EARLY EMBRYO DEVELOPMENT AND PARACRINE RELATIONSHIPS

Organizers: Susan Heyner and Lynn Wiley
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Culture of Mammalian Preimplantation Embryos

CC 001 ENERGY SUBSTRATE REGULATION OF PREIMPLANTATION EMBRYO DEVELOPMENT IN VITRO, Barry D. Bavister, Department of Veterinary Science, University of Wisconsin-Madison, Madison, WI 53706. Detailed knowledge about the energy substrate requirements for preimplantation development in vitro is limited to embryos of the mouse and rabbit. Embryos of most other species do not develop properly in vitro, often exhibiting overt "blocks to development" at certain stages of growth. Using a completely chemically-defined culture medium, we have studied embryos of the golden hamster, which block both at the 2-cell and 4-cell stages in vitro, to find the cause of these blocks (1,2). Initially, we found that removal of glucose from the culture medium allowed some development (27%) of 2-cell embryos to 4- or 8-cell stages. When phosphate was omitted, 70-80% of 2-cell embryos reached 8-cells, morulae or blastocysts. In the absence of phosphate, there was no inhibition by glucose. Amino acids are required by hamster embryos for growth in vitro; preliminary data indicate inhibitory as well as stimulatory effects. Presently, approx. 30% of hamster 2-cell embryos will grow to the blastocyst stage in vitro. Phosphate also blocked development of 4-cell embryos, at concentrations as low as 1μM; sulfate was not inhibitory up to 5.6 mM (H. Monis, unpublished). Both phosphate and glucose were also inhibitory to development of 8-cell hamster embryos but the effect was synergistic. Eight-cell embryos could use either amino acids or pyruvate or lactate as energy substrates for growth to blastocysts but in every case, addition of glucose was inhibitory. Hamster embryos were most sensitive to inhibition by phosphate (in the absence of glucose) at the 2- and 4-cell stages; early 8-cell embryos were markedly less inhibited by phosphate and mid to late 8-cell embryos had escaped from inhibition by phosphate alone, although increased concentrations of phosphate were effective in inhibiting development. These data show some novel characteristics of hamster preimplantation embryos grown in vitro: (i) they are not able to use glucose to support development at any stage; (ii) glucose inhibits development; (iii) inhibition of embryo development by glucose depends on the presence of inorganic phosphate; (iv) phosphate inhibits embryo development at very low concentrations, and the effect appears to be specific to this ion; (v) embryo development is influenced by amino acids in the culture medium. In these respects, hamster embryos differ from the classic metabolic model represented by preimplantation embryos from inbred mice. New information on the control of embryo development by energy substrates may be applicable to other species, including rodents and domesticated animals. Recently, it was found that glucose is partly responsible for the 2-cell block exhibited by embryos from outbred strains of mice (3). Furthermore, provision of unsuitable energy substrates and related compounds may contribute to the retarded growth and/or reduced viability typical of mammalian embryos grown in vitro. Supported by NIH grant no. HD22023.

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Metabolism of the Preimplantation Embryo

CC 002 ENERGY METABOLISM OF THE PREIMPLANTATION EMBRYO, Henry J. Leese, Department of Biology, University of York, Heslington, York, Yo1 5DD, U.K.

Preimplantation mouse embryos have an obligatory requirement for pyruvate to support the first cleavage division in culture. Glucose as sole energy substrate, is unable to support development until the 4-cell stage. This substrate preference is reflected in the pattern of nutrient uptake. Single preimplantation embryos from the mouse and the human consume pyruvate preferentially during early cleavage before switching to glucose at the 8-cell/morula stage. The formation of lactate increases as glucose becomes the predominant substrate such that in mouse blastocysts it accounts for 40% of the glucose consumed. The early block to glucose utilisation may reside at the level of transport across the plasma membrane and/or intracellularly.

Unfertilized and fertilized mouse oocytes and preimplantation embryos possess a facilitated system for pyruvate entry. A glucose transport system is present from the 2-cell stage onwards (1). A role for phosphofructokinase in limiting glucose metabolism has been postulated (2), and hexokinase, the activity of which is very low in unfertilized oocytes and early preimplantation mouse embryos before rising sharply at the morulae and blastocyst stages, may also be involved.

Nutrients are supplied to embryos by oviduct and uterine fluids (3), though there may be a contribution from cumulus cell metabolism around the time of fertilization (4).

Metabolic measurements may have potential in the assessment of embryo viability (5) and in the diagnosis of genetic disorders at the preimplantation stage.

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CC 003 CHANGES IN RNA AND PROTEIN SYNTHESIS DURING DEVELOPMENT OF THE PREIMPLANTATION MOUSE EMBRYO. G.A. Schultz+, D.A. Rappolee, R.A. Pedersen and Z. Werb, Department of Medical Biochemistry+, University of Calgary, Calgary, Alberta, Canada T2N 4N1 and Laboratory of Radiobiology and Environmental Health, University of California, San Francisco, CA 94143-0750. The transition from maternally-derived transcripts to zygote genome-derived transcripts occurs during the first cleavage of the fertilized mouse egg. There is a loss of maternal poly(A)+ RNA sequences from the egg to the 2-cell stage followed by progressive accumulation of newly synthesized mRNA, including specific mRNAs for histones and actins, from the 2-cell stage to the blastocyst. These changes in mRNA abundance are directly related to major changes in the pattern of protein synthesis during this transition period. The SnRNA molecules that are located in ribonucleoprotein particles (SnRNPs) within the nuclei of cells and that mediate post-transcriptional processing of newly synthesized mRNAs are not degraded during the 1-cell to 2-cell stage. Rather, maternal SnRNPs have been shown, by in situ hybridization and immunofluorescence microscopy, to be released into the cytoplasm of the unfertilized egg during germinal vesicle breakdown and meiotic maturation and subsequently to re-localize to pronuclei following fertilization. Since the SnRNA amount is constant during this period of development, pre-mRNA synthesized from the zygote genome at the 2-cell stage is probably spliced and processed by SnRNP machinery of maternal origin.

After the 2-cell stage, new SnRNA is also synthesized and accumulated and the transcription of a number of genes is activated as the embryo divides and differentiates into a blastocyst. Alkaline phosphatase is first detectable by histochemical staining at the 2- to 4-cell stage and receptors capable of binding insulin first appear at the post-compaction morula stage. Transcripts encoding enzymes or receptors are often in low copy number and are not readily detectable by traditional recombinant DNA methods within the small amount of embryonic material available. To examine these low abundance mRNA species, we have used a sensitive method for mRNA phenotype analysis that is based on the production of cDNA followed by amplification of specific target sequences by the polymerase chain reaction. As predicted from studies at the protein level, transcripts for alkaline phosphatase were first detected at the 2-cell stage whereas transcripts for insulin receptors were not detectable until the 8-cell stage. The method is sufficiently sensitive to detect these mRNA species within total RNA extracted from only one or a few preimplantation embryos. Such information provides a basis to test the functional role of such temporally regulated genes through approaches that cause mis-expression at appropriate points in the developmental program.

In Vitro Fertilization-Embryo Transfer

CC 004 OOCYTE MATURATION, FERTILIZATION AND EMBRYO DEVELOPMENT, Willard H. Eyestone and Neal L. First, Department of Meat and Animal Science, University of Wisconsin, Madison, WI 53706

Highly efficient systems for preparation of sperm for in vitro fertilization exist for most mammalian species studied. The principal limitations in producing embryos in vitro are limits in developmental competence of cultured oocytes and in embryo development in culture.

Mammalian oocytes undergo a period of growth, extensive transcriptional activity and storage of transcripts before reaching competence for meiosis, fertilization and embryo development. The last of these competences to be acquired is embryo development.

This limits the use of oocytes from small follicles for in vitro fertilization. The competence for embryo development can be acquired by bovine and ovine oocytes through co-culture with hormonally stimulated granulosa cells and is affected by source of oocyte and sperm. The competence factor(s) is unknown.

Fertilization and the first three to four cleavage divisions occur in the oviduct, after which the embryo passes into the uterus where compaction, blastulation and the balance of embryonic and fetal development occurs. Several landmark developmental events occur during this period, including the shift from meiosis to mitosis and the onset of embryonic gene expression. In many mammalian species, the study of early development is complicated by embryonic refractoriness to conventional in vitro culture methods, the rabbit and some inbred strains of mice being notable exceptions. In most species studied, cleavage in vitro arrests at specific cell stages (e.g., cow, sheep: 8-16; pig: 4-8; hamster, outbred mouse: 2). The cause of these blocks is unknown. However, they are not due to overt embryonic death, but seem to be due to a sublethal lesion induced by in vitro exposure during specific, vulnerable periods of early development. Although the connections are unclear, block stages coincide with the onset of embryonic transcription and abrupt increases in cell cycle length. In vitro blocks may be overcome by co-culturing embryos with oviductal tissue, or in medium conditioned by oviductal tissue, suggesting an embryonic dependence on tubal factors for normal cleavage.

