INTRODUCTION

Animal growth research has evolved during the past 50 yr resulting in incremental changes in fundamental knowledge and application, especially to meat animal production. Changes in production practices have resulted in heavier livestock at slaughter. In fact, the preslaughter weights of production animals have been increasing since the 1920s with the largest increase beginning in the 1980s (Cima, 2014). The working theory that efforts in improving meat production efficiency must optimize muscle growth and adiposity has been consistent for over a century of research efforts and farm practice. Key areas of experimental work to improve farm animal production included a progression of work in ruminant and nonruminant nutrition and feed harvesting and preservation/utilization technologies as well as in animal breeding, management, and reproductive biology (Harris and Newman, 1994; Emmerson, 1997; Webb, 1998; Lebret, 2008; Mellencamp et al., 2008; de Verdal et al., 2011). Laboratory-based research to determine critical aspects of cell growth and regulation has provided the basis for many farm-based changes in production management schemes.

Two elements, adipose and muscle stem cells, are of paramount importance to the production animal world and, therefore, have been a research focus for decades. This paper documents this journey and provides a historical overview of the transition of research from 50 yr ago to today. Six decades ago, researchers struggled to simply isolate stem cells with lofty goals of growing the cells and conducting repeatable in vitro experiments. Advances in research materials, including cell culture ware, media and media components, and instrumentation, have benefited...
advent of this field of study. Timely discoveries and fresh interdisciplinary ideas have also sped up knowledge gathering as novel hypotheses regarding the importance of adipose and muscle stem cells to the overall processes of development and growth, tissue regeneration, and final carcass composition have been addressed.

Emergence of Cellular Animal Growth Research in Animal Science

By the early 20th century, energy metabolism (Armsby, 1922; Brody, 1945; Kleiber, 1975) became a major focus in basic animal physiology/nutrition. Long-standing research in energy conversion helped develop present concepts and application of animal energy metabolism principles, such as the net energy system (Ferrell and Oltjen, 2008), to optimally feed animals with improved diet formulations. Concurrently, development of feed processing technologies and the determination of qualitative and quantitative requirements of all basic nutrients resulted in impressive performance improvements of beef cattle, sheep, dairy cows, poultry, and pigs by 1960 through the 1970s. For example, phased diets that differ in nutrient composition and particle size are currently common practice in the swine industry, and animal nutrition continues to be fine-tuned with the current focus on minimizing nutrient waste, improving food products for human consumption, and targeting the gastrointestinal tract microbiome.

Starting in the mid-1950s, new avenues of enhancing animal performance research indicated diethylstilbestrol could function as a growth-promoting hormone (Preston, 1960). Experimental findings indicated that protein synthesis and fat metabolism could be directly modulated with specific extrinsic agents. Research on a variety of agents was initiated and included steroid implants, β-adrenergic agonists, and somatotropin (Bergen and Merkel, 1991), which are available for use to improve production output. Initial work, in vivo, provided evidence on the potential of these agents on animal performance. Not only were BW gain, efficiency, and lean deposition increased, but these newer approaches could depress fat deposition during finishing periods.

The mechanisms whereby extrinsic agents affect muscle cells and adipocytes were studied to a limited degree in vivo (Cunningham et al., 1963; Ricks et al., 1984; Bergen et al., 1989; Halsey et al., 2011). Techniques using tissue explants in vitro (Mersmann and Hu, 1987; Peterla and Scanes, 1990) and both muscle- and adipose-cell culture proved invaluable as tools to understand the mechanism of action of specific extrinsic agents. Mersmann et al. (1976) used isolated porcine adipocytes in short-term incubations, whereas Hausman and Martin (1989) studied porcine preadipocytes differentiated in stromal vascular (SV) cell cultures and Weber et al. (1992) used a preadipocyte cell line not derived from a meat animal species. These and many other studies helped identify critical pathways along with similarities and differences between production animal physiology and those reported using other models including rodents and established cell lines used in molecular and human health research.

