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A J Watson

P H Watson

M Arcellana-Panlilio

D Warnes

S K Walker

See next page for additional authors

Authors
A J Watson, P H Watson, M Arcellana-Panlilio, D Warnes, S K Walker, G A Schultz, D T Armstrong, and R F Seamark

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A Growth Factor Phenotype Map for Ovine Preimplantation Development[1]

ANDREW J. WATSON,[2,3] PATRICIA H. WATSON,[4] MAYI ARCELLANA-PANLILIO,[5] DEIRDRE WARNES,[6] SIMON K. WALKER,[7] GILBERT A. SCHULTZ,[5] DAVID T. ARMSTRONG,[3,6] and ROBERT F. SEAMARK[6]

Department of Obstetrics and Gynaecology,[3] University of Western Ontario London, Ontario, Canada N6A 5A5
Lawson Research Institute,[4] St. Joseph's Health Center, London, Ontario, Canada N6A 4V2
Department of Medical Biochemistry,[5] University of Calgary, Calgary Alberta, Canada T2N 4N1
Department of Obstetrics and Gynaecology,[6] University of Adelaide, Adelaide, South Australia 5000
South Australia Research and Development Institute,[7] Turretfield Research Center, South Australia 5350

ABSTRACT

The reverse transcription-polymerase chain reaction (RT-PCR) was used to determine the patterns of expression for several growth factor ligand and receptor genes during ovine preimplantation development. Transcripts for insulin-like growth factor (IGF)-I, IGF-II, and the receptors for insulin and IGF-I were detected throughout ovine preimplantation development from the 1-cell to the blastocyst stage. Transforming growth factor α (TGFα) transcripts were also detected throughout ovine preimplantation development. The mRNAs encoding basic fibroblast growth factor (bFGF) were detected in all stages of the ovine preimplantation embryo, although the relative abundance of this transcript consistently decreased from the 1-cell to the blastocyst stage, suggesting that it may represent a maternal transcript in early sheep embryos. Transcripts encoding ovine trophoblast protein (oTP) were detected only within blastocyst-stage embryos. Primary ovine oviduct cell cultures express the transcripts for IGF-II, IGF-I, TGFα, bFGF, TGFβ1, and the receptors for insulin and IGF-I, suggesting that paracrine growth factor circuits may exist between the oviduct epithelium and the early ovine embryo. Transcripts for insulin, epidermal growth factor (EGF), and nerve growth factor (NGF) were not detected in any stage of the ovine preimplantation embryo or within the oviduct cell preparations. The expression of growth factor transcripts very early in mammalian development would predict that these molecules fulfil a necessary role(s) in supporting the progression of early embryos through the preimplantation interval. Our future efforts will be directed to understanding the nature of these putative regulatory pathways.

INTRODUCTION

It is possible to culture large proportions of mouse preimplantation embryos through to the blastocyst stage in relatively simple, defined salt solutions [1]. Similar culture environments have not been fully characterized for embryos of other species (e.g., rabbit, hamster, cow, sheep, pig), including the human [2, 3]. In these examples, culture media are still commonly supplemented with poorly defined constituents from conditioned medium or serum [3–18]. Some preimplantation embryos require the additional step of employing coculture on primary oviduct epithelial cell monolayers [9–12, 16–21]. However, in all cases, cultured mammalian embryos can be distinguished from their in vivo counterparts by their morphology, reduced cell number, impaired developmental rates, or lower pregnancy rates after embryo transfer (ET).

Recent efforts to characterize the appropriate molecular milieu required to successfully support development through the preimplantation interval in these species have focused upon the role(s) of growth factors in early development (for review see [22]). Growth factors play important roles in modulating embryonic and fetal growth and development [22–44]. This influence can be demonstrated as early as during the preimplantation interval, since physiological levels of several growth factors have been shown to affect murine development through increased protein and RNA synthetic rates, increased rates of cell division, increased cell number, and elevated blastocyst expansion and zona hatching rates [25, 26, 28–40].

Much less is known about the possible role(s) of growth factors in modulating early development in other, nonmurine, mammalian species [41–44]. We have demonstrated that bovine preimplantation embryos express a pool of growth factor mRNAs similar to those present in the mouse, but the stage-specific timing of expression for these transcripts varies between these species [45, 46].

The present study extends our investigation of the patterns of expression for these growth factors to include ovine preimplantation development. As in previous studies, the method of mRNA phenotyping by the reverse transcription-polymerase chain reaction (RT-PCR) was employed to examine whether transcripts for the insulin-like growth factor (IGF), epidermal growth factor (EGF), and transforming growth factor (TGF) families were detectable during ovine preimplantation development. The sheep represents an important model for the investigation of fetal development,

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[2]Correspondence: Dr. Andrew J. Watson, Department of Obstetrics and Gynaecology, University of Western Ontario, University Hospital, 339 Windermere Road, London, Ontario, Canada N6A 5A5. FAX: (519) 663-3388.

and extensive research has shown that growth factors such IGF-I and IGF-II play an essential role in regulating ovine fetal growth (for review see [47]). It is therefore of interest to investigate whether this growth factor-mediated influence is initiated during the preimplantation period. Our analysis also characterizes the expression of these transcripts within primary oviduct epithelial cells to identify putative paracrine regulatory circuits between the oviduct and the early embryo.

