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Reproductive biology in the era of genomics biology

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Abstract

Current and emerging technologies in reproductive biology, including assisted reproductive technologies and animal cloning, are discussed in the context of the impact of genomics era biology. The discussion focuses on the endocrinology associated with establishment and maintenance of pregnancy, fetal-placental development, lactation, and neonatal survival. Various aspects of uterine biology, including development during the neonatal period and function in adult females, are discussed with respect to reproductive efficiency. It is clear that combining strategies for use of conventional animal models for studying the reproductive system with new genomics technologies will provide exceptional opportunities in discovery research involving data integration and application of functional genomics to benefit animal agriculture and the biomedical community. New and emerging biotechnologies and comparative genomics approaches will greatly advance our understanding of genes that are critical to development of the reproductive system and to key events at each stage of the reproductive cycle of females and males.

Keywords: Reproductive biology; Pregnancy; Fetal-placental development; Biotechnology; Genomics

1. Introduction

Agricultural output of poultry and livestock in the USA exceeds $90 billion annually from about 9 million dairy cows, 5 million dairy heifers, 85 million beef cattle, and calves and approximately 100 million pigs [1]. Demand for animal protein is increasing as the standard of living of the world population improves [2]. The promise is that genomics era

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biology and the effective application of biotechnology will increase reproductive efficiency and overall production efficiencies in animal agriculture as new technologies are transferred to the marketplace and adopted by the livestock industries.

2. Biotechnology in animal agriculture

Modern breeds of livestock differ from their ancestors as a result of breeding strategies. For Holstein cows, milk production increased almost three-fold between 1945 and 1995 [3], primarily due to the use of semen from selected bulls in AI programs and improved management [4,5]. Similarly, breeding pressure resulted in lean, fast-growing pigs of uniform size [6]. In modern breeds of laying hens, annual egg production increased from 134 to 254 between 1940 and 1994, due mainly to genetic selection and use of AI. Further, commercial broilers took 84 days to reach a market weight of 1.8 kg in 1950, but now require only 43 days and one-half the feed [7,8]. Scientific breeding, better nutrition, and improved animal health have resulted in highly productive domestic animals. Current, research-driven biotechnologies in animal agriculture are based on improved understanding of reproductive physiology and endocrinology, embryology, genetics, and animal health at the cellular and molecular levels. Both established and emerging biotechnologies influence animal agriculture and serve as a benchmark for future progress as we enter the era of genomics biology.

3. Established technologies

3.1. Breeding and genetics

Improvements in production animal agriculture are uneven across species because of differences among producers to adopt new technologies. Nevertheless, advances have resulted from: (1) use of sophisticated statistical models to predict breeding values; (2) sire testing and selection; (3) crossbreeding; (4) marker-assisted selection; (5) identification of major genes and quantitative trait loci (QTL) of economic traits through genome mapping; (6) creation of transgenic animals by direct gene transfer or homologous recombination; (7) methods for producing transgenic animals through genetic engineering of sperm.

3.2. Reproductive management

Many aspects of reproductive management that have impacted animal agriculture also served to validate biotechnologies now used routinely in assisted reproductive technology clinics for women. These include: (1) artificial insemination with fresh or frozen semen; (2) use of sex hormones to control time of onset of estrus and/or time of ovulation; (3) use of sexed semen to control sex of offspring; (4) control of seasonality by melatonin; (5) sex reversal (in fish); (6) artificial incubation of eggs in chickens; (7) superovulation; (8) collection and transfer of embryos; (9) in vitro maturation and fertilization of oocytes and in vitro culture of embryos; (10) embryo splitting and cloning from blastomeres to produce

''copies''; (11) cloning adult animals by somatic cell nuclear transfer to produce ''copies''; (12) vaccinations and health technologies to prevent diseases; (13) climate control to reduce stress; (14) bovine somatotropin (BST) to increase milk production.

3.3. Artificial insemination

The use of frozen-thawed semen for AI, along with sire testing and 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% and increasing. With increased milk production, pregnancy rates to first insemination have decreased to an unacceptably low level [9,10]. Understanding this problem requires research at the cellular and molecular levels and will benefit from the application of new knowledge derived from understanding the bovine genome.

