Differential Expression, during the Estrous Cycle and Pre- and Postimplantation Conceptus Development, of Messenger Ribonucleic Acids Encoding Components of the Pig Uterine Insulin-Like Growth Factor System*

FRANK A. SIMMEN, ROSALIA C. M. SIMMEN, RODNEY D. GEISERT, FRANCOIS MARTINAT-BOTTE, FULLER W. BAZER, AND MICHEL TERQUI

Dairy Science (F.A.S.) and Animal Science (R.C.M.S., F.W.B.) Departments, University of Florida, Gainesville, Florida 32611; the Animal Science Department, Oklahoma State University (R.D.G.), Stillwater, Oklahoma 74078; and Laboratoire de Physiologie de la Reproduction, Institut National Recherche Agronomique (F.M.-B., M.T.), Nouzilly 37380, France

ABSTRACT. The temporal patterns of endometrial expression for mRNAs encoding insulin-like growth factor-I (IGF-I), IGF-II, IGF-binding protein-2 (IGFBP-2), and the type I IGF receptor (IGF-IR) were elucidated in cyclic and pregnant pigs. Peak levels of IGF-I mRNAs occurred on day 12 in cyclic and early pregnant gilts, while IGFBP-2 mRNA levels were lowest on day 10. Pregnant gilt endometrium had higher levels of both mRNA classes than the corresponding cyclic endometrium. IGF-II and IGF-IR mRNAs remained low during this period. In pregnant pig endometrium and rat uterus, levels of IGF-I mRNA decreased, while those of IGF-II and IGFBP-2 mRNAs increased with stage of pregnancy. Decreased endometrial production of IGF-I mRNA during pregnancy paralleled that in the myometrium. IGF-II mRNA tissue abundance was placenta > endometrium > myometrium. In contrast, IGFBP-2 mRNA levels were higher in endometrium than in placenta and myometrium. Endometrial expression of IGF-II mRNAs was limited to surface and glandular epithelial cells; epithelial and stromal cells expressed IGFBP-2 mRNAs at comparable levels. Expression of IGF-IR mRNAs was low and did not change with pregnancy. The endometria of two breeds of pigs that exhibit different levels of prolificacy were also examined for IGF mRNAs. On day 12, endometrium from the Large White breed with high conceptus mortality had higher levels of IGF-II and IGFBP-2 mRNAs than did endometrium from the Meishan breed with low conceptus mortality. Expression of IGF-I mRNAs was higher in endometria of Meishan than Large White gilts on day 12. The differential expression of IGF mRNAs with stage of gestation and the correlation of relative ratios of IGF mRNAs with prolificacy suggest an important role(s) for IGFs in conceptus and fetal development. (Endocrinology 130: 1547-1556, 1992)

THE INSULIN-like growth factors (IGF-I and -II) are implicated in control of proliferation and differentiation of the uterus in preparation for blastocyst implantation and during later feto-placental development (1–10). IGF-I and -II are abundantly expressed, at the level of their mRNAs, in the uteri of cycling and pregnant rodents, domestic species, and humans (1, 2, 6–8, 10). In addition, IGF-I and -II peptides are present at physiological levels in uterine secretions of a number of species during the perimplantation period (6, 9, 10).

Received October 10, 1991.
Address all correspondence and requests for reprints to: Dr. Frank A. Simmen, Dairy Science Department, Institute of Food and Agricultural Sciences, University of Florida, Gainesville, Florida 32611-0701.
* This work was supported by USDA Grant 89-37265-4545 (to F.A.S. and R.C.M.S.), a grant from the USDA Office of International Cooperation and Development, Division of Scientific and Technical Exchange (to F.W.B. and F.A.S.), and NIH Grant HD-21961 (to R.C.M.S.). This is Journal Series no. R-01974 of the University of Florida Agricultural Experiment Station.
IGF-I content, elongation in utero of spherical blastocysts to the filamentous morphology, and onset of conceptus secretion of estrogen, which is a paracrine regulator of endometrial function and the signal for maternal recognition of pregnancy (5, 6, 18–22). Low levels of IGF-II mRNAs are characteristic of porcine preimplantation endometrium, but uterine expression of these mRNAs increases by several orders of magnitude at an as yet undefined time postimplantation (12). Abundance of uterine IGF-I mRNAs, in contrast, declines after the implantation period (2, 6, 12). The hormonal and other regulatory signals that elicit the differential expression of uterine IGF-I and IGF-II mRNAs during pregnancy and in mature nonpregnant animals are not well defined. However, it is established that estrogens (in immature rats, immature pigs, and ovarioctomized mature pigs) and progesterone (in ovarioectomized mature pigs) can induce the accumulation of IGF-I mRNAs in the uterus without a concomitant increase in IGF-II mRNAs (1, 7, 8).