MATERIALS AND METHODS

In Vitro Maturation (IVM) and In Vitro Fertilization (IVF) of Ovine Oocyte Cumulus Complexes (OCC)

The methods outlined in Watson et al. [48] were employed in the production of preimplantation ovine embryos from follicular aspirates obtained from slaughterhouse ovaries. Ovarian follicles (2–8 mm) were aspirated from slaughterhouse ovaries within 4 h of removal from the animal. The OCC were pooled and washed a minimum of six times with collection medium (TCM-199 medium [Gibco, Grand Island, NY] + 2% [v/v] fetal bovine serum [FBS; Gibco] and 100 IU/ml heparin [Sigma, St. Louis, MO]). OCC were then placed into maturation medium (TCM-199 containing 10% [v/v] FBS supplemented with sodium pyruvate [35 μg/ml; Sigma], 5 μg/ml FSH [Follitropin; Vetrepharm, London, ON, Canada], 5 μg/ml LH [Vetrepharm], and 1 μg/ml estradiol-17β [Sigma]) for 24 h at 38.6°C in a humidified atmosphere containing 5% CO_2 in air [12].

For each insemination, frozen semen pellets were thawed and processed for "swim-up" procedures by layering the semen under 1 ml of prewarmed (38.6°C) Hepes-buffered modified Tyrode's solution (without BSA [49]) supplemented with 2% (v/v) heat-inactivated sheep serum for 2 h at 38.6°C. The OCC were removed from the maturation medium and washed through 100-μl drops of Hepes-buffered modified Tyrode's solution (without BSA) supplemented with 2% (v/v) heat-inactivated sheep serum. OCC were then allocated to pre-equilibrated fertilization drops (50 OCC/300-μl drop) consisting of modified bicarbonate-buffered Tyrode's medium supplemented with 2% (v/v) sheep serum under light paraffin oil (BDH Inc., Toronto, ON, Canada). After "swim-up," approximately 50 μl (or 50–75 × 10^3 motile spermatozoa) of sperm solution was added per each fertilization drop. The sperm/OCC droplets were incubated for 24 h at 38.6°C in a humidified 5% CO_2 atmosphere in air before removal of the remaining cumulus cell investment; the zygotes were then placed on primary ovine oviduct cell monolayers.

Oviduct Cell Monolayer Formation

All of the embryos utilized in the present study were produced through use of embryo coculture on primary oviduct epithelial cell cultures. The methods employed in the establishment of these cultures were as previously described [12, 48]. Briefly, oviducts were trimmed of connective tissue prior to trypsin digestion (0.05% trypsin solution in Ca^{2+}-, Mg^{2+}-free Hanks' balanced salt solution [HBSS; Gibco]) at 38.6°C for 20 min. The contents of the lumen were then squeezed into a small Petri dish (35 mm) with a pair of forceps. The cells were washed four times with HBSS and then resuspended in TCM-199 + 10% (v/v) FBS before being plated into 24-well plates containing 1 ml of TCM-199 + 10% FBS/well, at an approximate density of 1 × 10^6 cells per well.

Embryo coculture was maintained for a maximum of 8 days at 38.6°C in a humidified atmosphere of 5% CO_2 in air to allow for blastocyst formation to occur. The embryos were moved to fresh primary 48-h oviduct epithelial cell cultures every 48 h to optimize their developmental progress. In total, over 6000 preimplantation ovine embryos representing a developmental series from 1-cell zygotes to hatched blastocysts were utilized in the study.

RNA Extraction

Ovine zygotes (60–125) were pooled into one of the following groups: 1) zygotes; 2) 2-cell to 8-cell, early cleavage stage embryos; 3) morulae (embryos with a minimum of 30 cells); and 4) blastocysts (early cavitation to fully expanded blastocysts). Total RNA was extracted from embryo pools in the presence of 10 μg of *Escherichia coli* transfer RNA (Sigma) as previously described [27, 45]. Embryos were placed into 100 μl of extraction buffer (0.2 M NaCl, 1 mM EDTA, 25 mM Tris HCl, pH 7.4) and were vortexed with equal volumes of phenol and chloroform:isoamyl alcohol. After phase separation by centrifugation, the aqueous phase was re-extracted with chloroform:isoamyl alcohol, centrifuged, and then precipitated with 95% ethanol. RNA pellets were washed with 70% ethanol and suspended in 11 μl of sterile distilled water prior to reverse transcription into cDNA. Total RNA was extracted from 48-h primary oviduct epithelial cell monolayers by lysis in guanidinium isothiocyanate (Pharmacia, Quebec, PQ, Canada) followed by ultracentrifugation through CsTFA [50].

