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Short Contribution

Retinoids are endogenous to the porcine blastocyst and secreted by trophectoderm cells at functionally-active levels

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ABSTRACT Retinoic acid and its isomers are the major morphogens in vertebrate development. For mammals, it was previously considered that circulating retinoic acid was recruited from the uterine environment, to influence embryonic differentiation, morphogenesis and development. Here we report that retinoic acid is endogenous to the blastocyst of the domestic pig, *Sus scrofa*, as detected by high pressure liquid chromatography. Furthermore, using a continuous, normal line of porcine trophectoderm cells, TE1, we have identified the trophoblast as a major source of retinoids. Endogenous retinoic acid was found at a concentration of approximately 35 nM in extracts of entire blastocysts, and at a similar concentration in extracts of TE1 cells. Retinoids in explants of blastocysts and in conditioned medium from the TE1 cell line were found to be functionally-active, inducing gene expression from a retinoic-acid-responsive enhancer element in an *in vitro* assay system. In conclusion, we propose that there is a morphogenetic role for endogenous, and trophoblast-derived, retinoids in the early development of the pre-gastrulation porcine embryo; and that the TE1 cell line therefore provides a useful *in vitro* system for the study of retinoid metabolism. Furthermore, an implication of this study is that endogenous retinoids may play an active role in the pre-implantation embryology of other species, such as the human.

KEY WORDS: *blastocyst, differentiation, morphogen, pig, retinoic acid*

Retinoic acid (RA) and its isomers are major morphogens in vertebrate development (Pijnappel *et al.*, 1993; Kraft *et al.*, 1994; Horton and Maden, 1995). The role of RA in mouse development has been extensively studied, especially post-gastrulation (Gudas, 1994), and it is well-established that in the murine system retinoids influence development by activating specific genes, for example the homeobox genes, via retinoic-acid-responsive enhancer elements (RARE: Langston *et al.*, 1997). For earlier stages of development, RA is crucial also in cellular differentiation processes (Minucci *et al.*, 1996).

The source of RA that is functionally-active has been assumed to be circulatory; in fact the transport of the precursor, retinol, from maternal to foetal tissues is highly regulated, as variations in maternal intake of Vitamin A (retinol) have insignificant effects on foetal serum levels (Goodman, 1984). RA deficiency has been implicated as the cause of embryo mortality in the domestic pig, *Sus scrofa*, where the second week of pregnancy represents a crucial stage in development (Roberts *et al.*, 1993). Genetically-normal blastocysts are lost during the post-hatching, pre-implantation stage, possibly due to an asynchrony between the less developed blastocysts and the uterine environment (Wilde *et al.*, 1988). During porcine embryonic development, retinol is detectable in the uterine fluid at low levels until day 12 of pregnancy, whereafter levels are substantially increased. Prior to this elevation, at day 10, a retinol binding protein (RBP) becomes a major protein to be secreted by the blastocyst into the uterine fluid, specifically by the trophectoderm (Trout *et al.*, 1992; Yelich *et al.*, 1997). Thus it is of interest to study interactions between the blastocysts and the uterine environment in the porcine, and the possible involvement of retinoids and trophoblast during this critical stage. Although, in general, much is known concerning the pronounced effects of retinoids on cellular differentiation and embryonic development, little is known concerning the role of these compounds in blastocyst development, and concerning their metabolism in extra-embryonic tissues.

The trophoblast of the pre-implantation embryo is the first differentiated tissue, whose segregation is essential for embry-

Abbreviations used in this paper: EC, embryonal carcinoma; ES, embryonic stem; HPLC, high pressure liquid chromatography; RA, retinoic acid; RARE, retinoic acid-responsive element; X-gal, 5-bromo-4-chloro-3-indolyl-b-D-galactopyranoside.

