Emerging Technologies in Animal Reproduction

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Abstract. This lecture reviews current and emerging technologies in reproductive biology, including assisted reproductive technologies and animal cloning with regard to potential and pitfalls. The endocrinology of establishment and maintenance of pregnancy will be discussed in more detail with respect to fetal-placental development, lactation and neonatal survival. Also, regulation of uterine development and function will be discussed in the context of potential to influence reproductive efficiency. Biotechnologies likely to effect specific changes in the endocrinology of pregnancy and in neonatal uterine development will also be discussed.

Introduction. Agricultural output of poultry and livestock in the U. S. exceeds $90 billion annually from about 9 million dairy cows, 5 million dairy heifers, 85 million beef cattle and calves and approximately 100 million hogs (USDA, 2001). Demand for animal protein is increasing with an increasing world population and standard of living (Pinstrup-Andersen and Pandya-Lorch, 1999). The application of biotechnology in animal agriculture is expected to increase productivity, with enhanced reproductive efficiency being fundamental to this goal.

Biotechnology in Animal Agriculture. Biotechnology is the application of scientific and engineering principles to processing or production of materials by biologic agents to provide goods and services. Biotechnology likely began in Southwest Asia after the last ice age as humans trapped wild animals to breed in captivity for food, fiber and work. To date, fewer than 20 species of animals have been domesticated, including cattle, sheep, goats, pigs, horses and poultry (Diamond, 1999). These species are all relatively docile, flexible in their dietary habits, reach maturity at an early age on a herbivorous diet, breed readily in captivity, have a social structures that permit humans to establish dominance, and adapt to living in large groups.

Modern breeds of livestock differ from their ancestors as a result of breeding strategies. For Holstein cows, milk production increased almost threefold between 1945 and 1995 (Majeskie, 1996) due to use of semen from select bulls by A.I. and improved management (Hale, 1969; Diamond, 1999). Similarly, breeding pressure resulted in lean, fast-growing pigs of uniform size (Notter, 1999). In modern breeds of laying hens yearly egg production increased from 134 to 254 between 1940 and 1994 due mainly to genetic selection and use of A.I. Further, commercial broilers took 84 days to reach a market weight of 1.8 kg in 1950, but only 43 days and one-half the feed in 1988 (Pisenti et al., 1999; Lacy, 2000). Scientific breeding, better nutrition and quality veterinary care has resulted in highly productive domestic animals that are less genetically diverse. Current research-driven biotechnologies in animal agriculture are based on improved understanding of reproductive physiology and endocrinology, embryology, genetics, and animal health. Both established and emerging biotechnologies influence animal agriculture.

ESTABLISHED TECHNOLOGIES

Breeding and Genetics

Use of sophisticated statistical models to predict breeding values
Sire testing and selection
Crossbreeding
Marker-assisted selection
Identification of major genes and QTL's of economic traits through genome mapping
Transgenic animals by direct gene transfer or homologous recombination
Transgenic animals through genetic engineering of sperm

Reproductive Management

Artificial insemination with fresh or frozen semen
Use of sex hormones to control estrus and time of ovulation
Sexed semen
Control of seasonality by melatonin
Sex reversal (in fish)
Artificial incubation of eggs in chickens

Gamete and Embryo Manipulations

Superovulation, embryo collection and transfer
In vitro maturation and fertilization of oocytes and in vitro culture of embryos
Embryo splitting and cloning from blastomeres
Cloning of adult animals by somatic cell nuclear transfer to produce “copies”
Cloning of adult animals by somatic cell nuclear transfer to achieve genetic engineering

Environment Control

Vaccinations and health technologies to protect from microbial-based diseases
Use of antibiotics in feed
Control seasonality by artificial light regime (chickens, sheep)
Climate control

Physiological Manipulation

Steroid administration to improve weight gain
Bovine sommatotropin (BST) to increase milk production in dairy cows
Castration

EMERGING TECHNOLOGIES FOR ANIMAL AGRICULTURE

Genetic Engineering. Genetically engineered livestock are being used as bio-incubators for production of pharmaceuticals and other products, as a source of replacement organs for humans, and as models for human disease. The biotechnology to produce foreign proteins in milk by expressing novel genes in mammary glands of livestock has resulted in some products going to clinical trials and investments in production of enzymes, clotting factors, and other bioactive proteins (Murray and Maga, 1999). Pigs can provide replacement organs for humans, but a carbohydrate epitope (terminal α1,3-galactose residues) present on the surface of pig cells

