Endocrine, *vol. 8, no. 1, 45–50, February 1998* 0969–711X/98/8:45–50/$9.50

Concentration of mRNA Encoding 3β-Hydroxysteroid Dehydrogenase/Δ^5,Δ^4 Isomerase (3β-HSD) and 3β-HSD Enzyme Activity Following Treatment of Ewes with Prostaglandin $F_{2\alpha}$

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The objectives of these experiments were (1) to determine if prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$) decreased mRNA encoding 3β-hydroxysteroid dehydrogenase/d^5,Δ^4 isomerase (3β-HSD) specifically in large steroidogenic luteal cells, which contain the high affinity receptors for $PGF_{2\alpha}$; and (2) to determine if the decreased concentration of mRNA encoding 3β-HSD following administration of $PGF_{2\alpha}$ was associated with a decrease in 3β-HSD enzyme activity. Ewes on days 11 or 12 of the estrous cycle were administered $PGF_{2\alpha}$ (25 mg iv followed by 10 mg im 2 h later) and corpora lutea collected 4, 12, 24, or 48 h later (n = 4–5/time). Corpora lutea were also collected from non-injected (n = 4) or saline-injected (n = 4) control ewes. Administration of PGF_2 decreased ($P < 0.05$) steady-state concentrations of mRNA encoding 3β-HSD to 35, 15, 9, and 5 percent of the concentrations in the control group at 4, 12, 24, and 48 h, respectively. Concentrations of mRNA encoding 3β-HSD in large luteal cells were decreased to 43% of controls 4 h following injection, which was similar to the decrease seen in steady-state concentrations of this mRNA in total luteal mRNA (35%). However, 3β-HSD enzyme activity was not significantly decreased by 48 h after $PGF_{2\alpha}$ injection. Thus, the dramatic decreased in mRNA encoding 3β-HSD was not associated with an immediate decrease in 3β-HSD enzyme activity and, therefore, does not appear to be responsible for the acute decrease in secretion of progesterone from ovine luteal tissue during $PGF_{2\alpha}$-induced luteolysis.

Key Words: 3β-hydroxysteroid dehydrogenase/Δ^5,Δ^4 isomerase; mRNA; enzyme activity; corpus luteum; ovine.

Received September 15, 1997; Revised November 3, 1997; Accepted November 3, 1997.

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Introduction

Luteinizing hormone (LH) has long been considered the primary luteotropic hormone *(1)* whereas prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$) is the luteolytic hormone in sheep *(2,3)*. Growth hormone also appears to play a role in luteal development and function *(4)*. In most mammals studied to date, the corpus luteum is composed of at least two morphologically and biochemically distinct steroidogenic cell types *(1)*. Based on differences in size, these cells are commonly referred to as small luteal cells (thecal cell origin) and large luteal cells (primarily granulosal cell origin). It has been suggested that > 80% of the progesterone secreted from the ovine corpus luteum is of large luteal cell origin *(5)*.

In sheep, high-affinity receptors for $PGF_{2\alpha}$ ($PGF_{2\alpha}$-R), as well as mRNA encoding $PGF_{2\alpha}$- R, appear to be limited to large luteal cells *(6–8)*. Binding of $PGF_2\alpha$ to its receptor on large luteal cells activates protein kinase C (PKC), which is responsible for the antisteroidogenic effects of this hormone *(9,10)*. In addition, activation of $PGF_{2\alpha}$-R causes an increase in free intracellular calcium concentrations in large luteal cells *(11,12),* which may be responsible for luteal cell death through apoptotic mechanisms *(10,13)*. The mechanisms by which progesterone secretion from small luteal cells is decreased following exposure to $PGF_{2\alpha}$ are not clearly understood. Activation of PKC will decrease LH-stimulated secretion of progesterone from small luteal cells in vitro *(14,15),* but since small luteal cells do not possess receptors for $PGF_{2\alpha}$, the hormone(s) involved in physiological activation of PKC in small luteal cells is not known.

Biosynthesis of progesterone in luteal cells utilizes cholesterol as substrate, which is transported through the cell to the outer mitochondrial membrane. Cholesterol must then be transported from the outer to the inner mitochondrial membrane where it is cleaved to pregnenolone by cytochrome P450 side-chain cleavage enzyme complex ($P450_{scc}$). Pregnenolone is then converted to progesterone by 3β-hydroxysteroid dehydrogenase/Δ^5,Δ^4 isomerase (3β-HSD).