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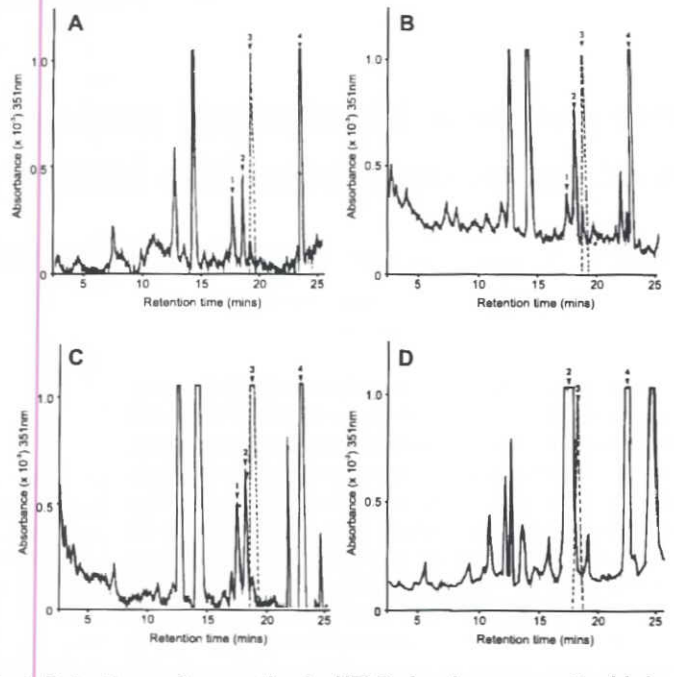

Fig. 1. Detection and separation by HPLC of endogenous retinoids in extracts of blastocysts, TE1 cells and uterine fluids. **(A)** *Intact, day 10 blastocysts;* **(B)** *TE1 cell line;* **(C)** *day 4 uterine fluid and* **(D)** *day 12 uterine fluid. Solid and dashed lines represent the data output from the UV detector and radioisotope detectors, respectively. Arrows denote the following retinoids; 13-cis-retinoic acid (arrow 1), retinol/9-cis-RA (arrow 2), all-trans-RA (arrow 3), and an internal standard, retinol acetate (arrow 4). Detection of radiolabeled all-trans-RA is indicated (dashed line).*

onic development (Pedersen, 1986). The pig is one of the few species for which stable lines of trophectoderm cells have been derived (Fléchon *et al.*, 1995); this may arise from the peculiarities of the prolonged, post-hatching, peri-implantation stage. The time-course of development of the porcine blastocyst is such that hatching occurs at day 6 of pregnancy, and the blastocysts become dispersed within the two uterine horns. By day 10 of gestation, the trophoblast becomes hyperplastic and is remodelled, eventually to form a distended, tube-like structure about 1 m in length. It consists of polarized epithelium, with a defined basement membrane, desmosomes, and tight junctions which seal the apical surfaces of trophectoderm cells (Reima *et al.*, 1993). During this time the inner cell mass (ICM) forms a quiescent embryonic disc, consisting of primary ectodermal cells. The trophoblast is non-invasive throughout gestation, and placentation is classified as epitheliochorial (Dantzer, 1985).

Detection of retinoids in the entire blastocyst, in TE1-cell extracts and in uterine fluid, by HPLC analysis

High pressure liquid chromatography (HPLC) was used to detect and separate retinoids in extracts of entire, day 10 blastocysts, in extracts of cultured TE1 cells, and in uterine fluids collected at days 4 and 12 of pregnancy (Fig. 1A-D). This method of analysis is based on absorbance spectra at 351 nm, and does not discriminate between 9-*cis*-RA and retinol (Horton and Maden, 1995); in Figure 1 absorbance peaks are indicated which correspond to 13-*cis*-RA (arrow 1), retinol (arrow 2), all-*trans*-RA (arrow 3), and an internal standard, retinol acetate (arrow 4). Radiolabeled all-*trans*-RA was added to samples as a second internal standard, and its elution position is indicated (dashed line).

From Figure 1A, retinoids are detected in extracts of day 10 blastocysts, with peaks of absorbance corresponding to 13-*cis*-RA (arrow 1), retinol (arrow 2), and all-*trans*-RA (arrow 3). These compounds were detected also in TE1-cell extracts (Fig. 1B), and in day 4 uterine fluids (Fig. 1C). For day 12 uterine fluids, the peaks for 13-*cis*-RA, all-*trans*-RA and retinol had merged (Fig. 1D) owing to a large increase in levels of retinol.