3.4. Assisted reproductive technologies

Embryo recovery and transfer allows valuable animals to contribute more offspring to the gene pool [11]. Embryos stored frozen before being used to initiate a pregnancy result in 40,000–50,000 beef calves per year [12]. Emerging technologies allow sexing of semen through cell sorting of X and Y sperms [13] or sexing embryos to control gender of offspring, as well as the use of genetic markers to identify embryos expressing desirable alleles for reproduction and production traits.

Hundreds or thousands of embryos can be produced using techniques for recovery and maturation of immature oocytes for about 1 day in medium containing hormones. The oocytes are fertilized with live spermatozoa or by injection of a single spermatozoa 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 being transferred to recipient females. These techniques can be used to produce normal human infants and offspring in animal agriculture [14]. 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, e.g., blocked oviducts [15]. The commercial application of IVF has resulted in as many as 4000 calves born in single year [12].

Splitting or bisecting embryos yields zygotic twins or clones [16] that are referred to as ''copies'' as they 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. However, only 1–2% of calves resulting from embryo transfer are produced in this manner [12].

Cloning by nuclear transfer from embryonic blastomeres [17] or from a differentiated cell of an adult [18,19] requires that the introduced nucleus be reprogrammed by the cytoplasm of the egg to direct development of a new embryo which is then transferred to a recipient mother to 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 mitochondrial DNA and

perhaps which nuclear genes are expressed. Cloning from blastomeres and somatic cells often results in large calves and lambs, termed the large offspring syndrome (LOS) [20,21]. More serious abnormalities may be associated with cloning from somatic cells [22].

The Holstein Association indicates that all nuclear transfers to date have been from blastomere nuclear transfer. Through 2001, a total of 2226 registered Holstein clones from embryo splitting (754 males and 1472 females) and 187 from blastomere 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 milk yield traits, but blastomere nuclear transfer clones were similar to, and clones from embryo splitting were slightly less genetically valuable than non-cloned full sibs [23].

4. Emerging technologies for animal agriculture

4.1. Genetic engineering

Reproductive technologies are required to produce genetically engineered livestock that may be used as bio-incubators for production of pharmaceuticals and other products such as silk, 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 [24]. Pigs can provide replacement organs for humans, but a carbohydrate epitope (terminal α1,3-galactose residues) present on the surface of porcine cells [25] results in the xenograft being rejected. Thus, pigs are being genetically engineered to silence the α1,3-galactosyl transferase enzyme genes [26,27]. Homologous recombination to specifically modify the genotype of an animal is now limited to mice, but specific gene knockouts in domestic animals can be achieved for use as models for human disease, e.g., sheep with a mutated collagen gene are a model for the human connective tissue disease *osteogenesis imperfecta* [28], and to understand gene function.

4.2. 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 1980's 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 the nucleus from a somatic cell into an oocyte from which the nucleus was removed [18,29]. Somatic cell nuclear transfer (SCNT) 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 transfer 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 [30,31].

Cloning by nuclear transfer, using present methods, is very inefficient. This is likely due to the limited ability of oocyte cytoplasm to reprogram somatic cell nuclear DNA, whereas it readily reprograms sperm cell nuclear DNA following normal fertilization [22]. Consequently, there are very high rates of embryonic, fetal, perinatal and neonatal deaths, as well as birth of offspring with various abnormalities. These losses are assumed to result from inappropriate expression of genes during various stages of development with only 0–4% of embryos reconstructed using adult or fetal somatic cell nuclei being born as live young. A very high percentage of conceptuses (embryo/fetus and associated membranes) that survive beyond the first 30–60 days of gestation in sheep and cattle have abnormal placental morphology (reduced vascularity, abnormal or few cotyledons) and functions (hydroallantois, hydroamnios), abnormal fetal development (enlarged liver, hydrops fetalis, dermal hemorrhage, swollen brain), and neonates often experience respiratory distress and cardiovascular abnormalities. In addition, there is evidence for abnormalities of the immune system, brain, and digestive system of cloned animals. Mice cloned from cumulus cells become obese, but this trait is not heritable after sexual reproduction, which is direct evidence that obesity in the clones result from epigenetic events [22,32].