In the ewe, concentrations of mRNA encoding 3β-HSD increased following ovulation and were expressed at maximum levels by day 3 of the estrous cycle *(16)*. Removal of pituitary luteotropins by hypophysectomy decreased concentrations of mRNA encoding 3β-HSD, and replacement of LH, but not GH, prevented this decrease *(4)*. Treatment of ewes with $PGF_{2\alpha}$ decreased concentrations of 3β-HSD mRNA within 3–5 h following initiation of luteolysis *(10,17)*. However, estimates of 3β-HSD enzyme activity indicate that this enzyme is in great excess during the estrous cycle *(18),* and in a preliminary report using ewes that had been induced to ovulate, it appeared that this enzyme decreased slowly in the first 24 h following treatment with $PGF_{2\alpha}$ *(19)*. Thus, the objectives of the following experiment were (1) to determine if $PGF_{2\alpha}$ decreased mRNA encoding 3β-HSD specifically in large luteal cells, which contain the high-affinity receptors for $PGF_{2\alpha}$ and (2) to determine the relationship between concentrations of mRNA encoding 3β-HSD and 3β-HSD enzyme activity following $PGF_{2\alpha}$ administration.

Results

The nucleotide sequence obtained from the ovine 3β-HSD 525-base pair cDNA was 95, 80, 83, 76, and 75% identical to the corresponding region of bovine *(20)*, human *(21)*, macaque *(22)*, rat *(23),* and mouse *(24)* 3β-HSD cDNA, respectively. The ovine 3β-HSD cDNA hybridized to a single transcript at approx 1700 bases in RNA isolated from steroidogenic tissue (adrenal and corpus luteum; Fig.1). No hybridization was seen in RNA isolated from other ovine tissues (Fig. 1).

Concentrations of progesterone in sera decreased ($P < 0.05$) within 4 h following injection of $PGF_{2\alpha}$ and remained low throughout the remainder of the experiment (Table 1). Concentrations of progesterone in luteal tissues and luteal weights were decreased ($P < 0.05$) by 24 h after treatment (Table 1). Steady-state concentrations of luteal mRNA encoding 3β-HSD decreased ($P < 0.05$) to 35% of controls within 4 h after treatment with $PGF_{2\alpha}$ and was less than 5% of controls at 48 h (Table 1). The amount of 3β-HSD mRNA in large luteal cells decreased ($P < 0.02$) from control values of 0.139 ± 0.011 to 0.060 ± 0.018 silver grains/μm^2 4 h after treatment with $PGF_{2\alpha}$ (Fig. 2). However, 3β-HSD activity did not change ($P = 0.16$) within the 48 h following treatment with $PGF_{2\alpha}$ (Table 1).

Discussion

The partial cDNA encoding 3β-HSD generated from ovine luteal tissue was similar in nucleotide sequence to the corresponding region of the cDNAs from other species *(20–24)*. This cDNA hybridized to a single transcript of approx 1700 bases in highly steroidogenic tissue, whereas no hybridization was observed in tissues in which little or no steroidogenesis occurs. The lack of detection of 3β-HSD mRNA in tissues known to produce low amounts of steroids, such as brain, was most likely owing to a lack of sensitivity of the current analysis. The size and pattern of tissue distribution of 3β-HSD mRNA transcript was similar to what we have previously observed in ovine tissues using a human 3β-HSD cDNA *(16,17)*.

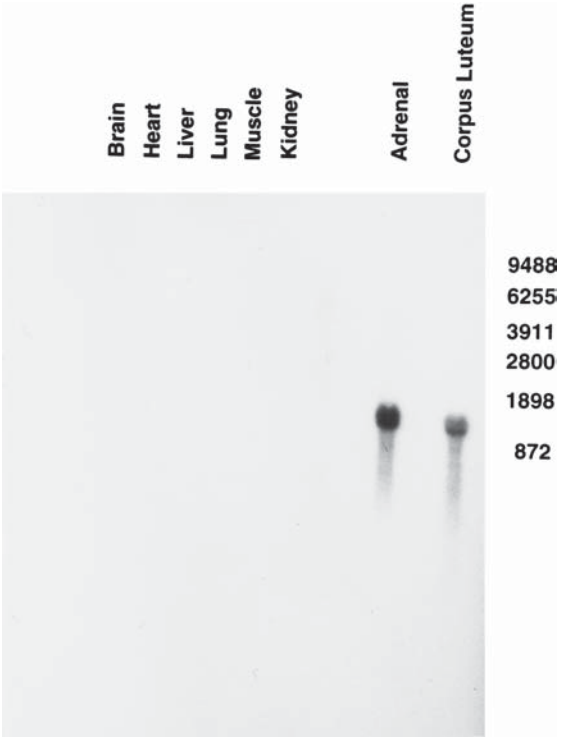

Fig. 1. Autoradioraph (2.5 h exposure) of total cellular RNA (20 μg) isolated from various ovine tissues hybridized to ovine 3β-HSD cDNA. Source of RNA is indicated at the top of each lane. Migration of RNA markers is on the right.

