3β-HSD Activity and Gestagen concentrations of Bovine Fetal and Maternal Placentae during Pregnancy and on Parturition

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Introduction

As bovine uterine arterio-venous progesterone (P) levels indicate no differences, P is reportedly not produced in placenta.[1,2] However, many documents[3-7] have demonstrated and confirmed the P synthesis in tissue cultures of placentae. In contrast to attenuated bovine corpus luteal functions in the later half of gestation,[4,8] the P level in blood indicates a substantial increase until near term.[9,10] Such an increase in P level at late period of gestation has been attributed to derive from the placenta[10,11] and the adrenal. Our present study therefore attempted to investigate P syntheses in both fetal and maternal placentae by measuring the synthesis-related enzyme, 3β-hydroxy steroid dehydrogenase (3β-HSD), and gestagen levels contained in the placente with respect to gestation progress.

Materials and Methods

Fetal and maternal placentae for the study were isolated respectively from 32 Holstein cows (28 gestations and 4 normal parturitions). The fetal and maternal placentae were then separated manually within 30 min either after slaughter or parturition. These isolated palcentae were washed with colded physiological saline and were frozen immediately by dry ice and stored at -80 ℃ before use. Fetal ages at months were derived from crown-runp length of fetuses according to Arthur et al.[12] and fetuses with fetal age at month 4 to 9 were separated accodingly on a monthly basis.

Determination of enzymatic activity was based on the substrate metabolism method of Seki et al.[13] NAD was purchased from Oriental Koubo (Japan). ^{14}C-prognenolone was obtained from New England Nuclear Co.(USA). Fetal and maternal placentae, 0.25g each, were homogenized in 9-fold volumes of 0.25 M sucrose-phosphate buffer by a teflon-glass homogenizor (Braun Melsungen) at 1,000 rpm for 45 sec. Enzyme preparations were obtained as precipitates between 9,000 and 105,000 g by a conventional differential centrifugation method (microsomal fraction). The composition of reaction medium induced 60 μl microsomal fraction, 740 μl of 5 mM $MgCl_2$ in 0.25 M sucrose-phosphate buffer and 100 μl of NAD (0.8 μM), and 100 μl of ^{14}C-pregnenolone (0.3 μCi). After aerating with CO gas 2 min, reaction was allowed continued for a further 10 min at 37 ℃ under constant agitation (120 times per min) and was stopped by adding 1 ml of 1N HCl. Non-reacted substrate and metabolites were removed using diethyl ether twice, and dried under a stream of N_2 gas. Pregnenolone was separated from P by using Silica gel G thin-layer chromatography with a development solvent of benzene:ethyle acetate (2:1). The scanner confirmed the site in thin-layer chromatography and P was removed by scrapping the resin at the site, followed by hormonal extraction with diethyl ether twice. The extract was determined by using liquid scintillation counter (Beckman, LS5801). As the present assay was induced only ^{14}C-P from ^{14}C-pregnenolone, and the quantity of ^{14}C-P produced was indexed by 3β-HSD activity. Enzymatic activity was calculated from the percent of product formed relative to the total radioactive steroid recovered. Recovery after extraction and chromatography was 64.4± 6.2% (n=15). The protein level in the sumple was determined by the Lowry's method.[14]

P, 20α-OHP and 17α-OHP levels were determined using the methods of Makino[15] and Tanemori et al.[16] with slight modifications. Labelled hormones such as [1,2-^{3}H]-

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P, [1,2,6,7-^{3}H]20α -OHP, [1,2,6,7-^{3}H]17α -OHP were products of Amersham Co., Ltd. (USA). Antisera against P-3-CMO-BSA, 20α -OHP-3-CMO-BSA and 17α -OHP-3-CMO-BSA were supplied by Teikoku Hormone Mfg. Co., Ltd. (Japan). Crossreactivities of antisera against P were 62.2% for 5α -pregnanedione and 6.3% for pregnenolone. Moreover, those of 20α -OHP antisera were 5.4% for 20β -OHP, and those of 17α -OHP antisera were 7.9% for P and 3.2% for 20α -OHP, respectively. To determine gestagen levels, the dried residue of supernatant from the ether-extract of homogenized sample (1～10 μ l) of the pre-ultracentrifugation was subjected to liquid chromatography elution with a Sephadex-LH-20 (Pharmacia Co., Ltd., Sweden) column by using a mobile-phase of hexane:benzene: methanol (82.5:10:7.5). Recovery after extraction and chromatography was 92.2± 4.2% for P, 82.5± 4.0% for 20α -OHP and 75.1± 3.8% for 17α -OHP (n=15). The lower limits of accurate quantitation were 10 pg/ tube under respective assay procedures for P, 20α -OHP and 17α -OHP. Variation coefficients of intra- and interassays (n=20) for P, 20α -OHP, 17α -OHP were 8.2, 9.8, 9.2% and 12.3, 14.8, 17.4%, respectively.

