with progesterone varied from 48 to 75%. Sufficient amounts of [^3H]20 α -dihydroprogesterone or [^3H]17 α -hydroxyprogesterone were not available for recrystallization.

Androstenedione was the major metabolite recovered after incubating CL from skunks with diapausing and activated blastocysts with [^3H]DHEA: 34–46% of the total radioactivity added had accumulated in this product after 180 min of incubation (Table 4). The [^3H]androstenedione was shown to be radiochemically pure by recrystallization to constant specific activity (data not shown). Small but significant ($P < 0.05$) quantities of [^3H]oestrone also accumulated after incubating activated CL but not 'delay' CL with [^3H]DHEA (Table 4). The identity of this product was not verified by recrystallization because all of the product was used for quantification purposes. There was no evidence that [^3H]DHEA was converted to androstenediol although the average recovery for this product was high (61%, data not shown).

[^3H]Testosterone was metabolized to oestradiol-17 β in low but significant ($P < 0.05$) amounts by activated skunk CL (Table 5). A similar test for the presence of aromatase activity was not conducted with 'delay' CL because insufficient tissue was available. The [^3H]oestradiol recovered was demonstrated to be radiochemically pure by recrystallization to constant specific activity (data not shown).

Table 4. Percentage of [³H]dehydroepiandrosterone metabolized ($\pm$ s.d., no. of observations in parentheses) to product by skunk CL

Product	Incubation time (min)	Blank	Blood cells	'Delay' CL	Activated CL
Androstenedione (66%)*	30	^a 11.8 $\pm$ 4.2 (4)	^a 9.4 $\pm$ 6.0 (2)		^a 6.6 $\pm$ 3.6 (3)
	60	^a 5.4 $\pm$ 2.0 (7)	^a 7.6 $\pm$ 5.1 (9)	^b 27.6 $\pm$ 8.3 (3)	^b 19.6 $\pm$ 9.7 (7)
	180	^a 6.8 $\pm$ 3.0 (7)	^a 5.8 $\pm$ 1.5 (10)	^b 34.0 $\pm$ 3.3 (3)	^b 46.7 $\pm$ 13.7 (7)
Oestrone (68%)*	30		^a 0.0 (3)		^a 0.2 $\pm$ 0.3 (9)
	60	^a 0.0 (10)	^a 0.0 $\pm$ 0.1 (9)	^a 0.1 $\pm$ 0.1 (3)	^b 0.2 $\pm$ 0.2 (7)
	180	^a 0.0 (8)	^a 0.0 $\pm$ 0.1 (10)	^a 0.1 $\pm$ 0.1 (3)	^b 0.2 $\pm$ 0.2 (5)

Different superscripts indicate significant differences ($P < 0.05$) across rows.

* Average recovery of product after chromatography and derivative formation.

Table 5. Percentage of [³H]testosterone metabolized ($\pm$ s.d., no. of observations in parentheses) to product by skunk CL

Product	Incubation time (min)	Blank	Blood cells	Activated CL
Oestradiol (64%)*	60	^a 0.1 $\pm$ 0.0 (3)	^a 0.1 $\pm$ 0.1 (3)	^b 0.4 $\pm$ 0.1 (3)
	180	^a 0.1 $\pm$ 0.0 (3)	^a 0.1 $\pm$ 0.0 (3)	^b 0.5 $\pm$ 0.2 (5)

Different superscripts indicate significant differences ($P < 0.05$) across rows.

* Average recovery of product after chromatography and derivative formation.

Packed blood cells were used as an additional control due to the highly vascular nature of the CL. In all cases, steroid metabolism by blood cells was not significantly different ($P > 0.05$) from controls without luteal tissue or cells.

Discussion

The most prominent steroid enzyme in skunk luteal tissue was found to be Δ^5 isomerase, 3β -hydroxysteroid dehydrogenase (3β -HSD) because incubation of skunk CL with [³H]pregnenolone for 3 h resulted in 36% of the precursor accumulating as progesterone. Studies of the ferret revealed progesterone to be the major steroid present in the CL (Dimond, Buchanan, Belbeck, Murphy & YoungLai, 1977). Earlier work with American badgers (Fevold & Wright, 1969) and ferrets (Kintner & Mead, 1983) demonstrated that CL produced large amounts of progesterone after incubation with [³H]pregnenolone. Our observation of high 3β -HSD activity in the skunk is therefore similar to the findings for badgers and ferrets. We found no evidence that progesterone was reduced to 5α -dihydroprogesterone and negligible quantities of 20α -dihydroprogesterone accumulated after 3-h incubations of 'delay' CL only. Similar observations regarding 5α -reductase and 20α -hydroxydehydrogenase activities have been reported for ferret CL (Kintner & Mead, 1983). Low plasma levels of 20α -dihydroprogesterone were reported for mink, suggesting that this enzyme may not be very active *in vivo* (Murphy & Moger, 1977). Another reaction catalysed by 3β -HSD, the metabolism of DHEA to androstenedione, proceeded with approximately the same amount of product accumulating (34–46%) as was observed in the conversion of pregnenolone to progesterone. These results suggest that if DHEA or androstenedione were made available from a non-luteal compartment of the ovary, they could be converted to testosterone or oestrogens within the CL. However, we found inconsistent evidence of 17β -hydroxysteroid dehydrogenase (17β -HSD) activity: conversion of [³H]DHEA to androstenediol did not occur and testosterone did not accumulate in incubations of CL with [³H]progesterone. Nevertheless, when CL were incubated

with [^3H]pregnenolone, 0.3–0.6% of this precursor accumulated as testosterone, but this product failed to reach constant specific activity after 4 recrystallizations (data not shown). Had sufficient material remained for a 5th crystallization it is quite likely that radiochemical purity of this product would have been demonstrated.