Treatment of ewes on day 11 or 12 of the estrous cycle with 25 mg $PGF_{2\alpha}$ iv followed by 10 mg $PGF_{2\alpha}$ im 2 h later rapidly and completely induced luteal regression as indicated by decreased concentrations of progesterone in sera and luteal tissues and decreased luteal weights. As we have previously reported in the ewe *(10,17)* and Tian et al. *(25)* have reported in the cow, mRNA encoding 3β-HSD decreases rapidly following $PGF_{2\alpha}$-induced luteolysis. It was proposed that this decrease in 3β-HSD mRNA may be occurring primarily in large luteal cells *(17)*, since these cells contain the high-affinity receptors for $PGF_{2\alpha}$ *(6–8)*. However, since the decrease in mRNA encoding 3β-HSD was similar in large luteal cells (43% of controls) and whole luteal tissue (35% of controls), and small and large luteal cells contain similar concentrations of mRNA encoding 3β-HSD *(16),* the decrease in mRNA encoding 3β-HSD was not limited to large luteal cells. Thus, binding of $PGF_{2\alpha}$ to its receptor on large luteal cells likely triggers the release of a factor(s) that results in decreased concentrations of mRNA encoding 3β-HSD in small luteal cells. A similar

Table 1
Concentrations of Progesterone (P_4) in Sera and Luteal Tissues, Luteal Weights, Luteal Concentrations of mRNA Encoding 3β-HSD and Luteal 3β-HSD Enzyme Activities in Untreated and Saline-Treated Ewes (control) and Ewes 4, 12, 24, and 48 h After Treatment with $PGF_{2\alpha}$

Time after $PGF_{2\alpha}$	Serum P_4,[e] ng/mL	Luteal P_4,[f] ng/mg tissue	Luteal wt,[e] mg	3β-HSD mRNA,[f] fmol/mg poly(A)$^+$ RNA	3β-HSD activity,[f] mg P_4/mg tissue/20 min
Control	2.03 ± 0.33[a]	25.4 ± 4.1[a]	556 ± 41[a]	21.0 ± 1.8[a]	4.23 ± 0.51[a]
4	0.76 ± 0.07[b]	14.1 ± 1.8[a]	434 ± 35[a,b]	7.4 ± 0.8[b]	4.19 ± 1.00[a]
12	0.86 ± 0.18[b]	15.0 ± 1.8[a]	459 ± 33[a,b]	3.1 ± 0.9[c]	4.10 ± 0.82[a]
24	0.18 ± 0.04[b,c]	5.4 ± 0.8[b]	399 ± 38[b]	2.0 ± 0.4[c,d]	3.27 ± 0.34[a]
48	0.12 ± 0.04[c]	1.5 ± 0.3[c]	204 ± 8[c]	1.0 ± 0.1[d]	2.00 ± 0.54[a]

[a,b,c,d]Within a parameter, means with different letters were different ($P < 0.05$).
[e]A portion of these data has been published *(8,32)*.
[f]Measurements were obtained from whole luteal tissue and are indicative of changes in both small and large luteal cells.