Statistical significance was verified by the Duncan's multiple range test, and the correlation coefficients were evaluated.

Results

To decide on the sample-quantity, microsomal fraction of gestation month 8 fetal placenta was determined 0.1～0.55 mg protein, a linear relationship was obtained (Fig.1). About 0.3 mg protein samples (wet weight=about 10 mg) were used in this study. On determination on 3β -HSD activities of the fetal placenta samples against time at 10-min intervals, a linear plot up till 20 min was obtained (Fig. 2). An incubation-time of 10 min was thus employed in our experiments. Michaelis constant (K_m) and Maximum 3β -HSD reaction velocity (V_{max}) for gestation month 8 fetal placenta were

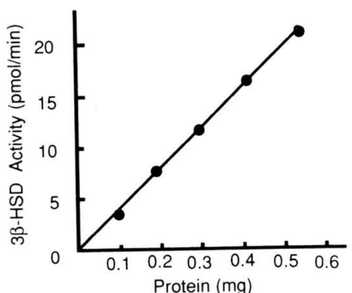

Fig. 1. 3β-HSD activity and protein content in the bovine fetal placenta at gestation month 8.

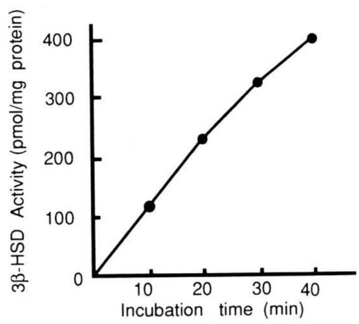

Fig. 2. 3β-HSD activity in relation to incubation time from 10 to 40 min using the bovine fetal placenta at gestation month 8.

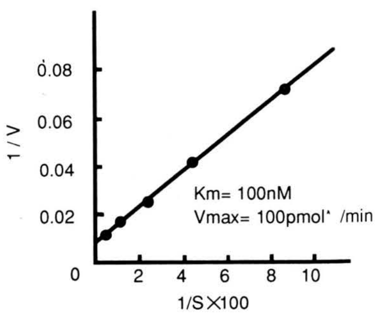

Fig. 3. Lineweaver-Burk plot depicting the relationship between bovine placental (microsomai fraction)3β-HSD activity (1/V) and [^{14}C] -pregnenolone concentations (1/S×100).

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derived from a Lineweaver-Burk plot on the enzyme activity against the substrate concentration. The K_m and V_{max} values in the fetal placenta were 100 nM and 100 pmol/min/mg protein, respectively (Fig. 3).

The 3β-HSD activity level (Mean $\pm$ S.E.) in the fetal placenta indicated 79.2$\pm$ 15.2 pmol/min/mg protein at gestation month 4, signficnatly (P<0.01) increased to 188.2$\pm$ 7.3 pmol/min/mg protein at gestation month 7, reached a peak of 276.2$\pm$ 43.6 pmol/min/mg protein at gestation month 8 and abruptly decreased thereafter to 85.4$\pm$ 10.9 pmol/min/mg protein immediately after purturition. The 3β-HSD level in maternal placenta showed 25.7$\pm$ 5.3 pmol/min/mg protein at gestation month 4 and maintained such a low level till immediately after parturition (Fig. 4).

P levels of fetal placentae indicated 6.8$\pm$ 1.6 nmol at gestation month 4, significantly (P<0.01) increased to 21.1$\pm$ 2.5 nmol at gestation month 7. This high level was maintained till gestaion month 9, and declined to 9.5$\pm$ 3.4 nmol immediately after parturition. In the maternal placenta, P levels indicated 2.8$\pm$ 0.7 nmol at gestation month 4, increased slightly to 3.9 $\pm$ 0.9 nmol at gestation month 7, and this P level was maintained till gestation month 9. However, P levels declined to 2.5$\pm$ 0.7 nmol immediately after parturition. On comparison of P levels in fetal and maternal placentae, the former manifested a consistently higher P level than the latter throughout the gestation period significantly (P<0.01)(Fig.5). Moreover, a significant correlation (P<0.01) between the P and 3β-HSD levels in the fetal placentae was verified.

Fetal placentae indicated a 20α-OHP level of 2.4$\pm$ 0.6 nmol at gestation month 4, and significantly (P<0.01) increased to 17.0$\pm$ 3.7 nmol at gestation month 7. The 20α-OHP level thereafter reached to 19.9$\pm$ 3.1 nmol at gestation month 8, howevere, cascaded to 14.2$\pm$ 3.7 nmol at gestation month 9, and registered below level of 7.0$\pm$ 3.1 nmol immediately after

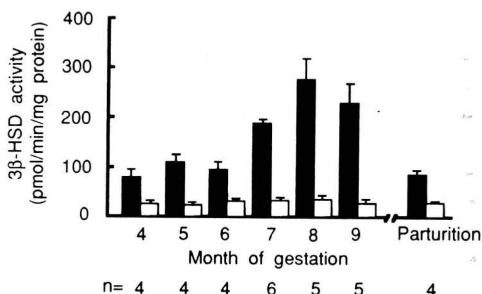

Fig. 4. 3β–HSD activity in fetal (■) and maternal (□) placentae during the bovine gestaion.