Wilmut et al. [22] reported that a number of cellular factors influence the outcome of cloned offspring. First, normal development depends on the embryo having normal ploidy that is achieved by coordinating stage of cell cycle of the recipient oocyte and donor nucleus. For donor cells, the G2/M/G1/G0 phases are associated with effective nuclear reprogramming; however, there are conflicting reports as to whether there is an advantage for donor cell nuclei being in G1 versus G0. Donor cells at metaphase seem to be most compatible with metaphase II oocytes, with respect to development of embryos to the blastocyst stage, but high rates of mortality occur later. This mortality likely reflects inappropriate expression of genes at different stages of development, which is lethal. For example, perturbation of expression of H10 and/or insulin-like growth factor II (IGF-II), as well as reduced expression of IGF-II receptor in liver, kidney, heart, and muscle, is associated with large lamb syndrome following embryo culture. Changes in expression of the IGF-II receptor gene has been associated with loss of methylation at a differentially methylated region involved with genetic imprinting. There are also reports of inappropriate expression of both imprinted and non-imprinted genes, inappropriate reactivation of the inactive X chromosome [33] and reprogramming of telomerase activity [34] to restore telomere length in cloned animals.

Inappropriate gene expression can result from defects in the organization of nuclear material, chromatin structure, and/or activity of regulatory molecules. Chromatin proteins regulate access of transcription factors to chromosomal DNA and expression patterns for individual genes [35]. Linker histone H1 and somatic cell histone H1 are important regulators of gene expression. Their expression changes from early embryonic development (absent to very low) to time of activation of the embryonic genome (low to moderate), and somatic cell histone H1 is lost from most mouse nuclei soon after transfer depending on cell cycle stage for donor and recipient cells. Enzymatic modifications of histones include phosphorylation, methylation, acetylation, and ubiquitination or removal of these modifications, which lead to epigenetic regulation of gene expression [36].

Expression of genes in higher organisms is regulated by histones H2A, H2B, H3, and H4, as well as histone H1 and other non-histone proteins, which result in the condensed state DNA which is inaccessible to transcription factors. Transcription of genes requires ''unraveling'' of DNA and a higher order chromatin structure. This is achieved by a family of ATP hydrolyzing enzymes that remodel chromatin by shuffling nucleosomes, and a family of enzymes that modify histones covalently by acetylation, phosphorylation, methylation, or ubiquination of histone tails. If this does not occur, the gene(s) is silent [37]. Widespread disruptions in DNA methylation occur in cloned embryos of mice and cattle, with abnormally high rates of methylation being retained through several cell divisions. In pigs, changes in methylation are initially similar for embryos resulting from in vitro fertilization, but by the 4- to 8-cell stage, embryonic DNA is more extensively methylated than for embryos resulting from oocytes fertilized in vivo.

Epigenetics is the study of factors that influence behavior of a cell without directly affecting its DNA or other genetic components. The epigenetic view is that cells undergo differentiation events that depend on correct temporal and spatial repression, derepression or activation of genes that affect the fate of cells, tissues, organs, and ultimately organisms. Thus, epigenetic changes in an organism are normal and result in alterations in gene expression. For example, epigenetic transformation of a normal cell to a tumor cell can occur without mutation of any gene.

Santos et al. [38] reviewed epigenetic marking that correlates with developmental potential of cloned bovine pre-implantation embryos. They indicated that reprogramming DNA methylation affects the entire genome of mammalian embryos: (1) Phase I during germline development when DNA methylation imprints are eliminated and (2) Phase II during preimplantation development of mammalian embryos. Phase II DNA reprogramming is initiated upon fertilization of the oocyte for remodeling of chromatin in the male pronucleus and selective demethylation of its DNA, while subsequent DNA demethylation in early cleavage stage embryos is passive. At the blastocyst stage, de novo methylation is lineage-specific as the inner cell mass (embryonic disc) becomes highly methylated and trophectoderm becomes hypomethylated. These epigenetic reprogramming events appear to be deficient in cloned embryos that have abnormal patterns of DNA methylation and gene expression.