Early Embryo Development and Paracrine Relationships

Blastocoele Formation

CC 005 GENE EXPRESSION REQUIRED FOR BLASTOCOELE FORMATION IN THE MOUSE, Gerald M. Kidder, Andrew J. Watson and Douglas J. Barron, Department of Zoology, University of Western Ontario, London, Ontario, Canada, N6A 5B7. Fluid accumulation to form the blastocoele in the mouse is known to depend on the development of two critical plasma membrane functions: intercellular communication via membrane channels (gap junctions), and trans-trophectodermal sodium transport driven by Na^+,K^+-ATPase. Embryos defective in gap junctional communication are unable to maintain compaction and subsequently fail to cavitate (1,2). Embryos treated with ouabain, an inhibitor of Na^+,K^+-ATPase, are likewise unable to accumulate blastocoelic fluid (3,4). We have begun to analyze the expression of the genes encoding these two functions using antibodies and recombinant DNA probes. We have found by immunoblotting that a 27 kD gap junction protein similar to connexin32, the major protein from liver gap junctions, is present in all preimplantation stages of development including the zygote; however, we have been unable to detect connexin32 mRNA on RNA blots probed at moderate stringency, suggesting either that connexin32 is present only as an oogenetic product, or that a different member of the connexin family is being detected by the antibody. Immunocytochemical studies with several different antibodies against the alpha (catalytic) subunit of Na^+,K^+-ATPase demonstrated that this enzyme becomes readily detectable in the late morula and assumes a juxtacoelic distribution in the early blastocyst, being restricted to the basolateral domain of mural trophectoderm consistent with its presumed role in fluid transport into the blastocoele (5). A 4 kb RNA was identified in 8-cell and later stages that hybridizes with a cDNA for the alpha subunit from rat brain; this mRNA was not detected in zygotes or 2-cell embryos. The fact that this mRNA accumulates after compaction raises the possibility that the expression of Na^+,K^+-ATPase genes is dependent on close cell apposition, an hypothesis which we are currently testing. This work is being supported by grants from NSERC Canada and NIH.

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Growth Factors in Early Development

CC 006 GROWTH FACTORS IN EARLY DEVELOPMENT. Eileen D. Adamson, La Jolla Cancer Research Foundation, La Jolla, CA 92037. This plenary session will examine the possibility of roles for growth factors in early mammalian embryo development. Although preimplantation mouse embryos develop into blastocysts in vitro independently of growth factors in the medium, such embryos are now known to contain and produce mRNA encoding a number of growth factors (1). Dr. Z.Werb will show that maternal mRNA already present in mouse zygotes contain messages that code for TGFα, TGFβ1 and PDGF-A. Later, the developing preimplantation embryo genome is also active in growth factor production, thus raising the possibility that autocrine stimulation is necessary for development. If autocrine stimulation occurs, it is likely to require specific receptors. Cell surface receptors for EGF/TGFα have been detected in outgrowing blastocysts in vitro (2), but there is a possibility that intracellular receptors could be functional: these are known to occur in embryonal carcinoma (EC) cells (3). Insulin receptors are also present on plasma membranes of EC cells and preimplantation embryos (4). The latter evidence will be presented by Dr. S. Heyner. The presence of TGF-like factors produced by preimplantation embryos was also detected several years ago by Dr. A. Rizzino (5) and recently he has described the production of an FGF-like factor produced by at least two EC cell lines (6). These early embryo equivalents, however, do not bind FGF until they have differentiated. A role for FGF and TGFβ has been suggested for frog development. These findings of growth factor production and growth factor receptors in embryos suggest roles for autocrine and paracrine stimulation of growth and development.

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CC 007 INSULIN AND INSULIN-LIKE GROWTH FACTORS IN EARLY MAMMALIAN DEVELOPMENT, Susan Heyner, Department of Obstetrics and Gynecology, Albert Einstein Medical Center, and Temple University School of Medicine, Philadelphia PA 19141
Development of the early mammalian embryo is governed primarily by the embryonic genome, although it requires a continuous supply of energy, growth factors and hormones. There is a considerable body of evidence to suggest that maternal endocrine influences are important in mammalian embryogenesis. Rapid cellular proliferation is characteristic of embryonic growth, and may be mediated by growth factors that bind to specific receptors prior to the initiation of cell division. Among candidates for this role are insulin and the insulin-like growth factors (IGFs). Previous studies have demonstrated that insulin is bound in a stage-specific manner to the mouse embryo, beginning at the morula stage, and that receptors for insulin, IGF-I and IGF-II are expressed by the preimplantation embryo. High resolution electron microscopy in conjunction with gold-labelled insulin has shown that insulin is bound specifically to the plasma membrane of the morula and blastocyst, and is endocytosed via coated pits. At the blastocyst stage, the ligand is translocated to the cells of the inner cell mass via the trophectoderm. Immunocytochemical studies do not support the idea that insulin is synthesized by the embryo, and we hypothesize that the source of insulin is maternal, and is present in oviductal fluid. Functional studies have shown that preimplantation embryos respond to physiological levels of insulin in culture media (4 ng/ml) by increasing the rate of synthesis of DNA and RNA. Supported by NIH grant HD23511

CC 008 PRODUCTION OF GROWTH FACTORS DURING EARLY MAMMALIAN DEVELOPMENT, Angie Rizzino, Jay Tiesman, David Kelly, Charles Kuszynski and Anita Meyer, Eppley Institute, University of Nebraska Medical Center, Omaha, NE 68105.
Previous studies from this laboratory determined that cultured mouse embryos produce growth factors that induce the soft agar growth of non-transformed cells (1). In an attempt to identify the embryonic growth factors responsible for this activity, we have used two model systems - cultured mouse embryos and embryonal carcinoma (EC) cell lines. Several years ago, we demonstrated that EC cells produce a growth factor closely related to platelet-derived growth factor (PDGF) (2). More recently, we determined that EC cells also produce a growth factor related to fibroblast growth factor (FGF). The FGF-related growth factor is a heat-labile heparin-binding growth factor that competes with FGF for binding to membrane receptors and is immunologically related to basic FGF (FGFb) (3). These findings are complemented by our recent finding that RNA from human EC cells contains transcripts that hybridize with probes for the PDGF A-chain and FGFb (4). To determine whether either of these growth factors is produced by early mouse embryos, we have examined conditioned medium collected from mouse blastocysts maintained in vitro for 72 hrs. To avoid the growth factors present in serum, the embryos were plated in a serum-free medium supplemented with insulin, transferrin, bovine serum albumin, high density lipoprotein (HDL) and the attachment factor fibronectin (FN). Under these conditions, FN promotes attachment and HDL induces trophoblast outgrowth. We examined the serum-free conditioned medium with a FGF bioassay and with antibodies that recognize FGFb. The results indicate that early mouse embryos, like EC cells, produce a growth factor that is immunologically related to FGFb. Studies are in progress to determine whether this factor is FGFb or a closely related member of the FGF family of growth factors. These findings and those obtained with EC cells strongly suggest that the production of several different growth factors is carefully regulated during early development. This work was supported by grants from NICHD (21568, 19837) and NCI (36727).

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CC 009 GROWTH FACTOR AND GROWTH FACTOR RECEPTOR GENE EXPRESSION IN PERI-IMPLANTATION MOUSE EMBRYOS. Zena Werb, Gilbert A. Schultz, Roger A. Pedersen, Karin Sturm, and Daniel A. Rappolee, Laboratory of Radiobiology and Environmental Health, University of California, San Francisco, CA 94143-0750. Control of growth and differentiation during mammalian embryogenesis may be regulated by growth factors from endogenous or maternal sources. Mouse preimplantation embryos grow in serum-free culture, suggesting that endogenous factors are sufficient to sustain growth. This growth progresses nearly exponentially from the time of fertilization through the 64-cell blastocyst. We have used a novel method for mRNA phenotype analysis based on production of cDNA followed by enzymatic amplification of specific fragments, to examine simultaneous expression of growth factor transcripts in single mouse embryos. Platelet-derived growth factor (PDGF-A), transforming growth factor-α (TGF-α), transforming growth factor-β1 (TGF-β1) and insulin-like growth factor-II (IGF-II) transcripts were found in blastocysts. PDGF-A and TGF-α transcripts were first found as maternal mRNA in unfertilized ovulated oocytes, disappeared during the degradation of maternal mRNA at the two-cell stage, and reappeared once the expression of the zygotic genome was initiated. IGF-II and TGF-β1 were expressed only after activation of transcription of the zygotic genome. Basic fibroblast growth factor (bFGF), epidermal growth factor (EGF), nerve growth factor-β (NGF-β), granulocyte-colony stimulating factor (G-CSF), insulin-like growth factor-I (IGF-I), and insulin mRNA transcripts were not found in blastocysts. Because transcription of growth factor mRNA is not invariably coupled with translation of these transcripts into protein, it was necessary to determine whether these transcripts were translated. PDGF, TGF-α and TGF-β1 antigens were also demonstrated in blastocysts by immunofluorescence. The expression of a unique subset of growth factors in mouse blastocysts suggests a role for these factors in the growth and differentiation of early mammalian embryos. The embryonic growth factors may be directed within the embryo or between the mother and the embryo. If the growth factors have embryonic targets, then growth factor receptor genes should be transcribed and translated in the embryo. We found that transcripts for the insulin receptor and IGF-I receptor appeared during the cleavage stages, and increased through the blastocyst stage. Taken together, these data suggest that growth factors may have endocrine effects in the earliest stages of mammalian development. Supported by NIH, US DOE-OHER, and MRC (Canada).

