Role of the Insulin-Like Growth Factor System in Uterine Function and Placental Development in Ruminants

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ABSTRACT

Maternal nutrition during pregnancy influences fetal and placental weights. The insulin-like growth factors (IGF) are also important determinants of fetal size. Furthermore, the expression of several components of the IGF system is regulated by nutrition. Effects of nutrition on fetal growth could therefore be mediated by the IGF system in the uterus and placenta. The oviductal mucosa produces IGF-I, which may influence oviductal secretions or act directly on embryonic type 1 IGF receptors. In the uterus, IGF-I mRNA is localized to the stroma surrounding the endometrial glands, which contain high concentrations of IGF type 1 receptors. Uterine IGF-I concentrations fall during pregnancy; therefore, glandular activity is more likely influenced by systemic than local IGF-I production. The IGF-II mRNA is present in both caruncles and fetal placental mesoderm, but concentrations are much higher in the latter. The actions of IGF-I and IGF-II on the endometrium and placenta are influenced by IGF-binding proteins. In the ewe, mRNAs for IGF binding protein-1 and -5 are located in the luminal and glandular epithelia, IGF binding proteins-2 and -4 are produced in the subepithelial stroma, and IGF binding protein-4 is also in the placentome capsule; IGF binding protein-3 is more widely expressed in both maternal and fetal tissues. The IGF binding proteins, therefore, form a major barrier to the passage of IGF between the fetal and maternal circulatory systems. (**Key words**: insulin-like growth factors, uterus, placenta, oviduct)

Abbreviation key: **GH** = growth hormone, **IGFBP** = IGF binding protein, **IGF-1R** = type 1 IGF receptor, **IGF-2R** = type 2 IGF receptor.

INTRODUCTION

Insulin-like growth factors are produced locally in many organs of the body, where they influence both metabolic and proliferative activities (21, 23). The synthesis of most components of the IGF system can in turn be regulated through nutrition (62). This relationship makes the IGF-I and IGF-II suitable intermediaries to match growth and development of an animal to its nutritional status. Both IGF-I and IGF-II have also been implicated as important regulators of both preimplantation and placental development. Local production of various members of the IGF system has been reported in the uterus and placenta of several species, including rat (73), human (19, 73), pig (56), and sheep (48, 59). Both IGF-I and IGF-II can stimulate the development of preimplantation cultured blastocysts (24) and are also thought to be important for fetal development (16).

Confirmation that the IGF system can control placental growth has come from gene deletion studies (1, 6) that showed a deficiency in placental growth of mouse embryos carrying null mutations for IGF-II; in contrast, IGF-I deletion did not influence placental weight. The placenta clearly acts as the interface between mother and fetus, and there is a strong positive correlation between placental and fetal weight at birth (3, 64). Placental insufficiency can retard fetal growth (3, 52). The ability of the placenta to transfer nutrients during late pregnancy is rate limiting to fetal growth (16) and is influenced by the surface area of the placenta and its current metabolic activity.

In the ewe, placental growth occurs during the first half of pregnancy, reaching a plateau by d 90; in contrast, 80% of fetal growth occurs during the last 8 wk of gestation. Therefore, factors that affect early placental development may have an important impact on the ability of the dam to supply sufficient nutrients for fetal growth near term. The allantochorion colonizes the maternal caruncles between d 25 to 30, so that growth restriction during this period can limit the eventual number of placentomes that form the

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placenta. Later in pregnancy, the size and morphology of the placentomes are affected rather than their number (53, 63, 64, 65).

For ewes, placental weight at term is influenced both by litter size and by maternal nutrient status. For a ewe carrying more than one lamb, the individual placental weights are reduced compared with that of singletons as each placenta has access to fewer maternal caruncles. There is, however, some weight increase of individual placentomes (36, 65). Placentome weights can also be increased by restricting access to maternal caruncles by partial uterine ligation (44). The effects of nutrition are more complex. Severe undernutrition reduces placental weight, but less extreme reductions in the maternal diet generally have the opposite effect (34, 53, 64). These studies suggest that a compensatory mechanism of fetal origin exists that acts to stimulate placental growth if the fetus is receiving an inadequate nutrient supply. Paradoxically, overfeeding of pregnant adolescent ewes causes a significant reduction in both fetal and placental weights because maternal growth is favored at the expense of the feto-placental unit (64).

This review focuses on the information that is currently available regarding the production, regulation, and function of the IGF system in the reproductive tract and fetal membranes of ruminants. This topic is discussed in relation to the possible importance of this system in altering placental development according to the nutritional status of the mother. Many other aspects of the IGF have been covered extensively in a number of recent reviews [e.g. (21, 23, 33, 35, 62)], and the reader is referred to these for more background information.

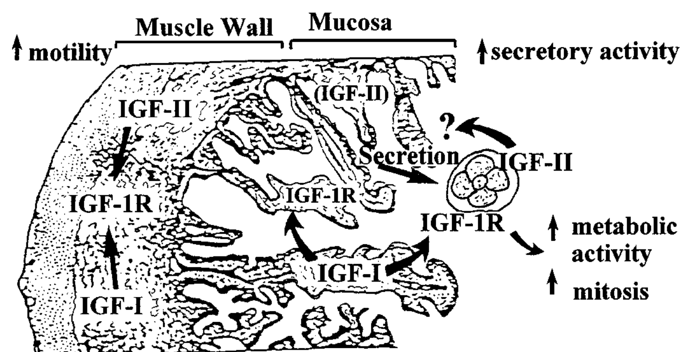

Figure 1. Diagram of the possible involvement of the IGF system in the oviduct: IGF-I is produced by the mucosa and could act on mucosal type 1 IGF receptors (IGF-1R) to stimulate secretory activity or be released into the lumen to have direct metabolic or mitotic effects on the early embryo. The IGF-II mRNA is principally located in the muscle wall and could potentially enhance metabolic activity necessary for motility.