Santos et al. [38] noted that epigenetic reprogramming is severely deficient in cloned bovine embryos and involves histone H3 lysine 9 (H3-K9). In control bovine embryos DNA methylation is reduced between the 2- and 4-cell stages followed by de novo methylation after the 8-cell stage. There is close correlation between H3-K9 methylation and overall DNA methylation. DNA methylation is up to 10-fold greater at all stages of development in cloned bovine embryos indicating that more genes are silent. Further, H3-K9 can be acetylated, indicating active gene expression, and cloned embryos are also hyperacetylated. These results suggest failure to both activate and to silence genes appropriately in cloned bovine embryos. In normal blastocyts there is lineage-specific hypermethylation of the inner cell mass compared to trophectoderm, but this asymmetry is not found in cloned embryos. Differences in acetylation of H3-K9 were also found, but they were not as dramatic as those for methylation. Failure of trophectoderm to be hypomethylated may explain aberrant placental development that is characteristic of cloned animals. Nuclei from bovine granulosa cells yield a higher percentage of cloned

blastocysts than nuclei from fetal fibroblasts and, as predicted, granulosa cell-derived clones had a higher proportion of normal DNA and histone methylation. One example of inappropriate expression of a gene is Major Histocompatibility Class I antigens that are not expressed by normal bovine trophectoderm/chorion. However, MHC Class I genes are expressed by trophectoderm/chorion of cloned conceptuses [39], which may put cloned conceptuses at risk for rejection by the maternal immune system.

Epigenetic programming is a normal part of development including differentiation of primordial germ cells, sperm, oocytes, and conceptus tissues. This programming allows for both temporal and spatial repression and derepression of genes and development of healthy offspring. There is no evidence that the basic genome of cloned animals differs from that of the donor cells used for somatic cell nuclear cloning. However, there is evidence that a very low percentage of cloned embryos survive, because of inappropriate silencing or over-expression of genes due to abnormal: (1) genome methylation; (2) histone assembly into nucleosomes; (3) chromatin remodeling by linker histones, polycomb group proteins, nuclear scaffold proteins and transcription factors. Most cloned animals do not survive, but those that do survive beyond the neonatal period have an apparently normal phenotype. In either case, the basic genome is not modified. Rather, temporal and spatial aspects of gene expression during the course of development are apparently flawed in cloned embryos and the severity of these epigenetic events likely dictate the fate of the clone.

4.3. Marker-assisted selection (MAS)

Marker-assisted selection 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. Thus, MAS is important for studying complex traits governed by many genes, and in animal breeding and selection strategies [40]. The use of 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 [41]. Eventually, MAS will be replaced by genotyping for genes or mutations of interest. Two examples from sheep are the Booroola gene [42] 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 Assaf dairy breeds [43] to increase ovulation rate.

4.4. 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@foodproduction-

daily.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%. 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 using embryo transfer. Another example of mapping identifying a QTL affecting reproductive traits in pigs is on the long arm of chromosome 8 of pigs for increased litter size and prenatal survival [44]. Although unconfirmed, the SPP1/Osteopontin gene was reported to be the QTL candidate gene. This is of special interest since osteopontin is highly expressed in the pig uterus and is very abundant at the interface between trophectoderm/chorion and uterine epithelia from the peri-implantation period through the last trimester of gestation in pigs [45]. Further, OPN −/− mice have significantly smaller fetuses at Days 10.5, 15.5 and 19.5 of gestation, but litter size was not different between OPN −/− and OPN +/+ mice [46].

The germline of animals may be genetically modified by introduction of transgenes. Germline refers to the lineage of cells that can be genetically traced from parent to offspring. It is possible to access the germline of animals using one of five methods [47]: (1) directly manipulating the conceptus after implantation; (2) manipulating the spermatozoa that produces the zygote; (3) manipulating early embryonic tissue in situ; (4) using embryonic stem cell lines in early embryos; (5) manipulating cultured somatic cells and transfer of their nuclei into enucleated oocytes.

Transfection is a method to introduce novel genes into animals in a manner similar to methods used for plants including: (1) microinjection of DNA into the nucleus of anchored cells; (2) electroporation of DNA through cell membrane pores by pulsed electrical charges; (3) polycationic neutralization of the cell membrane and DNA to improve passive uptake; (4) lipofection whereby DNA is taken up by cells; (5) sperm-mediated transfection, often used in conjunction with intracytoplasmic sperm injection or electroporation. As with plants, microinjection is a very inefficient means of creating transgenic animals. An incredibly small percentage of livestock embryos that undergo microinjection yield transgenic animals (Rexroad, 1994) and transgenic animals resulting from successful microinjection do not necessarily pass their transgenes on to their offspring [47].

The use of virus strains modified to carry genetic material into a cell differs from transfection in that after the novel DNA is delivered there is a requirement for viral replication processes to integrate the desired DNA into the host cell's genome. The use of transposable elements in animal cells has not been developed, as there are no known active and naturally occurring transposable elements in mammals. Therefore, transposable elements found in insects and fish are under investigation for potential use in animals.

