BIOLOGY OF REPRODUCTION 43, 210–218 (1990)

Expression of Trophoblastic Interferon Genes in Sheep and Cattle[1]

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ABSTRACT

The trophoblastic interferons ovine and bovine trophoblast protein-1 (oTP-1 and bTP-1, respectively) have been implicated as mediators of maternal recognition of pregnancy in sheep and cattle. The objective of this study was to describe the onset and duration of gene expression for oTP-1 and bTP-1 in preimplantation ovine and bovine conceptuses by in situ hybridization and Northern analysis. Sections from paraffin-embedded ovine conceptuses, collected on Days 10, 11, 12, 13, and 15 of gestation (n = 1, 3, 3, 2, 2), and bovine conceptuses, collected on Days 12/13, 15/16, and 19 (n = 2, 4, 5), were hybridized to specific [^{35}S]-labeled cDNA probes. Two different probes, one encompassing bases 442–918 and representing both coding and 3′-untranslated regions, and a second 3′-specific probe (bases 650–912) were used to detect oTP-1 mRNA. At all stages examined, oTP-1 mRNA was confined to trophectoderm of ovine conceptuses. Consistent with earlier studies, expression increased markedly at Day 13. oTP-1 mRNA was detected at low levels in seven of seven ovine conceptuses prior to Day 13 when the longer probe was employed. With the 3′-specific probe, however, oTP-1 mRNA was detected in only one of the seven ovine conceptuses prior to Day 13. Thus, although low amounts of oTP-1 mRNA may be present in ovine conceptuses prior to Day 13, massive induction of this mRNA occurs on Day 13 coincident with the initiation of maternal recognition of pregnancy. bTP-1 mRNA, detected with a coding plus 3′-noncoding region probe (bases 236–913), was confined to the trophectoderm and present in all conceptuses from Days 12/13–19. Maximal expression was noted at Day 15/16. These results were confirmed by Northern blotting of RNA extracted from bovine conceptuses on Days 15, 17, 19, 21, 23, and 25 of gestation. bTP-1 mRNA was detected on all days examined. Taken together, these data demonstrate that bTP-1 mRNA is present in conceptuses as early as Day 12 of pregnancy, with a marked increase in expression occuring on Day 15/16 of pregnancy and continuing through at least Day 25. The increase in expression of bTP-1 mRNA on Day 15/16 occurs coincident with elongation of the blastocyst and maternal recognition of pregnancy in this species.

INTRODUCTION

Maternal recognition of pregnancy in sheep and cattle has been defined as the critical period during which the conceptus must be resident within the uterus to prevent luteolysis and establish pregnancy (Short, 1969). The major conceptus secretory products responsible for initiation of maternal recognition of pregnancy in sheep and cattle are known as ovine trophoblast protein-1 (oTP-1) and bovine trophoblast protein-1 (bTP-1), respectively. These proteins are major secretory products of conceptuses between Days 13 and 21 of gestation in sheep (Godkin et al., 1982) and Days 15 and 24 of gestation in cattle (Bartol et al., 1985) and have been implicated in triggering a series of maternal responses to the presence of the conceptus that include extension of the lifespan of the corpus luteum (Godkin et al., 1984b; Knickerbocker et al., 1986a; Thatcher et al., 1989), changes in endometrial protein synthesis (Godkin et al., 1984a; Vallet et al., 1987; Salamonsen et al., 1988; Sharif et al., 1989), and inhibition of prostaglandin $F_{2\alpha}$ production by the uterus (Fincher et al., 1986; Knickerbocker et al., 1986b; Salamonsen et al., 1988). In addition, oTP-1 and bTP-1 have been identified as interferons (IFNs) belonging to the 172-amino acid IFNα_{II} subclass. oTP-1 and bTP-1 exhibit approximately 85% identity in cDNA sequence with bovine IFNα_{II} in their protein-encoding regions and approximately 70% sequence identity in their 3′-untranslated regions (Imakawa et al., 1987, 1989; Stewart et al., 1987). These trophoblast proteins also possess potent antiviral and antiproliferative activities (Roberts et al., 1989) which are similar to those of the extensively studies 166-amino acid IFNα_I subclass proteins. Furthermore, administration of IFNα_I can mimic some physiological actions of oTP-1 or bTP-1 including introduction of synthesis of specific ovine endometrial proteins in culture (Salamonsen et al., 1988) and prolongation of the interestrous interval after intrauterine infusion in nonpregnant cows (Plante et al., 1988, 1989; Thatcher et al., 1989) and ewes (Stewart et al., 1989).

Production of oTP-1 by ovine conceptuses as detected by two-dimensional gel electrophoresis, begins at approximately Day 13 of pregnancy (Godkin et al., 1982). By using a more sensitive radioimmunoassay, however, Ashworth and Bazer (1989) reported that oTP-1 is produced as early as Days 8 and 10 of gestation. Similarly, although major production of bTP-1 appears to begin at Days 15–17 of gestation (Bartol et al., 1985), bTP-1 has been reported to

Accepted March 16, 1990.

Received January 12, 1990.

[1]Research supported by NIH Grant #HD21896. C.E.F. supported by NIH Postdoctoral Fellowship #HD07217. This paper is #11,114 from the Missouri Agricultural Experiment Station.

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be produced as early as Day 12 on the basis of antiviral activity in media from groups of cultured bovine embryos (Betteridge et al., 1987).