IGF-I AND IGF-II

Preimplantation Period

In cattle and sheep embryos, mRNA for both IGF-I and IGF-II have been identified by polymerase chain reaction techniques throughout preimplantation development from the single-cell stage to the hatched blastocyst (69, 70). Both IGF-I mRNA and IGF-II mRNA are also present in oviductal cells (58, 70), raising the possibility that the beneficial effects of oviductal coculture on blastocyst development (13) could be mediated, at least in part, by the production of IGF.

Ruminant embryos reach the 8- to 16-cell stage in the oviduct (22) where the embryo is in intimate contact with the epithelial lining of the ampullary mucosa. In ewes, IGF-I mRNA concentrations were maximal in both the mucosa and muscle layers of the oviduct wall at estrus (58). Concentrations then gradually decreased during the luteal phase but were still elevated on d 2 to 3 when the embryo would be present. A similar peak in IGF-I mRNA activity around the ovulatory period has been reported in the cow oviduct (54). In sheep, IGF-II mRNA was also present in the mucosa, but the concentrations were much lower than for IGF-I and showed less cyclic variation. In contrast, the expression of IGF-II was high in the muscular layer (58). Therefore, locally produced IGF-I is more likely than IGF-II to influence this early stage of embryonic development. Two mechanisms are possible and are illustrated in Figure 1. The IGF-I may be released into the oviductal fluid to stimulate embryo growth directly. Alternatively, or in addition, IGF-I may stimulate secretory activity of the ampulla, which peaks during this period (42). A number of steroid-modulated glycoproteins are released, which have been postulated to enhance both fertilization rates and the developmental competence of the embryos (42).

The conceptus normally passes from the oviduct to the uterus about 3 to 4 d after ovulation (71), although the timing may be influenced by the maternal endocrine environment, particularly the time of the postovulatory increase in progesterone. The conceptus hatches from the zona pellucida on about d 7 and starts to elongate on d 12. Attachment to the uterine wall does not begin until d 16 for ewes (72) or d 19 for cows (72). Until attachment, the conceptus is free living in the uterine lumen and is entirely dependent on uterine secretions for meeting all of its developmental requirements.

In the ovine uterus, IGF-I mRNA concentrations peaked at estrus in both endometrium and myometrium (59). Concentrations fell about threefold during the early luteal phase, but low expression continued in both the caruncles and endometrial stroma underlying the luminal epithelium and surrounding the glands. Moderate expression of IGF-I mRNA also occurred in the bovine uterus during the luteal phase (14, 25). This expression was mainly present in a band of dense caruncular stroma that underlies the luminal epithelium (R. S. Robinson and D. C. Wathes, 1997, unpublished observations). In both species, IGF-I is secreted into the uterine lumen, particularly around estrus (14, 27), so that maximal concentrations are present during sperm transport but several days before the arrival of the embryo. The porcine uterus also has the capacity to synthesize and secrete IGF-I, but, in pigs, maximum concentrations in the uterine fluid are found on d 10 to 12 of pregnancy when conceptus elongation is initiated (18, 26, 61).

The endometria of both cows and ewes also express IGF-II mRNA during the preimplantation period (14, 59). In the ewe, expression was highest in the caruncles and was much lower in the surrounding endometrial stroma. We observed a gradual decline in expression during the luteal phase (59). The IGF-II is also found in uterine flushes but, although values in the cow were reported to be maximal on d 0 to 5 of the cycle, those in the sheep were higher on d 12 to 14 (27).

Although local production of the IGF by both the uterus and embryo is clearly important, the uterus will also be exposed to maternal circulating IGF. In mature cattle and sheep, IGF-II concentrations are about fivefold higher (11, 35) than those of IGF-I, although plasma IGF-I concentrations also increase at estrus (32, 57).

Placental Development

Following implantation in the ewe, maternal uterine IGF-I mRNA expression was low; highest concentrations were found in the maternal stroma surrounding the deep uterine glands (48). Concentrations in the caruncles decreased below the limit of detection after invasion of the fetal villi, and there was also no detectable hybridization to fetal placental tissue. This observation makes it unlikely that locally produced IGF-I is important in placental development. Studies in mice of IGF-I gene deletion support this view, because neither fetal nor placental development were altered (1). The IGF-II mRNA in the maternal caruncles also decreased following fetal interdigitation, but some expression continued in the maternal caruncles, placentome capsule, and endometrial stroma throughout the first half of gestation.

In contrast, mRNA expression in the fetal mesodermal tissue in the allantochorion was much more intense, particularly at the tips of the invading villi (48). Expression increased between d 14 and 35 and thereafter remained high until parturition (Figure 2A). Therefore, assuming that this IGF-II mRNA is translated into protein and can cross the fetal-maternal interface, the maternal uterine tissue will be continuously exposed to high local concentrations of IGF-II throughout gestation. The way in which the uterus responds will be governed by both receptor availability and IGF binding protein (**IGFBP**) concentrations.