(Sandrin et al., 1993) result in the xenograft being rejected. Thus, pigs are being genetically engineered to silence the α1,3 galactosyl transferase enzyme gene (Dai et al., 2002; Lai et al., 2002). Homologous recombination to specifically modify genotype of an animal is now limited to mice,but specific gene knockouts in domestic animals can allow them to be used to mimic human disease, e.g, sheep with a mutated collagen gene is a model for the human connective tissue disease *osteogenesis imperfecta (*McCreath et al. (2000).

Cloning. Cloning, originally used in horticulture to describe asexually produced progeny, means to make a copy of an individual or, in cellular and molecular biology, groups of identical cells, and replicas of DNA and other molecules. Monozygotic twins are clones. Animal cloning in the 1980s resulted from the transfer of nuclei from blastomeres of early cleavage-stage embryos to enucleated oocytes and, more recently, cloning of animals has resulted from transferring a nucleus from a somatic cell into an oocyte from which the nucleus has been removed (Wilmut et al., 1997;Westhusin et al., 2001). Somatic cell nuclear transfer can also be used to produce embryonic stem cells. Undifferentiated stem cells can be genetically matched to recipients for research and therapy that is independent of reproductive cloning. Cloned progeny from use of nuclei from either blastomeres or somatic cells are not exact replicas due to cytoplasmic inheritance of mitochondrial DNA from the donor egg, other cytoplasmic factors which may influence "reprogramming" of the genome of the transferred nucleus, and subsequent development of the cloned organism (Cummins, 2001; Jaenisch and Wilmut, 2001).

Assisted Reproductive Technologies. A.I., using frozen semen, along with sire testing and sire selection has markedly affected genetic quality of livestock, especially dairy cattle. Select bulls are tested for fertility and judged on the basis of milk production by their daughters. Inbreeding coefficients among modern Holstein and Jersey breeds are about 5 percent and increasing. With increased milk production, pregnancy rates to first insemination have decreased (Pryce et al., 2000; Royal et al., 2000).

Embryo recovery and transfer allows valuable animals to contribute more offspring to the gene pool (Seidel, 1984). Embryos stored frozen before being used to initiate a pregnancy result in 40,000 to 50,000 beef calves per year (NAAB, 1996). In this procedure, hormones are used to induce maturation and release of multiple oocytes (Driancourt, 2001) that are fertilized with sperm from a select bull and, as embryos, transferred to the reproductive tract of less recipient cows for gestation. Emerging technologies allow sexing of semen and sexing embryos to control gender of offspring. The production of single sex sperm, through cell sorting of X and Y sperm (Johnson, 2000), will greatly benefit the respective livestock industries.

Hundred or thousands of embryos can be produced using techniques for recovery and maturation of immature oocytes for about one day in medium containing hormones. The oocytes are fertilized with live sperm or by injection of a single sperm or sperm head either beneath the zona pellucida or directly into the cytoplasm (intracytoplasmic injection, ICSI). The resulting zygotes are cultured in vitro, usually to the blastocyst stage, before transfer to recipient females. These techniques are used to produce normal human infants and offspring in animal agriculture (First, 1991). Importantly, *in vitro* maturation of oocytes underpins cloning and transgenic technologies that require large numbers of competent, matured oocytes for nuclear transfer and pronuclear injection. *In vitro* fertilization is also used to preserve genomes of valuable animals

that have infertility problems such as blocked oviducts (Boland and Roche, 1993). The commercial application of IVF has resulted in as many as 4,000 calves born in single year (NAAB, 1996).

Control of the reproductive cycles of livestock by hormonal intervention allows synchronization of estrus and mating and superovulation through use of equine chorionic gonadotropin (eCG) or Follicle Stimulating Hormone (FSH) to mature a cohort of follicles that are ovulated simultaneously for use in assisted reproductive technologies (Nebel and Jobst, 1998).

Splitting or bisecting embryos yields zygotic twins or clones (Heyman et al., 1998) that are genetically identical in both their nuclear and mitochondrial genes. These embryos are placed in an empty zona pellucida and transferred to recipient females that carry them to term. Only 1 to 2 percent of calves resulting from embryo transfer are produced in this manner (NAAB, 1996).