Analysis of changes in oTP-1 messenger RNA levels during the period of maternal recognition of pregnancy by dot-blot and Northern techniques have demonstrated a marked increase in oTP-1 mRNA beginning on Day 13 or Day 14 of pregnancy followed by a decline between Days 16 and 22 (Hansen et al., 1988; Stewart et al., 1989). When the more sensitive method of in situ hybridization was used to analyze changes in oTP-1 mRNA concentrations, good agreement was found in that mRNA increased markedly on Day 13 of gestation and declined gradually through Day 23 (Farin et al., 1989). In contrast, however, oTP-1 mRNA was not detected in six conceptuses on Day 12 of gestation by dot-blot analysis (Hansen et al., 1988) but was detected in a single conceptus on Day 11 of gestation by in situ hybridization (Farin et al., 1989). No data have been reported regarding changes in bTP-1 mRNA levels in conceptuses.

The objectives of the present studies were, first, to analyze the onset of oTP-1 mRNA production, characterizing more precisely the initiation of trophoblastic IFN gene expression, and, second, to describe the localization and pattern of bTP-1 mRNA production during the period of maternal recognition of pregnancy.

MATERIALS AND METHODS

Experiment I

Eleven ovine conceptuses were collected by surgical flushing of the uterus of crossbed whiteface ewes using techniques described by Smith and Murphy (1987). Conceptuses were collected on Days 10 (n = 1), 11 (n = 3), 12 (n = 3), 13 (n = 2), and 15 (n = 2) of pregnancy (estrus = Day 0). Within 15 min of recovery, conceptuses were immobilized in blocks of 2% (w/v) low-melting agarose (Fisher Scientific, St. Louis, MO) dissolved in 0.01 M phosphate-buffered saline (PBS) and immersion-fixed in 4% (w/v) paraformaldehyde on ice for 2 h. Conceptuses were washed in PBS and embedded in paraffin. Messenger RNA for oTP-1 was detected by in situ hybridization (Farin et al., 1989) by using random-primed (Random Prime Labeling Kit, No. 1004760, Boehringer-Mannheim, Indianapolis, IN), [^{35}S]-labeled cDNA probes from either a coding plus 3′-untranslated region fragment of oTP-1 mRNA (oTP-560, a *Pst*I/*Pst*I restriction fragment including bases 442–918 of the oTP-1 cDNA; sp. act.: 1.2 × 10^9 dpm/μg) or a 266-base pair fragment from the 3′-untranslated region of oTP-1 mRNA (oTP-266, a *Bgl*II/*Ssp*I restriction fragment including bases 650–912 of the oTP-1 cDNA; sp. act.: 1.2 × 10^9 dpm/μg; Farin et al., 1989). For each conceptus, adjacent sections were hybridized (50% formamide, 0.6 M sodium chloride, 42°C) with either [^{35}S]-γ-actin cDNA (sp. act.: 9.7 × 10^8 dpm/μg; positive control) or [^{35}S]-pBS M13 plasmid (vector) DNA (sp. act.: 1.1 × 10^9 dpm/μg; negative control) probes. Hybridization signals were detected by autoradiography after 10 days of exposure at 4°C. The relative intensity of hybridization signals, quantified as optical density based on the reflectance of hybridized silver grains, was measured under dark-field illumination by computerized video image analysis (Bioquant System IV, R&M Biometrics, Nashville, TN). A single, randomly selected section was used to represent each conceptus in the analysis. The value for the hybridization signal represents the average pixel gray-scale value (optical density) based on measurement of all pixels included within the entire cross-sectional area of each type of tissue (i.e. trophectoderm, endoderm, yolk sac, embryonic disc) present in each randomly selected section. Measurements on all conceptus sections were done at the same magnification (12.5×). The coefficient of variation for repeated measures (n = 4) of a standard section of trophectoderm was 4.21%. For individual conceptuses the specific hybridization signals associated with oTP-1 and actin cDNA probes were determined by subtraction of the hybridization signals associated with the negative control (vector DNA) probe.

Experiment II

Holstein heifers were synchronized by injection of Lutalyse (Upjohn Co., Kalamazoo, MI) and bred at estrus by artificial insemination. Eleven bovine conceptuses were obtained by nonsurgical uterine flush on Day 12/13 (n = 2), Day 15/16 (n = 4), and Day 19 (n = 5) of pregnancy (estrus = Day 0). The flush medium used was Modified Dulbecco's PBS supplemented with pennicilin and streptomycin (Seidel et al., 1980). Conceptuses were recovered within 3 h of flushing and immersion-fixed in ice-cold 4% paraformaldehyde in PBS for 2 h. After washing in PBS, conceptuses were then embedded in paraffin. Messenger RNA for bTP-1 was detected by in situ hybridization (50% formamide, 0.6 M sodium chloride, 42°C) by using a [^{35}S]-labeled *Pst*I/*Ssp*I (bases 236–913) coding plus 3′-untranslated region bTP-1 cDNA probe (sp. act.: 1.2 × 10^9 dpm/μg; Day 13–19 conceptuses) or the [^{35}S]-oTP-266 cDNA probe described in Experiment I (Day 12 conceptus). The oTP-266 cDNA probe was derived from the unique 3′-untranslated region of the oTP-1 mRNA that shows >92% identity with the corresponding region of the bTP-1 mRNA (Imakawa et al., 1989) and hybridizes to bTP-1 mRNA (Farin, unpublished results). In addition, for each conceptus, positive (actin cDNA) and negative (vector DNA) in situ hybridization control sections were included. Specificity of the bTP-1 cDNA, and vector DNA probes was confirmed by Northern hybridization analysis with total RNA from Day 19 bovine conceptuses (Fig. 1) according to procedures described by Farin et al. (1989). In situ hybridization signals were detected and quantified as in Experiment I. Data from individual conceptuses were corrected for background hybridization by subtraction of signals associated with hybridization of the negative control (vector DNA) probe.