Levels of retinoids detected in Figure 1 were quantified, and are expressed in Table 1. Owing to light-induced isomerization of all-*trans*-RA to 13-*cis*-RA, which would have occurred during the collection of samples, levels of these isomers are expressed jointly as 'total RA'. Extracts from entire day 10 blastocysts and from TE1 cells contained similar levels of total RA, at concentrations of 36.2 nM and 35.4 nM, respectively. Levels of retinol in extracts of blastocysts and TE1 cells were 48.9 nM and 122.3 nM, respectively. The collection of uterine fluids by perfusion results in their dilution by an approximate factor of 20; and so the concentration of total RA in the uterine fluid at day 4 is calculated to be approximately 54 nM. For day 12 uterine fluid, however, the level of retinol was so high that the total RA levels could not be quantified. Adjusted concentrations of retinol in day 4 and 12 uterine fluids were 94 nM and 272 μM, respectively, in accordance with previous findings (Trout *et al.*, 1992).

TE1 cells and explants of blastocysts secrete RARE-activating factor(s)

To establish the trophectoderm as a source of functionally-active retinoids, and to determine functional concentrations, assays were performed using the F9-RARE-*lacZ* reporter system of Wagner *et al.* (1992). This assay uses F9 embryonal carcinoma (EC) cells which have been transformed with a reporter construct, Sil-REM/β-gal-NEO, comprising an *E.coli lacZ* gene under the control of a retinoic-acid-responsive element (RARE; de The *et al.*, 1990). Thus retinoids, either present in conditioned medium or secreted by explants of embryonic tissue, will activate expression of the *LacZ* gene when placed on a confluent monolayer of reporter cells, via the RARE. (This assay does not discriminate between isomers or metabolites of RA and retinol, but rather identifies functional retinoid activity.) From Figure 2, TE1-cell-conditioned medium induced an activity equivalent to treatment of reporter cells with 5 nM RA; explants of trophectoderm from day 10 blastocysts induced activity in reporter cells immediately below and adjacent to the explant, at levels equivalent to treatment with 10 nM all-*trans*-RA; and explants of embryonic discs induced local activity equiva-

TABLE 1

QUANTIFICATION OF TOTAL RETINOIC ACID (13-*CIS*-RA AND ALL-*TRANS*-RA) AND RETINOL BY HPLC

	Total RA (13-*cis*-, all-*trans*-)	Retinol
Day 10 blastocysts (n=1)	36.2 nM	48.9 nM
TE1-cell extracts (±s.e.m., n=4)	35.4 ± 0.5 nM	122.3 ± 25 nM
*Day 4 uterine fluid (n=2)	54.0 nM	94.0 nM
*Day 12 uterine fluid (n=1)	—	272.0 μM

*concentrations adjusted for a dilution factor of 20.

lent to treatment with 5 nM all-*trans*-RA. This latter, retinoid activity may have been produced by either residual trophectoderm or endoderm tissue, either of which might adhere to the embryonic discs, as well as by the primary ectoderm cells.

In the day 10 blastocyst the other cells types which are present are endodermal or primary ectodermal. Thus, the possibility arises that these cells also are sites of retinoid accumulation. However, these cell types are outnumbered by trophectoderm cells by approximately 100 to 1 at this stage of development (as revealed by differential staining techniques, results not shown); and from the amounts of retinoid secreted by primary trophectoderm and TE1 cells in culture (10 nM and 5 nM, respectively), we conclude that the trophectoderm is the most significant source of these compounds.

It should be emphasized here, in connection with Table 1 and Figure 2, that direct comparisons cannot be made between the levels of endogenous retinoids accumulated by trophectoderm cells in the intact blastocysts and those accumulated by TE1 cells in culture, owing to the difference in environments to which these cells were exposed. In the intact blastocyst, retinoids are accumulated from the uterine fluid, and in cultured TE1 cells, from the culture medium (in which RA-equivalent activity was present at a level of 10 nM, as determined by the reporter-cell assay). Rather, the data confirm the capacity of these tissues to accumulate and secrete retinoids.