Preimplantation and Implantation Signals

CC 010 STEROID HORMONAL MODULATION OF C-MYC AND EGF IN THE MOUSE UTERUS DURING THE PERI-IMPLANTATION PERIOD, Yvette M. Huet, Glen K. Andrews and S. K. Dey, Departments of Physiology & OB/GYN, and Biochemistry and Molecular Biology, University of Kansas Medical Center, Kansas City, Kansas 66103. Two major features of the implantation process are: (i) cellular proliferation and differentiation of luminal epithelium and stroma for induction of the receptive state and (ii) a prerequisite increased capillary permeability in the differentiated stroma at the site of the blastocyst. In the mouse, changing ovarian progesterone (P) and estrogen (E) secretion is central to these processes. Preovulatory ovarian E secretion causes proliferation, death, or differentiation of the luminal epithelial cells during the first few days of pregnancy. In contrast, rising P levels, superimposed on preovulatory E priming, shift the wave of proliferation from the luminal epithelium to stroma around the time of implantation. Stromal cells differentiate to decidual cells by preimplantation ovarian E secretion in the presence of the blastocyst following implantation. To test the hypothesis that steroid hormonal modulation of the uterus for implantation is mediated via altered expression of proto-oncogenes and/or in an autocrine/paracrine fashion via altered expression of growth factors, cell-specific localization of c-myc and epidermal growth factor (EGF) proteins by immunocytochemistry, and preproEGF mRNA by *in situ* hybridization was performed in the mouse uterus on days 1-5 of pregnancy (D1 = vaginal plug) and following E treatment. The c-myc protein was localized in the luminal and glandular epithelial cells on D1, while in stromal cells on D4 and D5. EGF was localized in the cytoplasm of the luminal and glandular epithelia on D1, but only in the apical border of the luminal epithelium on D4. On D5, EGF almost disappeared from the luminal epithelium, although blood vessel endothelia in the stromal cells were clearly labelled. EGF and c-myc proteins were scanty in the uterus on D2 and D3. In the estrogenized mouse, EGF first appeared in the luminal epithelium at 24h , whereas both luminal and glandular epithelia showed intense labelling at 48h. EGF disappeared at 72h after E withdrawal. The localization of preproEGF mRNA followed the same pattern as EGF protein. The results suggest that the presence of c-myc and EGF proteins on D1 is preovulatory E dependent and associated with cellular proliferation. The disappearance of these proteins on D2 and D3 could be associated with cell death and/or differentiation. EGF in the luminal epithelium on D4 and in the blood vessel endothelia on D5 could be related to prostaglandin synthesis and vascularization necessary for implantation. The accumulation of c-myc protein in the stroma on D4 and D5 is P dependent and could be associated with proliferation and differentiation of stromal cells to decidual cells.

Early Embryo Development and Paracrine Relationships

Decidualization

CC 011 SECRETORY ENDOMETRIAL AND DECIDUAL PRODUCTS OF THE BABOON (PAPIO ANUBIS) AND HUMAN. Asgerally T. Fazleabas, Harold G. Verhage and Stephen C. Bell*, Departments of Obstetrics and Gynecology, University of Illinois, Chicago, Illinois, 60612, and University of Leicester*, Leicester LE2 7LX, U.K.
Electrophoretic analysis of ^{35}S-methionine labelled endometrial culture media obtained from baboons and humans during the menstrual cycle demonstrated that the pattern of proteins synthesized during the follicular (FS) and mid-luteal (MLS) stages in these two species are very similar. The secretory pattern during these two stages of the cycle are characterized by the presence of an estrogen (E_2)-induced protein (M_r 33,000; pI 7.6) during the FS and a progesterone (P)-induced basic protein (M_r 40,000) during the MLS. At the late luteal stage (LLS) of the cycle the endometrial secretory activity in these two species differs to some extent. The major synthetic product of the human endometrium (α_2-PEG/PP14) is totally absent in baboons, however a minor synthetic product (α_1-PEG/PP12), also known as insulin-like growth factor binding protein (IGF-BP), is present in both species. This IGF-BP, synthesized by the non-pregnant endometrium and decidua of pregnancy, is biochemically and immunologically similar both species. Immunostaining using Mab's to IGF-BP indicated that in the non-pregnant baboon endometrium, this protein was localized predominantly in the glandular epithelium of the deep glands and was most intense during the LLS. In contrast, human endometrium from the same stage of the cycle showed minimal epithelial staining but strong stromal staining. During pregnancy, however, the hypertrophied stromal cells of the decidua from both species is the major site of synthesis of IGF-BP, thus suggesting that in the baboon synthesis switches from glandular to stromal in the presence of a conceptus. The IGF-BP produced by the decidua of pregnancy in both the human and the baboon is immunolocalized in the perinuclear region of the hypertrophied stromal cells, has a M_r between 29,000-33,000 and binds IGF-I with a Kd of approximately $1x10^{-9}$ M. A cDNA coding for this protein hybridizes a single mRNA transcript of 1.65kb in both species. These studies suggest that I) endometrial and decidual protein synthesis in the baboon and human are quite similar, II) the difference in the site of synthesis of IGF-BP during the LLS may play a role during implantation, and, III) endometrial and decidual IGF-BP may be associated with autocrine/paracrine actions of IGF in the uterus. (Supported by NIH HD 21991 and HD 20571).

Endometrial Signals

CC 012 *IN VITRO* IMPLANTATION: REGULATION OF NON-RECEPTIVITY ON POLARIZED UTERINE EPITHELIA, Stanley R. Glasser and JoAnne Julian, Department of Cell Biology and Center for Population Research and Studies in Reproductive Biology, Baylor College of Medicine, Houston, TX 77030
Blastocyst attachment is a hormonally regulated specialized function of uterine epithelial (UE) cells. *In vivo* attachment to the apical UE surface occurs only during a short period following estrogen (E) modulation of the progesterone (P) dominated rat uterus; 24-30 h after E the UE cells become non-receptive to embryo attachment. The temporal, spatial and biochemical constraints which regulate *in utero* attachment do not apply when the blastocyst is removed to ectopic or *in vitro* environs. Regardless of hormonal conditions blastocysts, *in vitro*, attach without discrimination to almost any surface, including UE cells. The inability to modulate UE receptivity to attachment has been attributed to the failure of UE cell cultures to develop polarity. Expression of blastocyst attachment, a specialized function of the UE cell, is dependent on its polarity.

Primary cultures have been developed in which UE cells maintain responsiveness to E. Cultured on EHS matrix-impregnated HA or CM Millipore filters in completely defined medium containing 2.5×10^{-9} M E_2 UE cells proliferate to confluence and exhibit morphological and functional polarity. Polar organization, separate membrane domains, functional tight junctions and trans-epithelial resistance are validated by ultrastructural and immunocytochemical evidence. Coordinated indices of functional polarity include preferential basal surface uptake of ^{35}S-methionine and apical preference for increased UE secretory activity. Development of polarity was monitored by analysis of protein and glycoconjugate profiles of apical and basolateral secretory compartments. Expression of CAM 105 (apical membrane integrated glycoprotein) and apical secretion of two proteins, induced in uteri of immature rats by E_2, validate the hormonal responsiveness of these polarized UE cells.

In the presence of E_2 95% blastocysts transferred directly or after prior culture with polarized UE cells attach within 24-48h and grow out on bare HA filters, EHS-impregnated HA or CM filters or on uterine stromal cell monolayers on EHS covered filters. Only when co-cultured with polarized UE cells in the presence of E_2 do blastocysts fail to attach. They remain viable and continue to develop. Together with CAM 105 expression and the secretion of uterine E_2 marker proteins the development of a UE cell surface non-receptive to blastocyst attachment is evidence that hormone sensitivity is characteristic of these polarized immature UE cells. (Supported by HD-07495 (Ctr. Grant), HD-25189).