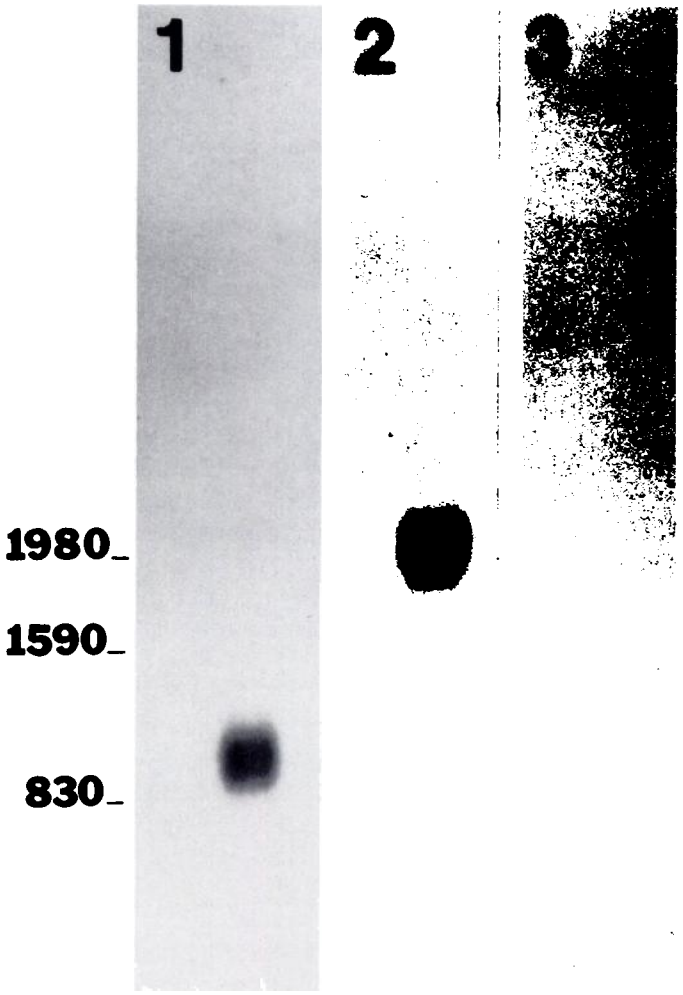


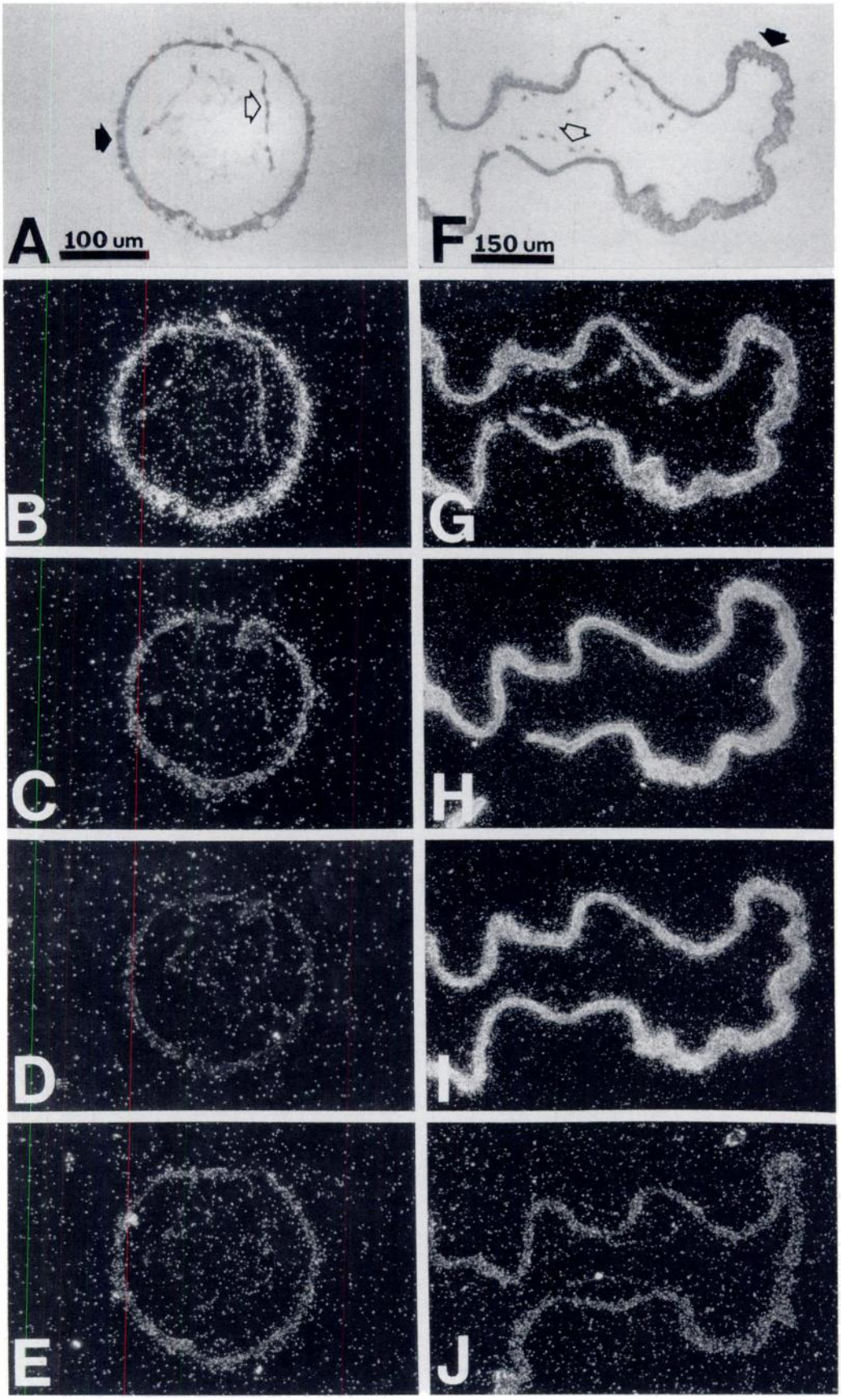
FIG. 1. Northern blot analysis of Day 19 bovine conceptus total RNA (2 μg/lane) demonstrating specificity of hybridization for the bTP-1 cDNA probe (Lane 1; bTP-1 mRNA approximately 1 kilobase [Imakawa et al., 1989]). γ-actin cDNA probe (Lane 2; actin mRNA approximately 2 kilobase [Gunning et al., 1983]), and lack of hybridization with the vector (negative) control DNA probe (Lane 3). Estimated base pair lengths, based on *Eco*RI-*Hind*III-digested lambda DNA, are indicated on the left margin. All cDNA probes used in this analysis were [^{32}P]-labeled by random prime procedures (Farin et al., 1989).