In conclusion, our study establishes that the hatched, pre-implantation porcine blastocyst contains all-*trans*-RA and its precursor, retinol. This is the first report of endogenous RA in a mammalian blastocyst: to date, it is only for blastocysts of *Xenopus laevis* (Chen *et al.*, 1994) and zebrafish embryos (Costaridis *et al.*, 1996) that endogenous retinoids have been reported. We have identified that the trophoblast is a major source of retinoids, in addition to factors of hormonal, metabolic and immunological importance in pregnancy (Chaouat *et al.*, 1983; La Bonnardière 1993). In the blastocyst, the trophectoderm forms an impermeable epithelium around the blastocoelic cavity (Ducibella *et al.*, 1975). This cavity therefore constitutes a microenvironment for the primary ectoderm, as well as for the exposed, basolateral surface of the trophectoderm. In theory, RA secreted by the trophectoderm into the blastocoele could affect the differentiation of the primary ectoderm, or the delamination of endoderm from ectoderm (Hogan *et al.*, 1981): it is well-established that RA-treatment of murine EC cells and ES cells induces endoderm formation (Mummery *et al.*, 1990), and murine ES cells cultured on monolayers of TE-1 cells were found rapidly to differentiate (not shown). Thus, we propose that the finding of endogenous retinoids in the porcine blastocyst is relevant to mechanisms involved in early differentiation of cells of the ICM, including both the differentiation of primitive endoderm and the formation of the embryonic disc, and to mesoderm specification and gastrulation (Knezevic *et al.*, 1995). Furthermore, these studies support the involvement of retinoid in the regulation of trophoblast morphogenesis in the pig (Yelich *et al.*, 1997), as was suggested for the human (Stephanou *et al.*, 1994).

Experimental Procedures

Collection of porcine blastocysts

All pigs used in this study were of the Large White strain, maintained at the Babraham Institute. Blastocysts were collected surgically from gilts on day 10 (where the day of oestrus is designated as day 0), by perfusion of uterine horns using modified Dulbecco's phosphate-buffered saline. Blastocysts were maintained in the perfusion medium at 37°C, without CO_2, prior to experimentation.

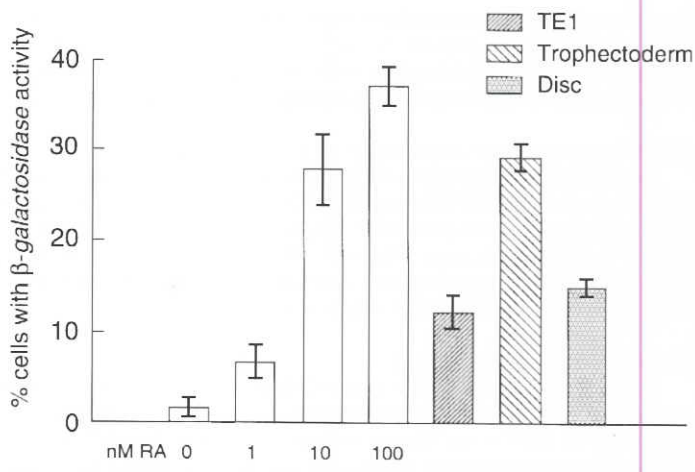

Fig. 2. Quantification of retinoids produced by explants of day 10 blastocysts, or present in TE1 cell-conditioned medium, by F9-RARE-*lacZ* reporter assay. *Reporter cells were assayed for b-galactosidase activity, and the percentage of b-galactosidase-positive cells were estimated by counting fields of 600 cells. Open bars indicate β-galactosidase activity in reporter cells treated with 0 to 100 nM all-trans-RA; 'TE1', reporter cells treated with TE1 conditioned medium; 'Trophectoderm', reporter cells cocultured with trophectoderm explants; 'Disc', reporter cells cocultured with embryonic discs. Each value is the mean ± s.e.m. of three estimations. The experiment was repeated once with similar results.*