Kintner & Mead (1983) also demonstrated a high percentage of conversion of [^3H]DHEA to androstenedione in ferret CL. Dimond *et al.* (1977) reported high levels of androstenedione in ferret CL, but because the amount of androstenedione was estimated by radioimmunoassay, it is not known whether this was produced from 17α -hydroxyprogesterone or DHEA. The fact that skunk CL readily metabolized DHEA to androstenedione suggests that the efficacy of androstenedione to induce implantation should be tested since androgens such as testosterone and 5α -dihydrotestosterone can induce implantation in mice (Roy, SenGupta & Manchanda, 1980). However, administration of three different doses of androstenedione to progesterone-primed ferrets ovariectomized on Day 6 of pregnancy failed to induce implantation by Day 13 (Kintner & Mead, 1983). There is therefore reason to doubt whether this might be the luteal factor which acts in conjunction with progesterone to induce implantation in mustelids.

Metabolism of [^3H]pregnenolone to 17α -hydroxypregnenolone and [^3H]progesterone to 17α -hydroxyprogesterone suggests the presence of C_{21} steroid 17α -hydroxylase in skunk CL. However, only very small quantities of these products accumulated. Minimal activity of this enzyme was also demonstrated in ferret CL (Kintner & Mead, 1983). There was a lack of accumulation of DHEA from 17α -hydroxypregnenolone and of androstenedione from 17α -hydroxyprogesterone, suggesting that $17\text{--}20$ desmolase might not be very active. However, the observation that [^3H]pregnenolone was converted to androstenedione suggests that this enzyme is indeed present in the skunk CL.

Aromatase activity, as estimated by measuring the accumulation of oestradiol- 17β from [^3H]testosterone, was present in activated CL. It has likewise been demonstrated that ferret CL contain this enzyme (Mead & Swannack, 1980) and that the precursor (testosterone) is present in ferret CL collected on Days 6 and 8 of pregnancy (Mead & McRae, 1982). Dimond *et al.* (1977) reported that significant quantities of androstenedione, testosterone and oestradiol were secreted into the culture medium during a 24-h incubation of ferret CL on Day 12 of pregnancy. Oestrogen levels measured throughout pregnancy in the spotted skunk ranged from 3 to 29 pg/ml and were lower a few days before implantation (3–8 pg/ml) than during the preceding period of embryonic diapause (Ravindra & Mead, 1984). Although oestrogen has been demonstrated to be an essential factor for implantation in the rat and mouse (Weichert, 1942; Cochrane & Meyer, 1957; Smithberg & Runner, 1960; Yoshinaga, 1961; Harper & Walpole, 1967), there is no evidence that oestrogen is required to induce implantation in any carnivore. The initiation of implantation with oestrogen or a combination of progesterone and oestrogen has so far failed in weasels (Wright, 1963; Shelden, 1973), European badger (Canivenc, 1966), mink (Cochrane & Shackelford, 1962), and ferrets (Wu & Chang, 1972, 1973; Mead & McRae, 1982). Murphy & Mead (1976) could not inhibit implantation in ferrets by daily injections of high titre oestrogen antibody on Days 3–13 of pregnancy.

It could be argued that the only enzyme (3β -HSD) which was observed to be highly active requires NAD whereas all the other enzymes, which require NADPH, exhibited lesser activity because inadequate NADPH was synthesized by luteal tissue in the absence of glucose in the medium. However, NADPH could not have been exhausted otherwise we would not have observed increased synthesis of products such as 17α -hydroxypregnenolone (Table 1) and 17α -hydroxyprogesterone (Table 3) with increased time of incubation.

Packed skunk blood cells were incubated as additional controls due to the highly vascular nature of the CL. Steroid metabolism by blood cells was not observed, suggesting that blood cells trapped within skunk CL do not contribute significantly to steroid metabolism. This finding differs slightly from the observations of Kintner & Mead (1983) who reported that ferret blood cells exhibited 20α -hydroxydehydrogenase and 5α -reductase activity.

The present study did not reveal any dramatic differences in steroid metabolism by CL during the two periods of pregnancy investigated. We do not believe this is due to inappropriate classification of the two types of CL. Prior studies have convincingly demonstrated that skunk blastocysts with diameters of <1.2 mm exhibit more depressed protein synthesis (Rourke & Mead, 1982) and RNA synthesis (unpublished observations) than those with larger diameters. The majority of animals in this study classified as activated had blastocyst diameters of ≥ 1.6 mm. Based upon empirical observations, we estimate that such blastocysts are less than 3 days away from implantation. Moreover, earlier studies suggest that morphological and biochemical changes in skunk CL can be detected several days before implantation (Sinha & Mead, 1975, 1976; Mead, 1981). Our results do not readily suggest which steroid(s) (in conjunction with progesterone) could be candidates for the unknown luteal factor which is believed to induce implantation in the skunk. In fact, these results could be interpreted as suggesting that the luteal factor is not a steroid.

This study was supported by a grant from the National Institutes of Child Health and Human Development (HD 06556). We thank Ms Peggy Kintner and Martha McRae for excellent technical assistance and Ms Linda Florence for typing the manuscript.

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Received 19 March 1984