IGF TYPE 1 AND 2 RECEPTORS

Background

The structure, function, and signaling pathways of the IGF receptors have been reviewed previously [e.g., (23, 28, 33)], and only a brief summary is included here. The majority of actions of both IGF-I and IGF-II are mediated by the type 1 IGF receptor (**IGF-1R**). This receptor is structurally similar to the insulin receptor, consisting of two α and two β subunits, which combine to form a heterotetramer; IGF binding activates a tyrosine kinase signal pathway. Both IGF-I and IGF-II have a low affinity for insulin receptors and only activate these if present in high concentrations. Hybrid insulin and IGF-1R can also develop if both genes are concurrently expressed in the same cell. These hybrid receptors have binding affinities similar to the IGF-1R, bind IGF-I preferentially over insulin, and have been identified in placental membranes (23).

In contrast, the type 2 IGF receptor (**IGF-2R**) is a monomeric receptor that is identical to the mannose-6-phosphate receptor. This receptor targets bound proteins to lysosomes and is now widely regarded as a clearance molecule that does not have a signaling function, but instead acts primarily as a degradative pathway to remove excess IGF-II from the circulation (28). The affinity of IGF-2R for IGF-I is 500-fold less than for IGF-II, and IGF-2R does not bind insulin. A circulating form, consisting of the extracellular domain of the receptor protein, is also present. Concentrations are extremely high in the plasma of fetal sheep, contributing up to 40% of the total IGF-binding capacity in fetal blood (12, 15). This situation is developmentally regulated as concentrations are significantly lower in the adult. The function of

the circulating IGF-2R is uncertain, although it must influence the bioavailability of IGF-II. Targeted disruption of the IGF-2R is lethal in mice; all fetuses died by d 15 (9). It was postulated that, in the absence of the clearance action of the IGF-2R, the elevations in IGF-II to which the fetuses would have been exposed were incompatible with normal development.

Localization of Receptors

The IGF-1R are present in preimplantation bovine embryos (69) so that these could potentially respond either to paracrine production of IGF or to secreted IGF released into the oviductal or uterine lumens. In the oviduct, IGF-1R mRNA is present in both the secretory epithelium and the muscle layer (58). Concentrations in both regions peaked on d 2 to 3 of the cycle, which was significantly later than maximal IGF-I expression, but when the embryo would be in transit.

In the uterus of cyclic cattle and sheep, IGF-1R mRNA is mainly localized to the epithelium of the endometrial glands [(48, 59); R. S. Robinson and D. C. Wathes, 1997, unpublished observations] (Figure 2B). The highest concentrations were in the deep glands throughout the cycle. In the superficial glands of the ewe, expression was up-regulated between estrus and d 2; in the cow, expression was highest in the midluteal phase. Lower concentrations were de-