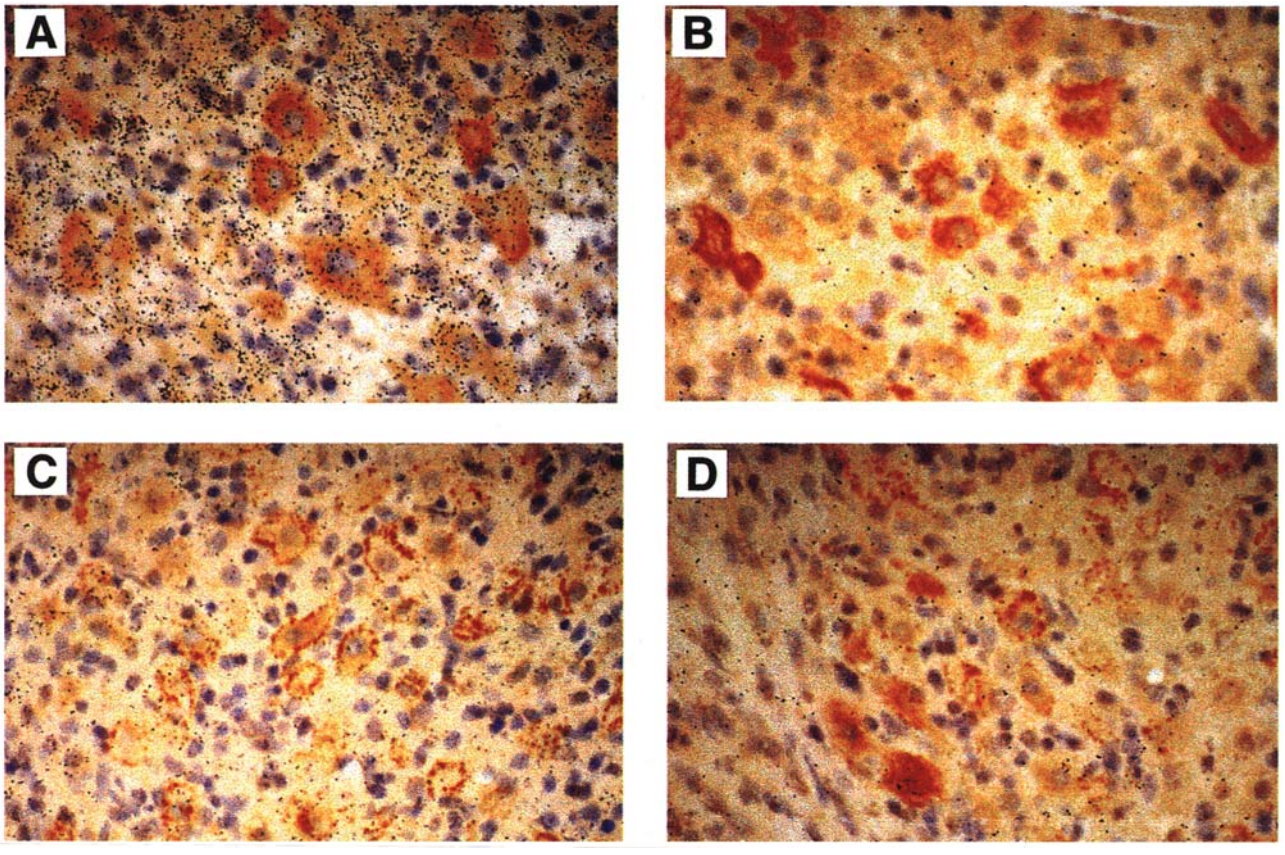

Fig. 2. Picture of ovine luteal tissue following immunostaining for TIMP-1 and hybridization with an ^{35}S-labeled 3β-HSD cRNA. Cells containing TIMP-1 protein (large luteal cells) stain dark brown (all panels). Panels **A** and **B** are sections of a corpus luteum collected from a control animal whereas panels **C** and **D** are sections of a corpus luteum collected 4 h after $PGF_{2\alpha}$ injection. Labeled antisense cRNAs for 3β-HSD were used for hybridization for panels A and C, whereas panels B and D were hybridized with labeled sense cRNAs for 3β-HSD.

situation is observed in ovine luteal tissue with $PGF_{2\alpha}$-induced downregulation of mRNA encoding the receptor for LH *(26)*, which is primarily found in small luteal cells.

Even though mRNA encoding 3β-HSD decreased rapidly following $PGF_{2\alpha}$-induced luteolysis, 3β-HSD enzyme activity was not decreased by 48 h following induction of luteolysis. At this time, concentrations of progesterone in sera and luteal tissues were approx 6% of control values. Thus, the acute decrease in 3β-HSD mRNA that occurs following administration of $PGF_{2\alpha}$ is not associated with a decrease in 3β-HSD enzyme activity and, therefore, is not a major cause of the decreased secretion of progesterone. The lack of an effect of $PGF_{2\alpha}$ on 3β-HSD enzyme activity through 48 h posttreatment generally confirms the prelimi-

nary results reported by McClellan et al. *(19)*. A similar decrease in 3β-HSD mRNA without a decrease in 3β-HSD enzyme, as measured by analysis of Western blots, occurs in bovine luteal tissue following $PGF_{2\alpha}$ administration *(25,27)*. Thus, in ruminants, although $PGF_{2\alpha}$ acutely decreases luteal concentrations of mRNA encoding 3β-HSD, decreased luteal secretion of progesterone is not owing to a decreased amount of 3β-HSD protein or reduced activity of this enzyme.

The proposed rate-limiting step in steroid biosynthesis is the transport of cholesterol across the mitochondrial membrane to P450scc *(28,29)*. Wiltbank et al. *(18)* have shown that this process appears to be the step that is downregulated by $PGF_{2\alpha}$. Recently a protein, termed steroidogenic acute regulatory protein (StAR), has been identified, which appears to control this step *(30,31)*. Treatment of ewes with $PGF_{2\alpha}$ rapidly reduced luteal concentrations of mRNA encoding StAR *(32)*. In contrast to 3β-HSD, StAR is a very labile protein with a half-life of minutes *(33)*. Thus, downregulation of mRNA encoding StAR likely causes acute decreases in concentrations of this protein and decreases secretion of progesterone.