Maintenance of the porcine trophectoderm cell line, TE1

The derivation and characterization of the porcine trophectoderm cell line, TE1, has been described (Fléchon *et al.*, 1995). This cell line was maintained on feeder layers of mitotically-inactivated, SNL mouse fibroblasts (provided by Dr. A. Bradley). The culture medium was Dulbecco's modified Eagle's Medium (DMEM), supplemented with 5% foetal-calf serum and 10% newborn-calf serum, both having been heat-inactivated at 56°C for 30 min. To this medium was added 0.1 mM β-mercaptoethanol, penicillin (50 units/ml) and streptomycin (50 μg/ml). Culture was at 37°C, with 5% CO_2. Tissue-culture dishes were pre-coated with a solution of 0.1% (w/v) type I swine-skin gelatin (Sigma). TE1 cells were passaged by disaggregation using 0.25% (w/v) trypsin in 0.04% (w/v) EDTA, and propagated onto fresh feeder layers. TE1 cells were passaged one in five, at weekly intervals, or at attainment of confluency.

Extraction and HPLC analysis of samples

Intact day 10 blastocysts were collected, washed twice in PBS, and immediately frozen under liquid nitrogen pending HPLC analysis. Uterine perfusion medium was collected at days 4 and 12 of pregnancy, by perfusion of uterine horns with 20 ml of perfusion medium, as described above. The perfusion medium was stored under liquid nitrogen pending HPLC analysis. Retinoids were extracted from approximately 30 entire blastocysts, from 10^7 TE-1-cells, and from uterine fluids according to Thaller and Eichele (1987), as follows: samples were sonicated in an equal volume of ice-cold stabilizing buffer, with 40 ng retinol acetate (Sigma) added as an internal standard, and extracted twice in 2 volumes of solvent (20 minutes/extraction) by continuous mixing on a vibromax. The solvent phases were pooled after low speed microcentrifugation, dried under nitrogen, and resuspended in 100 μl methanol. All solvents were HPLC grade (BDH. Inc., Lutterworth Leicestershire, UK). Finally, the extracts were transferred to HPLC autosampler vials, and approximately 2 nCi of ^{3}H-radiolabeled all-*trans*-RA (New England Nuclear, Boston MA) was added.

Separation and analysis of extracts was achieved using Beckman System Gold hardware and software. Briefly, 50 μl of tissue extract was auto-injected onto a 5 μm, C-18 reverse phase column (LiCrospher 100, Merck) with an equivalent pre-column, and separated at 1 ml/minute using

the following mobile phase B (acetonitrile:methanol, 3:1) rising linearly to 100% mobile phase B over 25 minutes. The eluent was monitored at 351 nm in series with a radioisotope detector equipped with a solid scintillant. This extraction method results in an average 70% recovery of retinoids.

Dissection of porcine blastocysts for explant culture

Embryonic discs and trophectoderm were micro-dissected from day 10 blastocysts, immediately following collection, and explanted into co-culture with monolayers of F9 reporter cells, see below. The processes of dissection of trophectoderm and embryonic discs took 5 min and 15 min, respectively. Either type of explant would attach loosely to the underlying monolayers of F9 reporter cells within 4 h of seeding.