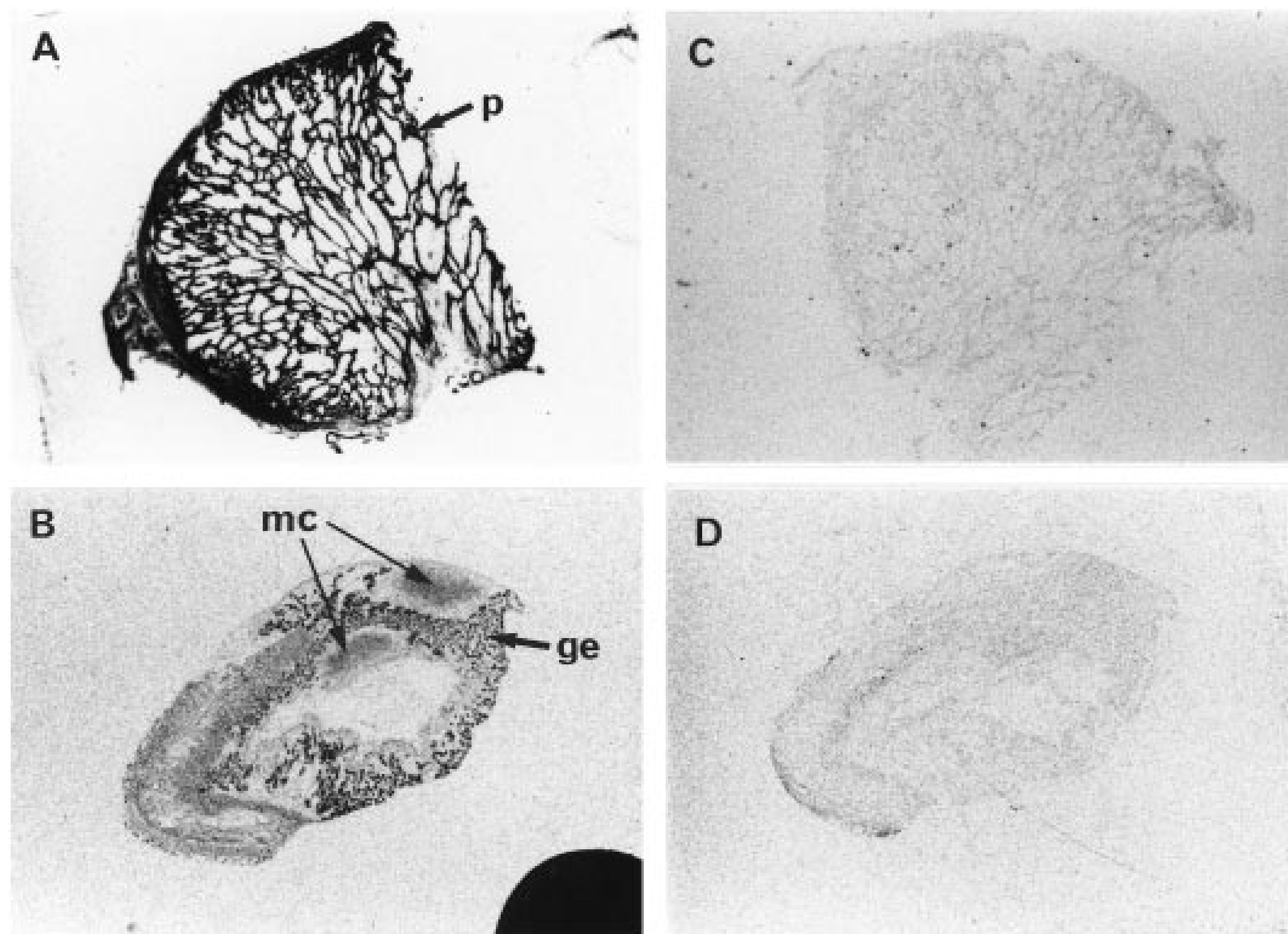

Figure 2. Localization of IGF-II mRNA (A) and the type 1 IGF receptor (IGF-1R) mRNA (B) within the pregnant ovine uterus, as shown by in situ hybridization using antisense oligonucleotide probes. The corresponding negative (sense) controls are also illustrated (C and D). A. There is intense expression of IGF-II mRNA in the fetal mesoderm within the placentome (p) (d 70 of pregnancy). ×3.7. B. The IGF-1R is expressed strongly in the glandular epithelium (ge) and at lower concentrations in the maternal caruncles (mc). Note that expression continues in the glands throughout pregnancy but becomes undetectable in the caruncles following interdigitation by the fetal allantochorion (d 34 of pregnancy). ×3.7.

tectable in the caruncles and myometrium. The pattern of high expression in the deep glands with somewhat lower expression in the superficial glands was maintained throughout pregnancy in the ewe. However, the initially low expression in the caruncles decreased following interdigitation by the fetal villi, and no signal could be detected in either the fetal or maternal components of the placentomes after about d 30. These data suggest that any putative actions of the high IGF-II concentrations within the placentomes cannot be mediated via the IGF-1R.

Several lines of evidence suggest the existence of another type of IGF receptor in placenta. The presence of such a receptor may explain the results of Lacroix et al. (29) who used affinity crosslinking studies to demonstrate what was apparently IGF-1R on trophoblast from ewes between d 40 to 75 of gestation. Binding studies using human placental membranes revealed a previously unrecognized common (putative type 3) high affinity IGF receptor. This receptor site had equal affinity for IGF-I and IGF-II and exhibited only slightly less affinity for insulin (20). Discrepancies in molecular mass of the IGF-1R purified from different sources and the ability of the monoclonal antibody αIR-3 to neutralize binding activity also led LeRoith et al. (33) to propose the existence of another receptor subtype. Strong additional support has come from studies of placental growth in mutant mice. Neither the Igf-I(–/–) nor the Igf1r(–/–) mutation affected placental weight; the Igf-2(p–) mutation caused a pronounced deficiency in placental growth (1). In addition, the placentas of mice with the Igf2(p–)Igf1r(–/–) double mutants were identical to those of the mice with the Igf2(p–) single mutation, again suggesting that the IGF-II must be acting through another receptor. This receptor must differ significantly from those already described in terms of genetic sequence and still awaits identification. The receptor does not appear to be a hybrid insulin-IGF receptor, as these would have been detected by the existing molecular probes.

The IGF-2R has also been identified in bovine embryos by polymerase chain reaction (69). In the ewe, IGF-2R mRNA was localized mainly to a band in the stromal tissue underlying the maternal luminal epithelium and glands (T. S. Reynolds and D. C. Wathes, 1997, unpublished observations) (Figure 3A). Receptor protein has been observed in fetal placental mesoderm by immunohistochemistry (29). Binding studies showed a decrease in the IGF-2R concentration in the placenta of ewes during pregnancy.

Receptor-Mediated Actions of IGF

In mice, both IGF-I and IGF-II increased the rate of development of cultured embryos (24). However, the two peptides had quite distinct effects on the pattern of individual proteins synthesized at both the 8-cell and expanded blastocyst stages (55). Detailed studies using ruminants are sparse, but IGF-II (but not IGF-I) increased both the cleavage rate and yield of viable bovine blastocysts cultured in synthetic medium (4). A combination of IGF-I and IGF-II to the medium also promoted the secretion of IFN-τ by the ovine conceptus (27).

The high concentration of IGF-1R on the epithelium of both the oviductal mucosa and the uterine endometrial glands suggests a rule for IGF-I and IGF-II in regulating their secretory activity. Protein secretion increases in both parts of the tract in the immediate postovulatory period, which is likely to be important in providing an environment capable of sustaining embryonic development (38, 42) because the preimplantation conceptus is dependent on such secretions for all nutritional support.

On about the 3rd or 4th d after estrus, the ampullary-isthmic junction releases the embryo, which then progresses to the uterus. The precise control mechanisms behind this process are uncertain, although the timing of the release is potentially important because it may alter the stage of development at which the embryo is first exposed to the uterine environment. Embryo transfer experiments have shown that embryos placed in an asynchronous environment have their potential to survive severely compromised. An earlier increase in luteal progesterone advances the stage of the uterus such that it can sustain an older but not a synchronous embryo (30, 37). This action is likely mediated, at least in part, by controlling glandular activity.