In conclusion, $PGF_{2\alpha}$ administration appears to decrease 3β-HSD mRNA in both small and large luteal cells, even though in the ovine corpus luteum, only large luteal cells contain high-affinity receptors for $PGF_{2\alpha}$. In addition, $PGF_{2\alpha}$ decreased total luteal concentrations of 3β-HSD mRNA to 35% of controls within 4 h of administration, and the amount of 3β-HSD mRNA decreased further during the remainder of the experiment. However, this decline in mRNA encoding 3β-HSD was not associated with a significant decrease in 3β-HSD enzyme activity. Thus, the decline in 3β-HSD mRNA does not appear to play a major role in promoting the initial attenuation of secretion of progesterone during $PGF_{2\alpha}$-induced luteolysis.

Materials and Methods

Experimental Design

Western range ewes exhibiting normal estrous cycles (18 ± 1 d; 80% with multiple corpora lutea) were used in this experiment. Ewes were observed for estrous behavior twice daily in the presence of vasectomized rams. Corpora lutea were collected surgically via midventral laparotomy. This experiment was approved by the Colorado State University Animal Care and Use Committee. To determine if $PGF_{2\alpha}$ was differentially affecting expression of mRNA encoding 3β-HSD in the two steroidogenic cell types and to determine if the decrease in mRNA encoding 3β-HSD was followed by a decrease in activity of 3β-HSD, 25 ewes on days 11–12 of the estrous cycle were assigned to receive $PGF_{2\alpha}$ or serve as controls. To induce luteolysis, 17 ewes received 25 mg $PGF_{2\alpha}$ (Lutalyse; Upjohn Company, Kalamazoo, MI) iv followed by 10 mg $PGF_{2\alpha}$ im 2 h later. The iv injection was given to allow precise timing of the onset of the action of $PGF_{2\alpha}$ whereas the im injection was administered to ensure complete luteolysis, since $PGF_{2\alpha}$ is cleared rapidly from the bloodstream. Corpora lutea were collected from ewes 4, 12, 24, or 48 h following the iv injection of $PGF_{2\alpha}$ (n = 4–5/time-point). In addition, corpora lutea were collected from two groups of control ewes (n = 4/group), which received no injections or saline injections 24 h prior to tissue collection. Corpora lutea were decapsulated, weighed; and a center section embedded in Lab-Tek O.C.T embedding medium (Miles Laboratories; Elkhart, IN). The remaining tissue was frozen in liquid nitrogen within 15 min of collection. Jugular blood samples were drawn prior to injection of $PGF_{2\alpha}$ or saline and at the time of luteal tissue collection. Concentrations of progesterone in sera and luteal tissues were determined in a single radioimmunoassay (*34*, coefficient of variation [CV] = 14%, sensitivity = 90 pg/mL or 10 pg/mg tissue).

Generation of a cDNA Encoding a Portion of Ovine 3β-HSD

Unless otherwise specified, all materials used were purchased from Sigma Chemical Company, St Louis, MO or Fisher Scientific, Denver, CO. To generate a cDNA encoding a portion of ovine 3β-HSD, complementary DNA was generated by reverse transcription of luteal RNA (isolated from corpora lutea collected from ewes on day 10 of the estrous cycle) with random hexamers as primers. A portion of the ovine 3β-HSD cDNA was amplified by polymerase chain reaction (GeneAMP, Perkin Elmer Cetus, Norwalk, CT; denaturing 95°C, 1 min; annealing 55°C 1 min; extension 72°C 2 min; 35 cycles) with primers specific for bases 269–288 and 774–793 of the bovine 3β-HSD sequence *(20)*. The resulting 525-bp fragment was ligated into pGEM^R-T (Promega, Madison, WI) according to the manufacture's instructions and the sequence determined by the dideoxy procedure (*35*; Sequenase Version 2.0; US Biochemicals, Cleveland, OH). The 525-bp insert was released from pGEM^R-T by digestion with SAC I and SAC II. The purified insert was then used for Northern and slot-blot analysis following incorporation of ^{32}P-dCTP (3000 ci/mmol, Amersham; Arlington Heights, IL) by the random primer method (*36*; specific activity of cDNA 0.5×10^9 dpm/μg DNA).