Cloning by nuclear transfer from embryonic blastomeres (see Willadsen, 1989) or from a differentiated cell of an adult (Wilmut et al., 1997; Polejaeva et al., 2000) requires that the introduced nucleus be reprogrammed by the cytoplasm of the egg to direct development of a new embryo which is transferred to a recipient mother for develop to term. The offspring are identical to their siblings and to the original donor animal in terms of their nuclear DNA, but differ in their mitochondrial genes and perhaps which nuclear genes are expressed. Cloning from blastomeres and somatic cells often result in large calves and lambs, the large offspring syndrome (LOS; Young et al., 1998; Sinclair et al., 2000). More serious abnormalities may also be associated with cloning from somatic cells (see Wilmut et al., 2002).

The Holstein Association indicates that all nuclear transfers to date have been from embryos rather than adult cells. Through 2001, a total of 2,226 registered Holstein clones from embryo splitting (754 males and 1,472 females) and 187 from nuclear transfer (61 males and 126 females) were reported. On the basis of milk production (total yield, fat and protein content, somatic cell score) and productive life-span, cattle selected for cloning were slightly superior genetically for yield traits, but nuclear transfer clones were similar to, and clones from embryo splitting were slightly less genetically valuable than non-cloned full sibs (Norman et al. 2002).

Bovine Somatotropin. Bovine somatotropin (bST), approved for use by FDA to increase milk yield from cows in 1993 (see Bauman, 1999), is marketed by Monsanto and increases milk production up to 30 percent in well managed herds. The greatest concerns about BST are related to mastitis which is higher in high-producing cows, but not exacerbated by bST (Judge et al., 1997). Collier et al. (2001) reported that bST had no effect on pregnancy rates, days open, twining, cystic ovaries, or abortions, but was associated with an increase in foot disorders. There is a trend toward breeding heifers and then sustaining milk production for up to 600 days using BST which results in fewer calves per lifetime in the herd than for first-calf heifers and a shortage of replacement heifers (Harlow, 2002).

Marker-Assisted Selection (MAS). MAS involves establishing a linkage between inheritance of a desirable trait, e.g, milk yield, and segregation of specific genetic markers which are coupled to that trait. MAS is important for studying complex traits governed by many genes, and in animal breeding and selection strategies (Georges, 2001). MAS is expected to increase

exponentially as genome sequencing projects increase the density of useful, segregating markers for economically important traits. Initially, animals will be screened for genes that control simple traits that may be undesirable, such as horns in cattle and halothane sensitivity/metabolic stress syndrome in pigs. In time, easily identifiable markers that accompany multiple genes controlling more complex traits such as meat tenderness and taste, growth, calf size, and disease resistance will become available to improve animal health and production traits (Dekkers and Hospital, 2002). Some suggest that short-term gains in productivity from MAS may inhibit longer-term improvements due to polygenic drag (see Dekkers and Hospital, 2002). On the other hand, MAS may encourage breeding strategies that maintain diversity among major genetic loci and permit exploitation of genetics of rare breeds and wild ancestors to improve traits for disease and parasite resistance and adaptability. Eventually, MAS will be replaced by genotyping for genes or mutations of interest. Two examples from sheep are the Booroola gene (Freking et al., 2002) in which identification of the single base change responsible for the Callipyge Muscle Hypertrophy Phenotype is the only known example of polar overdominance in a mammal. Another example of MAS in sheep is introgression of the Booroola gene into Awassi and the Assaf dairy breeds (Gootwine et al. (2001).

Application of Biotechnologies in Animal Agriculture to Enhance Reproductive Efficiency

Successful application of most biotechnologies depends on reproductive efficiency of the females. Low conception rates or high embryonic and fetal mortality undermine progress through animal breeding, gamete and embryo manipulations as they reduce the number of offspring produced per pregnancy or during the reproductive life of a female. By overcoming problems of reproductive inefficiency, the full impact of new technologies can be exploited. A major factor governing reproductive efficiency is uterine function; therefore, the subsequent and major focus of this paper is on research on uterine biology in sheep.