The change in IGF-1R localization from the entire epithelial layer at estrus to a predominant location deep in the gland during the luteal phase might affect either the amount or type of protein secreted. In ruminants, secretion of uterine histotroph, including uterine milk proteins, by the endometrial glands in the intercotyledonary regions is thought to continue to provide an important source of nutrients to the developing fetus throughout gestation (39, 51).

IGFBP

Background

The action of IGF-I and IGF-II on the reproductive tract is modulated by the IGFBP. The majority of

circulating IGF-I and IGF-II are complexed to binding proteins, in particular IGFBP-3 (21, 23). This complex serves to maintain a large and relatively stable reservoir of IGF. Both IGF-I and IGF-II complexed to IGFBP-3 are unable to cross the capillary endothelium. The other binding proteins, however, do leave the circulation, and the type of binding protein involved may target IGF-I and IGF-II to particular tissues (2).

Within the tissues, the IGFBP can have both inhibitory and stimulatory activity. The inhibitory action is achieved by competition with the IGF-1R for IGF binding, and any IGFBP present in a molar excess effectively eliminates IGF bioactivity. Stimulatory effects have also been reported for IGFBP-1, -2, -3, and -5 [reviews (21, 23)] that are probably achieved by providing local increases in IGF concentration and by delivering IGF to particular target cells. Both IGFBP-1 and IGFBP-2 contain the amino acid Arg-Gly-Asp integrin recognition sequence, which is involved in binding to cell surfaces. The IGFBP-3 associates with cells by an alternative mechanism involving interaction with cell surface glycosaminoglycans. The IGFBP-5 binds to the extracelluar matrix. In each case, bound IGFBP have lower affinity for IGF-I and IGF-II than do IGFBP in the circulation, which may provide a mechanism to allow dissociation of the IGF-I and IGF-II from the binding proteins in favor of the receptors.

Localization and Possible Actions of IGFBP in the Reproductive Tract

IGFBP-1. The most prevalent binding protein in the uterus during implantation in primates is IGFBP-1 (19, 60). Preliminary studies in pregnant ewes, however, have indicated that, although there is clear expression of IGFBP-1 mRNA localized to the luminal epithelium on d 13, concentrations subsequently dropped, becoming undetectable by d 21 (Figure 3B) (T. S. Reynolds, 1997, unpublished observations). This pattern suggests a species difference in either importance or function. In baboons, IGFBP-1 is thought to facilitate trophoblast penetration at the maternal interface (60), something that does not occur in the less invasive ruminant placenta. In serum-free culture systems, IGFBP-1 is inhibitory to IGF action, but potentiation effects have also been reported when low concentrations of serum are present (23).

IGFBP-2. We were unable to detect IGFBP-2 mRNA in the ovine oviduct (58) or uterus during the estrous cycle. Expression appeared in the uterus from d 29 of gestation onward, localized as an intense band in the dense caruncular-like stroma underlying the luminal epithelium (48). Similar results have been reported for the rat (73). In contrast, IGFBP-2 mRNA has been found in the bovine oviduct and uterus, and concentrations increased between d 10 to 18 (14, 25). This instance is one of the few encountered so far of an apparent difference in the IGF system in the reproductive tract of sheep and cows, and the reason is currently unknown. Often IGFBP-2 mRNA is found in association with IGF-II mRNA in rapidly proliferating tissues (7). Again, under cell-free conditions, IGFBP-2 is a potent inhibitor of both IGF-I–stimulated and IGF-II–stimulated mitogenesis in cultured fibroblasts, although IGFBP-2 has also been shown to enhance glucose transport in microvascular endothelial cells (23).

IGFBP-3. The IGFBP-3 mRNA is present in ovine and bovine oviducts in both the mucosa and muscle layers; a clear peak in expression occurred during the preovulatory stage of the cycle (25, 58). In the uterus, expression in the luminal epithelium was high at estrus and during the luteal phase, but concentrations in this location dropped following implantation. In the cow, there was also low expression in the endometrial stroma, and concentrations in this location were higher in nonpregnant than in pregnant cows on d 16 (R. S. Robinson, 1997, unpublished observations). If IGFBP-3 is inhibitory, this could potentially increase the availability of free IGF if an embryo is present. The IGFBP-3 has been measured in ovine uterine luminal fluid (31). Proteolytic activity, which cleaved IGFBP-3 into smaller fragments, was also present in the fluid, apparently under regulation by progesterone. Proteolysis represents another mechanism by which the concentration of the protein can be regulated. During pregnancy, the distribution in the ewe of uterine IGFBP-3 mRNA was extended into the caruncles, endometrial stroma, placentome capsule, and, for some ewes only, also the allantochorion.

In the uterus, iodinated IGF-I and des-IGF-I both showed pronounced binding to blood vessel walls (59). Because no IGF-1R mRNA could be detected in this location, binding could have involved IGFBP; IGFBP-3 was the most likely candidate. This likelihood was supported by in situ hybridization studies of IGFBP-3 mRNA in which the high level of expression in the maternal component of the placentomes was localized to the blood vessel walls (48). Cultured bovine endothelial cells can synthesize and secrete IGFBP-2, -3, and -4 (40), and IGF-I can stimulate

DNA, RNA, and protein synthesis in arterial smooth muscle cells (46). Furthermore, close arterial infusions of IGF-I increase local blood flow to the caprine mammary gland (47). Therefore, endothelial cell-binding proteins, in particular IGFBP-3, may be important in regulating both the rate of placental blood flow and the rate of transfer of IGF-I and IGF-II from the maternal circulation to the uterus.