Tissue distribution and size of the ovine 3β-HSD transcript was determined by Northern analysis *(37)*. Total cellular RNA was isolated from ovine corpus luteum, brain, heart, liver, lung, muscle, kidney, and adrenal by organic extraction *(38)*. Twenty micrograms of total cellular RNA were separated through a 1.5% agarose-formaldehyde-3[*N*-Morpholino]propanesulfonic acid gel, transferred to a nylon membrane (Hybond, Amersham; Arlington Heights, IL) by capillary action, and crosslinked by ultraviolet light. Filters were prehybridized for 2–12 h at 42°C in hybridization buffer (5X SSC: 750 m*M* NaCl and 75 m*M* sodium citrate; 1X Denhart's; 0.1% [w/v] Ficoll 400, 0.1% [w/v] polyvinylpyrrolidone, 0.1% [w/v] BSA [US Biochemicals]; 0.1% SDS; 50% deionized formamide; 50 m*M* sodium

phosphate, and heat-denatured salmon sperm DNA [100 µg/mL]). Filters were hybridized in 10 mL hybridization buffer containing $2.0–3.0 \times 10^7$ cpm of random primer-labeled ^{32}P-cDNA encoding 3β-HSD for 24 h at 42°C. The final wash was in 0.1X SSC, 0.1% SDS at 65°C for 30 min. Filters were exposed to Kodak X-AR film (VWR Scientific, Denver, CO) for 2.5–24 h.

Quantification of mRNA Encoding 3β-HSD

Since the cDNA encoding 3β-HSD hybridized to a single band in ovine luteal RNA, mRNA encoding 3β-HSD was quantified by slot-blot analysis. Individual samples of luteal tissue were homogenized in lysis buffer (0.2*M* NaCl, 0.2*M* Tris, 1.5 m*M* $MgCl_2$, 2% SDS, and 0.4 mg/mL proteinase K), and poly A^+ RNA isolated *(39)* by binding to oligo dT cellulose (Collaborative Biomedical Products; Bedford, MA). There was no indication of mRNA degradation in experimental samples as assessed by Northern analysis. To quantitate mRNA encoding 3β-HSD, 30 ng poly A^+ RNA isolated from each luteal sample were applied to a nylon filter in duplicate using a slot-blot apparatus. To generate standard curves, sense RNAs for 3β-HSD were synthesized by transcribing the linearized plasmid containing the 3β-HSD partial cDNA with T7 polymerase. Varying quantities (5, 10, 30, 50, 100, 300, and 500 pg) of appropriate sense mRNA, positive-control RNA (10, 30, 50, and 100 ng pooled luteal poly A^+ RNA), and negative-control RNA (30 ng ovine liver poly A^+ RNA) were also included on each filter. All RNA was crosslinked to the nylon filter with UV light. Conditions for prehybridization, hybridization, and washing of the filter were as described for Northern analysis. Comparison of hybridization of 3β-HSD ^{32}P-cDNA to samples and standards as measured by densitometric scanning (Hoefer Scientific Instruments, San Francisco, CA) of autoradiographs was used to calculate amount of mRNA encoding 3β-HSD in individual samples. In a preliminary slot-blot analysis, concentrations of mRNA encoding 3β-HSD were 10.0, 12.7, 12.0, and 10.3 fmoles/µg poly A^+ RNA when measured in 10, 30, 50, and 100 ng pooled luteal poly A^+ RNA. Recovery of transcribed 3β-HSD standard mRNA, when added to poly A^+ RNA isolated from ovine liver, was 111%. The standard curves for synthesized 3β-HSD mRNA were linear from 10–300 pg ($r^2 = 0.99$). Intra-assay CV was 8%, sensitivity of the assay was 0.84 fmol of 3β-HSD mRNA/µg poly A^+ RNA.

To determine if equal amounts of poly A^+ RNA were applied to each slot, the filter was hybridized to ^{32}P-end-labeled dT (18 mer) generated with T_4 polynucleotide kinase (New England Biolabs, Beverly, MA) and ^{32}PATP (3000 Ci/mmol; Amersham; *16*). There was a high correlation between amount of pooled luteal RNA applied to the filters and densitometric reading following autoradiography ($r^2 = 0.95$). Coefficient of variation of densitometric values after hybridization to the end-labeled dT averaged 13%. Therefore, no corrections were made for differences in loading of poly A^+ RNA.

Quantification of 3β-HSD mRNA in Large Luteal Cells

Antisense and sense ^{35}S-UTP-labeled cRNAs were synthesized using SP6 polymerase with 3β-HSD containing plasmid digested with SAC II or T7 polymerase with 3β-HSD containing plasmid digested with SAC I *(40)*. Sections (10 µm) from corpora lutea collected from noninjected ewes and from ewes 4 h after injection of $PGF_{2\alpha}$ were thaw mounted onto 3-aminopyroxysilane-coated slides and processed for immunostaining for detection of tissue inhibitor of metalloproteinases 1 (TIMP-1) protein and mRNA encoding 3β-HSD as described *(8)*. Tissue inhibitor of metalloproteinases 1 protein has been shown to colocalize with mRNA encoding $PGF_{2\alpha}$-R *(8)*. One animal from each group was not analyzed because of loss of tissue. Number of silver grains was determined in an average of 25 TIMP-1-positive cells/animal. Diameters of cells were also measured for determination of number of grains/µm^2. Number of background silver grains was determine in two areas of the slide not containing luteal tissue, and corrected values were used for statistical analysis.