In the present study we characterized tissue-specific expression of several components of the uterine IGF system by monitoring in parallel, steady state levels of the mRNAs encoding IGF-I, IGF-II, IGFBP-2, and IGF type I receptor (IGF-IR) tyrosine kinase during the estrous cycle and pregnancy. In particular, we have elucidated 1) the temporal aspects of IGF mRNA accumulation in uterine endometrium of cycling and pregnant pigs, 2) the cell type-specific expression of IGF mRNAs in uterus at midgestation, and 3) the differential expression of IGF mRNAs in preimplantation uterine endometrium of prolific Chinese Meishan (MS) pigs compared to those in the less prolific European Large White (LW) pigs.

**Materials and Methods**

**Materials**

Specialty materials, reagents, and vendors used were: X-OMat RP and AR films (Eastman Kodak, Rochester, NY), Rapid Hybridization Buffer and nick-translation kits (Amer sham Corp., Arlington Heights, IL), NA45 (DEAE-cellulose) paper (Schleicher and Schuell, Keene, NH), Gene Clean II (Bio 101, Inc., LaJolla, CA), [α-32P]dCTP (3000 Ci/mmol; ICN Radiochemicals, Irvine, CA), yeast RNA (Sigma Chemical Co., St. Louis, MO), BioTrans nylon membranes (0.2 μm; ICN), and restriction endonucleases (Promega Corp., Madison, WI).

**Animals**

Yorkshire × Duroc × Hampshire gilts were allowed to experience at least two estrous cycles before assignment to the experiment. Gilts were mated when detected in estrus and 12 and 24 h later on days 0 and 1 of the estrous cycle. Day of pregnancy or cycle was determined by assigning day of onset of estrus as day 0. On the appropriate days postestrus, gilts were hysterectomized, and uterine endometrium and myometrium were obtained (5, 7). Placentas were manually stripped from pregnant uterus between days 30–105. Gilts of the MS and LW breeds were inseminated artificially 24 and 36 h or 12 and 24 h, respectively, after the onset of estrus (23, 24). All gilts experienced a minimum of three estrous cycles before insemination. Day 0 was considered the day of first insemination. Gilts were slaughtered, the reproductive tracts were immediately placed in ice, and oviducts were obtained. Uteri were flushed with 20 ml/horn 0.9% (wt/vol) saline to recover conceptuses, except on day 30, when each conceptus was removed by dissection. The presence of conceptuses in uterine flushings confirmed pregnancy. Endometrium was dissected from myometrium, and tissues were frozen in liquid nitrogen and stored at -70 C. Endometrial epithelial and stromal cells were isolated using a method developed for the bovine uterus.1 The upper one third sections of gravid uterine horns, stripped of the placenta, were used to isolate epithelial and stromal cells.

**Hybridization probes**

Purified cDNA fragments (Table 1) were radiolabeled with [α-32P]dCTP (3000 Ci/mmoll by nick translation. After incubation at 14 C, 100 μg yeast RNA were added, and the samples were extracted with phenol. Unincorporated [α-32P]dCTP was removed from DNA by gel filtration on a Sephadex G-50 column.

**RNA analysis**

RNA was isolated using the method of Puissant and Houdebine (28). This material was purified further by phenol extraction and ethanol precipitation and was resuspended in water and quantified by absorbance at 260 nm. RNA preparations were subjected to dot blot hybridization as follows. Total RNA (10 or 20 μg) was diluted in 250 μl 50% formamide-6% formaldehyde-20 mM Tris, pH 7.0, and heated at 65 C for 5 min. To each sample were added 250 μl 20 × SSC (1 × SSC = 150 mM NaCl and 15 mM sodium citrate, pH 7.0), followed by vortex mixing and centrifugation in a microcentrifuge for 5–10 sec. A BioTrans nylon membrane, prewetted in water and then in 10 × SSC, was placed in a microsample filtration device (Schleicher and Schuell) according to the manufacturer’s instructions. To each well were filtered in sequence, 500 μl 10 × SSC, RNA sample, and 500 μl 20 × SSC. Filters were air dried and baked at 80 C for 1–2 h.