CC 013 ROLE OF UTERINE SECRETORY ACTIVITY IN THE SUPPORT OF THE PREIMPLANTATION EMBRYO, R. Michael Roberts, Departments of Animal Science and Biochemistry, University of Missouri, Columbia, MO 65211. The epithelium which lines the surface and glands of the uterus is active in secretion. These secretions (or histotroph) contain protein components different from those in plasma and whose quality and amount are correlated with circulating levels of maternal estrogen and progesterone (P_4). A variety of functions for uterine secretory proteins (USP) in pregnancy have been proposed but few have been convincingly demonstrated. In my laboratory, emphasis has been placed on species with an epitheliochorial placentation, such as the pig, where uterine endometrium under the influence of P_4 is particularly active in secretion and where USP are believed to provide a sustained embryotrophic environment for much of pregnancy. All of the major P_4-induced proteins of porcine uterine secretions have been purified and at least partially characterized. The sequences of two (uteroferrin and the uteroferrin-associated polypeptides) have been inferred from their cDNA's. The best characterized USP is uteroferrin (Uf), an iron-containing acid phosphatase with a deep purple color. Evidence suggests that Uf, rather than functioning as an acid phosphatase, transports iron to the conceptus. Uf mRNA and protein are induced within a few days of P_4 replacement therapy in ovariectomized gilts, but maximum synthesis of Uf does not occur until about day 60 of pregnancy. Three basic polypeptides, which are found noncovalently associated with Uf and which originate from a common precursor, are members of the large serpin superfamily of proteins that includes a number of serine protease inhibitors and ligand transport proteins. Their function in the uterus is presently unknown. A group of Mr=14000 basic protease inhibitors (Kunitz type) have been isolated and possess considerable sequence homology to bovine pancreatic trypsin inhibitor (aprotinin). Finally a family of retinol binding proteins have been purified. Like Uf, these proteins seem to be responsible for transport of an essential nutrient molecule to the conceptus. It thus appears that the functions of the major USP of the pig are in nutritional support of the conceptus and in controlling protease activity within the uterine environment. Supported by USDA grant 87-01316 and NIH grant HD21980.

Late Addition

CC 014 IMPAIRED MACROPHAGE T CELL RESPONSES TO UTERINE LISTERIA IN NONPREGNANT DECIDUOMA-BEARING MICE, *V.E. Papaioannou, **C.Y. Lu, & **R.W. Redline, *Dept. of Pathology, Tufts University Schools of Medicine and Veterinary Medicine, Boston, MA 02111; and **Depts. of Pathology & Medicine (Renal Division), Brigham and Women's Hospital & Harvard School, Boston, MA 02115

Pregnant mice develop overwhelming fetoplacental listeriosis due to an inability to recruit macrophages and T-cells to the infected decidua basalis (J Clin Invest 79: 1234, 1987). This local susceptibility may be due to immunoregulatory mechanisms protecting the allogenic fetus from rejection. To determine whether fetal cells play a role we have analyzed the uterine response to Listeria monocytogenes (Lm) in nonpregnant mice with deciduoma. Such deciduoma mimic the decidua basalis but lack fetal trophoblast. Deciduoma were created by introducing 200 Lm into the uteri of oophorectomized hormonally prepared mice. Mice with deciduoma were unable to prevent uterine Lm growth (log Lm ± SEM; deciduoma 6.6 ± 0.29 vs. control 3.9 ± 0.45). Prior immunization augmented resistance to Lm in controls but not in deciduoma-bearing animals (deciduoma 6.2 ± 0.22 vs. control 1.9 ± 0.42). The hormones had no effect on the systematic response to Lm. Infected deciduoma contained granulocytes and Lm, not no macrophages or T-cells by immunostaining. Infected control uteri had granulomas with Ia-bearing macrophages and T-cells. The parallels between the anti-Lm response in deciduoma and the placenta suggest that susceptibility to infection is due to properties of decidualized maternal tissue and not fetal trophoblast.

Posters

CC 100 CELL DIVISION RATE OF RABBIT ONE-CELL EMBRYOS IS INCREASED BY CO-CULTURE WITH RABBIT OVIDUCT EPITHELIAL CELLS, Edward W. Carney and Robert H. Foote, Department of Animal Science, Cornell University, Ithaca, NY 14853

Rabbit embryos develop more slowly in culture than they do within the oviduct. We have co-cultured rabbit 1-cell embryos with rabbit oviduct epithelial cells (OEC) to determine if OEC can stimulate embryos to divide more rapidly in vitro. Primary OEC were established according to Black et al. (Biol. Reprod. 32 Supp.1:363) and were cultured in Ham's F10 + 5 ug/ml insulin and transferrin, 5 ng/ml selenium, 10 ng/ml EGF, and 1.5 % BSA, at 39°C in an atmosphere of 5% CO_2/95% air. One-cell embryos were added to freshly collected or to 4-day-old cultures of OEC which had been seeded in plastic culture wells or on collagen membranes (Cellagen^R, ICN). One-cell embryos cultured in similar vessels without OEC served as controls. After 65 h in culture, embryos were stained with Hoechst 33342 to count the number of nuclei per embryo. Mean nuclei per embryo were as follows (26-31 embryos/treatment, mean squared error=880, n=8 rabbits):

Control (no OEC)		Freshly recovered OEC		Four-day-old OEC	
Plastic	Cellagen	Plastic	Cellagen	Plastic	Cellagen
51	60	73	73	96	75

Cell numbers were significantly ($p<0.01$) higher in all co-culture treatments when compared to controls. Optimal development was obtained by co-culture with 4-day-old OEC grown on plastic. Although development was slower than would be expected for embryos developed in vivo (250 cells/embryo at this time), these results demonstrate that co-culture with OEC can stimulate the cell division rate of rabbit one-cell embryos in vitro.

CC 101 AN IMPROVED CULTURE MEDIUM PROMOTES DEVELOPMENT OF 1-CELL MOUSE EMBRYOS IN VITRO, Clare L. Chatot and Carol A. Ziomek, Worcester Foundation for Experimental Biology, Shrewsbury, MA. 01545.

One-cell embryos (CF-1 X B6SJLF1/J) have exhibited a 2-cell block to in vitro development in our lab in M16, modified Whitten's medium, and EBSS with EDTA. However, culture of these embryos in CZB medium, a modified BMOC-2 containing a lactate/pyruvate ratio of 116, 0.1mM EDTA and 1mM glutamine but lacking glucose, promoted development of 83% of embryos beyond the 2-cell stage with 63% morulae by 72 h of culture. In order to allow these embryos to develop from morula to blastocyst, it was necessary to wash embryos into CZB with glucose at 48 h (3-4-cell stage). Under these conditions, embryos developed to the morula(10%) or blastocyst(48%)(N=451) stage at 96 h. The average cell number per blastocyst was 33.7(N=160). The presence of glucose in the medium for the first 48 h of culture was inhibitory to embryo development with only 10% morula or blastocysts at 96 h compared to 58% in its absence. Glutamine exerted a beneficial effect on embryo development when present in the medium for the first 48 h of culture with 65-68% of embryos developing to morula or blastocyst compared to 30-33% in its absence. Embryos cultured using this protocol showed comparable patterns of glutamine uptake compared to in vivo until the blastocyst stage when in vitro uptake was reduced(31.1±1.9 fmol/embryo/h) compared to in vivo(43.8±3.4 fmol/embryo/h). Glycogen was accumulated by embryos in culture as determined by PAS staining in the presence or absence of amylase. M16 was modified to lack glucose and contain 0.1mM EDTA and 1mM glutamine like CZB. Under these conditions, M16 was also capable of supporting development of 68% of 1-cell embryos to morula and blastocyst stage(N=75). Supported by NIH Grant#HD21942.

CC 102 EGF-RECEPTOR EXPRESSION IN HUMAN CYTOTROPHOBLASTS: POSITIVE REGULATION BY LIGAND ACTION, Manjusri Das, Department of Biochemistry and Biophysics, University of Pennsylvania School of Medicine, Philadelphia, PA 19104

The EGF-receptor is a transmembrane glycoprotein with a ligand stimulatable tyrosine kinase site. This receptor interacts with both EGF and TGF-α, the latter being an embroyonically expressed EGF-family protein. We have been studying ligand induced changes in the expression of EGF-receptor in primary cultures of human embroyonal cytotrophoblasts. EGF-receptor specific cDNAs and monoclonal antibodies were used in these analyses. The cytotrophoblasts were found to express functional EGF-receptors that were down-regulated, i.e. degraded at saturating concentrations of EGF. However at low nanomolar concentrations of EGF there was a specific stimulation of receptor protein biosynthesis in these cells. The effect was pre-translational, i.e., due to an increase in receptor mRNA. Subsequent nuclear run-on experiements and studies on mRNA stability showed that the ligand-induced increase in receptor mRNA was largely an consequence of increase in mRNA stability and that there was only a small increase in receptor gene transcription. These results indicate a positive regulatory role of the ligand in EGF-receptor action in an embryo derived cell type, and imply the importance of paracrine interactions in the maintenance of expression and activity of this regulatory protein.

