Histochemical Activities of Hydroxysteroid Dehydrogenases and Lipids in the Goat Corpus Luteum during Late Pregnancy

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Abstract The histochemistry of lipids, NADH and NADPH dehydrogenases, 4^5 - 3β -, 3α -, 11β -, 17β -, 20α - and 20β -hydroxysteroid dehydrogenases (HSD) has been studied in the goat corpus luteum during late pregnancy. The activity of lipids was moderate with Sudan black B and weak with oil red O. The activities of NADH and NADPH dehydrogenases were very strong. The 3β -HSD activity, when pregnenolone was used as substrate, was moderate. When dehydroepiandrosterone was used, the activity was strong. The 3α -HSD activity was observed to be weak. The enzymes 11β -, 17β -, 20α - and 20β -HSD were not detectable in the corpus luteum. These results suggest that the goat corpus luteum can synthesize progesterone during late pregnancy. The present histochemical findings seem to be correlated with the importance of the corpus luteum for the maintenance of pregnancy in the goat.

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Ovariectomy or removal of the corpora lutea at any time during pregnancy will lead to the termination of pregnancy in the cow, pig and goat unless appropriate replacement therapies with progesterone or estrogen are initiated, except for the sheep in which the presence of the corpus luteum is not essential during the second half of pregnancy^{1,2)}. Plasma progesterone of pregnant goats was measured³⁻⁵⁾. Plasma progesterone levels in the ovarian or peripheral venous blood increased gradually by the 90th day of pregnancy and then fell⁵⁾. Plasma estrogens in pregnant goats were also determined and the levels increased steadily with advancing pregnancy⁶⁾.

Since the histochemical activities in the ovary correlate fairly well with the biochemical results⁷⁾, the histochemical demonstration of steroidogenic enzyme activities has been made to aid the interpretation of the ovarian function. Some histochemical studies on the ovaries of pregnant farm animals have been reported⁷⁻¹⁴⁾. However, the histochemical demonstration of the hydroxysteroid dehydrogenase activity in the pregnant goat ovary has not been reported except for one investigation¹³⁾ in which the observed ovaries were only of very early pregnancy. The purpose of the present investigation was to examine the activity of hydroxysteroid dehydrogenases and lipids (steroid hormone precursors) in the goat corpus luteum during late pregnancy.

Materials and Methods

Japanese native goats were used in this study. The ovaries were obtained from

three goats at 110–130th day of pregnancy. They were immediately frozen in pre-chilled isopentane and stored with dry ice until the histochemical procedures were performed. They were sectioned at 15 μ m in a cryostat maintained at about -20°C and the sections were mounted on glass slides.

The activities of the following steroidogenic enzymes were studied: Δ^5 -3 β -(3 β -HSD), 3 α -(3 α -HSD), 11 β -(11 β -HSD), 17 β -(17 β -HSD), 20 α -(20 α -HSD) and 20 β -hydroxysteroid dehydrogenases (20 β -HSD). Hydroxysteroid dehydrogenases, substrates and cofactors are shown in Table 1. The sections were incubated at 37°C

Table 1. Histochemical methods for dehydrogenases

Dehydrogenase	Substrate	Cofactor	Reference
NADH dehydrogenase	NADH	—	BARKA and ANDERSON ¹⁵⁾
NADPH dehydrogenase	NADPH	—	BARKA and ANDERSON ¹⁵⁾
3 β -HSD	pregnenolone	N A D	BLAHA and LEAVITT ¹⁶⁾
3 β -HSD	dehydroepiandrosterone	N A D	WATTENBERG ¹⁷⁾
3 α -HSD	androsterone	N A D	FOWLER and FELDMAN ¹⁸⁾
11 β -HSD	androstenedione	N A D	BAILLIE <i>et al.</i> ¹⁹⁾
17 β -HSD	estradiol-17 β	N A D	ISHIDA and NIIMURA ²⁰⁾
17 β -HSD	testosterone	N A D	ISHIDA and NIIMURA ²⁰⁾
20 α -HSD	20 α -dihydroprogesterone	N A D P	BALOGH ²¹⁾
20 β -HSD	20 β -dihydroprogesterone	N A D	BAILLIE ²²⁾

for 1 hr. Then the sections, except for 17 β -HSD, were fixed in 10% neutral formalin. Those for 17 β -HSD were fixed in 80% alcohol. All series of sections were mounted with glycerol. The control sections were incubated in a medium without any steroidal substrate.

