Ovarian Dehydrogenase Activities During Pregnancy in the Rabbit (36629)

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It is difficult to assess ovarian function on morphological and histological grounds alone. Therefore, we sought reliable enzymic markers of ovarian endocrine activity with which we could evaluate the process of luteolysis. In the rat, regression of the corpora lutea coincides with a dramatic rise in ovarian 20 α-hydroxysteroid dehydrogenase (20α-OH-SDH) content (1–3). This enzyme is responsible for the conversion of progesterone to the 20α-hydroxyl derivative, 20α-hydroxypregn-4-en-3-one (20α-OH-P), a biologically weak progestin (4). In the rat ovary, 20α-OH-SDH activity is localized primarily in regressing luteal tissue (1, 2, 5). These relationships suggest that 20α-OH-SDH can serve as a sensitive index of luteolysis in the rat and that functional luteolysis may be effected by increasing ovarian catabolism of progesterone to 20α-OH-P.

The purpose of this investigation was to study the relationship between luteal function and 20α-OH-SDH activity during pregnancy in the rabbit. Glucose-6-phosphate dehydrogenase (G-6-PDH), isocitrate dehydrogenase (ICDH), 3β- and 17β-hydroxysteroid dehydrogenases were measured for comparison.

Materials and Methods. Mature New Zealand rabbits (3–4 kg) were isolated for at least 21 days before use. Animals were killed in estrus and at various times after mating. The ovaries were removed, trimmed of adherent fat, weighed, and corpora lutea were then dissected from the interstitial tissue. In the first series of animals, both luteal and interstitial tissues were prepared for enzyme assays by previously described methods (6). In the second series, similar methods were used except that tissue was homogenized in distilled water (50 mg/ml) and the homogenates were centrifuged at 30,000*g* for 30 min. 20α-OH-SDH and G-6-PDH assays were performed by the methods reported by Strauss, Foley and Stambaugh (6). The 17β- and 3β-OH-SDH assays were modified from the 20α-OH-SDH assay in the following respect. In the 17β-OH-SDH assay testosterone (0.347 μmoles) served as substrate and in the 3β-OH-SDH assay 5β-androstan-3β-ol-17-one (0.344 μmoles in 0.1 ml acetone) served as substrate and NAD (2 μmoles) was substituted as cofactor. ICDH was assayed spectrophotometrically at 37°. The reaction cuvette consisted of 100 μmoles Tris buffer, 3.9 μmoles sodium DL-isocitrate, 10 μmoles $MnCl_2$, 0.60 μmoles NADP and the pH of the incubation mixture was adjusted to 7.5 with 1 *N* HCl. After 5 min preincubation at 37°, the reaction was initiated by addition of enzyme preparation to a final cuvette volume of 3.0 ml. The control cuvette contained all reagents except the substrate. Protein was determined by the method of Lowry *et al.* (7).

Tissue for histology was fixed in Bouin's solution and paraffin sections were stained with hematoxylin and eosin. Tissue for histochemistry was frozen in liquid nitrogen and sections 20 μ thick were cut on a cryostat. 20α-OH-SDH was studied by the method of Balogh (8) and 3β-OH-SDH was stained by the technique of Wattenberg (9) using 3β-hydroxyandrost-5-en-17-one (dehydroepiandrosterone) as substrate.

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TABLE I. 20α-OH-SDH and G-6-PDH Activities in Rabbit Ovarian Tissue During Pregnancy.[d]