Prehybridizations were performed in Rapid Hybridization Buffer supplemented with 200 μg/ml purified yeast RNA. Incubation (3–6 ml/filter) was performed at 61 C for 1–2 h. Overnight hybridization was performed at 61 C in fresh Rapid Hybridization Buffer containing 200 μg/ml yeast RNA and the radiolabeled denatured cDNA fragment (2 × 106 to 2 × 107 cpm/ml). After hybridization, filters were washed in 2 × SSC-0.1% sodium dodecyl sulfate at room temperature for 1–2 h and in 0.1 × SSC-0.1% sodium dodecyl sulfate at 61 C (high stringency) for 0.5–1 h. Hybridization was quantified by scanning densitometry of the autoradiograms. Differences in mRNA abundance were evaluated by use of Student’s t test (29). On

---

TABLE 1. Isolation of cDNA fragments used for nick translation

<table>
<thead>
<tr>
<th>Subclone</th>
<th>Restriction endonuclease</th>
<th>Length (basepairs)</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Porcine IGF-I (sigf.3)</td>
<td>EcoRI</td>
<td>580</td>
<td>2</td>
</tr>
<tr>
<td>Rat IGF-II (in pUC12)</td>
<td>PstI</td>
<td>780</td>
<td>25</td>
</tr>
<tr>
<td>Rat IGFBP-2 (in pGEM3)</td>
<td>HindIII</td>
<td>1200 (~530 basepairs of cDNA)</td>
<td>26</td>
</tr>
<tr>
<td>Human IGF-I receptor (pIGF-I-R.8)</td>
<td>EcoRI</td>
<td>700</td>
<td>27</td>
</tr>
</tbody>
</table>

Plasmid DNAs were purified via CsCl-ethidium bromide density-gradient centrifugation, and the corresponding cDNA fragments were excised by restriction endonuclease digestion and gel purified by use of Gene Clean II or electrophoresis onto DEAE-cellulose paper.

In most filters, yeast RNA was used as a control for nonspecific hybridization.

Results

Steady state mRNA levels were examined by dot blot hybridization of cDNA probes to total cellular RNA (see Materials and Methods). We chose to use this method since it allows simultaneous analysis of large numbers of RNA samples with multiple hybridization probes in the absence of a high degree of intra- and interassay variation. In addition, this method is readily optimized for use with heterologous nucleic acid probes, thereby eliminating the requirement for cloning of homologous DNA sequences. We modified the method to include use of Rapid Hybridization Buffer and high stringency (61°C; 0.1 X SSC) washes (see Materials and Methods) to facilitate the detection of low abundance mRNA transcripts with minimal or no background signal (6, 7). The level of sensitivity of our modified method is comparable to that of RNase protection assays (6, 7, 30). All cDNA probes were previously validated for specific hybridization to appropriately sized porcine mRNAs by Northern analysis (2, 7, 10, 30) (Lee, C.-Y., and F. A. Simmen, unpublished data).

Initially, total cellular RNAs extracted from 15 different tissues of a day 60 (midgestation) pregnant gilt were hybridized with IGF-I, IGF-II, and IGFBP-2 cDNAs to establish the extent of tissue IGF mRNA variation (Fig. 1). IGF mRNA levels across tissues were examined by applying a constant amount of total RNA to each membrane and comparing intensities of autoradiographic signals after hybridization with a given probe. Substantial tissue variation in IGF mRNA levels was apparent (Fig. 1). Colon, mammary glands, and whole uterus exhibited the highest levels of IGF-I mRNAs; in contrast, the highest levels of IGF-II and IGFBP-2 mRNAs were observed in whole uterus (Fig. 1).

The autoradiograms shown in Fig. 2 demonstrate hybridization of IGF cDNA probes to blots containing total RNA preparations from endometrial tissues of individual cycling and early pregnant gilts that were hysterecomitized on comparable days after estrus. Each day of the cycle or of pregnancy was represented by three animals (one RNA dot = one gilt) to monitor the temporal variation in steady state mRNA levels. Four replicate membranes were hybridized with the indicated DNA probes of specific activities in the range of 10^7-10^8 cpm/μg DNA.