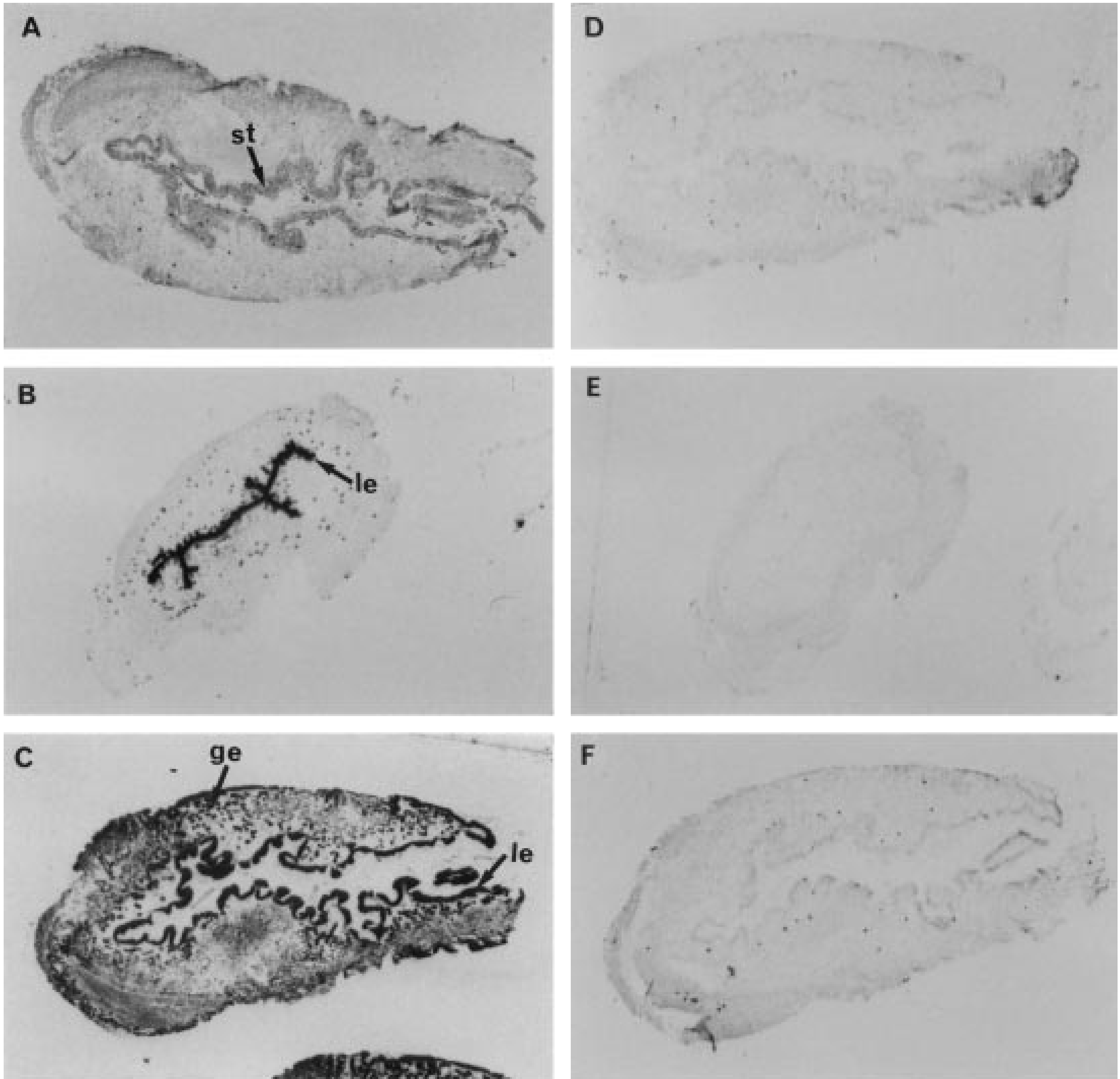

Figure 3. Localization of the type 2 IGF receptor (IGF-2R) mRNA (A), IGF binding protein (IGFBP)-1 mRNA (B), and IGFBP-5 mRNA (C) within the pregnant ovine uterus, as shown by in situ hybridization using antisense oligonucleotide probes. The corresponding negative (sense) controls are also illustrated (D, E, F). A. Expression of IGF-2R mRNA is found mainly in a band of stromal tissue (st) underlying the maternal uterine luminal epithelium (d 40 of pregnancy) ×4.7. B. The IGFBP-1 mRNA is strongly expressed in the luminal epithelium (le) before implantation but becomes undetectable after d 21 (d 13 of pregnancy). ×4.7. C. Expression of IGFBP-5 mRNA is in both the luminal and glandular epithelia (ge) throughout pregnancy (d 40 of pregnancy). ×4.7.

IGFBP-4. In the oviduct, IGFBP-4 mRNA was expressed in both the stromal fibroblasts of the ampulla and the surrounding smooth muscle cells. Variation in mRNA concentrations during the estrous cycle was significant; transcript was present from the midfollicular to the midluteal phases, but became undetectable during the late luteal and early follicular phases (58). In pregnant ewes, IGFBP-4 mRNA expression was colocalized with IGFBP-2 to the caruncular stroma lining the luminal space and was also present at high concentrations in the placentome capsule and at lower concentrations in the caruncular stroma (48). The action of IGFBP-4 appears to be purely inhibitory (21, 23). Therefore, its localization to the caruncular capsule and the caruncular stroma underlying the luminal epithelium may suggest a role in inhibiting fetally derived IGF-II from spreading outside the placentomes.