Experiment III

Crossbred beef cows were artificially inseminated 12 h after detection of estrus (Day 0). On Days 15 (n = 1), 17 (n = 3), 19 (n = 3), 21 (n = 3), and 25 (n = 3) of gestation, the reproductive tracts were removed at slaughter, placed on ice and returned to the laboratory where conceptuses were recovered by uterine flushing (Bartol et al., 1985; Helmer et al., 1987). Total RNA was isolated from these conceptuses by using a modification of the methods described by Chirgwin et al. (1979) and Chomczynski and Sacchi (1987). Briefly, individual conceptuses were homogenized in 4 M guanidine thiocyanate, 25 mM potassium citrate (pH 7), 0.5% (v/v) sarcosyl, and 0.1 M 2-mercaptoethanol (GTC) and precipitated in the presence of 25 mM acetic acid and ethanol at −20°C. RNA was collected by centrifugation (5 000 × *g*, 20 min, 4°C) and resuspended in 1–2 ml GTC solution. After addition of sodium acetate (pH 4) to 0.2 M, proteins were extracted sequentially with 1 volume of phenol (water-saturated) and 0.2 volumes of chloroform-isoamyl alcohol (24:1). Samples were centrifuged (10 000 × *g*, 20 min, 4°C) and RNA was precipitated from the aqueous phase with 1 volume of isopropanol. After centrifugation (10 000 × *g*, 10 min, 4°C) the resulting RNA pellets were dissolved in 0.3 volumes of GTC and precipitated again with 1 volume of isopropanol. The RNA pellets were washed with 80% ethanol, sedimented, vacuum-dried, dissolved in 0.5% (w/v) SDS and stored at −80°C. RNA concentrations were determined spectrophotometrically.

Five micrograms of total RNA were pooled from each conceptus for each day of pregnancy for Northern blot analysis. Thereafter, 7-μg samples of each pool of total RNA from Days 17, 19, 21, 23, and 25 were subjected to electrophoresis and transferred to nylon membranes (Zetaprobe, Bio-Rad Laboratories, Richmond, CA) for hybridization analysis. Because of low recovery of total RNA from the single conceptus collected on Day 15, only 4 μg total RNA was analyzed. Blots were hybridized with a nick-translated, *Pst*I/*Eco*RI restriction fragment (bases 241–1035) of bTP-1 cDNA (Imakawa et al., 1989), which included coding, 3′-untranslated, and poly(A)$^+$ regions of the bTP-1 cDNA. In addition, blots were hybridized with γ-actin cDNA (Gunning et al., 1983). Actin was considered to be a 'housekeeping gene' whose expression was used to assess mRNA integrity. bTP-1 and actin cDNA inserts were purified on a Nensorb column and were nick-translated (Maniatis et al., 1982) in the presence of [α-^{32}P]-deoxycytididine triphosphate (dCTP) to specific activities of approximately 0.5 × 10^8 and 1.0 × 10^8 cpm/μg, respectively. *Hind*III/*Eco*RI-digested lambda DNA standards were end-labeled with [^{32}P]-ATP (Maniatis et al., 1982) and used to determine the size of message. In addition, the bTP-1 cDNA insert was subcloned (Maniatis et al., 1982) into a pBS M13 transcription vector (Stratagene Inc., San Diego, CA) and cRNA transcripts were synthesized by standard protocols outlined by the manufacturer. cRNA transcripts were used as a positive control in the Northern analysis. The conditions for hybridization and autoradiography were as described previously (Hansen et al., 1988). Following autogradiography of the bTP-1 hybridization, nylon membranes were washed in 0.15 M sodium chloride/0.015 M sodium citrate, 0.5% (v/v) SDS at 95°C for 20 min to remove bound bTP-1 cDNA probe. These membranes were subsequently rehybridized with the γ-actin cDNA probe.