Since NADH and NADPH dehydrogenases are prerequisites for the histochemical localization of NAD- and NADP-linked dehydrogenases, the activity of these dehydrogenases was also determined by the method of BARKA and ANDERSON¹⁵⁾. Lipids were stained with Sudan black B or oil red O. The activity of enzyme reaction was expressed as 0 (negative), 1 (very weak), 2 (weak), 3 (moderate), 4 (strong), and 5 (very strong).

Results

The activities of lipids and dehydrogenases in the goat corpus luteum during late pregnancy are summarized in Table 2. The activity of lipids was moderate with Sudan black B (Fig. 1) and weak with oil red O. The corpus luteum contained only moderate sparse lipids and did not show the accumulation of coarse lipid droplets (Fig. 1). The activities of NADH (Fig. 2) and NADPH dehydrogenases were very strong.

The 3 β -HSD activity with pregnenolone as substrate was moderate (Figs. 3, 4) and the activity with dehydroepiandrosterone was strong (Fig. 5). The 3 β -HSD activity in the ovary was restricted to the corpus luteum except for the theca interna

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Table 2. Histochemical activity of dehydrogenases and lipids in the goat corpus luteum during late pregnancy

Dehydrogenase/lipid		Activity
Lipids	Sudan black B	3
	oil red O	2
Dehydrogenases	NADH dehydrogenase (NADH)	5
	NADPH dehydrogenase (NADPH)	5
	3 β -HSD (pregnenolone)	3
	3 β -HSD (dehydroepiandrosterone)	4
	3 α -HSD (androsterone)	2
	11 β -HSD (androstenedione)	0
	17 β -HSD (estradiol-17 β)	0
	17 β -HSD (testosterone)	0
	20 α -HSD (20 α -dihydroprogesterone)	0
	20 β -HSD (20 β -dihydroprogesterone)	0

The enzyme activity was rated on an arbitrary scale from 0 to 5 : 0 (negative), 1 (very weak), 2 (weak), 3 (moderate), 4 (strong) and 5 (very strong).

of antral follicles in which very weak activity was observed. This enzyme was most active hydroxysteroid dehydrogenase encountered in the present study. The 3 α -HSD activity was found to be weak (Fig. 6). The enzymes 11 β -, 17 β -, 20 α - and 20 β -HSD were not detectable in the corpus luteum. To the contrary, the 17 β -HSD was weakly present in the granulosa cells and theca interna of antral follicles.

Discussion

In many farm animals including the goat, the ovary is the main source of progesterone secretion during late pregnancy^{4,5,23}) and can not be removed at any stage of pregnancy without causing abortion^{1,2,24}). However, in the sheep the placenta is the main source of progesterone during late pregnancy^{4,5}).

The enzyme 3 β -HSD is active in the conversion of pregnenolone to progesterone and in the conversion of dehydroepiandrosterone to androstenedione. The occurrence of moderate 3 β -HSD activity with pregnenolone as substrate in the corpus luteum, therefore, suggests the capacity of goat corpus luteum during late pregnancy to synthesize progesterone. In the present study 3 β -HSD activity in the ovary was restricted to the corpus luteum except for the theca interna of antral follicles in which very weak activity was observed. These histochemical results suggest that the corpus luteum is the major site of progesterone production among the ovarian components in the late pregnant goat. The results are consistent with the presence of plasma progesterone in the ovarian venous blood found by biochemical methods³⁻⁵) and with the importance of the corpus luteum for the maintenance of pregnancy in the goat²⁴).

The corpus luteum contained only moderate sparse lipids. Since the accumulation of coarse lipid droplets in steroid hormone-producing glands is usually thought to be

inversely related to secretory activity²⁵), the absence of coarse lipid droplets in this study may suggest the active function of the goat corpus luteum during late pregnancy. Since lipids are steroid hormone precursors, these results may be related to the presence of 3 β -HSD activity in the corpus luteum and plasma progesterone in the ovarian venous

Explanation of Figures

Enzymatic preparations of goat corpus luteum during late pregnancy.