BIOTECHNOLOGIES TO ENHANCE REPRODUCTIVE EFFICIENCY

Uterine Biology. All mammalian uteri contain endometrial glands that synthesize and secrete or transport a complex array of proteins and related substances termed histotroph to nourish the conceptus (embryo/fetus and associated extraembryonic placental membranes (see Gray et al., 2002). Available evidence from studies of livestock, rodents, primates and subprimate species indicates an unequivocal role for histotroph as a primary regulator of conceptus survival, development, production of pregnancy recognition signals, and implantation/placentation. In rodents, leukemia inhibitory factor and calcitonin, expressed exclusively by endometrial glands, are essential for establishment of uterine receptivity and embryo implantation (Bagchi et al. 2001; Carson et al., 2002). Uterine secretions from endometrial glands are particularly important for conceptus survival and development in domestic animals due to the prolonged peri-implantation period that precedes superficial attachment and placentation.

Adenogenesis, the process of uterine endometrial gland morphogenesis, can be disrupted in ewe lambs by exposure to a progestin from birth to post-natal day (PND) 56. This ablates endometrial gland differentiation and results in an epigenetic “uterine gland knockout” (UGKO) phenotype in adult ewes that are unable to experience normal estrous cycles or support conceptus

development beyond the pre-implantation period (Day 14). Partial to complete UGKO phenotypes in heifers exposed from birth to progesterone plus estradiol benzoate (P+E) have low pregnancy rates. In mares, infertility and sub-fertility are associated with fibrotic lesions in the endometrium that compromise the integrity and functionality of endometrial glands. In sheep, cattle, and pigs, endometrial glands undergo extensive hyperplasia and hypertrophy during pregnancy in response to increasing demands of the developing conceptus for histotroph. In ewes, a servomechanism appears to regulate endometrial gland morphogenesis and function during pregnancy. The servomechanism involves sequential actions of ovarian steroid hormones, the pregnancy recognition signal interferon tau, placental lactogen and placental growth hormone from the placenta. Unexplained rates of peri-implantation embryonic losses in humans and livestock may reflect unrecognized defects in endometrial adenogenesis, whereas knowledge of endometrial gland developmental biology is suggesting mechanisms to increase uterine capacity in domestic animals.

Uterine adenogenesis involves a variety of endocrine, cellular and molecular mechanisms that include: (1) site-specific alterations in cell proliferation and movement; (2) paracrine, cell-cell and cell-extra-cellular matrix (ECM) interactions; and (3) specific endocrine-, paracrine-, and juxtacrine-acting factors and their receptors. Uterine development and function depends upon epithelial-mesenchymal interactions for local control and coordination of morphogenetically important cell behaviors including movement, adhesion, differentiation and proliferation of cells. Molecules involved in key epithelial-mesenchymal interactions include ECM such as glycosaminoglycans (GAGs) and sulfated GAGs, including chondroitins and heparans, and nonsulfated GAGs such as hyaluronic acid, that accumulate in morphogenetically active sites, such as the tips of proliferating glands, and affect patterns of branching morphogenesis through control of the cell cycle, apoptosis, and related changes in stromal and epithelial gene expression that define such developmental programs. Interactions between growth factors and their receptors can involve elements of the ECM. Peptide growth factors implicated in uterine development include FGF-7, FGF-10, hepatocyte growth factor (HGF) and insulin-like growth factors one (IGF-I) and -II, and their respective receptors (Taylor et al., 2001). IGF-I and IGF-II are expressed in the stroma surrounding nascent and proliferating endometrial GE, which is both IGF-I receptor (IGF1R) and estrogen receptor-α (ER-α) positive. Cross-talk between ERα and IGF1R signaling pathways results in synergistic growth stimulation, likely involving the mitogen-activated protein kinase (MAPK) pathway and direct serine phosphorylation. The developing ovine uterine endometrial glands have high levels of phosphorylated MAPK. The roles of ovarian steroids and/or growth factors in ovine neonatal uterine development are not clear, but a requirement for ERα in ovine uterine adenogenesis is based on evidence that progestin-induced ablation of endometrial gland genesis involves suppression of epithelial ERα expression. The ERα receptor can be activated by estrogens, in a ligand-dependent manner, or by growth factor-coupled pathways, in a ligand-independent manner (Taylor et al., 2000).