Measurement of 3β-HSD Enzyme Activity

Amount of 3β-HSD enzyme activity was determined as described *(41),* except tissue that had been frozen was used. In a preliminary assay the amount of 3β-HSD enzyme activity measured in tissue that had been frozen was linear with increasing time ($y = -2.573 + 2.6417x$; $r^2 = 0.96$) or tissue concentrations ($y = 3.6567 + 285.75x$; $r^2 = 0.99$). Amount of progesterone synthesized from added pregnenolone was similar between fresh and frozen tissue (1.18 vs 1.26 µg progesterone in 20 min from 1 mg tissue). Amount of 3β-HSD enzyme activity was determined in all samples in a single assay. Tissues were assayed in four groups and a standard luteal sample was included at the beginning and end of each group. Coefficient of variation was 19%. Amounts of progesterone synthesized by luteal tissues were determined in a single radioimmunoassay (CV = 14%, sensitivity = 0.06 µg progesterone/mg tissue/20 min).

Statistical Analysis

Amount of 3β-HSD mRNA in large luteal cells in 0 and 4 h groups was compared with a *t*-test using SAS *(42)*. All other analyses were performed using the general linear models procedures of SAS *(42)*. Owing to nonnormality of data or heterogenous variance, data for all parameters, except 3β-HSD enzyme activity, were transformed (square-root or log) for statistical analysis; however, means ± SEM of raw data are presented. There were no differences in any parameter measured between noninjected and saline-injected control ewes; therefore, data from these groups were combined for final statistical analysis and are referred to as controls. Differences among means were evaluated by Tukey's method *(42)*.

Acknowledgments

We appreciate the invaluable technical assistance of Michael Gallegos, Carol Moeller, and Kit Sutherland, and the secretarial assistance of Kathy Thomas. This research was supported by grants from NIH (HD11590; G. D. N.) and USDA (CSRS 92-37203-7950; M. F. S.) and E. W. M. was supported by a USDA National Needs Fellowship. We would like to thank D. Haworth and W. Mellion for their help with tissue collection.