F9-RARE-lacZ reporter assay

Reporter cells (provided by Dr. M. Wagner and Dr. C. Redfern) were maintained routinely on gelatinized dishes and in selective medium, consisting of DMEM (as above) supplemented with 0.8 mg/ml G418 (Gibco). For retinoid assays, confluent monolayers of reporter cells were incubated in serum-free, RPMI 1640 medium, containing 2 μl/ml N3 supplement (Hank's Balanced Salt Solution supplemented with 1 mg/ml Bovine Serum Albumin, 20 mg/ml Human Transferrin, 3.2 mg/ml Putrescine dihydrochloride, 1 μg/ml Sodium Selenite, 2 μg/ml Triiodothyronine, 1 mg/ml Insulin, 1.2 μg/ml Progesterone, 4 μg/ml Corticosterone Grade A; Sigma). Either explants of porcine embryos, previously washed in serum-free medium, or serum-free medium conditioned by TE1 cells overnight, were added to the cultures, and cultures were incubated overnight. For each experiment, the dose-response of reporter cells to retinoid was established by treatment with all-*trans*-RA at concentrations of 1 to 100 nM (using a stock solution of all-*trans*-RA (Sigma) in DMSO, aliquoted and stored at -20°C). As reported by Wagner *et al.* (1992), concentrations of RA higher than 100 nM RA resulted in a decrease in the percentage of β-*galactosidase*-positive cells (not shown); and we observed that the maximum response was induced with 100 nM RA. Our limit of detection was 1 nM all-*trans*-RA (not shown). After incubation, explants were removed carefully, and reporter cells were fixed and assayed for *β-galactosidase* synthesis using the conventional X-gal assay (Lim and Chae, 1989), where *LacZ* gene expression is inferred from blue staining resulting from exposure of the transfected cells to the substrate 5-bromo-4-chloro-3-indolyl-β-D-galactopyranoside (X-Gal). Retinoid was quantified as RA-equivalent activity, by counting the proportion of 600 cells expressing *β-galactosidase* in a given area, or immediately below and adjacent to explants, compared to RA-treated control samples. Three areas were analyzed per sample.

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References

CHAOUAT, G., KOLB, J.P. and WEGMANN, T.G. (1983). The murine placenta as a immunological barrier between the mother and the fetus. *Immunol. Rev. 40: 31-60.*

CHEN, Y., HUANG, L. and SOLRUSH, M. (1994). A concentration gradient of retinoids in the early *Xenopus laevis* embryo. *Dev. Biol. 16*: 70-76.

COSTARIDIS, P., HORTON, C., ZEITLINGER, J., HOLDER, N. and MADEN, M. (1996). Endogenous retinoids in the zebrafish embryo and adult. *Dev. Dynamics 205: 41-51.*

DANTZER, V. (1985). Electron microscopy of the initial stages of placentation in the pig. *Anat. Embryol. 172:* 281-293.

DE THE, H., DEL MAR VIVANCO-RUIZ, M., TIOLLAIS, P., STUNNENBERG, H. and DEJEAN, A. (1990). Identification of a retinoic acid responsive element in the retinoic acid receptor β gene. *Nature 343:* 177-180.

DUCIBELLA, T., ALBERTINI, D.F., ANDERSON, E. and BIGGERS, J.D. (1975). The preimplantation mammalian embryo: characterization of intercellular junctions and their appearance during development. *Dev. Biol. 45: 231-251.*

FLÉCHON, J-E., LAURIE, S. and NOTARIANNI, E. (1995). Isolation and characterization of a feeder-dependent, porcine trophectoderm cell line obtained from a nine-day blastocyst. *Placenta 16:* 643-658.

GOODMAN, D.S. (1984). In *The Retinoids* vol. 2 (Eds. M.N. Sporn, A.B. Roberts, A.B. and D.S. Goodman). New York: Academic Press. pp. 42-88.

GUDAS, L.J. (1994). Retinoids and Vertebrate Development. *J. Biol. Chem. 269:* 15399-15402.

HOGAN, B.L.M., TAYLOR, A. and ADAMSON, E. (1981). Cell interactions modulate embryonal carcinoma cell differentiation into parietal or visceral endoderm. *Nature 291:* 235-237.

HORTON, C. and MADEN, M. (1995). Endogenous distribution of retinoids during normal development and teratogenesis in the mouse embryo. *Dev. Dynamics 202: 312*-232.