In epitheliomesenchymal organs such as mammary gland, prolactin stimulates epithelial differentiation and development that may be facilitated by cooperative ECM signaling. In neonatal ewes, serum PRL concentrations are high at birth, increase between PND 1 and 14 and then decline to PND 56; the period of onset of endometrial gland proliferation in uteri of neonatal ewes. Expression of prolactin receptors (PRLR) in nascent and proliferating endometrial GE increases 7-fold between PNDs 7 and 56. The PRLR gene is also expressed

exclusively by GE in ewes during pregnancy as endometrial glands undergo hyperplasia and hypertrophy. Intrauterine administration of placental lactogen (PL), a member of the PRL/growth hormone (GH) family, stimulates proliferation of endometrial glands, particularly the terminal ends of coiled and branched glands in the stratum spongiosum. Proliferation of uterine GE and genesis of uterine glands may involve PRLR-dependent, estrogen-independent activation of ERα and stimulation of MAPK signaling resulting in serine phosphorylation, ligand-independent activation of ER-α, and up-regulation of ERα, as well as IGF1R gene expression (Carpenter et al., 2003).

Mechanisms regulating endometrial gland morphogenesis and function in the adult uterus. During pregnancy in sheep, the endometrium is exposed sequentially to estrogen, progesterone, IFNt, oPL and oGH, that may activate and maintain endometrial remodeling, secretory function, and uterine growth. Estrogen from ovarian follicles increases expression of progesterone receptors (PR) in endometrial epithelia, while progesterone from the corpus luteum (CL) increases expression of a number of progesterone-responsive genes in endometrial LE and/or GE, as well as stromal cells. However, continuous exposure of the endometrium to progesterone down-regulates expression of the PR in endometrial LE and then GE, but not stromal cells. Interferon tau (IFNτ), the maternal recognition of pregnancy signal in ruminants, is produced by mononuclear cells of the conceptus trophectoderm between Days 8 to 21 in sheep (maximally on Days 14 to 16), and acts in a paracrine manner on the endometrium to abrogate development of the uterine luteolytic mechanism and stimulate expression of many interferon stimulated genes.

Progesterone down-regulation of PR expression in LE and GE is necessary for expression of certain secretory genes, such as uterine milk proteins (UTMP) and osteopontin (OPN) by endometrial GE. Secretion of oPL by binucleate cells of conceptus trophectoderm begins on Day 16 of pregnancy, with peak secretion between Days 120 and 130 of gestation. The oPL can bind to a PRLR homodimer or a heterodimer of a PRLR and GH receptor, expressed exclusively by GE. Placental GH is secreted by the ovine placenta between Days 35 to 70 of gestation and binds to endometrial GHR. Sequential intrauterine administration of ovine IFNτ to ewes from Days 11 to 15 post-estrus, followed by ovine PL from Days 21 to 25, increases proliferation of GE in the deep stratum spongiosum, while infusion of oGH into the uterine lumen from Days 21 to 25 increased uterine gland density in the deep stratum spongiosum and size of endometrial glands in the shallow stratum spongiosum. These results suggest a developmentally programmed sequence of events, mediated by specific paracrine-acting factors at the conceptus-endometrial interface, that supports both endometrial remodeling and increased uterine secretory activity (i.e., expression of UTMP and OPN genes) during gestation. Whether similar gestational servomechanisms regulate uterine gland development and function in other species remains to be determined. However, strategic manipulation of such mechanisms may provide therapeutic schemes to improve uterine capacity, conceptus survival and reproductive health.

Prolactin Enhances Endometrial Adenogenesis in Ewes. In neonatal ewes, pituitary PRL, estradiol-17β, and stromal cell-derived growth factors, including FGF-7, FGF–10, HGF, and IGF-I and -II, with their respective epithelial cell receptors are likely regulatory factors controlling endometrial adenogenesis. Further, circulating levels of PRL are high on PND 1, reach a maximum on PND 14, and decline slightly to PND 56, while PRL receptors (PRLR) are exclusive to nascent uterine GE buds on PND 7 and proliferating and differentiating GE from