In cycling gilts, IGF-I mRNA abundance in endometrium was lowest at estrus (day 0), increased ~6-fold by day 12, and thereafter declined by days 15 and 18 (Fig. 2). In pregnant gilts, endometrial IGF-I mRNA abundance was highest on day 12 and declined by days 15 and 18 after estrus (Fig. 2). Comparison of mean endometrial IGF-I mRNA levels between day 12 cycling and pregnant gilts indicated a higher level of IGF-I mRNAs in the pregnant animals (P < 0.05).

Temporally associated changes in endometrial IGFBP-2 mRNA abundance contrasted with those noted in IGF-I mRNA. In cycling gilts, IGFBP-2 mRNA abundance was highest around estrus (days 0 and 18), declined 6- to 8-fold by day 10 of the cycle, and began to increase at the approach of the next estrus (Fig. 2). In pregnant gilts, endometrial levels of IGFBP-2 mRNA were lowest on day 10, increased ~6-fold by day 15, and remained at this level on day 18. On day 15 postestrus, the endometrium of pregnant gilts had a 4-fold greater level of IGFBP-2 mRNA than did the corresponding nonpregnant gilt endometrium (P < 0.01; Fig. 2). In contrast, levels of the mRNAs encoding IGF-II and IGF-IR were low and relatively invariant in endometrium during the estrous cycle and early pregnancy (Fig. 2).

Hybridization analysis of IGF mRNAs in endometrium, placenta, and whole fetuses of a day 30 (early postimplantation) pregnant gilt was presented in Fig. 3. IGF-I mRNA levels were not different among these three tissues. Levels of IGF-II transcripts in placenta and fetuses were comparable and exceeded those in the corresponding endometrium. The relative abundance of IGFBP-2 mRNA was endometrium > fetuses > placenta. Levels of IGF-IR mRNAs were low and similar among these tissues.

RNA dot blot hybridizations were also performed to characterize the tissue-specific expression of the four IGF transcript classes in pig uterus and placenta with
FIG. 1. Steady state levels of IGF mRNAs in tissues of a midpregnant gilt, as determined by RNA dot blot hybridization and autoradiography. Three replicate RNA-containing nylon membranes were hybridized with the indicated radioactive cDNA probes (see Materials and Methods). Each dot represented 10 μg total cellular RNA applied to the membrane. Hybridization probes varied in specific activity, and autoradiographic exposure times differed for each panel.

Stage of pregnancy and the uterine cell type-specific expression on day 60 of pregnancy (Fig. 4). RNA preparations from pooled whole uteri of late pregnant rats were included for comparison. IGF-I mRNA transcripts in endometrium were highest during the preimplantation period, but were undetectable by midgestation (Fig. 4). Epithelial and stromal cells isolated from the endometrium of a day 60 pregnant gilt did not have detectable IGF-I mRNA expression. Levels of IGF-I mRNAs in the myometrium exceeded those in the endometrium during preimplantation stages and remained detectable later in pregnancy (days 75 and 105; Fig. 4). A lower level of IGF-I mRNAs was observed in the placenta than in the myometrium postimplantation. IGF-I mRNA abundance in rat uteri declined by ~41% from days 14 to 19 of pregnancy (Fig. 4).

Levels of IGF-II mRNA in endometrium and myometrium were highest during midgestation (Fig. 4). However, on all days of pregnancy examined, the relative tissue expression of IGF-II mRNAs was placenta > endometrium > myometrium (Figs. 3 and 4). In the endometrium during midgestation, IGF-II transcripts were significantly more abundant in isolated endometrial surface and glandular epithelial cells than in isolated endometrial stromal cells. Rat uteri expressed IGF-II mRNAs, the levels of which increased by ~83% with progression of late pregnancy.

In endometrium, IGFBP-2 mRNAs were also expressed at the highest levels during midgestation (Fig. 4). On all days examined, IGFBP-2 mRNA levels in endometrium exceeded those in the corresponding myometrium and placenta (Figs. 3 and 4). Isolated endometrial epithelial and stromal cells had comparable levels of this mRNA (Fig. 4). Rat uteri expressed IGFBP-2 mRNA, the levels of which increased by about 32% with progression of late pregnancy. The abundance of IGF-IR transcripts was low and did not vary by tissue, uterine cell type, or day of pregnancy (data not shown).