RT-PCR

RNA from embryos was reverse transcribed into cDNA according to the protocol provided with the SUPERSCRIPT® RNase H-Reverse transcriptase kit (Gibco BRL, Gaithersburg, MD). Briefly, 0.5 μg of oligo$(dT)_{12-18}$ primer (Gibco BRL) was added to each suspended embryo RNA sample prior to a 10-min incubation at 70°C. After this incubation, the RNA was reverse transcribed by combining 200 U of SUPERSCRIPT® (Gibco BRL) with 5-strength reaction buffer (250 mM Tris HCl [pH 8.3], 375 mM KCl, and 15 mM $MgCl_2$), 10 mM dithiothreitol (DTT; Gibco BRL), and 0.5 mM each of dATP, dCTP, dGTP, and dTTP (Pharmacia) for an incubation at 43°C for 1 h. The reaction was termi-

nated by heating at 92°C for 5 min and quickly cooling on ice. The cDNA sample was further diluted by the addition of 30 μl of sterile distilled water before storage at −20°C or immediate amplification by PCR.

PCR [51] was performed as described previously [23, 31, 45]. Aliquots of embryo cDNA (5 μl) were amplified with 1 U of *Taq* DNA Polymerase (Bresatec, Adelaide, Australia) in a final volume of 50 μl containing 10-strength *Taq* reaction buffer (670 mM Tris HCl [pH 8.8], 166 mM $(NH_4)_2SO_4$, 2 mg/ml gelatin, and 4.5% Triton X-100; Bresatec), 2 mm $MgCl_2$, 0.2 mM of each dNTP, and 1 μM of each sequence-specific primer. The mixture was overlaid with mineral oil and then amplified by PCR for up to 40 cycles in a DNA thermal cycler (BioQuest DNA Thermal Cycler, Sydney, Australia); each cycle consisted of denaturation at 94°C for 1 min, re-annealing of primers to target sequences at 55–60°C for 2 min, and primer extension at 72°C for 2 min. An aliquot of the PCR products was run on 2% agarose gels containing 0.5 μg/ml of ethidium bromide, and the resulting amplified DNA fragments were photographed.

PCR Primers

Primer pairs were obtained from the regional DNA synthesis Laboratory, University of Calgary with the exception of the ovine IGF-I and IGF-II (oIGF-I; oIGF-II) primer pairs, which were synthesized by the Department of Microbiology, University of Adelaide, Adelaide, South Australia. The sequences of the primers used and the sizes of the expected PCR fragments were exactly as reported previously [45] with the exception of the oIGF-I and oIGF-II primer sets. The oIGF-I primer sequences target a region of the oIGF-I cDNA near the 3′ end of the molecule, result in an amplified fragment of 261 bp, and represent the following sequences: 1) 5′-CACATCCTCCTCGCATCTCTT-3′ for the upstream primer and 2) 5′-TTGAGAGGCGCACAGTACATC-3′ for the downstream primer [52]. The oIGF-II primer sequences target a region of the oIGF-II cDNA also near the 3′ end of the molecule and result in an amplified fragment of 318 bp. The sequences are 1) 5′-CTTCTACTT-CAGCCGACCATC-3′ for the upstream primer and 2) 5′-TGACTCTTGGCCTCTCTGAGC-3′ for the downstream primer [53]. All of our PCR experiments included a control reaction utilizing a set of primers designed to bracket an intron/exon border of the rodent β-actin gene [45, 54]. This primer pair can be used to detect the presence of both β-actin mRNA (cDNA) via the predicted 243-bp PCR fragment and contaminating genomic DNA (if genomic DNA is present a second fragment, larger than the one expected, will appear due to the inclusion of the intervening sequences). Also, the target sequence bracketed by these β-actin primers is 2000 bp from the 3′ end of the cDNA. Thus, detection of a β-actin PCR fragment also indicated that the reverse transcription of this transcript was virtually complete and that it produced cDNAs of at least 2 kb in length.

To confirm the identity of the PCR products, the amplified DNA fragments either were cut with an appropriate diagnostic restriction enzyme (RE) according to the supplier's instructions or were transferred to a nylon membrane and probed with the appropriate sequence as previously described [31, 45]. Probes were labeled in the presence of α-^{32}P-dCTP (3000 Ci/mmol) to specific activities of approximately 5×10^9 cpm/μg by means of the PCR reaction [55]. The mouse TGFα and TGFβ1 DNA fragments for amplification and labeling were derived from an RT-PCR clone originally generated from 11.5-day mouse embryo RNA. The insulin and IGF-I receptor cDNA clones used for probe preparation have been described by Ullrich et al. [56] and Telford et al. [31], respectively.

RESULTS

The sensitive technique of mRNA phenotyping by RT-PCR was applied to total RNA samples isolated from pools of staged in vitro-matured, in vitro-fertilized, and in vitro-cocultured (IVMFC) ovine preimplantation embryos. In this manner we have searched for detectable levels of the transcripts encoding insulin, IGF-I, IGF-II, EGF, TGFα, basic fibroblast growth factor (bFGF), TGFβ1, ovine trophoblast protein (oTP), and nerve growth factor (NGF) and also for the insulin, IGF-I, and IGF-II receptor mRNAs during preimplantation ovine development. The assays were repeated a minimum of six times with different pools of staged embryos representing a total of 6000 embryos. Each reverse-transcribed embryo cDNA sample was tested with the β-actin primers for the presence of contaminating genomic DNA. Genomic actin sequences give rise to a PCR DNA band larger (approximately 400 bp) than the expected 243-bp band that is derived from β-actin mRNA (cDNA). No PCR product for actin genomic DNA was detected in any of the growth factor phenotyping RT-PCR assays.