KNEZEVIC, V., RANSON, M. and MACKEM, S. (1995). The organizer-associated chick homeobox gene, GNOT1, is expressed before gastrulation and regulated synergistically by activin and retinoic acid. *Dev. Biol. 171: 458-470.*

KRAFT, J.C., SCHUH, T., JUCHAU, M. and KIMELMAN, D. (1994). The retinoic X receptor X receptor ligand, 9-*cis*-retinoic acid, is a potential regulator of early Xenopus development. *Proc. Natl. Acad Sci. USA 91:* 3067-3071.

LA BONNARDIERE, C. (1993). Nature and possible functions of interferons secreted by the preimplantation pig blastocyst. *J. Reprod. Fertil. (Suppl.) 48: 157-170.*

LANGSTON, A.W., THOMPSON, J.R. and GUDAS, L.J. (1997). Retinoic acid-responsive enhancers located 3' of the Hox A and Hox B homeobox gene clusters. *Functional analysis.J. Biol.Chem. 272: 2167-75.*

LIM, K. and CHAE, C.B. (1989). A simple assay for DNA transfection by incubation of the cells in culture dishes with substrates for betagalactosidase. *Biotechniques 7:* 576-579.

MINUCCI, S., BOTQUIN, V., YEOM, Y.I., DEY, A., SYLVESTER, I., ZAND, D.J., OHBO, K., OZATO, K. and SCHÖLER, H. (1996). Retinoic acid-mediated down-regulation of *Oct3/4* coincides with loss of promoter occupancy *in vivo. EMBO J. 15:* 888-899.

MUMMERY, C.L., FEYEN, A., FREUND, E. and SHEN, S. (1990). Characteristics of embryonic stem cell differentiation: a comparison with two embryonal carcinoma cell lines. *Cell Differ. Dev. 30:* 195-206.

PEDERSEN, R.A. (1986). Potency, Lineage, and allocation in preimplantation mouse embryos. In *Experimental Approaches to Mammalian Embryonic Development* (Ed. J. Rossant and R.A. Pedersen). Cambridge University Press, Cambridge, pp. 3-34.

PIJNAPPEL, W.W.M., HENDRICKS, H.F., FOLKERS. G.E., VAN DEN BRINK, C.E., DEKKER, E.J., EDELENBOSCH, C. VAN DER SANG, P.J. and DURSTON, A.J. (1993). The retinoid ligand 4-oxo-retinoic acid is a highly active modulator of positional specification. *Nature 366:* 340-344.

REIMA, I, LEHTONEN, E, VIRTANEN, I and FLÉCHON, J-E. (1993). The cytoskeleton and associated proteins during cleavage, compaction and blastocyst differentiation in the pig. *Differentiation 54:* 35-45.

ROBERTS, R.M., XIE, S. and TROUT, W.E. (1993). Embryo-uterine interactions in pigs during week 2 of pregnancy. *J. Reprod. Fertil. (Suppl.) 48:* 171-186.

STEPHANOU, A., SARLIS, N.J., RICHARDS, R. and HANDWENEGER, S. (1994). Expression of retinoic acid receptor subtypes and cellular retinoic acid binding protein-II mRNAs during differentiation of human trophoblast cells. *Biochem. Biophys. Res. Comm. 202:* 772-780.

TROUT, W.E., HALL, J.A., STALLINGS-MANN, M.L., GALVIN, J.M., ANTHONY, R.V. and ROBERTS, R.M. (1992). Steroid regulation of the synthesis and secretion of retinol-binding protein by the uterus of the pig. *Endocrinology 130:* 2557-2564.

WAGNER, M., HAN, B. and JESSEL, T.M. (1992). Regional differences in retinoid release from embryonic neural tissue detected by an *in vitro* reporter assay. *Development 116:* 55-66.

WILDE, M.H., XIE, S., DAY, M.L. and POPE, W.F. (1988). Survival of small and large littermate blastocysts in swine after synchronous and asynchronous transfer procedures. *Theriogenology 30:* 1069-1074.

YELICH, J.V., POMP, D. and GEISERT, R.D. (1997). Detection of transcripts for retinoic acid receptors, retinol-binding protein, and transforming growth factors during rapid trophoblastic elongation in the porcine conceptus. *Biol. Reprod. 57: 286-294.*

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