References

1. Niswender, G. D. and Nett, T. M. (1994). In: *The Physiology of Reproduction, Vol 1.* Knobil, E., and Neill, J. D. (eds.). Raven, New York.
2. McCracken, J. A., Glew, M. E., and Scaramuzzi, R. J. (1970). *J. Clin. Endocrinol. Metab.* **30,** 544–546.
3. McCracken, J. A., Carlson, J. C., Glew, M. E., Godin, J. R., Baird, D. T., Green, K., et al. (1972). *Nature (New Biol,)* **238,** 129-134.
4. Juengel, J. L., Nett, T. M., Tandeski, T.R., Eckery, D. C., Sawyer, H. R., and Niswender, G. D. (1995). *Endocrine* **3,** 323–326.
5. Niswender, G. D., Schwall, R. H., Fitz, T. A., Farin, C. E., and Sawyer H. R. (1985) *Recent Prog. Horm. Res.* **41,** 101–151.
6. Fitz, T. A., Mayan, M. H., Saywer, H. R., and Niswender, G. D. (1982) *Biol. Reprod.* **27,** 703–711.
7. Balapure A. K., Caicedo, I. C., Kawada, K. Watt D. S., Rexroad C. E. Jr, and Fitz, T. A. (1989) *Biol. Reprod.* **41,** 385–392.
8. Juengel, J. L., Wiltbank, M. C., Meberg, B. M., and Niswender, G. D. (1996) *Biol. Reprod.* **54,** 1096–1102.
9. Wiltbank, M. C., Diskin, M. G., Flores, J. A, and Niswender, G. D. (1990) *Biol. Reprod.* **42,** 239–245.
10. McGuire, W. J., Juengel, J. L., and Niswender, G. D. (1994) *Biol. Reprod.* **51,**800–806.
11. Wiltbank, M. C., Guthrie, P. B., Mattson, M. P., Kater, S. B., and Niswender, G. D. (1989) *Biol. Reprod.* **41,** 771–778.
12. Davis, J. S., Weakland, L. L., Weiland, D. A., Farese, R. V., and West, L. A. (1987) *Proc. Natl. Acad. Sci. USA* **84,** 3728–3732.
13. Juengel, J. L., Garverick, H. A., Johnson, A. L., Youngquist, R. S., and Smith, M. F. (1993) *Endocrinology* **132,** 249–254.
14. Hoyer, P. B., and Marion, S. L. (1989) *J. Reprod. Fertil.* **86,** 445–455.
15. Wiltbank, M. C., Knickerbocker, J. J., and Niswender, G. D. (1989) *Biol. Reprod.* **40,** 1194–1200.
16. Juengel, J. L., Guy, M. K., Tandeski, T. R., McGuire, W. J., and Niswender, G. D. (1994) *Biol. Reprod.* **51,** 380–384.
17. Hawkins, D. E., Belfiore, C. J., Kile, J. P., and Niswender, G. D. (1993) *Biol. Reprod.* **48,** 1185–1190.
18. Wiltbank, M. C., Belfiore, C. J., and Niswender, G. D. (1993) *Mol. Cell. Endocrinol.* **97,** 1–7.
19. McClellan M. C., Abel, J. H. Jr., and Niswender, G. D. (1977) *Biol Reprod* **16,** 499–512.
20. Zhao, H-F., Simard, J., Labrie, C., Breton, N., Rheaume, E., Luu-The, V., et al. (1989) *FEBS Lett.* **259,** 153–157.
21. Luu-The, V., Lachance, Y., Labrie, C., Leblanc, G., Thomas, J. L., Strickler, R. C., et al. (1989) *Mol. Endocrinol.* **3,** 1310–1312.
22. Simard, J., Melner, M. H., Breton, N., Low, K. G., Zhao, H-F., Periman, L. M., et al. (1991) *Mol. Cellul. Endocrinol.* **75,** 101–110.
23. Zhao, H-F., Labrie, C., Simard, J., de Launoit, Y., Trudel, C., Martel, C., et al. (1991) *J. Biol. Chem.* **266,** 583–593.
24. Bain, P. A., Yoo, M., Clarke, T., Hammond, S. H., and Payne, A. H. (1991) *Proc. Natl. Acad. Sci. USA* **88,** 8870–8874.
25. Tian, X. C., Berndtson, A. K., and Fortune, J. E. (1994) *Biol. Reprod.* **50,** 349–356.
26. Guy, M. K., Juengel, J. L., Tandeski, T. R., and Niswender, G. D. (1995) *Endocrine* **3,** 585–589.
27. Rodgers, R. J., Vella, C. A., Young, F. M., Tian, X. C., and Fortune, J. E. (1995) *Reprod. Fertil. Dev.* **7,** 1213–1216.
28. Crivello, J. F., and Jefcoate, C. R. (1980) *J. Biol. Chem.* **255,** 8144–8151.
29. Jefcoate, C. R., BiBartolomeis, M. J., Williams, C. A., and McNamara, B. C. (1987) *J. Steroid Biochem.* **27,** 721–729.
30. Clark, B. J., Wells, J., King, S. R., and Stocco, D. M. (1994) *J. Biol. Chem.* **269,** 28,314–28,322.
31. Lin, D., Sugawara, T., and Strauss, J. F. III. (1995) *Science* **267,** 1828–1831.
32. Juengel, J. L., Meberg, B. M., Turzillo, A. M., Nett, T. M., and Niswender, G. D. (1995) *Endocrinology* **136,** 5423–5429.
33. Stocco, D. M, and Sodeman, T. C. (1991) *J. Biol. Chem.* **266,** 19,731–19,738.
34. Niswender, G. D. (1973) *Steroids* **22,** 413–424.
35. Chen, E. Y., and Seeburg, P. H. (1985) *DNA* **4,** 165–170.
36. Feinberg, A. P., and Volgelstein, B. (1983) *Anal. Biochem.* **132,** 6–13.
37. Sambrook, J., Fritsch, E. F., and Maniatis, T. (1989) *Molecular Cloning: A Laboratory Manual, 2nd ed.* Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY.
38. Chomzynski, P., and Sacchi, N. (1987) *Anal. Biolchem.* **162,**156–159.
39. Badley, J. E., Bishop, G. A., St John, T., and Frelinger, J. A. (1988) *Biotechniques* **6,** 114–116.
40. Melton, D. A., Krieg, P. A., Rebagliati, M. R., Maniatis, T., Zinn, K., and Green, M. R. (1984) *Nucleic Acids Res.* **12,** 7035–7056.
41. Caffrey, J. L., Nett, T. M., Able, J. H. Jr., and Niswender, G. D. (1979) *Biol. Reprod.* **20,** 279–287.
42. SAS (1987) *SAS User's Guide, Statistics.* Statistical Analysis System Institue, Inc., Cary, NC.