4.5. Transgenic spermatozoa

New procedures are being explored to use spermatozoa as the vector for transgenesis in animals. These include: (1) incubation of isolated spermatozoa with DNA before use in fertilization of oocytes; (2) microinjection to transfect male germ cells within the seminiferous tubules; (3) tranfection of male germ cells in vitro followed by transfer to a recipient's seminiferous tubules [48–50]. Lavitrano et al. [51,52] described efficient production of sperm-mediated gene transfer (SMGT) of human decay accelerating factor

(hDAF) to produce transgenic pigs. Success in achieving DNA uptake by spermatozoa requires that one adhere to specific techniques. However, even under the current ''optimum conditions'', the efficiency of SMGT to produce DNA positive transgenic pigs varied from 0 to 88% and of those that were DNA positive expression of the RNA varied between 41 and 100%. It is evident that SMGT offers a relatively efficient and inexpensive method for producing transgenic animals, and that the transgene can stably integrate into the genome and be transmitted to the next generation. However, stringent evaluation of each potentially transgenic animal is required due to the substantial among animal variation in expression of the transgene, perhaps due to the fact that the insertion site of a transgene is random and subject to positional effects at the site of insertion that influence expression of the transgene. An unintended effect of SMGT could be altered expression of one or more genes in embryos resulting from fertilization of an oocyte with a transgenic sperm. Indeed, human Growth Hormone expression driven by pVLCN (plasmid, viral long terminal repeat retrotransposon construct with neo sequence for integration via ribosome entry site) an expression vector derived from VL30, a family of murine retrotransposons, was not well tolerated and exerted lethal effects on embryos soon after implantation when more than 50 ng foreign DNA per one million spermatozoa was used [53]. A plasmid cytomegalovirus, human Growth Hormone (pCMVhGH) construct was tolerated at up to 1000 ng foreign DNA per one million sperm, but pregnancy rate and pups born was significantly reduced at 500 ng and 1000 ng foreign DNA per one million spermatozoa compared to 100 ng foreign DNA per one million spermatozoa. This technology holds much promise, but requires further development.

4.6. Use of ectopic DNA

Biotechnology can be used to modify the endocrinology of domestic animals to affect reproduction, lactation, and growth. For example, instead of modifying hypothalamic-specific expression of Growth Hormone Releasing Hormone (GHRH), one can use ectopic expression of a cDNA for this neuropeptide that is driven by a synthetic muscle-specific promotor to elicit increases in both GH and IGF-I [54]. This technology is effective in pigs and rats to achieve pulsatile and physiological release of GHRH [55]. 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 plasma IGF-I concentrations. Interestingly, pituitaries of pups from dams receiving the GHRH cDNA had more somatotrophs and lactotrophs and, at 3 weeks of age, 2.5 times more GH and prolactin mRNA in their pituitaries. These results suggest that modification of cell lineage differentiation has intergenerational effects on growth, development and reproduction. Perhaps 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 also has the potential to affect the reproductive system through expression of specific hormones and growth factors during critical developmental periods to enhance uterine capacity or to increase milk production by the dam.

5. Biotechnologies to enhance reproductive efficiency

5.1. Uterine biology and pregnancy

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 [56]. 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 [57,58]. 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 of the epitheliochorial and syndesmochorial types.

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). Comparing gene expression between UGKO and control ewes during adenogenesis and during the preimplantation period of pregnancy has identified key genes for study to understand uterine biology and early pregnancy.

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 regulates endometrial gland morphogenesis and function during pregnancy through sequential actions of ovarian steroid hormones, the pregnancy recognition signal interferon tau, placental lactogen and placental growth hormone [59]. Unexplained rates of peri-implantation embryonic losses in humans and livestock may reflect unrecognized defects in uterine and/or placental functions that, when understood at the genomic level, will suggest mechanisms to increase uterine capacity in domestic animals.

Genomics approaches to understanding key genes and molecular mechanisms responsible for uterine adenogenesis, fertility, and prolificacy are expected to reveal molecular markers for use in animal agriculture, veterinary medicine and human medicine to enhance reproductive efficiency.