IGFBP-5. Preliminary observations in the ewe using in situ hybridization have revealed IGFBP-5 expression in the luminal and glandular epithelia, and concentrations increased as gestation progressed (Figure 3C). There was also expression in the caruncular stroma, but not in any fetal placental tissue (50). The IGFBP-5 has the unique property of adhering to fibroblast extracelluar matrix and can then potentiate the action of IGF-I and IGF-II.

IGFBP-6. There is currently no information available concerning IGFBP-6 in the ruminant reproductive tract.

REGULATION OF THE UTERINE IGF SYSTEM

Relatively little is known about the factors regulating either the concentration or tissue-specific localization of IGF-I and IGF-II within the reproductive tract. Only control of mRNA expression is discussed here, although regulation can occur at other levels. In particular, the activity of the IGFBP can be influenced by the degree of phosphorylation and glycosylation of the expressed protein. The degradation of IGFBP is caused by proteolytic enzymes, the local regulation of which also appears to be tightly controlled (21, 23).

IGF-I

The up-regulation of IGF-I mRNA in both the oviduct and uterus at estrus is driven by the preovulatory increase in circulating concentrations of estradiol (41, 57). In the ewe, estradiol receptor concentrations in the tract also peak at this time (67) and are highly correlated with the IGF-I mRNA concentration. This peak is quite short lived. Cyclic ewes were injected with cloprostenol in the midluteal phase to induce estrus; IGF-I mRNA concentrations were low 24 h later, increased markedly at 48 h, and had already returned to basal concentrations by 65 h, at about the time of ovulation (57). Similarly, in ovariectomized ewes treated with steroids, estradiol administration following progesterone withdrawal resulted in high expression, but this expression could not be maintained by continued treatment with low concentrations of estradiol (57).

In chronically ovariectomized ewes, IGF-I mRNA transcript concentrations are similar to those measured in the early luteal phase, suggesting that basal concentrations are maintained in the absence of ovarian stimuli (57). In liver, IGF-I is principally regulated by growth hormone (**GH**) GH (8) and GH receptor mRNA has been identified in the bovine uterus on d 17 of pregnancy with lower concentrations in the oviduct. However, although long-term GH treatment tended to decrease GH receptor concentrations, it had no apparent effect on IGF-I expression (25). Concentrations of IGF-I mRNA in the cow uterus on d 16 to 17 tended to be higher in pregnant cows than in inseminated cows from which no embryo was recovered [(25); R. S. Robinson, G. E. Mann, G. E. Lamming and D. C. Wathes, 1997, unpublished observations]. It was, however, postulated that this apparent increase was more likely to represent a beneficial effect of high IGF-I concentrations on embryo survival than a mechanism indicating conceptus regulation of maternal IGF-I production. This view is supported by our work with ewes in which there was no difference in the IGF-I mRNA concentration between unmated and pregnant ewes during the luteal phase or between the pregnant and nonpregnant horns of ewes with a transected uterus carrying a unilateral pregnancy (57).

The nutritional control of IGF-I secretion has been reviewed by Thissen et al. (62). In general, dietary restriction of cattle and sheep decreases serum IGF-I and raises circulating concentrations of GH. Maternal starvation and hypoglycemia also reduce circulating concentrations of IGF-I in fetal plasma (12). Insulin at physiological doses increases IGF-I concentrations in cultured ovine luteinized granulosa cells (68), supporting the view that insulin may be a direct regulator of IGF-I gene transcription (10). A number of other hormones, including glucocorticoids, gonadotropins, and thyroid hormones, have been shown to influence IGF-I production (23). However, the relevance of these factors to the uterus has not been investigated.

Taken together, the available data show that a brief surge of locally produced IGF-I occurs in the ruminant reproductive tract at estrus. This surge is

stimulated by estradiol and might be important for promoting the survival of the spermatozoa or early embryo either directly or indirectly by enhancing uterine secretions. Thereafter, uterine IGF-I mRNA concentrations gradually decline during early pregnancy, although the putative inhibitor remains to be identified. Local production of IGF-I is therefore unlikely to be important in placental development. There is, however, a positive correlation between fetal and maternal concentrations of IGF-I in late gestation and placental weight (5), which suggests that maternal nutrition may influence placental growth via an alteration in circulating concentrations of IGF-I.

IGF-II

Less is known about the regulation of IGF-II in the reproductive tract. Although mRNA concentrations in caruncular stroma gradually decline during the luteal phase from the peak expression reached at estrus, this decline did not reveal any obvious relationship with either estradiol or progesterone concentrations in plasma or with receptor concentrations (57). In both cattle (14) and sheep (59), concentrations of IGF-II are higher during the late luteal phase in pregnant animals than in nonpregnant animals, apparently because of a marginal delay in the timing of down-regulation, as caruncular concentrations had declined further by d 21 of pregnancy. Following implantation, there was a visible gradient in the IGF-II mRNA concentration across the caruncles, from high expression near the base to lower expression adjacent to the fetal membranes (48). Thus, the fetal tissue may in fact inhibit maternal IGF-II expression, but the mechanism is unknown.