Fig. 1. Corpus luteum showing moderate lipid activity. Only sparse lipids are present. Note the absence of the accumulation of lipid droplets. Stained with Sudan black B. $\times 100$.

Fig. 2. Very strong NADH dehydrogenase activity is observed. $\times 40$.

Fig. 3. Moderate 3 β -HSD activity when pregnenolone is used as substrate. $\times 40$.

Fig. 4. A high magnification ($\times 200$) of Fig. 3. Moderate 3 β -HSD activity is seen in the cytoplasm of luteal cells. No staining of the nuclei.

Fig. 5. Strong 3 β -HSD activity when dehydroepiandrosterone is used as substrate. $\times 100$.

Fig. 6. Weak 3 α -HSD activity is observed. $\times 40$.

blood during late pregnancy in the goat⁸⁻⁵).

The enzyme 3 α -HSD influences steroidogenesis and catalyses reversibly the interconversion of androsterone and androstanedione. The weak activity of this enzyme in the goat corpus luteum during late pregnancy seems to show that a weak androgen metabolism is occurring in these luteal cells.

Plasma estrogen levels in pregnant goats increased steadily with advancing pregnancy⁶). The enzyme 17 β -HSD is reversibly active in the interconversion of estradiol-17 β and estrone, or of testosterone and androstenedione. This enzyme was not detectable in the corpus luteum in this study, suggesting the lack of capacity of the corpus luteum to synthesize estrogens. As the granulosa cells and theca interna of antral follicles in the late pregnant goat exhibited weak 17 β -HSD activity, antral follicles are likely to contribute to estrogen synthesis.

The ovary of the cow and pig produces 20 β -dihydroprogesterone, while that of the sheep produces 20 α -dihydroprogesterone²³). Furthermore, 20 β -dihydroprogesterone was identified in the corpus luteum of the cow and pig²³). The interconversion of 20 α -dihydroprogesterone and progesterone is catalyzed by 20 α -HSD and that of 20 β -dihydroprogesterone and progesterone by 20 β -HSD.

DAVIES *et al.*²⁶) reported no 20 α - and 20 β -HSD activity in the cow corpus luteum whereas LOBEL and LEVY¹⁰) observed the occurrence of 20 β -HSD in the cow corpus luteum. No positive 20 α - and 20 β -HSD activities were obtained in this study. The negative histochemical activities in the corpus luteum in this study could not be explained by lack of tetrazolium reductase⁷), as both NADH and NADPH dehydrogenase activities were obtained. As very weak 20 α -HSD activity was observed in the non-pregnant goat corpus luteum (unpublished data), perhaps these enzyme activities in the goat corpus luteum during late pregnancy were too weak to be detected with the histochemical method used, or the corpus luteum during late pregnancy lacked any of these enzymes.

References

- 1) CATCHPOLE, H. R., in *Reproduction in Domestic Animals*. 3rd ed. (COLE, H. H. and P. T. CUPPS, eds.) 341-368. Academic Press. New York. 1977.
- 2) ROBERTSON, H. A., in *Reproduction in Domestic Animals*. 3rd ed. (COLE, H. H. and P. T. CUPPS, eds.) 475-498. Academic Press. New York. 1977.
- 3) RAESIDE, J. I. and C. W. TURNER, J. Dairy Sci., **38**: 1334-1343. 1955.
- 4) LINZELL, J. L. and R. B. HEAP, J. Endocr., **41**: 433-438. 1968.
- 5) BLOM, A. K. and O. LYGSET, Acta Endocr., **66**: 471-477. 1971.
- 6) CHALLIS, J. R. G. and J. L. LINZELL, J. Reprod. Fert., **26**: 401-404. 1971.
- 7) BJERSING, L., Histochemie, **10**: 295-304. 1967.
- 8) FOLEY, R. C., R. P. REECE and J. H. LEATHEM, J. Anim. Sci., **13**: 131-137. 1954.
- 9) RUBIN, B. L., H. W. DEANE and J. A. HAMILTON, Endocrinology, **73**: 748-763. 1963.
- 10) LOBEL, B. L. and E. LEVY, Acta Endocr., **59**: Suppl. **132**: 1-63. 1968.
- 11) NAKAMA, S., Bull. Univ. Osaka Pref., Ser. B, **28**: 19-98. 1976.
- 12) GOODE, L., A. C. WARNICK and H. D. WALLACE, J. Anim. Sci., **24**: 955-958. 1965.
- 13) MIYAMOTO, H., M. IRIE, T. ISHIBASHI and K. UTSUMI, Jpn. J. Zootech. Sci., **50**: 870-878. 1979.
- 14) MIYAMOTO, H., T. ISHIBASHI and K. UTSUMI, Jpn. J. Zootech. Sci., **51**: 582-587. 1980.
- 15) BARKA, T. and P. J. ANDERSON, in *Histochemistry*. 296-333. Harper and Row Publishers. New York. 1963.