5.2. Placental lactogen affects conceptus development and mammary gland function

High circulating concentrations of ovine placental lactogen (oPL) in pregnant ewes is well known, but its biological roles are not clear. Ovine placental lactogen, a member of the GH/PRL gene family, has both somatogenic and lactogenic activities and is produced by binucleate cells of trophectoderm/chorion from Day 16 to Days 120 to 140 of pregnancy when concentrations may be 1 ug/mL. However, concentrations of oPL in fetal plasma are maximum on Day 90 of pregnancy (ng/mL concentrations). 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 elucidated the physiological role(s) of oPL during pregnancy and subsequent lactation after active immunization of ewe lambs against recombinant oPL (roPL) [60]. 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, 3 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 serum from 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 serum from immunized ewes.

Immunization of ewes did not affect lambs born per ewe lambing, but lambs born to immunized ewes were significantly heavier and this differential in weight of individual lambs increased as number of lambs born per ewe increased from singles to triplets. This effect could not be attributed to differences in IGF-I levels in serum or milk composition. In immunized ewes, higher concentrations of oPL in the 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 weaned growing lambs and in lactating ewes [61]. In two experiments of 35 days each, 2-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 enhanced lamb growth by 10–25%. 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)). The roGH significantly increased daily milk production by up to 55%, whereas roPRL had no effect [61]. 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 concentrations, 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 [62].

5.3. Transcriptional profiling to unravel complex physiological mechanisms

The following discussion is based on a review by Lewin [63] in which he states: (1) that traditional animal and veterinary sciences have been based on systems biology including genetics, nutrition, immunology, reproductive biology, lactation, and environmental physiology; (2) that we are now in an era of systems biology that begins at the genome level

to predict biological responses rather than studying a phenotype or a biochemical response in an attempt to identify the responsible genetics and/or biochemical mechanism(s); (3) that combining conventional modeling strategies with new genomics technologies will provide exceptional opportunities in discovery research involving data integration and application of genomics biology in livestock species for functional genomics to enhance our understanding of systems biology to benefit animal agriculture and the biomedical community.

The modern era of cattle genomics is claimed to have begun in 1997 with the production of cattle-hamster radiation hybrid panels [64] that led to the identification of polymorphic markers and the ability to score their segregation into large families [63]. Subsequent progress in producing expressed sequence tags (ESTs) and search engines, such as BLAST algorithm, transformed cattle genomics after it was established that this was a powerful resource for comparative mapping to identify human orthologs [63]. Of great importance to animal agriculture and veterinary medicine is the expectation that coordinate maturation of comparative functional genomics and system biology will result in a resurgence of interest in and funding for domestic animal models for biomedical research targeted to reproduction, nutrition, immunotherapeutics, lactation, growth, and development and interactions among the various aspects of systems biology. The thought-provoking paper by Lewin [63] validates the notion that we are now in the era of genomics biology and that only those programs that embrace this fact will continue to make substantial contributions to animal agriculture, veterinary medicine, and human medicine.

5.4. Veterinary medicine in the era of genomics biology

Genomics biology can impact practicing veterinarians in a number of ways. First, molecular geneticists will provide ‘‘genomic tools’’ in the form of gene markers to identify desired production and health traits in livestock and genes related to performance in sports animals. Food safety and biosecurity demands require development and validation of methods for real-time DNA identification for traceability of animals and animal products in the food chain. As one example, there is great interest in building on the abilities of the veterinary profession to identify sheep that are resistant to scrapie and retain those ewes and rams in the breeding herd. Scrapie is the ovine homologue of bovine spongioform encephalopathy (BSE), so veterinarians should be key participants in efforts to eliminate BSE. Similar approaches will be used to identify genes in cattle that predispose resistance to other diseases, such as Johne’s disease, bovine tuberculosis, foot and mouth disease, and Rift Valley Fever, and make our livestock industry less vulnerable to potential weapons of agricultural bioterrorism. Identifying, mapping, and understanding the function of genes in livestock will make the nation’s food supply safer by providing methods for genetic tracking of animals and animal products, selecting animals with reduced risk for disease, and decreasing the use of antibiotics. Practicing veterinarians will be partners in genomics era biology as they engage in activities ranging from collection of white blood cells for DNA analyses, obtaining tissue biopsies for DNA analyses, conducting molecular tests in the laboratory and helping livestock producers interpret results of DNA analyses. This information will be central to breeding programs designed to produce livestock that are resistant to disease and parasites and have desired production traits.

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