There is strong expression of IGF-II in the fetal placenta throughout pregnancy. Placental lactogen has been suggested as a possible controlling factor and, in the pregnant ewe, IGF-II concentrations correlate with placental lactogen concentrations (17). Using a culture system, we have found that the fetal mesoderm of the allantochorion continues to produce IGF-II mRNA in vitro and that the concentration can be increased by treatment with dbcAMP (K. R. Stevenson, P. F. Whitelaw, and D. C. Wathes, 1997, unpublished observations). Cortisol is a possible inhibitor: the prepartum cortisol rise alters the cell cycle from proliferation to differentiation, in part by switching fetal IGF-II to IGF-I production (10).

IGF-1R

Distribution of IGF-1R was specific to the tissue and the time in the reproductive tract during the establishment of pregnancy. Concentrations in most regions of the ovine uterus and oviduct peaked on d 0 to 3 of the cycle. The pattern of glandular expression showed no obvious correlation with either circulating estradiol or progesterone concentrations or with the tissue distribution of their respective receptors (57, 59). As already discussed, IGF-1R mRNA is lost from the caruncular stroma following implantation (48). It is possible that this process could be regulated by the high fetal IGF-II production because IGF-II was shown to down-regulate IGF-1R mRNA in human breast cancer cells (45).

Nutrition has also been proposed as a potential modulator. This proposal was supported by a trial using pregnant adolescent lambs fed a complete diet. The daily ration was calculated to achieve either a rapid (approximately 300 g/d) or moderate (approximately 50 g/d) maternal growth rate. In some lambs, the diet was switched between the high and low planes of nutrition on d 52. The uterine IGF-1R mRNA concentrations were highest for young ewes in which the growth rate was decreased from rapid to moderate in midgestation (49). Feed deprivation has also been reported to increase IGF-1R concentrations in various tissues of the rat (33) and in ovarian follicles of ewes (66), but this increase may be secondary to a decrease in circulating concentrations of IGF-I. In cultured cells, increasing the IGF-I concentration also tends to decrease the receptor number (33).

Therefore, at the present time, concentrations of IGF-I and IGF-II appear to be the most likely candidates to control IGF-1R numbers in the tract. However, the localization data suggest that additional tissue-specific regulatory mechanisms must be important, but it is uncertain what these mechanisms are.

IGFBP

Fetal concentrations of IGFBP-1 and IGFBP-2 are higher than those in the adult, but concentrations of IGFBP-3 are lower. Several previous studies of fetal sheep have examined the nutritional regulation of circulating IGFBP. In summary, starvation increases IGFBP-1 and IGFBP-2 levels; this rise is mediated by a fall in insulin concentration and is inversely correlated with the fetal glucose concentration. The reverse is true for IGFBP-3 and IGFBP-4, which fall when nutrient supply is limiting (11, 12, 43).

Measurements in plasma may not, however, reflect more subtle changes at a cellular level. We have therefore started to use in situ hybridization to study the expression of placental IGFBP in pregnant ewes

Figure 4. Diagram of the localization and possible actions of the IGF system within the ovine placenta. Local production of IGF-I is very low; thus, circulating concentrations of IGF-I are more likely to play a regulatory role. The concentration of IGF-I within the circulation will be controlled by the maternal metabolic hormones insulin, glucose, and growth hormone (GH). There is intense expression of IGF-II mRNA by the fetal mesenchyme and lower concentrations in the maternal caruncular stroma, but the receptor on which IGF-II is likely to act within the placentomes has not yet been identified. The IGF-II concentration may possibly be regulated through either placental lactogen (PL) or ACTH acting through cyclic AMP (cAMP). The type 1 IGF receptors (IGF-1R) are located principally in the maternal endometrial glands, where they are likely to influence secretory activity. The IGF binding proteins (IGFBP)-1 to -5 mRNA and the type 2 IGF receptors (IGF-2R) have all been localized to the maternal epithelium and the band of connective tissue stroma that underlies this, in addition to the placentome capsule. The IGFBP-3 mRNA was also present in the blood vessel walls within the caruncles, which would suggest that any transfer of IGF between the maternal and fetal circulations will be regulated extremely tightly.

following dietary manipulation. In the study of ewe lambs fed to achieve rapid or normal maternal growth rates, IGFBP-3 mRNA expression was higher in the endometrial glands of the lambs that were growing more slowly. In contrast, a change from a rapid to a normal growth rate in midgestation, a treatment that reduces placental weight at term, increased IGFBP-3 expression in fetal trophoblast (49).

CONCLUSIONS

All of the components of the IGF system so far investigated are present in the ruminant reproductive tract or placenta at some stage of pregnancy. Variations in both temporal and spatial expression suggest the presence of local regulatory factors, which, in most cases, have not yet been defined clearly. Our current investigations show that maternal nutrition has the potential to influence the expression of several components of the IGF system within the placenta; some differences were observed in the response of fetal and maternal tissues. This work may in the future help us to understand how placental growth can be modulated by both the mother and fetus according to their individual perceptions of the nutritional environment. The fact that all of the binding proteins so far studied (IGFBP-1 to IGFBP-5) are synthesized either in the uterine luminal epithelium or in the subepithelial stromal cells immediately underlying it strongly suggests that the IGFBP are important for regulating the transfer of IGF-I and IGF-II between the fetal and maternal compartments. Presumably the production of each IGFBP is controlled somewhat differently, and each has a variety of effects in modulating IGF action actually within the placental tissues. A summary of the information currently available concerning the localization and control of the IGF system in the ovine placenta is given in Figure 4. At our present level of understanding, it is hard to predict how alterations to this complicated mixture of IGF-I and IGF-II, receptors, and binding proteins in close proximity will influence placental growth. This topic remains a challenge for the future.

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