Examples of PCR results are summarized in Figure 1, which displays the typical pattern of expression for these growth factor transcripts during preimplantation development. The expression pattern within 48-h primary oviduct epithelial cell cultures is shown in Figure 2. Transcripts for β-actin were detected within all of the embryo stages and oviduct cell cultures. IGF-II expression was observed throughout ovine preimplantation development and also within oviduct cell cultures. The identity of the expected 318-bp PCR fragment was confirmed by subjecting the PCR fragment to a diagnostic RE digest with the restriction enzyme *Hpa* II (Fig. 3A), which resulted in the cleavage of the oIGF-II PCR fragment, as expected, into two equal halves (158 and 160 bp). The expression of IGF-II receptor mRNA was not detected within ovine preimplantation embryos or within the 48-h oviduct epithelial cell RNA preparations. A strong 186-bp IGF-II receptor fragment was amplified in mouse ovary RNA preparations, suggesting that our PCR primers were capable of amplifying the homologous IGF-

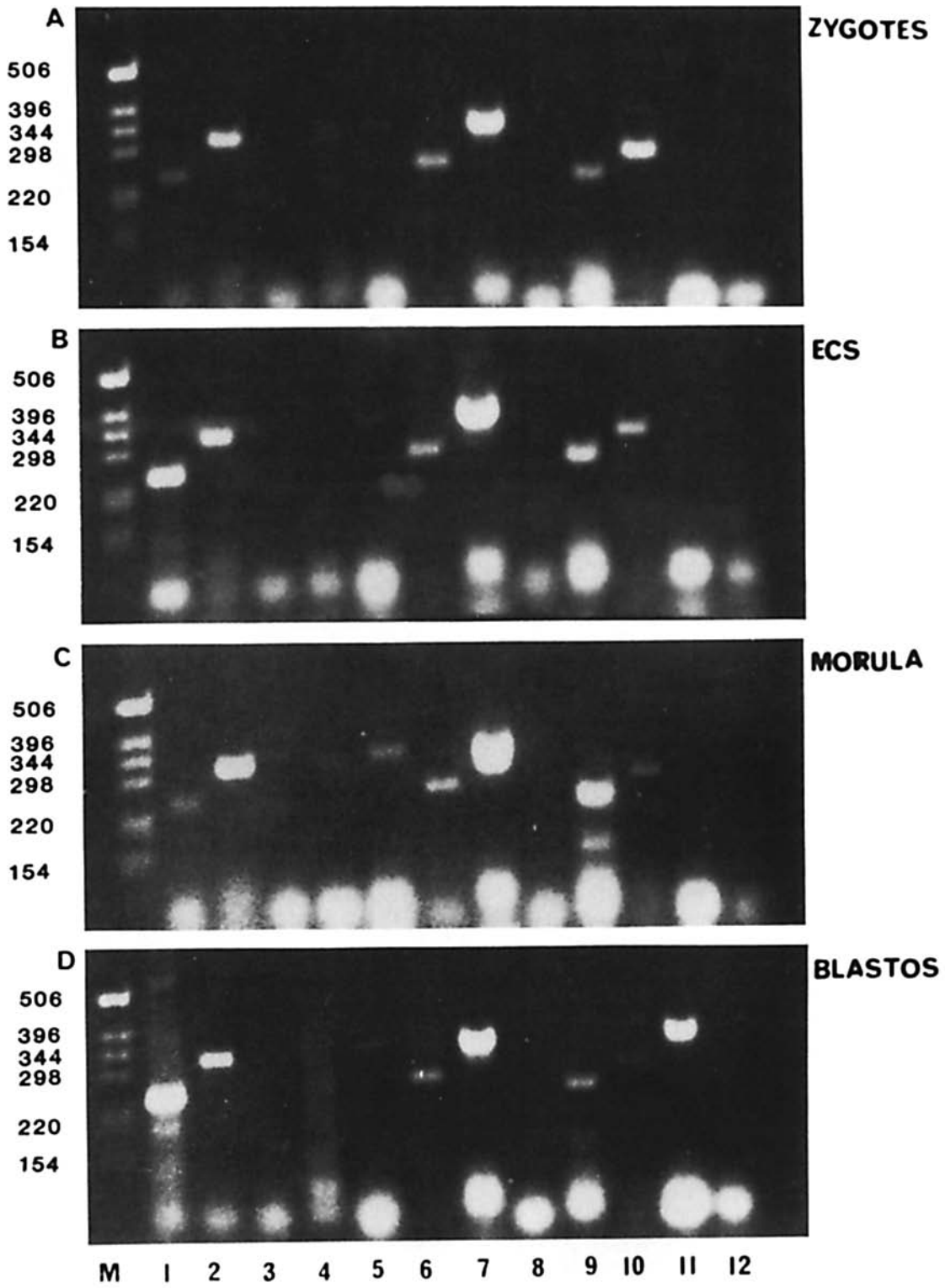

FIG. 1. Detection of growth factor transcripts within ovine preimplantation embryo RNA transcription products. Each lane represents the growth factor PCR products derived from the equivalent of 10 preimplantation embryos: (A) one-cell zygotes; (B) early cleavage stage embryos (2-cell to 8-cell stage); (C) morulae (> 30 cell embryos); and (D) blastocysts (from early to fully expanded blastocoel cavities). Part of the total PCR product (20 μl) was resolved on 2% agarose gels along with 2 μg of 1-kb DNA ladder (Gibco BRL; lanes marked M). The expected PCR fragments are: 1) β-actin (243 bp), 2) oIGF-II (318 bp), 3) IGF-II-R (186 bp), 4) insulin (301 bp), 5) insulin-R (324 bp), 6) oIGF-I (261 bp), 7) IGF-I-R (354 bp), 8) EGF (247 bp), 9) TGFα (239 bp), 10) bFGF (282 bp), 11) bTP (358 bp), and 12) NGF (401 bp).

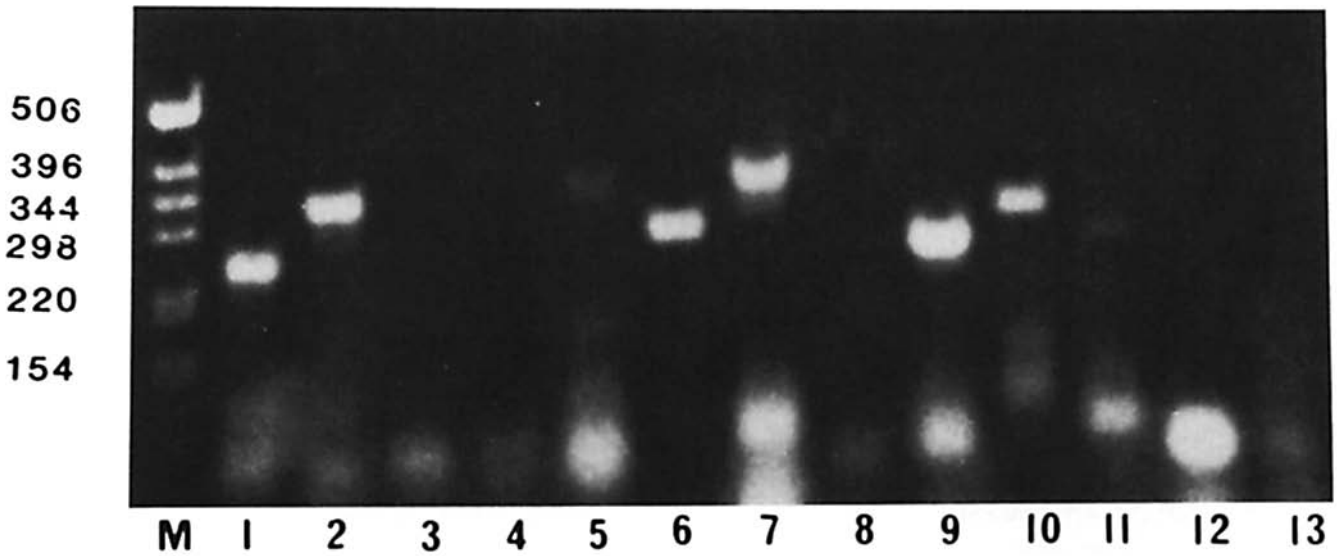

FIG. 2. Detection of growth factor transcripts within 48-h ovine oviduct epithelial cell cultures. Each lane represents the PCR product derived from amplifying the cDNA aliquot produced from 100 ng of purified oviduct epithelial cell RNA. The PCR product (20 μl) was resolved on 2% agarose gels along with 2 μg of 1-kb DNA ladder (lane M). The PCR products are as follows: 1) β-actin, 2) oIGF-II, 3) IGF-II-R, 4) insulin, 5) insulin-R, 6) oIGF-I, 7) IGF-I-R, 8) EGF, 9) TGFα, 10) bFGF, 11) TGFβ1 (244 bp), 12) bTP, and 13) NGF.