IGF mRNA levels in preimplantation endometrium of two pig breeds known to differ in prolificacy were also determined. The European LW breed is characterized by a high degree of conceptus mortality in early pregnancy; in contrast, conceptuses from the prolific Chinese MS breed exhibit more rapid and uniform development and greater survival rates in early pregnancy (23, 24, 31). Endometrial and oviductal RNA preparations from gilts of each breed on the indicated days of pregnancy were subjected to RNA dot blot hybridization (Fig. 5). IGF-I mRNA levels for endometrium were highest on day 12, were nearly undetectable by day 30 (13.5-fold difference in signal; day 12 vs. day 30), and did not differ among the two breeds, except for day 12 tissues (MS > LW, P < 0.05; Fig. 5). Oviductal IGF-I mRNA levels were lower than endometrial IGF-I mRNA levels, except for day 30 tissues, and did not vary by day of pregnancy between the two breeds.

On day 12 of pregnancy, levels of IGF-II mRNAs in endometrium of LW gilts exceeded those in endometrium of MS gilts (P < 0.01; Fig. 5). Endometrial IGF-II mRNA levels were greater than those in the oviduct; however, breed differences in oviductal IGF-II mRNA abundance were not apparent. In both breeds, endometrial IGFBP-2 mRNA abundance declined from days 4-10 and then increased by day 30 (Fig. 5). Endometrial IGFBP-2 mRNA abundance was greater, however, for LW than for MS gilts (P < 0.05) on day 12. In oviducts, IGFBP-2 mRNA abundance appeared to increase from days 10-12 and then decline by day 30, with no breed differences observed (Fig. 5). IGF-IR transcript levels did not differ among days, tissues, or breeds (data not shown).

Discussion

The uterus undergoes growth as well as morphological and functional differentiation during the estrous cycle.
FIG. 2. Hybridization analysis of endometrial RNAs from cycling and early pregnant pigs. Four replicate RNA-containing dot blots (A-D) were individually hybridized with the indicated radioactive cDNA probes (see Materials and Methods). Each dot represents 10 μg endometrial RNA from a single animal, with three animals used on each of days 0, 5, 10, 12, 15, and 18 of the estrous cycle and days 10, 12, 15, and 18 of pregnancy. DNA probes were of variable specific activity, and autoradiographic exposure times differed for each panel. Autoradiograms were subjected to densitometry in order to quantitate fold differences in signal among the samples within a filter. For each probe, the group of dots with the lowest mean signal intensity was assigned a value of 1, and all other group means were expressed as a ratio to the lowest value (E). Also included (E) are the reported values for circulating estradiol and progesterone in cyclic pigs (47) on the days studied here.

and pregnancy (1, 3, 18, 20). The potential importance of polypeptide growth factors in regulating uterine and conceptus growth has been the subject of much recent research (reviewed in Refs. 11–13). In an effort to define further the involvement and interactions of the IGFs in these processes, we have characterized the steady state...
levels of mRNAs transcribed from IGF-I, IGF-II, IGFBP-2, and IGF-IR genes in porcine uterus during the estrous cycle and pregnancy. The results of our previous studies indicated the differential expression of IGF-I, IGF-II, and IGFBP-2 genes in whole uteri of early pregnant gilts, with the latter two genes being highly expressed, at the level of their mRNAs, postimplantation (2, 6, 7, 12). However, a study of IGF mRNA expression in the uterus throughout the estrous cycle and in distinct uterine tissue compartments and corresponding placenta during pregnancy has not been reported. Similarly, uterine expression of mRNAs encoding the IGF-IR had not been examined.

Previously, we established that endometrial IGF-I mRNA abundance in pregnant gilts increases from days 8–12, decreases by day 14, and declines further by day 30 (6). The changes in levels of endometrial expression of IGF-I mRNAs from days 8–14 are paralleled by comparable changes in uterine luminal fluid IGF-I content (5, 6). The present results demonstrate that the rise in IGF-I mRNA levels, with a peak on day 12 and a subsequent decline by days 14–15, is also characteristic of the nonpregnant pig endometrium. These temporal changes probably represent a hormonally programmed process in the uterus that is specific for IGF-I mRNAs, since mRNAs encoding IGF-II, IGFBP-2, or IGF-IR did not change in a similar fashion. IGF-I mRNA levels can be correlated with the changes in circulating progesterone concentration during the luteal phase (days 7–17) of the estrous cycle. In contrast, uterine IGF-I mRNA abundance in cycling rats is highest at proestrus, coincident with maximal levels of circulating estrogens (8).