CC 103 THE EFFECT OF FREE RADICAL GENERATING AGENTS ON CRANIAL NEURAL CREST CELLS (CNCC) IN CULTURE, W.L. Davis, R.E. Dill, G.R. Farmer, L.Crawford, B.C. Nelson, J. Cooper and J.L. Matthews, Department of Anatomy, Baylor College of Dentistry, Dallas, TX 75246

Craniofacial development depends upon the viability of CNCC. Congenital anomalies have been linked to deficiences in CNCC. Many agents (teratogens) perturb the function of CNCC. We decided to test teratogens on CNCC in vitro. Cranial neural tubes were microdissected from stage 9-11 chick embryos and explanted to culture dishes containing supplemented Medium 199 (Davis et al., 1989). After 2 days the tubes were removed and the CNCC cultured an additional 48-72 hrs. Viability was >94% by dye exclusion. The effects of x-rays, ETOH, hyperoxia, H_2O_2, N_2O_2 and PHT (Dilantin) on CNCC were tested. All depressed viability (<10% in some instances, ETOH). All generated free radicals (FR), primarily superoxide anions. Untreated cells were tested for superoxide dismutase (SOD). The enzyme was not detected. The absence of SOD was confirmed by immunohistochemistry. EM studies with La or HRP as tracers showed increased membrane "leakiness" in treated CNCC. SOD "scavenges" FR. DNA and membranes are at high risk from FR. Environmental agents (pesticides, cigarette smoke, electromagnetic irradiation, aromatic hydrocarbons) have been linked to craniofacial anomalies. All generate FR. Thus, the decreased viability of CNCC in vitro may be related to their overt sensitivity to endogenously generated FR and their apparent lack of SOD activity during early development.

CC 104 Metabolism of Platelet Activating Factor in Rabbit Uterine Tissue Amelia L. Dean and Marjorie A. Jones, Depts. of Chemistry and Biological Sciences, Illinois State University, Normal, Illinois 61761. Platelet activating factor (PAF), a biologically active phospholipid, has been shown to modulate increased vascular permeability. Angle et al. (1988) have shown that PAF is found in rabbit uterine tissue and the levels change in response to the reproductive status. PAF is implicated in maternal-embryo interactions, therefore, its concentration is of interest. Breakdown of the PAF molecule has received little attention. In tissues, such as kidney, PAF is degraded to lyso-PAF by the enzyme, acetylhydrolase. The lyso-PAF has little biological activity. We now report studies of the acetylhydrolase in the uterus. Estrous rabbit uterine mince was incubated at 37oC with 0.05 uCi of [3H]-PAF in 0.1M NaP (pH 8.0) buffer, for various times. The reaction was terminated using the Folch method followed by lipid extraction. The separation of [3H]-PAF and lyso-PAF was by TLC followed by scintillation spectrometry. Results show that 85% of the PAF was degraded when incubated with a uterine tissue mince (500 mg) for 10 minutes. Several different products were seen by TLC. The lyso-PAF product was 8.8% of the total giving a specific activity of 56 fmol lyso-PAF/min/wet wt of tissue. At least two other products were formed. Longer incubations resulted in very little lyso-PAF and more acyl-PAF and other products. Therefore, we conclude that uterine tissue contains enzymes which rapidly degrade PAF. Since multiple enzymes are active in uterine tissue, specific enzyme inhibitors are currently being studied. (Supported by Phi Sigma and Graduate Student Association Research Grant, ISU).

CC 105 STUDIES ON GENE EXPRESSION IN EARLY MOUSE EMBRYOS USING ANTISENSE RNA TECHNIQUES, Robert P. Erickson, Arturo Bevilacqua, and Brian Levy, Department's of Humans Genetics and Pediatrics, University of Michigan School of Medicine, Ann Arbor, MI 49109-0618.

We have found that the mammalian preimplantation embryo will tolerate up to 20 pg of RNA injected into the cytoplasm without harmful effects on development. If this is a capped RNA transcript, it persists for reasonably long periods of time. We have injected a number of sense and antisense RNAs and shown that antisense RNA to several genes results in inhibition of expression of their products, sometimes with marked effects on the morphology of development. Specifically, antisense RNA to a cDNA for β-glucuronidase can inhibit expression up to 75%. The inhibition can reach 95% when the antisense RNA is to a 5' fragment including the start codon. Antisense RNA to a liver gap junction protein cDNA which includes 5' transcribed material has dramatic effects on preimplantation development leading to delays and failure of compaction when injected throughout the embryo. If injected into a single blastomere at a later stage, the blastomere is excluded from the embryo. That these effects of the antisense RNA are due to effects on gap junction communication is shown by studies with lucifer yellow. We are currently using an antisense RNA to int-1, a c-oncogene showing homology to the Drosophila developmental gene wingless, to try and determine if int-1 has an important role in early mammalian development. In addition, we are currently using DNA constructs in which antisense RNA is driven by the metallothionein promoter to create antisense transgenics; currently the level of inhibition is not as great as that achieved with RNA injections.

CC 106 EXPOSURE OF PREIMPLANTATION RABBIT EMBRYOS TO LABORATORY LIGHT AND ROOM TEMPERATURE, Bernd Fischer, Armin Schumacher, Christa Hegele-Hartung and Henning M. Beier, Department of Anatomy and Reproductive Biology, RWTH School of Medicine, Aachen, Federal Republic of Germany

Rabbit 1-c stages (Day 1 p.c.) and morulae (Day 3 p.c.) have been exposed to light (L; 1600 lx) and room temperature (RT; 23°C). Ultrastructural analysis after 24h RT exposure indicates an altered cytoskeleton organization, a possible reason for an observed impairment of cleavage. Following L exposure cell degeneration and cell death are predominating. Measured by thymidine incorporation DNA synthesis is statistically significant reduced (a) in 1-c embryos after 1h L and 8h RT exposure and (b) in morulae after 8h L and 3h RT exposure. Simultaneous exposure to both stressors shortens tolerance time to 1h in both stages. Autoradiographic analysis of exposed embryos reveals comparable labelling indices of trophoblast and embryoblast cells after exposure to L or RT, indicating no specific inhibition of one cell line by the investigated stressors. An astonishing regulatory capacity of preimplantation embryos is demonstrable after embryo transfer: Despite the reported damages embryos implant (L) or develop into viable young (RT) after exposure times of up to 24h. Supported by DFG grant Fi 306/1-3

CC 107 IN VITRO INDUCED DECIDUAL CHANGES IN HUMAN ENDOMETRIAL STROMAL CELLS, Irwin, J.C. and Gwatkin, R.B.L., Reproductive and Developmental Biology, Research Institute and Gynecology, The Cleveland Clinic Foundation, Cleveland, OH 44106

The decidualization of the human endometrium during the secretory phase of normal ovulatory cycles involves a morphological transformation of stromal cells which is associated with increased proliferation, prolactin secretion and with the production of laminin and fibronectin. Stromal cells, derived from proliferative phase endometria cultured in medium supplemented with steroid-depleted fetal bovine serum, grew as monolayers and had ultrastructural characteristics of mesenchymal cells, with an average assortment of organelles and no specialized intercellular junctions. In the absence of steroids, stromal cultures did not produce detectable levels of prolactin as measured by ELISA (< 0.4 ng/ml) and showed only occasional areas of weak reactivity for fibronectin and laminin by immunofluorescence. Treatment of confluent cultures with 30 nM estradiol + 0.3 µM progesterone for 10-19 days induced prolactin production (25-94 ng/day/10^7 cells) and stimulated proliferation, resulting in multilayering with an increase of the saturation density (2-3 x control values). Transmission electron microscopy showed cells which formed gap junctions and had abundant cytoplasm with a well developed rough endoplasmic reticulum and golgi. Immunofluorescence revealed a dense fibrillar pericellular matrix of immunoreactive fibronectin and discrete deposits of laminin reactivity along the cell borders. These results show that human endometrial stromal cells in culture respond to physiological doses of ovarian hormones with ultrastructural, proliferative and biochemical changes which parallel those seen during decidualization in vivo.

CC 108 CHANGES IN PROTEIN PHOSPHORYLATION ASSOCIATED WITH MITOSIS AND RADIATION INDUCED G_2-BLOCK IN CLEAVAGE MOUSE EMBRYOS, T.Jung, G.Luscher and C.Streffer, Institut für Medizinische Strahlenbiologie, Universitätsklinikum Essen, D-4300 Essen, Germany

Protein phosphorylation in the 2nd cleavage of normally dividing and of X-irradiated mouse embryos was assessed by ^{32}P incorporation and 1 and 2D electrophoresis.

Overall protein phosphorylation increased with progessing cell cycle from G_2 to mitosis. X-irradiation (4Gy) in G_2 led to a block of about 4h in the same cell cycle phase, which coincided with a 4h retardation in the increase in protein phosphorylation.

Correlated with beginning mitosis was the phosphorylation of a 49kDa protein in normally dividing embryos. In X-irradiated embryos the ^{32}P labelled 49kDa protein appeared after releasing the block with the onset of cell division. X-irradiation diminished ^{32}P incorporation in a 29kDa protein to undetectable levels within 1h. The reappearence of this phosphorylated protein was followed by mitosis 4h later.