II receptor sequences. Insulin ligand expression was also not detected within any of these ovine embryo stages or in the oviduct cell preparations. It was, however, possible to detect the expected 301-bp insulin DNA fragment in ovine pancreatic cell RNA preparations (data not shown). The PCR product corresponding to the 324-bp insulin receptor was detected in all preimplantation embryo stages and also within the 48-h oviduct cell cultures. The identity of this PCR fragment was confirmed by Southern blot analysis (Fig. 3B), which consisted of transferring the PCR fragment to a nylon membrane and then probing the blot by hybridization with a radiolabeled human insulin receptor cDNA clone [56]. The probe hybridizes to the insulin receptor PCR fragment and, because of sequence similarity to the IGF-I receptor, also cross-hybridizes with the latter product even at high-stringency washes. The hybridization is, however, specific for these two products. IGF-I ligand mRNAs were detected from the 1-cell stage to the blastocyst stage and also within the oviduct cell cultures. The identity of the expected 261-bp DNA fragment was confirmed by conducting an RE digest of the PCR fragment with *Hpa* II; this resulted in the cleavage of the IGF-I PCR product, as expected, into two fragments of 214 bp and 47 bp (Fig. 3C). Transcripts for the IGF-I receptor were also amplified from the 1-cell stage through to the blastocyst stages of preimplantation development. Similarly, this transcript was also localized within oviduct epithelial cell RNA preparations. The identity of this 354-bp DNA fragment was confirmed by blotting and hybridization with a mouse IGF-I receptor cDNA ([31]; Fig. 3D). In this case, the hybridization of the radiolabeled IGF-I receptor probe was specific for the IGF-I receptor PCR product when the blots were washed at high stringency (0.1-strength saline-sodium citrate [SSC], 70°C). Cross-hybridization with the insulin receptor PCR product was observed as expected when washes were carried out under less stringent conditions.