	Days of pregnancy						
	Estrus	2–5	8–11	14–17	20–23	26–29	
abbits	4	4	5	5	5	5	
wt ± SE)	517 ± 50[a]	465 ± 58[a]	693 ± 70[b]	712 ± 58[b]	795 ± 17[b]	736 ± 26[b]	
us luteum ng ± SE)		6.2 ± 1.2[ae]	17.9 ± 1.9[b]	18.8 ± 0.9[b]	17.2 ± 0.4[b]	18.1 ± 0.7[b]	11
-SDH activity							
protein ± SE		1.4 ± 0.3[ae]	2.5 ± 0.5[a]	2.8 ± 0.4[af]	3.3 ± 0.4[a]	4.2 ± 1.1[a]	14
tissue ± SE		0.11 ± 0.03[ae]	0.13 ± 0.03[a]	0.16 ± 0.01[a]	0.19 ± 0.03[a]	0.26 ± 0.08[a]	0
tial							
protein ± SE	52.8 ± 8.5[a]	24.4 ± 11.0[b]	48.8 ± 6.3[ab]	48.7 ± 6.1[ab]	42.6 ± 9.9[ab]	39.9 ± 2.3[ab]	88
tissue ± SE	3.16 ± 0.54[a]	1.77 ± 0.74[a]	3.13 ± 0.48[a]	3.04 ± 0.40[a]	2.51 ± 0.54[a]	2.59 ± 0.20[a]	6
H activity							
protein ± SE		182 ± 5[abe]	233 ± 21[a]	181 ± 14[bf]	183 ± 18[b]	168 ± 14[b]	
tissue ± SE		13.9 ± 0.7[ae]	12.8 ± 1.5[a]	10.3 ± 0.4[ab]	10.1 ± 1.1[ab]	9.8 ± 0.8[b]	7

unit of enzyme activity is defined as the formation of 1 μmole NADPH/min. Means with different superscripts [a, b] or [c] are signi
at the 5% level by Duncan's new multiple range test.
values missing.
value missing.

TABLE II. Luteal Dehydrogenase Activities During the Peripartal Period.

	Pregnancy, days 28–30 ($n = 4$)	Postpartum, days 1–2 ($n = 6$)	p Value
Av corpus luteum wt (mg ± SE)	17.3 ± 1.1	11.6 ± 0.5	<.001
20α-OH-SDH	2.6 ± 0.3[a]	9.4 ± 0.7	<.001
17β-OH-SDH	2.6 ± 0.2	8.8 ± 0.8	<.001
3β-OH-SDH	11.5 ± 5.3	5.7 ± 2.6	NS
G-6-PDH	124 ± 14	140 ± 7	NS
ICDH	670 ± 92	619 ± 63	NS

[a] mU/mg protein ± SE.

Data were analyzed for statistical significance by analysis of variance and Duncan's multiple range test (10) or Student't t test.

Results. Throughout pregnancy, the average interstitial 20α-OH-SDH activity was always 6- to 19-fold greater than luteal enzymic levels. The interstitial 20α-OH-SDH content varied from animal to animal, even among rabbits at a similar stage of pregnancy. Despite the variation, the average activity in interstitial tissue was relatively constant during pregnancy, except for a transient decline between Days 2–5, and the activity was comparable to that observed in the ovaries of estrous animals (Table I). On the first postpartum day (PP), interstitial 20α-OH-SDH activity was significantly above the average levels seen during pregnancy.

20α-OH-SDH activity in luteal preparations increased slightly as pregnancy progressed (Table I). On Day 1 PP, a significant increase in luteal 20α-OH-SDH activity occurred with 20α-OH-SDH levels rising to more than 3-fold those observed during pregnancy. Luteal G-6-PDH activity declined during gestation with highest activities occurring on Days 2 to 11 and lowest levels on Day 1 PP (Table I).

In a second series of animals, 20α- and 17β-OH-SDH activities were found to increase significantly in luteal tissue during the peripartal period. In contrast, 3β-OH-SDH, G-6-PDH and ICDH showed no significant change in activity during this time (Table II). In corpora lutea, ICDH activity was greater than G-6-PDH, which was in turn greater than the hydroxysteroid dehydrogenase activities. Interstitial tissue from pregnant and postpartum animals displayed a similar pattern of enzymic activities (Table III).

Attempts to demonstrate 20α-OH-SDH histochemically in rabbit ovarian tissue were unsuccessful. No positive reaction greater than that observed on control slices was seen in tissues known to contain high 20α-OH-SDH activity by biochemical assay. However, 3β-OH-SDH could be readily stained in rabbit interstitial tissue.

Corpora lutea weights increased steadily from Days 2 to 11 and then remained constant until Day 1 PP when a significant fall occurred (Tables I and II). Histological evaluation of representative corpora lutea revealed that only trace amounts of interstitial tissue contaminated luteal homogenates. The luteal cells of early pregnancy had granular cytoplasm and the nuclei were large with clearly visible nucleoli. With age, luteal cells enlarged and cytoplasmic vacuoles became prominent. Sections of corpora removed on Day 1 PP revealed marked vacuolization, widespread nuclear pyknosis and increased connective tissue.