CC 109 INSULIN-LIKE GROWTH FACTOR-1 IN THE PREGNANT PIG: DEVELOPMENTAL EXPRESSION DURING UTERINE AND EMBRYONIC GROWTH, L.R. Letcher, R.C.M. Simmen, F. W. Bazer* and F. A. Simmen, Departments of Animal Science, The Ohio State University, Wooster, OH 44691 and The University of Florida* at Gainesville, FL. Swine embryos in utero change dramatically from spherical (3-10 mm) to elongated filamentous (up to 1000 mm) forms between d 10 and 14 of gestation. To elucidate the possible role of insulin-like growth factor-1 (IGF-1) in early embryonic development of this species, we have analyzed maternal endometria and embryonic tissues for the presence of IGF-1 peptide and messenger RNAs. Corresponding embryos, endometria and uterine luminal fluid (ULF) were collected from sows during d 8-14 whereas endometria and allantoic fluid were obtained on d 30 of pregnancy. ULFs and tissues were acid extracted and evaluated by IGF-1 specific radioimmunoassay. IGF-1 mRNA levels in tissues were monitored by blot-hybridization with a porcine IGF-1 cDNA probe. ULF IGF-1 content was low on d 8, peaked on d 12 and precipitously declined by d 14. Allantoic fluid from d 30 of pregnancy was devoid of IGF-1. The IGF-1 content of endometrial tissue remained constant between d 8-14 but declined by d 30 of pregnancy. Embryonic tissue had a much lower amount of IGF-1 compared to maternal tissues. IGF-1 mRNA levels in endometria were similar during d 8-14 but decreased by d 30. These results demonstrate relatively high levels of IGF-1 peptide and mRNA in the maternal endometrium during the critical early embryonic phase of development. The peak in luminal IGF-1 on d 12 coincides with the production of estrogen by elongating blastocysts, suggesting a possible local effect of this steroid on the endometrial release of IGF-1 peptide.

CC 110 METHYLATIONS IN THE EARLY EMBRYO. *Ado Met synthesis and effect of transmethylation inhibitors*, Menezo Y. and Khatchadourian C. INRA - LA 23203 - INSA - 69621 Villeurbanne Cedex - France.

In early embryo, *de novo* methylation is involved in *gamete imprinting* and inactivation of artificially introduced genes. Using radiolabelled 35 S Methionine (50 µ M), we studied, from the oocyte to the blastocyst the biosynthesis of S Adenosyl Methionine (Ado Met) : the universal effector of Methylation. In a second time, two transmethylation inhibitors (ethionine and homocysteine) were tested on early embryo development.

In the Mouse, Ado Met synthesis, is continuous from the unfertilized egg to the blastocyst. The rate rises abruptly at the compacting morula. The synthesis is limited by the saturation of the Methionine endogenous pool. We observed no differences between C 57 BL and Swiss strains especially in relation to the 2 cell block stage. Experiments carried on unfertilized and polyploïd human eggs, rejects from an *in vitro* fertilization program, show a higher methionine uptake associated with a higher conversion to AdoMet (*Ex : unfertilized eggs , mean met. uptake : 500 fmoles/hr/egg ; conversion rate to Ado Met : 4,1 %).*

Both ethionine and homocysteine adversly affect the progression of one or 2 cell embryos to blastocysts *in vitro* , but ethionine effect is more severe and is seen at lower doses. Homocysteine decreases the methylation index but also induces a progressive efflux of methionine from the embryo. Homocysteine might be used to modulate nucleic acid methylation levels in the early embryo *via* Ado Met depletion, as already obtained with other antimethylating agent in somatic cells.

CC 111 PDGF A CHAIN IS EXPRESSED IN EARLY MOUSE EMBRYOS AND IS MATERNALLY ENCODED IN XENOPUS EMBRYOS, Mark Mercola, Clare Brownlee, Doug Melton*, Ursula Drager**, and Charles D. Stiles, Dana-Farber Cancer Institute and the **Department of Neurobiology, Harvard Medical School, Boston, MA 02115, and *Harvard University, Cambridge, MA 02138.

We show that the A chain of platelet-derived growth factor (PDGF) is the predominant form of PDGF in developing mouse and *Xenopus* embryos. In the mouse, A chain and PDGF receptor mRNA are present in embryonic tissue as early as the onset of gastrulation (6.5 days after fertilization). Immunohistochemical localization studies show that the A chain protein is present in the embryonic ectoderm and is most abundant at 6.5 days, decreasing thereafter.

Xenopus oocytes and embryos contain a mRNA encoding the A chain. This mRNA is present throughout the early cleavage stages and increases in abundance at gastrulation following the activation of the embryonic nuclei.

Cloning, sequence, expresseion and mRNA analyses shows that the A chain sequence is remarkably well conserved in both species and that the *Xenopus* protein functions as a mitogen for mouse cells. Also, in both species, alternative splicing gives rise to different forms of the A chain protein. The presence of the A chain and lack of the B chain (the c-*sis* proto-oncogene product) in early mouse development and as a maternally encoded mRNA in *Xenopus* suggests a unique and phylogenetically conserved role for the A chain in early development.

CC 112 CONSTRUCTION OF A NOVEL EXPRESSION VECTOR FOR LARGE SCALE PRODUCTION OF RECOMBINANT UTEROGLOBIN IN E. coli, Lucio Miele, Eleonora Cordella-Miele and Anil B. Mukherjee, Section on Developmental Genetics, Human Genetics Branch, NICHD, NIH, Bethesda, Maryland 20892

Uteroglobin (UG) or blastokinin is a steroid dependent, low molecular weight secretory protein with many immunomodulatory, and antiinflammatory effects. Many of these biological effects may be related, at least in part, to its ability to inhibit phospholipase A_2 activity and consequent reduction of proinflammatory eicosanoid levels in various tissues including the uterus. It has been suggested that this protein may be instrumental in providing protection of implanting embryos from maternal immunological assault. More recently, a nonapeptide from the region of highest homology between UG and lipocortin-I has been identified which seem to be the putative PLA_2 inhibitory site of UG and in vivo this peptide is a potent antiinflammatory agent (Nature, In Press). To study the structure-function relationship of UG and PLA_2 enzyme it was necessary to develop an efficient expression system in E. coli for site-directed mutagenesis studies. Here, we describe the construction of a novel vector for a very high efficiency expression of UG in E. coli. The dimeric nature of UG posed a special problem for bacterial expression, since to our knowledge, there is no published report on the expression of proteins with quarternary structure in E. coli in their natural form. Our newly designed vector (plasmid) directing the expression of UG is designated pLE103-1. Our expression system combines the advantages of preexisting T7 promoter vectors (i.e. high efficiency and specificity of transcription) with those of "ATG vectors" based on E. coli promoters (i.e. built-in Nco-I site with ATG codon, 4 base overhang, possibility of blunt-end cloning with preservation of the ATG and possible use of the Pst I site for cDNA cloning). High quantities of recombinant UG or other similar proteins of scientific and medical importance could easily be produced in their natural form using this vector.

CC 113 DE NOVO SYNTHESIS AND SECRETION OF PROLACTIN-LIKE PROTEIN-B BY RAT PLACENTAL EXPLANTS, Susan Ogilvie, Mary L. Duckworth*, William C. Buhi and Kathleen T. Shiverick, University of Florida, Gainesville, FL 32610, USA and University of Manitoba*, Winnipeg, R3E OW3 Canada

Mid to late gestation rat placenta expresses three prolactin-related mRNA's (Duckworth *et al.*, 1988, Mol. Endocrinol., in press). *In situ* hybridization studies have localized rat placental lactogen II (rPLII) mRNA in the giant cells of the basal zone and the mRNA's for rat prolactin-like protein A (rPLP-A) and rat prolactin-like protein B (rPLP-B) in the basophilic cytotrophoblast cells of the basal zone. The protein product of rPLII mRNA has been characterized, but the protein products of the rPLP-A and rPLP-B mRNA's have not been identified. The molecular weight of a non-secreted, non-glycosylated rPLP-B protein based on the mRNA sequence would be 27,145. Antisera was generated against a chemically synthesized oligopeptide inferred from a specific region of the rPLP-B cDNA. Our studies have identified proteins synthesized and secreted by rat basal zone explants that crossreact with this antisera. Basal zone tissue for cultures was obtained on day 16 of gestation and incubated in Eagle's modified MEM (175 mg/5 ml) with or without tunicamycin (10 μg/ml) for 24 hours at 37 C. After 18 hours of incubation 40 μCi of ^{35}[S]methionine was added and the cultures terminated at 24 hours. ^{35}[S]methionine labeled proteins secreted by basal zone explants were analyzed by two dimensional, sodium dodecyl sulfate, polyacrylamide gel electrophoresis (2D-SDS-PAGE), followed by either fluorography or immunoblot analysis. Three to four distinct ^{35}[S]methionine labeled proteins cross-reacted with the rPLP-B antisera. The relative molecular weight (Mr) of these immunoreactive proteins is approximately 30 K with a pI varying from 6.1 to 6.6. The protein products were not synthesized by the explant tissue in the presence of tunicamycin, suggesting the proteins are glycosylated, which agrees with the presence of one potential N-glycosylation site derived from the rPLP-B mRNA sequence. In other studies paraffin sections from placentas of day 16 pregnant rats were treated with anti-rPLP-B followed by avidin-biotin peroxidase complex. These experiments show staining of basophilic cytotrophoblast cells, confirming the *in situ* mRNA hybridization studies. Although no physiological role has been established for rPLP-B, synthesis and secretion of this protein by cells only in contact with maternal circulation suggests a hormonal role.