The expression of EGF transcripts was not observed during early development or within the 48-h oviduct cell cultures, although the expected 247-bp DNA fragment was detected within murine and ovine ovary RNA preparations (data not shown). The expression of TGFα was detected throughout ovine preimplantation development and also within oviduct epithelial cell cultures. The PCR product was identified as a TGFα DNA fragment by blotting and hybridizing with a radiolabeled mouse TGFα DNA probe (Fig. 3E).

Expression of bFGF was detected throughout ovine preimplantation development and within the oviduct cell RNA preparations. It was, however, observed to consistently decline in its apparent abundance from the 1-cell to the blastocyst stage. That the PCR fragment represented the bFGF transcript was confirmed by subjecting a sample of the amplified product to an RE digest with *Hin*fI that resulted in the cleavage of the bFGF fragment into the expected 138- and 144-bp DNA cleavage products (Fig. 3F). The transcripts for TGFβ1 were detected throughout early development, although the product was very difficult to resolve by RT-PCR (data not shown). It was however, clearly expressed within the 48-h epithelial cell cultures. The identity of this PCR product was confirmed by specific hybridization with a radiolabeled mouse TGFβ1 cDNA probe after membrane transfer (Fig. 3G).

As was expected, the transcripts for oTP were detected only within the ovine blastocyst embryo cDNA preparations and were never apparent within samples of embryos at ear-