FIG. 2. Experiment I. In situ localization of oTP-1 and actin mRNAs in ovine conceptuses from Day 10 (A–E) and Day 13 (F–J) of pregnancy. (A and F) Bright-field micrograph of Day 10 and Day 13 ovine conceptuses, respectively; trophectoderm (closed arrow), and endoderm (open arrow). (B and G) Hybridization with [^{35}S]-γ-actin cDNA (dark-field optics). (C and H) Hybridization with [^{35}S]-oTP-560 cDNA. (D and I) Hybridization with [^{35}S]-oTP-266 cDNA. (E and J) Hybridization with [^{35}S]-vector DNA.



Statistical Analyses

For Experiments I and II, after correction for background hybridization levels, changes in relative hybridization signals on different days of pregnancy were analyzed by one-way analysis of variance within each probe type. When a significant F-statistic was encountered, means were separated by Least Significant Difference (Snedecor and Cochran, 1967).

RESULTS

Experiment I

Consistent with previous observations (Farin et al., 1989), mRNA for trophoblastic interferon was localized exclusively in the trophectoderm of the developing conceptuses and was not present in either endoderm (Fig. 2) or embryonic disc (data not shown). Trophoblastic interferon mRNA was detected at low levels in all seven conceptuses between Days 10 and 12 of gestation when the oTP-560 cDNA probe was used for hybridization (Fig. 2c, Fig. 3). In contrast, use of the oTP-266 cDNA probe resulted in a positive hybridization signal in only one of three Day 11 ovine conceptuses and in none of the conceptuses from Days 10 or 12 (Fig. 2d, Fig. 3). Thus, prior to Day 13, only one of seven conceptuses contained detectable amounts of oTP-1 mRNA as assessed by use of a specific cDNA probe that recognizes the unique 3′-noncoding region of the oTP-1 mRNA. An increase ($p < 0.01$) in the hybridization signal intensity for both the oTP-266 and oTP-560 cDNA probes occurred on Day 13 of gestation (Fig. 2, Fig. 3). Messenger RNA for oTP-1, as detected by both oTP-1 cDNA probes, remained elevated on Day 15 of gestation at concentrations not significantly different from that on Day 13 (Fig. 3). There was no significant change in the hybridization signals associated with actin mRNA between Days 10 and 15 of gestation (Fig. 3).

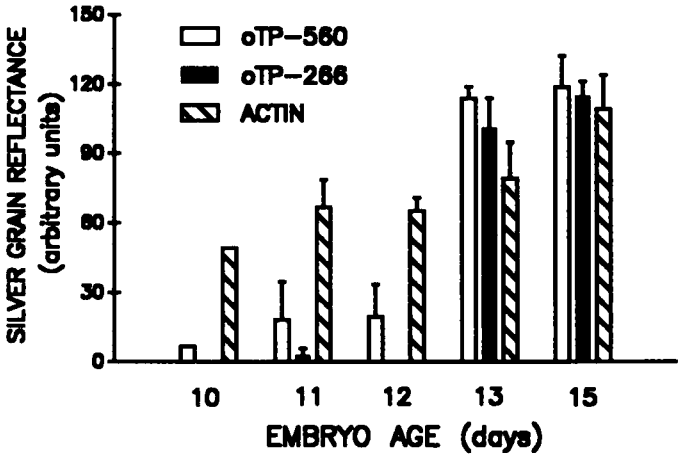


FIG. 3. Changes in relative hybridization signals (silver grain reflectance; Mean ± SE) for oTP-1 and actin mRNAs in ovine conceptuses from Day 10 to Day 15 of pregnancy. Changes in oTP-1 mRNA was assessed by using both the oTP-560 and oTP-266 cDNA probes (see *text* for details). All data corrected for background hybridization signals associated with vector (negative control) DNA.

Experiment II

Messenger RNA for bTP-1 was detected in all conceptuses examined from Days 12–19 of pregnancy. Messenger RNA for bTP-1 was localized in the trophectoderm (Fig. 4) but was not found in either endoderm or yolk sac tissues where hybridization signals were not significantly different from background, as determined by analysis of conceptuses from Days 15–19 of pregnancy. Because ^{35}S was used for labeling the bTP-1 cDNA probe, it was not possible to determine if the single binucleate trophectoderm cells scattered among the more numerous mononucleate trophectoderm cells exhibited differences in the intensity of hybridization signal.

Changes in the relative amounts of bTP-1 and actin mRNA in the trophectoderm of bovine conceptuses from Days 12–19 of pregnancy are shown in Figure 5. bTP-1 mRNA was found at low but detectable levels as early as Day 12 of gestation. The level of bTP-1 mRNA increased on Day 15/16 of pregnancy and remained elevated through Day 19 (Fig. 5). These differences were not significant, however, due to substantial variability in the developmental stage of conceptuses on Day 15/16. Of the four conceptuses recovered, one was spherical whereas the remaining three were filamentous forms of varying length. The spherical conceptus was most likely retarded in development and not degenerate since it appeared to be normal when examined histologically. The relative hybridization signal for bTP-1 mRNA in this conceptus was substantially lower than that for the three filamentous conceptuses recovered on Day 15/16 (17.4 vs. 73.4 ± 2.8, for the spherical vs. filamentous conceptuses, respectively). If data for the spherical Day 15/16 conceptus are not included in the data set, the increase in bTP-1 mRNA between Days 12/13 and 15/16 becomes even more pronounced (19.2 ± 11.1 vs. 73.4 ± 2.8, respectively). The level of actin mRNA in conceptuses did not differ with stage of gestation ($p > 0.4$).