CC 114 INSULIN STIMULATES THE UPTAKE AND INCORPORATION OF ^{3}H-THYMIDINE AND ^{3}H-URIDINE INTO PREIMPLANTATION MOUSE EMBRYOS, L.V. Rao, Maria L. Wikarczuk and Susan Heyner, Department of Obstetrics and Gynecology, Albert Einstein Medical Center, Philadelphia PA 19141.

The regulation of growth and differentiation in mammalian embryogenesis may be influenced by hormones and growth factors from either embryonic or maternal sources. Insulin is one of the most important hormones that influences fetal growth and metabolism. Specific binding sites for insulin have been demonstrated recently in mouse preimplantation embryos (Mattson et al., Diabetes 37:585, 1988). The present study was undertaken to investigate the effect of insulin on nucleic acid synthesis in preimplantation embryos. Embryonic stages (2-, 8-cell, morula and blastocyst) were incubated in media containing no insulin for 1 hr to ensure that insulin receptors would be unoccupied. Embryos were then incubated for 1 hr in in the presence (4 ng/ml) or absence of insulin. This was followed by a 2 hr incubation in ^{3}H-thymidine or ^{3}H-uridine with or without insulin (4 ng/ml). Insulin significantly increased the rate of synthesis of RNA and DNA in embryos at the morula and blastocyst stages of development.

Supported by NIH grant HD 23511

CC 115 AN ENDOGENOUS GROWTH FACTOR-RECEPTOR CIRCUIT IN PRE-IMPLANTATION MAMMALIAN DEVELOPMENT? Daniel A. Rappolee, Gilbert A. Schultz, Roger A. Pedersen, Karin Sturm, and Zena Werb, Laboratory of Radiobiology and Environmental Health, University of California, San Francisco, CA 94143-0750. Control of early growth and differentiation during mammalian embryogenesis may be regulated by growth factors from maternal or embryonic sources. To examine simultaneous expression of growth factor ligand and receptor expression in preimplantation mouse embryos, we have used a novel method for mRNA phenotype analysis based on production of cDNA followed by enzymatic amplification of specific fragments. We found that insulin-like growth factor (IGF)-I receptor, IGF-II receptor, and insulin receptor transcripts are expressed after compaction in mouse morulae. This is in agreement with published observations on the binding specificities for insulin, IGF-I, and IGF-II, in early mouse embryos (Mattson et al., *Diabetes*, 37, 585). In contrast, transcripts for the ligand, IGF-II, but not for IGF-I and insulin are expressed in preimplantation mouse embryos. This suggests that IGF-II ligand may form an endogenous circuit in early mouse embryos through one of its receptors; IGF-II receptor or IGF-I receptor. Experiments are under way to test this hypothesis. Although effects of insulin on preimplantation mouse embryos have been observed for insulin, IGF-I, and IGF-II *in vitro* (Harvey and Kaye, *Endocrinology*, 122, 1182), this may reflect effects of maternal insulin *in vivo*. Transforming growth factor-α, platelet-derived growth factor A-chain, and transforming growth factor-β1 transcripts and antigens have also been observed in early mouse embryos. Recent data also suggest that ks/hst-type fibroblast growth factor, which may be a secreted form, is expressed by mouse embryos at least as early as the first expression of the zygotic genome. These four growth factors are similar in possessing signal sequences, indicating the capability to mediate paracrine or autocrine effects in the early embryo. Studies aimed at demonstrating the effects of these ligands in early mouse embryogenesis are underway. Supported by NIH, US DOE-OHER, and MRC (Canada).

CC 116 MONOCLONAL ANTIBODIES AGAINST ENDOMETRIAL/DECIDUAL INSULIN-LIKE GROWTH FACTOR BINDING PROTEIN, Eeva-Marja Rutanen and Fredrika Pekonen, Department of Obstetrics and Gynecology, University of California, Los Angeles, California 90024, Minerva Institute for Medical Research, Kauniainen, 02740 Finland.
We have previously demonstrated that human secretory phase endometrium and pregnancy decidua synthesize and secrete a 27-34K insulin-like growth factor binding protein (IGF-BP), also called PP12. This IGF-BP species competes for IGF-I with cell membrane IGF receptors, suggesting that it functions as a paracrine regulator of IGF action in the endometrium or trophoblast. Monoclonal antibodies raised against PP12 were used in immunoblotting studies of endometrium and pregnancy decidua. Samples of endometrium and decidua were run on SDS-PAGE under nonreducing conditions. To determine the relationship of PP12 and IGF-I binding components in the tissues, duplicate lanes of each sample were studied by immunoblotting and by probing with ^{125}I IGF-I. A single immunoreactive band corresponding to a molecular mass of 28K or double bands at 27-29K were seen in immunoblots of secretory phase and pregnancy endometrium, but not in proliferative phase endometrium. In duplicate gels, ^{125}I IGF-I bound to bands with molecular masses identical with those of immunoreactive bands. Our data indicate that these monoclonal antibodies specifically recognize the endometrial/decidual IGF-BP and thus may provide valuable tools for clinical application and further studies on IGF and IGF-BP action in the endometrium.

CC 117 COMPARISON OF HAMSTER EMBRYO CULTURE MEDIUM-I AND -II FOR SUPPORTING IN VITRO DEVELOPMENT OF HAMSTER 2- AND 8- CELL EMBRYOS, Polani B. Seshagiri and Barry D. Bavister, Department of Veterinary Science, University of Wisconsin, Madison, WI 53706.
In hamsters, 'the 2-cell embryo block to development in vitro' was overcome by using hamster embryo culture medium (HECM) - I. This differs from HECM-II that supports ≈90% blastocyst development starting from 8-cell embryos. While HECM -I contains 20 amino acids, HECM-II contains only Phe, Ile, Met and Gln. In addition, phenol red and penicillin G are absent in HECM-I but present in HECM-II. With a view to develop an optimal culture medium that can support development of hamster 1-cell embryos to viable blastocysts, the two media were compared for their relative ability to support in vitro development of 2- and 8-cell embryos. Hamster 2-cell embryos developed to ≥4-cell stage to a comparable extent in both media, but the no. of 8-cell embryos (83.5%) and blastocysts (26.4%) obtained in HECM-I were significantly ($p \leq 0.003$) more than in HECM-II (62.5% 8-cell embryos and 13.5% blastocysts). Interchange of media after 24 h. culture did not enhance the ability of cultured embryos to become blastocysts. Development obtained in media that differed by the presence and absence of two components, i.e., phenol red and penicillin-G, did not show appreciable difference in terms of no. of blastocysts formed. Comparatively, blastocyst formation was better in HECM-I than in HECM-II, indicating that the presence of four amino acids (as in HECM-II) is not sufficient and additional amino acids (as in HECM-I) are required for 2-cell embryo culture. When hamster 8-cell embryos were cultured in HECM-I and II, there was no significant difference in total no. of blastocysts formed in both media (≈ 90%). However, of these total blastocysts, late blastocysts were significantly ($p \leq 0.0004$) more in HECM II (77.4%) than in HECM-I (38.9%), showing that four amino acids are sufficient to obtain late blastocysts. The low % late blastocysts observed in HECM-I could be attributed to the presence of some inhibitory amino acids. Hence, optimum medium may contain greater than four and less than 20 amino acids. (Support: NIH grant No. HD 22023).

CC 118 IMMUNOLOCALIZATION OF TGF-B_2 IN MOUSE PREIMPLANTATION-EMBRYOS, H.G. Slager, A.J.M. van den Eijnden-van Raaij and C.L. Mummery, The Hubrecht Laboratory, Netherlands Institute for Developmental Biology, Uppsalalaan 8, 3584 CT Utrecht, The Netherlands. An antibody specific for transforming growth factor beta type 2(TGF-B_2) was raised against a synthetic peptide corresponding to the first 29-N terminal amino-acid sequence of TGF-B_2. This antibody appeared to bind specifically TGF-B type 2 and not type 1 as judged by various immunological assays (van den Eynden-van Raaij; submitted for publication). With this antibody mouse preimplantation embryos were screened for the presence of TGF-B_2 protein by immunofluorescence- and immunogold labeling techniques. Blastocyst stage embryos cultured in vitro were also subjected to this investigation. Embryos processed directly for fluorescence labeling showed intensive labeling in trophoblast (TB) cell cytoplasm but not in inner cell mass (ICM) cells, indicating selective presence of TGF-B_2 protein. Label intensity sometimes varied between TB cells. Immuno-electronmicroscopy confirmed these findings at higher resolution: TB cell cytoplasm showed uniformly dense labeling while the nucleus did not. To a lesser extent ICM cells displayed the same labeling features. The embryos cultured in vitro developed endodermal cell outgrowths which were specifically stained by fluorescently labeled anti-TGF-B_2 antiserum. Selected trophoblast cells were also stained. These data indicate a tissue-specific presence of TGF-B_2 protein in preimplantation mouse embryos which could possibly have a role in early development.