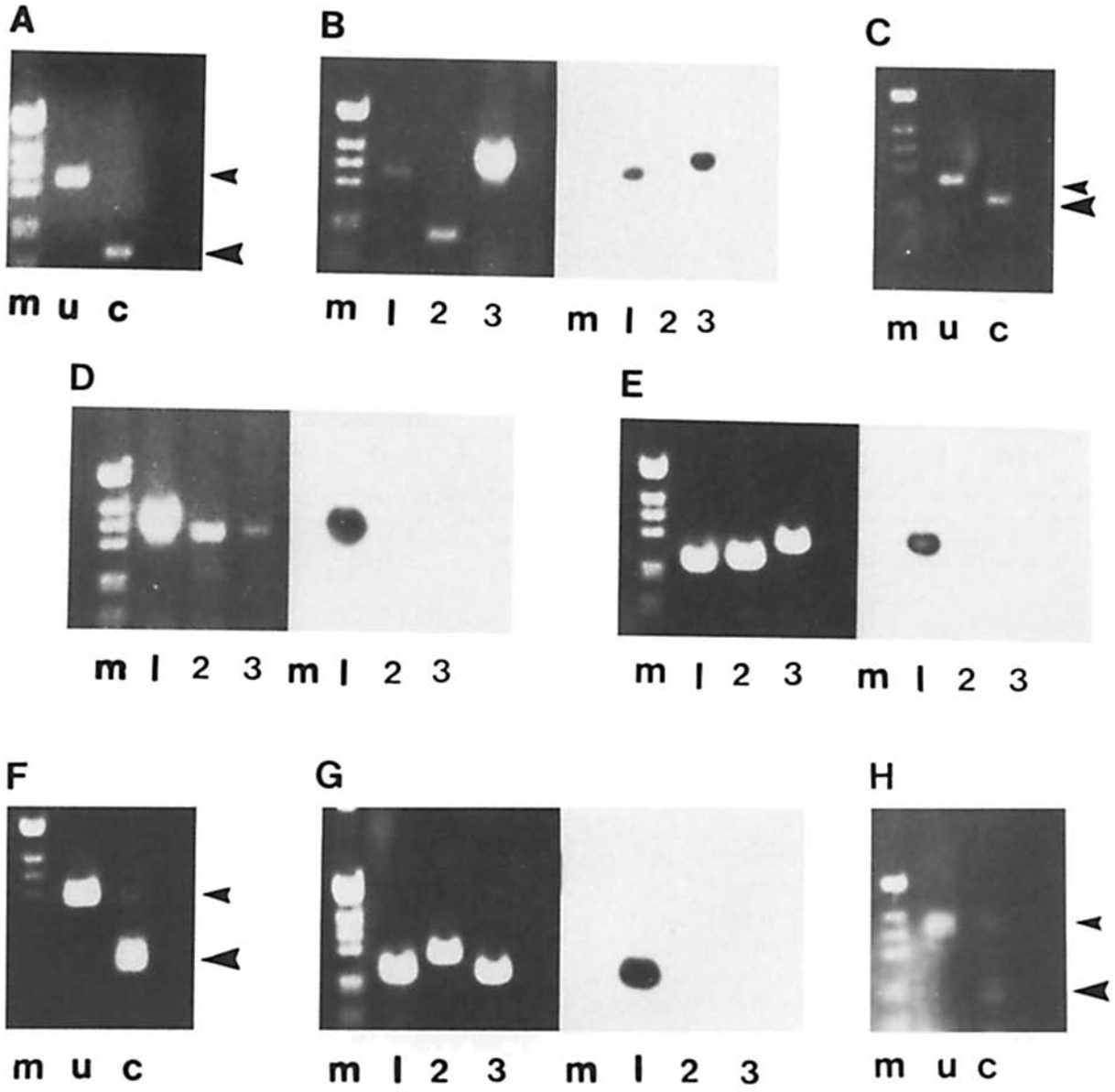

FIG. 3. *Verification of growth factor RT-PCR products.* PCR fragments for identification purposes were produced from ovine oviduct and preimplantation embryo cDNA preparations. In all cases (with the exception of the oIGF-II and oIGF-I primer pairs, which are unique to this study), the PCR product sizes were identical to those observed within murine and bovine preimplantation embryos [31, 45]. A) The 318-bp oIGF-II PCR product (small arrowhead) was cleaved with *Hpa* II, which produced the expected 160- and 158-bp DNA fragments (large arrowhead) when run on a 2% agarose gel. M, markers; U, uncut; C, cut. B) The 324-bp insulin receptor fragment (lane 1) was run together with the PCR products from the murine IGF-II-R (lane 2) and ovine IGF-I-R (lane 3) before blotting and hybridization with a radiolabeled insulin receptor cDNA clone [62]. The autoradiogram is shown next to the ethidium bromide-stained gel and demonstrates that only the insulin receptor PCR product and the IGF-I receptor PCR product (which have similar sequences) hybridize with the labeled probe. C) The 261-bp oIGF-I DNA fragment (small arrowhead) was cleaved with *Hpa* II, producing the expected 214-bp (large arrowhead) and 47-bp (not visible in figure) fragments when run on a 2% agarose gel. M, markers; U, uncut; C, cut. D) The 354-bp IGF-I receptor PCR product (lane 1) was run along with the PCR products for IGF-II ligand (lane 2) and the insulin receptor (lane 3), blotted, and hybridized with a ^{32}P-labeled IGF-I receptor clone. The autoradiogram (adjacent to the ethidium bromide-stained gel) shows that the radiolabeled probe hybridizes specifically to the IGF-I receptor lane. E) The 239-bp TGFα PCR fragment (lane 1) was run together with the TGFβ1 (lane 2) and bFGF (lane 3) PCR products before blotting and hybridization with a radiolabeled partial human TGFα cDNA clone. The autoradiogram (next to the ethidium bromide-stained gel) clearly shows that only the TGFα lane is capable of hybridizing to the cDNA probe. F) The 282-bp bFGF PCR DNA fragment (small arrowhead) was cleaved with *Hinf* I, resulting in the expected 144- and 138-bp fragments (large arrowhead) when resolved on a 2% agarose gel. M, markers; U, uncut; C, cut. G) The 244-bp TGFβ1 PCR product (lane 1) was resolved on an ethidium bromide-stained agarose gel along with the PCR products for bFGF (lane 2) and TGFα (lane 3) before blotting and hybridization with a radiolabeled mouse TGFβ1 cDNA probe. The autoradiogram (to the right of the gel) shows that only the TGFβ1 PCR product is capable of hybridizing to the cDNA probe. H) The 358-bp bTP PCR fragment (small arrowhead) was cleaved with *Apa* I, yielding the expected 190- and 168-bp DNA fragments (large arrowhead). M, markers; U, uncut; C, cut.

lier stages or within the oviduct cell cDNA samples. The identity of the oTP transcript was confirmed by cleaving the oTP PCR product with the restriction enzyme *Apa* I to yield the expected 190- and 168-bp products (Fig. 3H). The expected 401-bp DNA fragment for NGF was not detected within any ovine embryo stage or within the oviduct cell cultures. The primers did, however, successfully amplify the appropriate NGF PCR product within liver and ovary RNA preparations (data not shown).

DISCUSSION

In the study reported here, we detected the expression of eight different growth factor ligand and receptor genes during ovine preimplantation development and in primary ovine oviduct epithelial cell cultures. These include IGF-II, IGF-I, TGFα, bFGF, TGFβ1, oTP, insulin receptor, and IGF-I receptor. With the exception of the oIGF-I, oIGF-II, and oTP primer pairs, the oligonucleotide primers were designed through use of mouse or human cDNA sequences [45]. Their effectiveness in amplifying specific ovine embryo cDNAs was assured in all cases by observing the expected size of the amplified DNA fragment and then either cleaving with a diagnostic RE at a known sequence at the correct site or blotting and hybridizing with the complementary mouse or human cDNA probe.