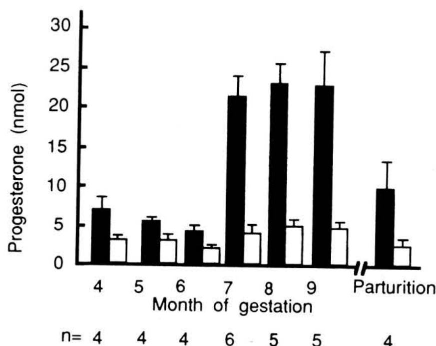

Fig. 5. Progesterone levels in fetal (■) and maternal (□) placentae during the bovine gestaion.

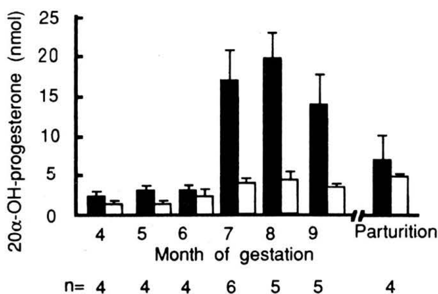

Fig. 6. 20α-OH-progesterone levels in fetal (■) and maternal (□) placentae during the bovine gestaion.

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parturition. As in the case of the maternal placenta, 20α -OHP showed a level of 1.3± 0.2 nmol at gestation month 4, peaked at 4.4± 0.8 nmol at gestation month 8 and plateaued at this level until gestation month 9. The level indicated a sudden decrease to 1.2± 0.1 nmol immediately after parturition. On comparison of 20α -OHP levels in the fetal and maternal placentae, the former indicated a persistently higher level than the latter throughout the gestation period (Fig. 6). P and 20α -OHP levels indicated a high correlation of r=0.872 (P<0.01) in the fetal placentae.

As regards to 17α -OHP levels in fetal placentae, a value of 10.7 ± 1.3 nmol was registered at gestation month 4, and decreased to 5.4 ± 0.8 nmol at gestation month 5 with no aparent changes observed till immediately after parturition. In maternal placentae, 17α -OHP levels showed 4.3± 0.8 nmol at gestation month 4, decreased to 2.3± 0.3 nmol at gestation month 5, and indicated gradual increases thereafter to gestation month 8 to reverse again to low levels immediately after parturition (Fig. 7).

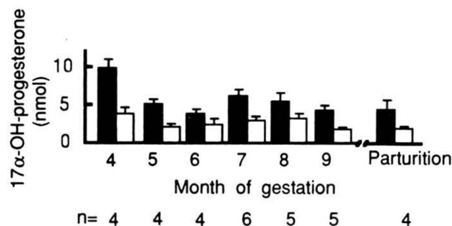

Fig. 7. 17α-OH-progesterone levels in fetal (■) and maternal (□) placentae during the bovine gestaion.

Discussion

3β -HSD activities of bovine placentae coincided with previous findings that this activity displays a higher value in the fetal than maternal placentae.[7,17] Placental hormone synthesis may have been more predominant in the fetal than maternal placentae. Though Comley & Ford[7] have recognized P synthesis in maternal placentae to registere 1/3 that of fetal placentae in tissue-cultures, a leakage of P from the latter to the former was possible, denying thus P synthesis in maternal placentae. On placental detachment, less than 10% of fetal placentae was confirmed to attach to the maternal placenta [unpublished data], binucleated cellls that function as the major cells responsible for P synthesis [6,17] are infliltrated from fetal to maternal placentae, but it is no denying the maternal placentae is possible as P synthesis-site. Further, the maternal placenta as a much lager system than the fetal placenta in size should also be considered.

Documentations on P synthesis in relation to gestation progress are limited. Inaba et al.[5] have demonstrated increases in P synthesis from gestation months 7.5 to 9 in cows, while the sample number is only one case a month. The finding much resembles the tendency of 3β -HSD activity in our present study. Moreover, P levels included in fetal placentae were very similar to the fluctuation of 3β -HSD activity. In luteoectomized pregnant cases exceeding 200 gestation days, miscarriages are not found in many.[18] This may be due to the functional role of P released from the placenta rather than the maternal adrenal. P levels in maternal placentae were much lower than those found in fetal placenta, implicating little difference in estrogen levels existed between maternal and fetal placentae [unpublished data].