PNDs 14 to 56. Given the central role of PRL and PRLR in mammary gland morphogenesis and function, experiments tested the hypothesis that circulating PRL activates PRLR signaling in nascent and proliferating endometrial GE stimulate and maintain coiling and branching morphogenesis of uterine glands in neonatal ewes. The experiments determined: (1) effects of hypoprolactinemia on uterine adenogenesis; (2) effects of hyperprolactinemia on uterine adenogenesis; (3) effects of postnatal age on expression of STATs 1, 3 and 5 and IRF-1 proteins; and (4) if PRL stimulates STAT and MAPK signal transduction pathways in the developing uterus. Results indicated that:

1) control (CX) ewes had coiled and branched glands in intercaruncular endometrium;

2) endometrium of bromocryptine-treated (BROMO) ewes had 35 percent fewer coiled and branched glands (CX vs BROMO: 695$\pm$30 vs 455$\pm$30 glands per section);

3) hyperprolactinemic ewes had more endometrial glands on PND 14 (CX vs roPRL: 36 $\pm$7 vs 61$\pm$7 total glands per uterine section) and this difference was greater PND 56 (CX vs roPRL: 450 $\pm$ 32 vs 732 $\pm$ 32 glands);

4) STAT 1 protein, detected in all cell types on PND 1, was more abundant in LE, present in developing GE from PND14, decreased in stromal cells with age, and expressed in nascent and proliferating glands;

5) STAT 3 protein, detected in LE and stroma , was abundant in LE and nascent and developing GE; and

7) STAT 5 protein detected in all uterine cell types on PND 1 was most abundant in stroma and LE and in nascent and developing GE throughout development.

Thus, PRL regulates endometrial gland morphogenesis in neonatal ewes which may determine functional capacity of the adult uterus. The challenge is to develop practical therapies for enhancing endometrial adenogenesis.

Ovarian Factors Affecting Uterine Endometrial Adenogenesis. Ovaries of neonatal ewe lambs contain growing and vesicular ovarian follicles at birth that decline to PND 14, increase to peak numbers on PND 28, remain high from PND 42 to PND 56, and then decline (see Tassell et al., 1978). These changes in ovarian follicles correlate with the ontogeny of endometrial gland development in ewe lambs. Ovariectomy of ewe lambs at birth does not affect uterine weight or initial stages of endometrial adenogenesis on PND 14 ([Bartol et al., 1988), but does affect uterine growth after PND 14 (Liefer et al., 1972). Postnatal uterine growth and endometrial adenogenesis are not dependent on estrogen (Carpenter et al., 2003 submitted); however, the role of the ovary in uterine adenogenesis after PND 14 has not been investigated in ewes.

The neonatal ovine uterus may be influenced by follistatin, activins, and activin receptors as autocrine and paracrine regulators of endometrial gland morphogenesis [Hayashi et al., 2003]. Activins and inhibins, members of the transforming growth factor (TGF) β superfamily, regulate growth and differentiation of many branched epitheliomesenchymal organs via autocrine, paracrine and perhaps endocrine mechanisms. Activin acts via receptor complexes Type IA (ActRIA) or Type IB (ActRIB) and Type II (ActRII). A feature that distinguishes effects of activins from those of TGFβ is that binding of activins to their receptors can be blocked if activin binds to follistatin or if inhibin α-subunit binds to activin receptors. Follistatin binds to activins with high affinity to neutralize its activity. Inhibin α-subunit, activins and follistatin are synthesized and secreted by ovarian follicles in neonatal and adult ewes; therefore, they could

act as endocrine regulators of uterine endometrial adenogenesis to compliment paracrine effects of the activin-follistatin system within the neonatal ovine uterus. Studies with neonatal ewe lambs determined: (1) effects of removing the ovary at PND 7 on subsequent uterine growth and endometrial adenogenesis on PND 56; (2) effects of ovariectomy on the uterine activin-follistatin system; and (3) expression of the activin-follistatin system in the neonatal ovary. Results indicated that:

1) Ovariectomy (OVX) did not affect circulating levels of estradiol-17β or prolactin; however, uterine weight was reduced by 52 percent in OVX ewes;

2) Thickness of the endometrium and myometrium, total number of endometrial glands, and endometrial gland density in the stromal stratum spongiosum were reduced in uteri of OVX ewes, but superficial ductal gland invaginations and gland density in the stromal stratum compactum were not affected by ovariectomy;

3) uteri of OVX ewes contained less activin βA-subunit, activin receptor ActRIA, ActRIB and follistatin mRNA and protein, but more activin βB-subunit;