The expression patterns of growth factor mRNAs in early ovine embryos are similar to those reported for other species. For example, transcripts for IGF-I ligand were detected throughout ovine preimplantation development just as reported previously for oocytes and preimplantation embryos for both the rat [57] and cow [45]. Although IGF-I transcripts were not observed in early studies on preimplantation mouse embryos [24], reexamination of IGF-I expression in murine embryos has established that transcripts are detectable by RT-PCR from oocyte to blastocyst stages in this species as well (R. Schultz, personal communication). IGF-I is an essential, direct regulator of ovine fetal growth [47, 58]. It exerts this influence by a variety of actions in many fetal cell types and tissues in vitro, including stimulation of cell proliferation, protein synthesis, glycogen synthesis, and differentiation (for review see [47]).

Basic FGF mRNAs were also detected within preimplantation ovine embryos. Although bFGF transcripts have not been detected in preimplantation mouse embryos, kFGF transcripts are present in early mouse embryos [24]. The apparent accumulation of bFGF mRNA within both ovine and bovine [45] 1- to 8-cell embryos would predict a possible maternal origin for these transcripts. However, even when growth factor transcripts are expressed in common between ovine and murine preimplantation embryos (as has been observed for IGF-II, insulin receptor, IGF-I receptor, and TGFβ1), there are differences in the stage-specific timing of appearance and thus possibly the origin (maternal or embryonic) of the growth factor transcript [22–24, 45]. The switch from maternal to embryonic control of preimplantation development (as measured by the onset of embryonic transcriptional activity) varies markedly among the mammalian species, with a very early onset in the mouse embryo at the 2-cell stage and a delayed initiation at the 8–16-cell stage in both the ovine and bovine embryo (for review see [59]). If we predict that a particular transcript is likely to be of embryonic origin if it is not expressed prior to the 2-cell stage in the mouse embryo, then the mRNAs for IGF-II, insulin receptor, IGF-I receptor, and TGFβ1 would all be products of the embryonic genome in the mouse but would be products of both the maternal and embryonic genomes in ovine and bovine embryos [45]. As in the mouse and bovine preimplantation embryos, TGFα expression was detected throughout ovine preimplantation development [23, 45]. The mRNAs encoding EGF, NGF, and insulin have not been detected during preimplantation development within any of these mammalian species [22–24, 35, 45].

The apparent lack of expression for the IGF-II receptor during ovine preimplantation development should be interpreted with caution: it was not possible to determine the extent of homology of our murine primers with the oIGF-II receptor sequences since the oIGF-II receptor has not to our knowledge been cloned and sequenced. Therefore, it was not possible to redesign new oIGF-II receptor oligodeoxynucleotide primer pairs and determine whether the absence of expression was simply due to the use of nonhomologous primer pairs and thus poor efficiency in the PCR reaction. This is the most probable explanation, since transcripts for IGF-II receptor have been reported throughout bovine preimplantation development [45] and also in the early mouse embryo as an embryonic transcript [24–27]. Clarification of the ovine expression pattern for IGF-II receptor must await the publication of the oIGF-II receptor cDNA sequences.

Bovine and ovine trophoblast proteins (bTP; oTP) are both classed as trophoblast interferons and play an important role in facilitating maternal recognition of pregnancy [60–62]. The cDNAs for these molecules show a high degree of homology (> 85% identical), particularly in the 3′ untranslated regions (> 90%) [60]; thus it appears certain that our bTP PCR primers have amplified an identical region of the oTP within the ovine blastocysts [62]. We investigated the expression of bTP/oTP within ovine preimplantation development to provide evidence that our in vitro ovine blastocysts were capable of expressing this important early molecular signal for the establishment of pregnancy. In addition, failure to detect oTP transcripts until the blastocyst stage is precisely as expected, and this result contributes to the validity of these assays.

The expression of growth factor transcripts very early in mammalian development would predict that these molecules fulfil a necessary role(s) in supporting the progression of early embryos throughout the preimplantation interval. It is now clear that members of the IGF family

(IGF-I, IGF-II, insulin), EGF family (EGF, TGFα), and TGFβ family collectively are involved in influencing a number of events during early murine development; among these events are RNA and protein synthesis, cleavage rates and embryo cell number, and blastocoel expansion and proportion of blastocysts hatching from the zona pellucida in vitro [25–30, 32–39].

Growth factors also certainly play a role in facilitating the early development of other mammalian species. When TGFβ and bFGF are added to bovine embryo culture, up to 38.8% of in vitro-fertilized zygotes progress beyond the 16-cell stage with 24.6% continuing on to the blastocyst stage [41]. Furthermore, 39.1% of cleaved bovine zygotes can progress beyond the 16-cell stage in medium supplemented with platelet-derived growth factor (PDGF), while TGFα is effective in increasing the proportion of bovine zygotes capable of undergoing blastocyst formation [42]. Similar studies have not yet been carried out on preimplantation ovine development, but the detection of growth factor transcripts in early ovine embryos should support such investigations.

The expression of transcripts for IGF-II, IGF-I, TGFα, bFGF, and TGFβ1 within 48-h ovine oviduct epithelial cell cultures extends our previous observations within bovine oviduct cell cultures [45] and further supports a role for the oviduct in facilitating early mammalian development by the establishment of paracrine growth factor circuits. Our future efforts will be directed to identifying the nature of these putative regulatory pathways.

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