Discussion. The rabbit ovary is known to secrete several neutral steroids including progesterone and 20α-OH-P (11). The major source of 20α-OH-P is believed to be the interstitial gland and the corpora lutea are thought to contribute the greater portion of the progesterone (11). The findings of the present study are consistent with these interpretations since ovarian interstitial tissue was shown to be rich in 20α-OH-SDH while luteal homogenates contained relatively low

TABLE III. Dehydrogenase Activities in Ovarian Interstitial Tissue from Pregnant and Postpartum Rabbits.

20α-OH-SDH	77.3 ± 6.1[a]
17β-OH-SDH	62.6 ± 4.1
3β-OH-SDH	131 ± 19
G-6-PDH	187 ± 15
ICDH	1027 ± 10

[a] mU/mg protein ± SE ($n = 11$).

enzymic activity. In addition to 20α-OH-SDH, the interstitial tissue was shown to contain high 3β- and 17β-OH-SDH activities, confirming an earlier report by Davenport and Mallette (12). The amount of interstitial gland present in the rabbit ovarian stroma increases as the animal ages and the ovaries of immature rabbits have low levels of hydroxysteroid dehydrogenase activity (unpublished). The inconstant quantity of interstitial gland is probably the major factor causing the variability in interstitial enzymic activity observed in our study.

In the rat ovary, 20α-OH-SDH activity rises dramatically in the peripartal period (1, 3). A similar increase in 20α-OH-SDH content has been reported to occur in the parturient mouse (13). In the present work, 20α-OH-SDH activity was found to rise more than 3-fold in luteal tissue during the peripartal period and a significant increase in interstitial enzyme levels was also observed. This increase in rabbit ovarian 20α-OH-SDH activity coincided with the appearance of morphological and histological signs of luteal regression. These relationships indicate that 20α-OH-SDH activity can serve as a sensitive index of luteolysis in the rabbit and that 20α-OH-SDH may play a role in regulating the progestational content of ovarian secretions during functional death of the corpus luteum. A correlation between rising 20α-OH-SDH activity and luteolysis is also found in the pseudopregnant rabbit (6). 17β-OH-SDH levels were shown to increase in corpora lutea during the peripartal period. This enzyme appears to be associated subcellularly with the 20α-OH-SDH and could possibly function in the metabolism of estrogen, which is an important component of the luteotropic complex of the rabbit (11).

G-6-PDH activity in luteal tissue declined during gestation. This enzyme may be of importance in the generation of NADPH for steroidogenesis and the declining levels of activity during pregnancy suggest diminishing steroid production. ICDH was also present in high levels in both luteal and interstitial tissue. This dehydrogenase may have a related function in the production of reduced cofactor for steroid synthesis. Since neither of these enzyme activities changed significantly during the peripartal period, it would seem that they are not controlling factors in the process of functional luteolysis.

20α-OH-SDH could not be demonstrated by histochemical methods in rabbit ovarian tissue, but 3β-OH-SDH was clearly stained. Davies *et al.* (14) have also reported difficulties in detecting 20α-OH-SDH by histochemical techniques. The causes for the discrepancy between the biochemical and histochemical data are obscure.

Summary. 20α-Hydroxysteroid dehydrogenase (20α-OH-SDH) activity was 6- to 19-fold greater in interstitial than luteal tissue throughout pregnancy. During the peripartal period, 20α-OH-SDH activity in luteal tissue increased more than 3-fold and a significant rise in activity in interstitial tissue was also observed. The increase in ovarian dehydrogenase levels coincided with the appearance of morphological and histological signs of luteolysis. These relationships indicate that increasing 20α-OH-SDH activity can serve as a sensitive index of luteolysis in the rabbit ovary. 17β-OH-SDH activity in luteal tissue also increased during the peripartal period, but the significance of this increase remains to be determined. In contrast to 20α-OH-SDH, glucose-6-phosphate dehydrogenase in luteal tissue declined during gestation and no significant changes in isocitrate dehydrogenase or 3β-OH-SDH were observed during the peripartal period.

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