Experiment III

The bTP-1 and γ-actin cDNA probes used for Northern analysis hybridized to mRNA bands of the expected size of approximately 1.1 kilobases for bTP-1 mRNA and 2 kilobases for actin mRNA (Fig. 6a,b). The majority of bTP-1 cRNA transcripts (positive control, Fig. 6a, Lane C) were shorter than the full-length bTP-1 mRNA. This was an expected result since only bases 241–1035 of the bTP-1 cDNA were subcloned into the transcription vector.

The Day 15 conceptus sample, which had less total RNA loaded (4 μg) than the conceptus samples from Days 17–25, gave a relatively low hybridization signal (Fig. 6a). bTP-1 mRNA was present at all stages of pregnancy examined from Day 15 to Day 25 (Fig. 6a). Although the washing procedures failed to remove all of the bTP-1 cDNA probe from

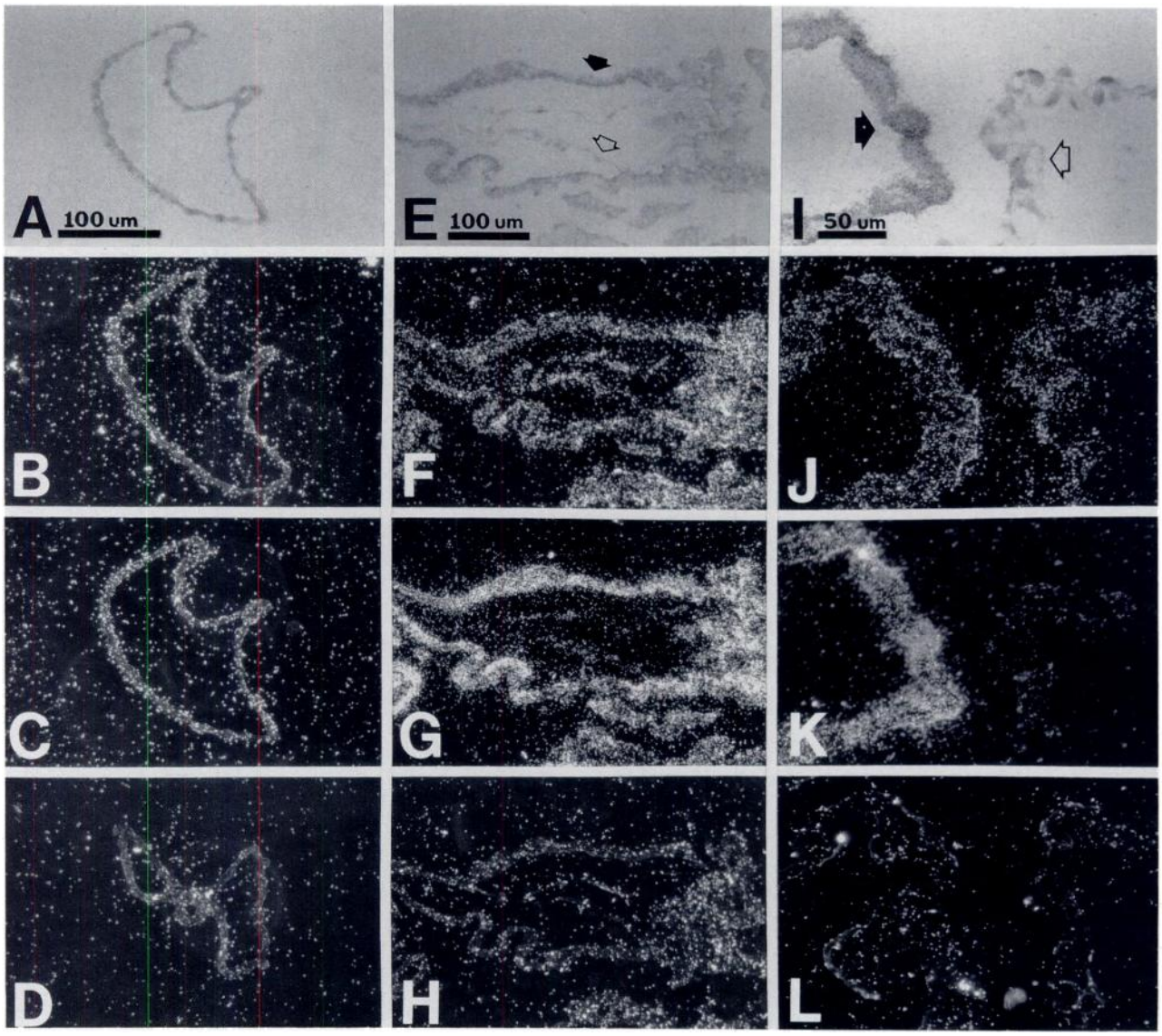


FIG. 4. Experiment II. In situ localization of bTP-1 and actin mRNAs in bovine conceptuses on Day 12 (A–D) and Day 19 (E–L) of pregnancy. (A, E, I) Bright-field micrograph of (A) Day 12 conceptus, (E) Day 19 conceptus trophectoderm (closed arrow) with endoderm (open arrow), and (I) Day 19 conceptus trophectoderm (closed arrow) with yolk sac tissue (open arrow), respectively. (B, F, J) Hybridization with [^{35}S]-γ-actin cDNA (dark-field optics). (C) Hybridization with [^{35}S]-oTP-266 cDNA (92% identity with bTP-1 mRNA), (G, K) Hybridization with [^{35}S]-bTP cDNA. (D, H, L) Hybridization with [^{35}S]-vector DNA.

the nylon membrane, it was possible in a subsequent hybridization to detect an actin signal at each of the days of gestation studied (Fig. 6b), because the actin mRNA is considerably larger than the bTP-1 mRNA.