- 16) BLAHA, G. C. and W. W. LEAVITT, *Biol. Reprod.*, **3**: 362-368. 1970.
- 17) WATTENBERG, L. W., *J. Histochem. Cytochem.*, **6**: 225-232. 1958.
- 18) FOWLER, E. H. and M. K. FELDMAN, *Gen. Comp. Endocr.*, **14**: 484-490. 1970.
- 19) BAILLIE, A. H., M. M. FERGUSON, K. C. CALMAN and D. McK. HART, *J. Endocr.*, **33**: 119-125. 1965.
- 20) ISHIDA, K. and S. NIIMURA, *Jpn. J. Zootech. Sci.*, **48**: 373-375. 1977.
- 21) BALOGH, K., *J. Histochem. Cytochem.*, **12**: 670-673. 1964.
- 22) BAILLIE, A. H., K. C. CALMAN, M. M. FERGUSON and D. McK. HART, *J. Endocr.*, **32**: 337-339. 1965.
- 23) HENRICKS, D. M. and D. T. MAYER, in *Reproduction in Domestic Animals*. 3rd ed. (COLE, H. H. and P. T. CUPPS, eds.) 79-117. Academic Press. New York. 1977.
- 24) MEITES, J., H. D. WEBSTER, F. W. YOUNG, F. THORP, JR. and R. N. HATCH, *J. Anim. Sci.*, **10**: 411-416. 1951.
- 25) DEANE, H. W., M. F. HAY, R. M. MOOR, L. E. A. ROWSON and R. V. SHORT, *Acta Endocr.*, **51**: 245-263. 1966.
- 26) DAVIES, J., G. R. DAVENPORT, J. L. NORRIS and P. I. C. RENNIE, *Endocrinology*, **78**: 667-671. 1966.

妊娠後期山羊黄体のステロイド水酸基・脱水素 酵素と脂質活性

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妊娠後期の山羊卵巣におけるステロイドホルモン合成に関して検討するために、妊娠110～130日の在来種山羊黄体中のステロイド水酸基・脱水素酵素(HSD)と脂質の活性を組織化学的に検出した。調べたHSDは、 3β - (基質はpregnenoloneまたはdehydroepiandrosterone), 3α -(androsterone), 11β -(androstenedione), 17β -(estradiol- 17β または testosterone), 20α -(20α -dihydroprogesterone) および 20β -HSD (20β -dihydroprogesterone) であった。なお、HSDの組織化学的反応は、NADH または NADPH 脱水素酵素に依存しているので、これらの酵素活性についても検討した。黄体中の脂質活性は、Sudan black B では中等度であり、oil red O

では弱かった。NADH と NADPH 脱水素酵素の活性は非常に強かった。 3β -HSDの活性に関しては、pregnenolone を基質として用いると中等度の活性があり、dehydroepiandrosterone を基質とすると強い活性が認められた。弱い 3α -HSD 活性も出現した。 11β -, 17β -, 20α - および 20β -HSD 活性は検出されなかった。これらの成績は、妊娠後期の山羊黄体がprogesteroneの合成能をもっていることを示し、山羊の妊娠維持には黄体が必要であるという、これまでの実験結果と一致していると思われる。

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