4) neither inhibin α-subunit mRNA or protein were detected in the neonatal ovine uterus, but βA- and βB-subunits were expressed in endometrial LE and GE and myometrium;

5) ActRIA, ActRIB and ActRII were expressed in all uterine cell types with highest levels in endometrial LE and GE and myometrium;

6) between PND 0 and PND 14, follistatin was detected in all uterine cell types, but between PND 21 and PND 56 its expression was limited to stroma and myometrium; and

7) follistatin, inhibin α-subunit, βA-subunit, βB-subunit, ActRIA, ActRIB and ActRII were expressed in granulosa cells and cumulus oocyte complexes of the neonatal ovary between PNDs 0 and 56.

These results indicate that the ovarian activin-follistatin system influences endometrial adenogenesis during the period of increased in ovarian weight and numbers of ovarian follicles (Tassell et al., 1978). Follistatin levels increase in the fetus during parturition and remain high in neonatal ewe lambs (McFarlane et al., 2002), whereas inhibin levels are low at 2 weeks and decline to 15 weeks of life (Braw-Tal et al., 1997). These factors likely regulate follicular development in the prepubertal ovary by affecting granulosa cell proliferation and release of pituitary gonadotropins (see Welt et al., 2002). Follistatin, activins and inhibins can regulate growth and differentiation of many branched epitheliomesenchymal organs via autocrine, paracrine and perhaps endocrine mechanisms [see Jones et al., 2002]. Thus, follistatin and activins from both ovary and uterus may stimulate endometrial adenogenesis in ewe lambs.

Inhibin α-subunit mRNA and protein were not expressed in the neonatal ovine uterus, but activin βA- and βB-subunits are expressed LE, GE and myometrium between PNDs 0 and 56. Also, ActRIA, ActRIB and ActRII are expressed in all uterine cell types, but highest in endometrial LE, GE and myometrium. Between PND 0 and PND 14, follistatin can be detected in all uterine cell types, but between PNDs 21 and 56 its expression is restricted to stroma and myometrium. Follistatin regulates epithelial branching morphogenesis in a number of organs and its expression may be regulated by activins. In the developing prostate, for example, follistatin can neutralize inhibitory effects of exogenous activin A and modulate branching morphogenesis.

Placental Lactogen Affects Conceptus Development and Mammary Gland Functions. High circulating concentrations of ovine placental lactogen (oPL) in pregnant ewes is well known, but

its biological roles are not clear. oPL, a member of the GH/PRL gene family, has both somatogenic and lactogenic activities. oPL is produced by placental chorionic binucleate cells and can be detected in maternal plasma after Day 16 of pregnancy and by Day 120-140 concentrations may be 1 ug/ml. However, concentrations of oPL in fetal plasma are maximum by Day 90 of pregnancy in tens of ng/ml. oPL may enhance fetal growth by affecting nutrient repartitioning to the fetus and/or stimulating the fetus to use nutrients more efficiently.

Active immunization against 'self' hormone molecules can inhibit or potentiate endogenous hormonal activity and has been use to investigate physiological roles of hormones. Studies have determined the physiological role(s) of oPL during pregnancy and subsequent lactation after active immunization of ewe lambs against recombinant oPL (roPL) (Liebovich et al., 2000). Booroola-Assaf and Assaf ewe lambs were immunized before 6 months of age and mated. Blood samples were collected from control and immunized ewes prior to immunization, three weeks after the second boster (post immune samples) and on Days 60, 80 and 130 of pregnancy to determine antibody titers and bioactivity of oPL using both the Nb2-11C lymphoma cell proliferation bioassay and radioimmunoassay. Antibodies to oPL were not detected in serum of any control ewe, but were detected in all immunized ewes. Titers peaked at Day 60 and then declined to Day 130 of pregnancy. Immunoreactive oPL was always higher than bioactive oPL in the same samples and, at Day 130 of gestation, bioactive oPL was higher in plasma from immunized ewes.

Immunization of ewes did not affect lambs born per ewe lambing, but lambs born to immunized ewes were significantly heavier and the differential in individual lamb weights increased as number of lambs born increased from singles to triplets. This effect could not be attributed to differences in IGF-I levels or milk composition. In immunized ewes, higher concentrations of oPL in maternal circulation not bound to antibodies and higher oPL bioactivity in late gestation were associated with both heavier lambs and greater milk production. Thus, via an unidentified mechanism, oPL stimulates growth and development of both the fetus and mammary glands.