DISCUSSION

From the results presented earlier (Farin et al., 1989) and extended herein, expression of oTP-1 mRNA occurs with rapid onset during embryonic development. On Days 10, 11, and 12 of pregnancy, mRNA for trophoblastic interferons, although present, was expressed at concentrations at least 8-fold lower than found on Day 13. From Days 13–15, oTP-1 mRNA was strongly expressed, although concentrations of mRNA fall shortly thereafter (Farin et al., 1989). The dramatic increase in oTP-1 gene expression on Day 13 correlated closely with the morphological transition of the ovine conceptus from a 1–2-mm-diameter sphere on Days 10–12 to a 3–5-mm tubular form usually found on Day 13 of pregnancy. Similarly, increased production of bTP-1 mRNA by conceptuses at the time of maternal recognition of pregnancy also coincided with a morphological change from a spherical to filamentous form.

Although both the oTP-560 and oTP-266 cDNA probes hybridized to oTP-1 mRNA, only the former gave a consistent signal with conceptuses younger than Day 13. Under the hybridization conditions used in the present study (Tm oTP-560: 59.07°C), it was possible that the oTP-560 cDNA

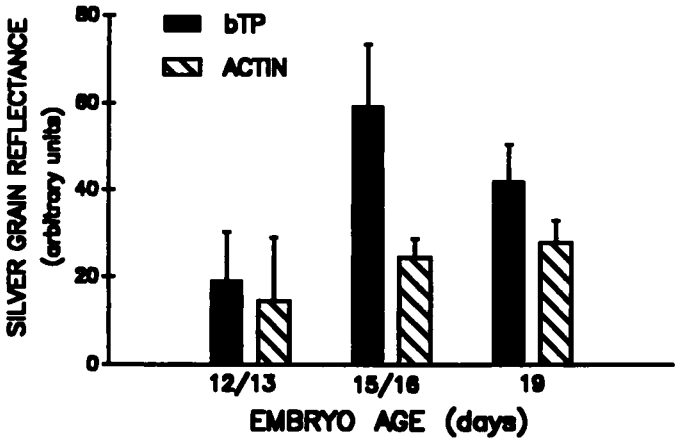


FIG. 5. Changes in the relative hybridization signals for bTP-1 mRNA in trophectoderm of bovine conceptuses between Days 12/13 and 19 of pregnancy (Mean ± SE). All data corrected for background hybridization signals associated with vector (negative control) DNA.

probe was able to recognize other interferons within the $IFN\alpha_{II}$ class and not just oTP-1, since there is approximately an 85% identity of sequence between the coding regions of the embryonic interferons (oTP-1 and bTP-1) and the $IFN\alpha_{II}$s (Imakawa et al., 1989). Under the conditions used, mRNA sequences with 75% identity to the oTP-560 probe could be distinguished, whereas theoretically, no distinction should be possible between sequences 85% alike.

The oTP-266 cDNA probe (Tm oTP-266: 56.6°C) represented that segment of the oTP-1 mRNA which was least identical to other $IFN\alpha_{II}$ mRNAs showing only about 70% sequence identity with the $IFN\alpha_{II}$ 3′-untranslated region (Imakawa et al., 1989). Under the hybridization conditions used in this experiment, only sequences of greater than 80% identity would be hybridized. Thus, because it represented the highly conserved 3′ end of oTP-1 (and bTP-1) mRNA, the oTP-266 probe was most likely more specific, distinguishing oTP-1 and bTP-1 mRNAs from other $IFN\alpha_{II}$ mRNAs. However, it should be recognized that although both the oTP-560 and oTP-266 cDNA probes were of comparable specific activities ($\sim 1 \times 10^9$ dpm/μg), the oTP-560 probe was approximately twice the length of the oTP-266 probe and could provide up to twice as much signal for each mRNA molecule hybridized. Therefore, a positive hybridization might be more easily visualized with the oTP-560 probe, particularly in conceptuses with very low amounts of oTP-1 mRNA.

The results of our study are consistent with the observation that ovine conceptuses as early as Days 8 and 10 of pregnancy produce an oTP-1-like protein, as determined by RIA of conceptus culture media (Ashworth and Bazer, 1989).