CC 119 PLACENTA AS WITNESS OF THE FATE OF THE EMBRYO IN HUMAN SPONTANEOUS ABORTION. Aron E. Szulman, Department of Pathology, Magee-Womens Hospital and School of Medicine, University of Pittsburgh, Pittsburgh, PA 15213
The fate of the embryo is of special interest in first trimester human abortion since its early demise leads to spontaneous uterine emptying most often delayed by several weeks. Whereas chromosomal anomalies are demonstrable in over 50% of all early abortions, the proportion reaches to 85% in empty intact gestational sacs and in developmentally disorganized embryos whose survival is the shortest. The embryo-fetus is, however, only rarely available for examination because of its small size, autolysis, fragility and application of suction-curettage. The placenta accordingly stands as witness of the operative pathologic process mainly by its ability to disclose the time-frame of embryonic demise.
The method involved is based on the following: 1. the normal appearance of early, primitive, poorly hemoglobinized erythrocytes at 4 1/2 weeks ovulation age (O.A.) (vessels, formed in situ, appear empty of blood before that), 2. the subsequent rapid hemoglobinization of cytoplasm, 3. the slow condensation of the nuclei prior to their massive intravascular extrusion during the 9th week O.A., 4. the contraction of the lumens of villous capillaries upon cessation of the heart-beat with eventual loss of endothelium but prolonged persistence (up to 8 weeks) of intact RBCs whose further maturation is arrested on stoppage of the circulation, 5. concomitant slow fibrosis and rather fast progressing edema of villous stroma.

CC 120 TRANSFORMING GROWTH FACTOR-β PROMOTES THE GROWTH OF MOUSE UTERUS AND VAGINA, *IN VIVO* Tsuneo Takahashi, Karen Nelson, LeMarquis Goods and John A. McLachlan, National Institute of Environment Health Sciences, Laboratory of Reproductive and Developmental Toxicology, Research Triangle Park, NC 27709
The studies described here were initiated to determine whether TGF-β functioned as a regulator of growth of mouse uterus and vagina. Cholesterol-based pellets containing TGF-β (2.5 - 125 ng) were transplanted under the kidney capsule or in the 4th mammary fat pad of ovariectomized female mice. TGF-β induced a dose-dependent stimulation of DNA synthesis in uterine luminal and glandular epithelial cells as measured by [^{3}H]-TdR autoradiography. Vaginal growth was also enhanced by TGF-β as evident by enhanced [^{3}H]-TdR incorporation into basal keratinocytes, increased cell number and increased keratinization at the higher TGF-β doses. Prior exposure of mice to TGF-β modified the estrogen-induced growth response of the uterus and vagina. Pretreatment of mice for 48 hrs with TGF-β significantly potentiated estradiol induced uterine luminal epithelial cell DNA synthesis as well as induced the growth of uterine stromal cells, which was not usually enhanced by estradiol alone. TGF-β also potentiated estrogen-induced vaginal epithelial cell DNA synthesis. Our results suggest that TGF-β is not only a growth factor for mouse uterine and vaginal cells when given alone; but also, TGF-β enhances the responsiveness of these cells to estrogen. Although the exact physiological role of TGF-β and its mechanism of action is not known, our data provides evidence that TGF-β may have an important role in regulating normal reproductive function and estrogen action.

CC 121 INSULIN IS ENDOCYTOSED BY PREIMPLANTATION MOUSE EMBRYOS:PATTERN OF UPTAKE AND TRANSLOCATION

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Previous studies have shown that preimplantation mouse embryos express receptors that bind insulin (Mattson et al., Diabetes 37:585, 1988). We have extended these studies, using colloidal gold-labelled insulin (gold-insulin) in conjunction with high-resolution electron microscopy to examine the location and fate of occupied insulin receptors in preimplantation mouse embryos. Internalization of gold-insulin appeared first at the morula stage. Labelled ligand was internalized via coated pits, in which it was concentrated four-fold in comparison to the surface membrane. At the blastocyst stage there was evidence of translocation to the inner cell mass via coated endocytotic vesicles. Controls consisted of embryos incubated in an equivalent concentration of gold-labelled BSA; these embryos showed only background levels of labelling. Supported by NIH grants DK 19525 and HD 23511.

CC 122 HORMONAL CONTROL OF MACROPHAGE ACCUMULATION IN THE MURINE UTERUS, **Gary W. Wood and Mamata De**, Department of Pathology, University of Kansas Medical Center, Kansas City, KS 66103

Recent studies, confirmed in our laboratory, have demonstrated progesterone (P_4) and estrogen (E_2)-dependent production of the macrophage-specific growth factor, CSF-1, by uterine epithelial cells during pregnancy. During the present study of the influence of P_4 and E_2 on macrophage accumulation in normal uterus, macrophages were quantitated both in cell suspensions and in tissue sections with a monoclonal rat anti-mouse macrophage antibody (F4/80). Those studies established that the normal uterus contained macrophages (7-12% of total cells) evenly distributed through the endometrium and in myometrial stroma, that there were no quantitative differences between stages of the estrous cycle, but that numbers of macrophages increased significantly during pregnancy (20-35% of total cells). Even though uterine cellularity decreased following ovariectomy, the numbers of macrophages decreased disproportionately to 1.5-2.5% of total uterine cells. Ovarian transplants beneath the kidney capsule restored macrophage levels to 6-7%. Six days after ovariectomy a single intraperitoneal injection of 100ng E_2 significantly increased uterine macrophages (to 5-6%), when tested 24-48 hrs later. 2mg P_4, similarly administered, also increased macrophage percentages to 5-6%. E_2 and P_4, injected simultaneously or sequentially, increased the macrophage percentage to 13% at 24-48 hrs. Those studies demonstrated that ovarian hormones have a regulatory effect on macrophage accumulation in the non-pregnant murine uterus and suggest that one of the effects of P_4 and E_2 stimulation of CSF-1 production and release during pregnancy is to stimulate macrophage accumulation. Supported by NIH grant # HD17678.

CC 123 CHARACTERIZATION OF A PROBABLE "ONCOFETAL" TRANSFORMING GROWTH FACTOR, HST1, A MEMBER OF FGF-INT2-HST1 FAMILY, Teruhiko Yoshida, Kiyoshi Miyagawa, Hiromi Sakamoto, Osamu Katoh, Takashi Sugimura and Masaaki Terada, Genetics Division, National Cancer Center Research Institute, Tsukiji 5-chome, Chuo-ku, Tokyo 104, Japan.

Many genes and their products playing specific roles in embryogenesis are also recruited in the process of carcinogenesis. We propose that HST1, an oncogene which we identified by NIH3T3 focus formation assay is involved in both embryogenesis and carcinogenesis on the basis of following observations; (1) the HST1 protein is 40-50% homologous to fibroblast growth factors and to mouse Int-2 protein, constituting an oncogene-growth factor superfamily. At least two members of this family are supposed to be involved in embryogenesis: basic FGF is a mesoderm-inducer in amphibian development, and Int-2 is normally expressed only in the early-stage embryos. (2) Mouse Hst-1 is not detectably expressed in adult tissues, but is expressed in mid-gestation period of mouse embryos just preceding the expression of Int-2. (3) Expression of Hst-1 is correlated with the differentiation status of the cell, since the gene is down regulated when F9 mouse teratocarcinoma cells are differentiated to parietal endoderm-like cells; in contrast, expression of Int-2 is turned on only in the F9-derived parietal endodermal cells. (4) HST1 is rarely expressed in many cancer cells, but, specifically, it is expressed in surgical specimens of human testicular germ cell tumors. (5) Normal HST1 product has transforming activity to NIH3T3 cells. We also found that two homologous oncogenes, HST1 and INT2 are located close each other in human and mouse genomes with the distance of 40-50 kb or less.

CC 124 CHANGES IN G-PROTEIN EXPRESSION DURING OOCYTE MATURATION AND PREIMPLANTATION DEVELOPMENT IN THE MOUSE, Carol A. Ziomek, Ann Allworth and John Hildebrandt, Worcester Foundation for Experimental Biology, Shrewsbury, MA 01545.

During oocyte maturation and preimplantation development, eggs and embryos are exposed in vivo to a variety of external stimuli (hormones, growth factors, cell-cell contact, etc.) that may influence their developmental program. Since several GTP-binding proteins (G-proteins) mediate the transduction of external signals across the plasma membrane in many different cell types, their presence in oocytes and embryos was determined. Germinal vesicle (GV)-stage, unfertilized and fertilized oocytes, and 2-cell, 4-cell, morula and blastocyst stage embryos were assayed for G-proteins by a pertussis toxin ADP-ribosylation assay, by immunoblotting using antibodies to subunits of several G-proteins (αi, all major α's, β) and by immunofluorescence. G-proteins were found in all eggs and embryos examined but with a stage-specific pattern to their expression. GV stage oocytes contained the most ADP-ribosylatable G-protein which decreased in the unfertilized egg, remained constant until the late 2-cell stage, then gradually decreased up to the 8-16 cell stage with some recovery at the blastocyst stage. There were two most likely explanations for this result: (1) a change in absolute amount of G-protein or (2) a change in subunit/receptor associations. Immunoblotting demonstrated that some G-protein subunits changed in a stage specific manner whereas at least one did not. This research was supported by grants from the Andrew Mellon Foundation, the Edward John Noble Foundation and HD-17674.