Fluctuation and level of estrogen in both placentae are relatively similar [unpublished data] through differences in P were

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evidently observed in the respective placentae (Fig. 5). According to Heap & Flint,[19] fetal placenta convert estrogen precursor derived from porcine mother's body, to estrogen, and the estrogen formed is then conveyed to the maternal placenta subsequently. Even in bovine fetal placentae, estrogen synthesized would eventually be transported to the mother's body, suggesting any P was hardly transferred to the mather's body from the fetus.

During gestation months 7～9, 20α-OHP levels in fetal placentae increased with a high correlation manifested between P and 20α-OHP levels in the placenta. Fetal erythrocytes in sheep and cows indicate the existence of enzyme(s) responsible for converting P to 20α-OHP, advocating conversion of P synthesized to the inactive 20α-OHP.[20] In our present investigation, such a conversion was evidently demonstrated in the fetal placentae. It is known that the P level is very low in bovine fetal plasma.[21,22] For the fetus to display a low P level is exactly a situation beneficial for it own development and survival, whereas estrogen levels in the fetal plasma are relatively high.[21]

Though fetal placentae indicated a high 17α-OHP level at gestation month 4, no changes accompanied the progress of fetal development thereafter. Moreover, there was no apparent correlations between 17α-OHP and P levels, implicating 17α-OHP was more likely to have derived from 17α-OH-pregnenolone.

From the above findings, increases of placental 3β-HSD activity persisted from gestation months 7 to 9 with P synthesized in the fetal placenta converted concurrently to 20α-OHP before P was transferred into fetal blood during the period.

Summary

Progesterone (P) synthesis, and P, 20α-hydroxy-progesterone (20α-OHP) and 17α-hydroxyprogesterone (17α-OHP) concentrations in bovine fetal and maternal placentae were determined by substrate metabolism method and radioimmunoasssay. A very higher 3β-hydroxy steroid dehydrogenase (3β-HSD) activity in fetal than maternal placentae was shown throughout the gestation period. 3β-HSD activity in fetal placentae increased extreamly during third trimester (gestation month 7 to 9) and decreased on parturition. Fluctuation of P and 20α-OHP concentrations in fetal placentae were similar to those of 3β-HSD activities, showing almost same concentrations. Unlike P and 20α-OHP, no changes of 17α-OHP concetrations in both placentae were observed. From our above findings, placental 3β-HSD activity level enhanced during the third trimester with P synthesized in fetal placenta converted concurrently to 20α-OHP before P was transferred into fetal blood.

References

1. Comline, R.S. et al. (1974) J. Endocr. 63: 451-472. 2. Ferrell, C.L. et al. (1983) J. Anim. Sci. 56:656-667. 3. Ainsworth, L. & Ryan, K.J. (1967) Endocrinology 81:1349-1356. 4. Shemesh, M. et al. (1983) Biol. Reprod. 29:856-862. 5. Inaba, T. et al. (1983) Jpn. J. Anim. Reprod. 29:88-93. 6. Reimers, T.J. et al. (1985) Biol. Reprod. 33:1227-1236. 7. Conley, A. & Ford, S.P. (1987) J. Anim. Sci. 65:500-507. 8. Erb, R.E. et al. (1968) J. Dairy Sci. 51:401-410. 9. Short, R.V. (1958) J. Endocr. 16:426-428. 10. Stabenfeldt, G.H. et al.(1970) Am. Physiol. 218:571-575. 11. Gomes, W.r. & Erb, R.E. (1965) J. Dairy Sci. 48:314-330. 12. Arthur, G.H. et al. (1982) In Veterinary & Obstetrics, 5th edn. PP.49-84. Eds. Arthur, G.H., Noakes, D.E. & Pearson, H., Bailliere Tindall, London. 13. Seki, M. et al. (1987) Acta Obst. Gynec. Jpn. 39:1571-1578. 14. Lowry, O.H. et al. (1951)

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J. Biol. Chem. 193:265-275. 15. Makino, R. (1972) Folia Endocr. Jpn. 49:629-646. 16. Tanemori, K. (1978) Bull. Nihon Univ. 37:1245-1255. 17. Gross, T.S. & Williams, W.F. (1988) J. Reprod. Fert. 83:565-573. 18. Estergreen, V.L. et al. (1967) J. Dairy Sci. 50:1293-1295. 19. Heap, R.B. & Flint, A.P.F. (1984) Pregnancy. in Hormonal Control of reproduction. 2nd eds. pp.158, Ed. Austin, C.R. & Short, R.V., Cambrige Univ. Press, London. 20. Nancarrow, C.D. (1983) Aus. J. Biol. Sci. 36:183-190. 21. Challis, J.R.G. et al. (1974) J. Endocr. 60:107-115. 22. Hoffman, B. et al. (1976) Biol. Reprod. 15:126-133.

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