In pregnant gilts, endometrial IGF-I mRNA is undetectable at mid- to late gestation. However, myometrial expressed IGF-I transcripts are abundant at least through midgestation. This finding indicates the differential production and/or stability of IGF-I mRNAs in uterine myometrium vs. endometrium during progression of pregnancy. In nonpregnant rats, IGF-I mRNAs are expressed in myometrium and in endometrial stroma and epithelium (32). Our data indicate a decline in rat uterine IGF-I mRNA levels during late pregnancy. This mimics the reported temporal changes in rat placental IGF-I mRNA levels, which decline after day 10 of pregnancy (33).

A marked induction of uterine IGF-II mRNA accumulation in the pregnant pig occurs after implantation, similar to that reported for IGF-II mRNA in human trophoblasts (34). In pregnant rats, placental expression of IGF-II mRNAs is undetectable before day 10, increases beginning on day 13, and reaches maximal levels at days 17–20 (33). Rat uterine IGF-II mRNA levels are also elevated in late pregnancy (this study). Thus, in the uterus and placenta of the pig and rat, IGF-I, rather than IGF-II, mRNA appears to predominate in early pregnancy.

IGFBP-2 mRNA abundance in endometrium varied markedly with day of the estrous cycle. The levels of IGFBP-2 mRNA were highest and lowest, respectively, at estrus and during the luteal phase, concomitant with the highest and lowest ratios of plasma estrogen to progesterone, respectively. Indeed, the increase in IGFBP-2 mRNA abundance during days 12–18 of pregnancy can be correlated with conceptus estrogen synthesis (18, 20–22). In ovaries of cycling gilts, IGFBP-2 mRNA levels do not differ in the midluteal, late luteal, or late follicular stages (35). Similarly, levels of oviductal IGFBP-2 mRNA levels do not change in concert with the corresponding endometrial mRNAs in pregnant gilts (this study). Thus, the cyclicity in steady state levels of IGF-1 and IGFBP-2 mRNAs appears to be an endometrium-specific process. A recent report (36) has described the stimulatory effects of progesterone and estrogen on IGFBP-2 secretion by human endometrial stromal cells in vitro, further supporting a role for steroid hormones in IGFBP-2 biosynthesis.

IGFBP-1, an IGFBP distantly related to IGFBP-2, is
FIG. 4. Temporal, cell type-specific, and tissue-specific expression of IGF mRNAs. Replicate RNA containing dot blots were hybridized with the indicated DNA probes. Dots represented 20 μg RNA from the various tissues and cell sources that are schematically indicated in the key. RNA preparations were from endometrium and myometrium of two day 12 pregnant pigs; endometrial surface epithelial cell, glandular epithelial cell, and stromal cell preparations from a day 60 pregnant pig; endometrium, myometrium, and placenta of one day 75 and one day 105 pregnant pig; pools of whole uteri of day 14, 16, and 19 pregnant rats; and yeast.
a well characterized secretory product of the progestational uterine endometrium (3, 4, 37). Antibodies to IGFBP-1 stain the uterine glandular epithelium of cycling baboons (4) and the luminal epithelium of preimplantation stage sheep endometrium (38). The uterine expression of IGFBP-2 is less characterized. Results from the present study demonstrate that expression of IGFBP-2 mRNA in pig uterus is relatively specific to the endometrium; little or no expression of this mRNA was detected in myometrium and placenta. In the rat at late gestation, however, IGFBP-2 mRNA is more abundant in placenta than in whole uterus (39). The differential expression of IGFBP-2 and other uterine-expressed genes in the pig is also noteworthy. For example, uteroferrin, a transplacental iron transport protein, is highly expressed at the level of mRNA in postimplantation pig uterus (40). Uteroferrin mRNAs, unlike IGFBP-2 mRNAs, are present at comparable levels in endometrium and myometrium, but are absent in placenta (41). Similarly, mRNAs encoding the protease inhibitor antileukoproteinase are expressed at low levels in porcine preimplantation uterus and at much higher levels in mid- and late gestation endometrium and myometrium, but not in placenta (41, 42). In contrast, IGF-IR mRNAs were constitutively expressed in endometrium and myometrium throughout the estrous cycle and pregnancy, which agrees with the reported constitutive nature of functional IGF-IR proteins in membranes of porcine uterus (17). Thus, IGFBP-2 is somewhat unique in its apparent restricted expression within the endometrium.