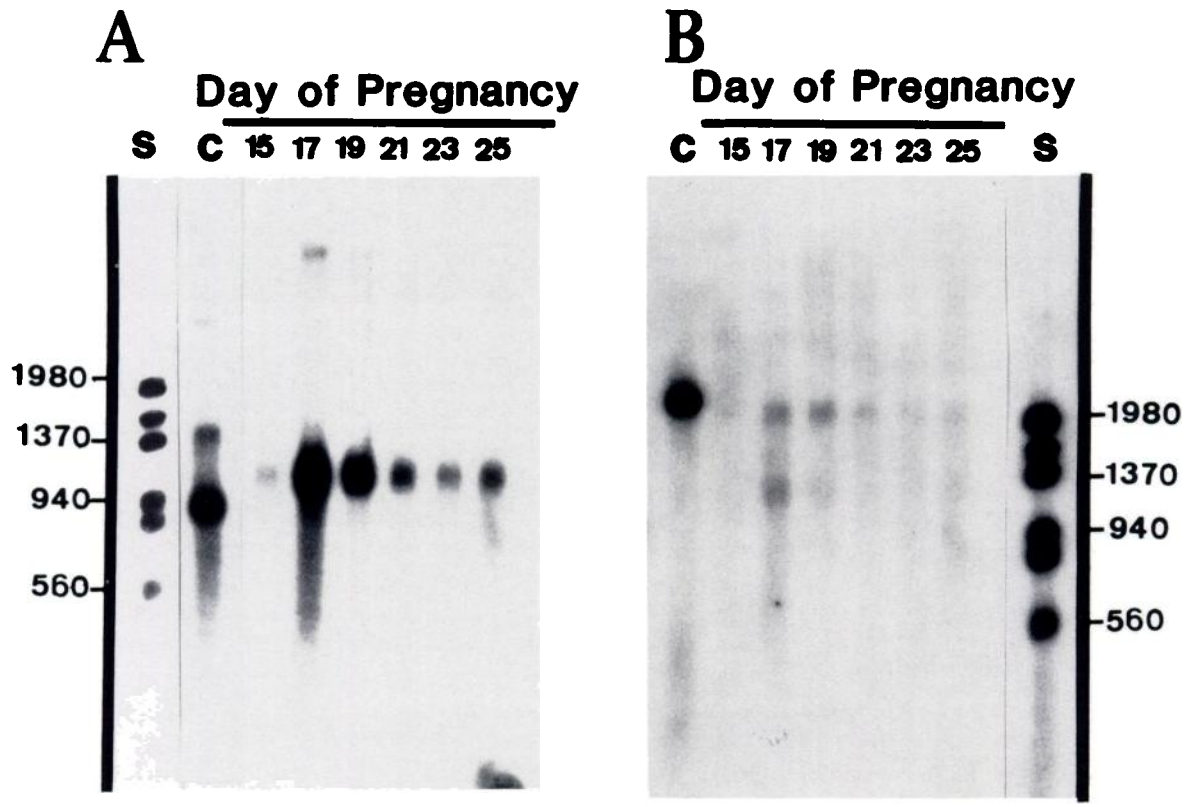


FIG. 6. Northern blot analyses of bTP-1 mRNA (A) and actin mRNA (B) in bovine conceptuses between Days 15 and 25 of pregnancy. Lane S represents molecular sizing standards; Lane C is bTP-1 cRNA transcripts used as a positive hybridization control (2 ng). In B, the same nylon filter was used as in A, but was probed with γ-actin cDNA. Lane C (Panel B) represents hybridization to synthetic γ-actin cRNA transcript (2 ng), which was used as a positive control.

Production of this protein appears extremely low in conceptuses at this stage of pregnancy since the amount of protein produced per conceptus per hour of culture was 100- to 1000-fold less than that produced by Day 14 and Day 16 conceptuses, respectively. The extent to which the antiserum used by Ashworth and Bazer (1989) cross-reacted with ovine IFNα_{II}s other than oTP-1 is unclear.

The results of Experiments II and III demonstrated that the bovine conceptus, like the ovine conceptus, produced low amounts of bTP-1 mRNA at least three days prior to the time of maternal recognition of pregnancy, which is considered to be Day 15/16 of gestation (Betteridge et al., 1978, 1980; Northey and French, 1980). These results are consistent with detection of antiviral activity as early as Day 12 of pregnancy by cultured bovine trophoblast tissues (Betteridge et al., 1988). Unlike the ovine conceptus, however, the bovine conceptus produced bTP-1 mRNA for an extended interval, with message detected as late as Day 25 of pregnancy in this study. Furthermore, immunoblot analysis of proteins secreted by cultured bovine conceptuses demonstrated that proteins cross-reactive with antisera to oTP-1 (i.e. bTP-1) are secreted at least through Day 36 of pregnancy (Godkin et al., 1988). The function associated with this extended period of production of bTP-1 is unclear.

bTP-1 mRNA was localized exclusively in the trophectoderm of the bovine conceptus and was not found in either endodermal or yolk sac tissues. These data are consistent with the localization of mRNA for oTP-1 in sheep conceptuses (Farin et al., 1989). Because ^{35}S was used for radiolabeling of the bTP-1 cDNA probe in the present study, it was not possible to determine if binucleate cells present in the trophectoderm of the Day 19 bovine conceptuses were differentially labeled compared to the surrounding mononucleate cells. However, on the basis of immunocytochemical localization, bTP-1 can be found in both binucleate and mononucleate cells of the trophoblast, with binucleate cells showing less intense staining than mononucleate cells (Lifsey et al., 1989).

In summary, expression of trophoblastic IFN genes occurred as early as Day 10 of pregnancy in sheep and Day 12 of pregnancy in cattle. However, the concentrations of oTP-1 and bTP-1 mRNA transcripts increased sharply at the time of maternal recognition of pregnancy in both species. This increased expression coincided with the morphological transition of the conceptus from a spherical to an elongated form. Expression of bTP-1 mRNA in bovine conceptuses continued from Day 12 through at least Day 25 of pregnancy.

ACKNOWLEDGMENTS

The authors would like to acknowledge Dr. L. Kedes (Department of Medicine, Stanford University, Palo Alto, CA) for generously contributing the γ-actin cDNA used in this study. In addition, the authors express their gratitude to the Upjohn Company (Kalamazoo, MI) and CEVA Laboratories, Inc. (Overland Park, KS) for donating materials used for synchronization of estrus.

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