In a related study, somatogenic and galactopoietic effects of roPL were compared to those of roGH in post-weaned growing lambs and in lactating ewes (Liebovich, et al., 2001). In two experiments of 35 days each, two-month-old lambs were given daily subcutaneous injections of (0.1 mg/kg live weight) of either roPL or roGH and growth rates compared to those of control lambs. Both roGH and roPL had similar growth-stimulating effects by enhancing lamb growth by 10 to 25%. In two other experiments, lactating ewes were injected with either roGH or roPL for 14 days during mid-lactation (0.1 mg/kg live weight/day). roGH significantly increased daily milk production by up to 55%, whereas similar treatment with roPRL had no effect (Leibovich et al., 2001). Treatment with roPL also increased milk yield by 25%, but this effect was not statistically significant. In all experiments, treatment of lambs or lactating ewes with roGH increased IGF-I levels, whereas roPL did not affect IGF-I levels. Therefore, roPL and roGH exert similar somatogenic effects in lambs, but only roGH had significant galactopoietic effects in lactating ewes. However, *in vivo* effects of roGH and roPL indicated comparable mammogenic activities in pseudopregnant ewes (Kann *et al.,* 1999). Receptor activation studies (Herman *et al.*, 1999; Biener et al. (2003) demonstrated that oPL stimulates transient heterodimerization of GHR and PRLR, whereas oGH or oPRL stimulate, respectively, homodimerization of GHR or PRLR. Homodimerization or heterodimerization of PRLR and GHR had no differential effect upon activation of STAT5, MAPK or phosphorylation of Jak2.

However, oPL stimulated prolonged phosphorylation of STAT1 and, in particular, STAT3, suggesting that oPL transduces a signal which differs from that induced by homodimeric receptor associations.

Biotechnologies for Enhancing Expression of Desirable Genes. Sequencing genomes of animals important to agriculture will define genes that influence reproductive efficiency. For example, a growth hormone receptor variant on bovine chromosome 20 affects milk yield and composition (see simon.pitman@foodproductiondaily.com, February 21, 2003). It is expected to increase milk production by 200 kg per lactation and decrease milk fat from 4.4 to 3.4 percent. The goal of MTT Agrifood Research Finland and University of Liege, Belgium is to biopsy bovine embryos and determine expression of this gene to enhance genetic progress.

Biotechnology can be used to modify the endocrinology of domestic animals and effect reproduction, lactation and growth. For example, hypothalamic-specific expression of Growth Hormone Releasing Hormone (GHRH) is not essential since ectopic expression of a cDNA for this neuropeptide can be driven by a synthetic muscle-specific promotor to elicit increases in both GH and IGF-I (see Khan et al., 2002). This technology is effective in pigs and rats and GHRH release is pulsatile and physiological (see Draghia-Akli, 2002). Intramuscular electroporated injection of a protease-resistant GHRH cDNA into pregnant rats on Day 16 of gestation resulted in pups that were heavier at 2, 3 and 10 weeks of age, and had higher circulating levels of IGF-I. Interestingly, pituitaries of pups from dams receiving the GHRH cDNA had more somatotrophs and lactotrophs and, at 3 weeks of age, these pups had 2.5 times more GH and prolactin mRNA in their pituitaries. These results suggest modification of cell lineage differentiation that may have intergenerational effects on growth, development and reproduction. It is of interest that maternal endocrine status influences development of the pup's endocrine system and expression of hormones during fetal development and perhaps throughout life. The use of an intramuscular cDNA for GHRH is one example of a biotechnology that can be used to increase growth and lactation performance in animals. This biotechnology has the potential to affect the reproductive system through use of specific hormones and growth factors during critical developmental periods to enhance uterine capacity and to increase milk production by the dam.

Most modern technological developments are used in intensive production systems of developed countries, but seldom applied in animal agriculture of developing countries in which low production efficiencies and low fertility are common. According to FAO 2002, 73% of the world population is living in Asia and Africa which produce only 36% of the world's milk and 45% of the worlds meat. Thus, the maximal impact of new technological development demands that it be both efficient and relatively cheap.

REFERENCES

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