The molecular basis for differences in prolificacy between the Chinese MS and European LW breeds of pigs

Fig. 5. Hybridization analysis of IGF mRNAs in uterine endometria and oviducts of pregnant LW and Chinese MS pigs. Each dot represented 20 μg RNA isolated from endometrium or oviduct of an individual pig. With the exception of the day 4 MS endometrium group (n = 3) and the day 30 groups (n = 2/breed), all groups had four gilts. Spatially, the position of endometrial RNA matched that of the corresponding oviductal RNA of each individual animal, and this organization was consistent for each replicate filter (A–C). Total RNA from yeast (four dots directly below those for the day 30 endometrial RNA preparations) constituted controls for background hybridization. Autoradiograms were subjected to scanning densitometry to quantitate relative hybridization for each group of samples (see text).
is unclear (23, 24, 31). A combination of maternal and fetal traits is thought to account for these differences, although the contributing genes remain unknown (31). As shown previously, the more rapid trophoblast elongation of MS conceptuses in utero compared to that of LW conceptuses is related to the correspondingly greater uterine luminal fluid content of estrogens and interferons, which are products of the elongating conceptuses (43, 44). Since conceptus elongation and production of estrogens are coincident with estrogen-stimulated endometrial release of IGF-I (7), a physiological role for IGFs in conceptus development may be hypothesized. The close temporal association of these biological events in the MS gilts (5) is consistent with this hypothesis.

Increased levels of endometrial IGF-II and IGFBP-2 mRNAs were noted for LW vs. MS gilts on day 12 of pregnancy. This contrasts with the greater levels of endometrial IGF-I mRNAs and ULF IGF-I proteins in MS than in LW gilts on the same day of pregnancy (Ref. 5 and this study). IGF-I, which acts as a placental growth factor (16, 33, 45), is a known stimulator of pig conceptus P450 aromatase activity (22). IGF-II, also postulated to function as a placental growth factor, has recently been shown to inhibit human placental P450 aromatase activity in vitro (33, 34, 45, 46). Thus, we speculate that the ratio of IGF-I to IGF-II content in uterine luminal fluid, rather than absolute concentrations of either growth factor, regulates the level of secretion of conceptus estrogens. Given the importance of these estrogens in the temporal progression of development of conceptuses in utero, it is interesting to speculate that the relative levels of IGF-I and IGF-II may be responsible in part for the different rates of development of conceptuses between the two breeds.

In summary, the differential expression of endometrial IGF-I and IGF-II mRNAs during pregnancy suggests preferential roles for IGF-I at preimplantation and for IGF-II at postimplantation stages, respectively. IGF I may function to regulate endometrial remodelling during the oestrous cycle and implantation, while IGF-II is likely to mediate growth and differentiation of endometrium and placentas during fetal development. Endometrium-expressed IGFBPs, rather than the constitutively expressed IGF-IR, may play a major part in modulating the actions of the IGFs at the fetomaternal interface and within the individual tissue compartments.

Acknowledgments

We thank Cheryl Feinstein and Frank Michel for expert technical assistance, Mary Ellen Hissem for secretarial support, Kal Feinstein for facilitating import of RNA preparations into the U.S., other members of our laboratories for assistance with animal management and surgeries, Michael Zavy for sharing his procedure for the isolation of uterine cell populations before publication, Kathleen Shiverick and Susan Ogilvie for providing rat uterine tissues, Matthew Rechler for use of IGF-II and IGFBP-2 cDNA clones, and Axel Ullrich for use of the IGF-IR cDNA clone.

References

between preimplantation development of the pig blastocyst and uterine endometrial secretions. Biol Reprod 27:925–939
34. Wahlstrom T, Seppala M 1984 Placental protein 12 (PP12) is induced in the endometrium by progestrone. Fertil Steril 41:781–784
47. Wahlstrom T, Seppala M 1984 Placental protein 12 (PP12) is induced in the endometrium by progestrone. Fertil Steril 41:781–784
61. Van De Wiel DFM, Erkens J, Koops W, Van Landeghem...