Therefore, work with muscle/satellite cells and adipose cells was initially divided into 2 major topics. First, in vitro or cell culture approaches were used to explore the effect of extrinsic agents on differentiated cells. Second, these procedures were used to study regulation of cellular hyperplasia and differentiation of muscle and fat cells from food-producing animals. Subsequent studies became very sophisticated and focused on cellular/intracellular signaling and gene regulation/expression during different physiological states. As molecular biology techniques, including genomics, transcriptomics, and proteomics, emerge, there has been a return to in vivo approaches (McNeel and Mersmann, 2003; Hoque and Suzuki, 2009; Halsey et al., 2011) with less focus on in vitro evaluations. Nonetheless, in vitro research is still essential as it provides unique insights and mechanistic understanding that may be unavailable in whole-animal studies. One example is the vast interest in understanding stem cell regulation.

Stem Cell Research is Providing Mechanistic Insights to Tissue Development and Regulation

Stem cells are a unique population of progenitor cells characterized by a virtually unlimited capacity for self-renewal and the ability to differentiate into a variety of terminally differentiated somatic cell types. Stem cells may be of 2 origins, embryonic or adult. Pluripotency, defined as the potential of a cell to differentiate into any of the 3 germ layers, is restricted to the zygote, early embryonic cells, primordial germ cells, and the stem cells derived from embryonic carcinomas in mammals. This pluripotency appears to allow both adult and embryonic stem (ES) cells the ability to restore or replace tissue that has been damaged by disease or injury (Gimble et al., 2007; Monaco et al., 2011; Liu et al., 2013). Embryonic stem cells are derived from the inner cell mass of the blastocyst. In contrast to adult stem cells (ASC), ES cells are pluripotent, contribute to all 3 primary germ layers (endoderm, mesoderm, and ectoderm), indefinitely proliferate, and maintain an undifferentiated phenotype. The ability to isolate embryonic cells from pre-implantation mouse and human embryos and maintain them, in vitro, has provided a powerful research tool to study mammalian development.
In contrast, ASC have been isolated from many differentiated tissues, including fat, bone marrow, muscle, liver, skin, and brain. As a result of stem cell activity, adult tissues are constantly renewed to ensure maintenance of cell type throughout the life of the animal. In vitro studies using pluripotent cell lines offer an opportunity to study the early stages of embryonic development not accessible in utero, the development of differentiated cell phenotypes such as adipose tissue, and the effects of animal genome modification. Recent studies of swine adipose-derived ASC (Monaco et al., 2009) demonstrate the vast opportunities to study gene expression (Monaco et al., 2010, 2012) during adipocyte differentiation as well as their potential for use in regenerative biology/medicine (Monaco et al., 2011).

Almost a decade of research has been conducted using murine ES cells. Research shows ES cells can serve as vectors for the transfer of foreign DNA in the production of transgenic mice (Gossler et al., 1986; Robertson, 1991; Robertson et al., 1986) and they are proving to be a significant aspect of biomedical research and regenerative biology. Embryonic stem cell–mediated transgenesis has some distinct advantages over other transgenic methods. First, the efficiency of producing transgenic animals may be significantly increased with this technology (Gossler et al., 1986; Rexroad and Pursel, 1988). Second, these cells can be transformed with foreign DNA in vitro (Thomas and Capecchi, 1987; Capecchi, 1989; Robertson, 1991). Genetically transformed ES cells can be selected and individual cell lines (clonal lines) can be derived from a single cell. Once isolated, these individual cell lines can be screened for recombination (homologous or nonhomologous) of exogenous DNA into chromosomal DNA. This provides the opportunity to establish the stable incorporation of the desired gene before chimera production (Gossler et al., 1986; Thomas and Capecchi, 1987) or before use as nuclei donors for nuclear transfer (Dai et al., 2010). Establishment of ES cells from various species will allow more flexibility in gene regulation studies and developmental biology research. In addition to the mouse, isolation of embryo-derived cell lines has also been reported from preimplantation embryos of a number of mammals including the pig (Evans et al., 1990; Notarianni et al., 1990, 1991; Piedrahita et al., 1990; Strojek et al., 1990; Gerfen et al., 1991; Chen and Wu, 1993; Onishi and Youngs, 1993; Talbot et al., 1993; Wheeler, 1994; Gerfen and Wheeler, 1995; Vackova et al., 2007; Park et al., 2013; Vassiliev and Nottle, 2013). The culture of ES cells to confluence or induction of differentiation with retinoic acid or dimethyl sulfoxide results in morphological differentiation into fibroblasts, adipocytes, and epithelial, neuronal, and muscle cells (Wheeler, 1994). Efforts to establish embryo-derived cell lines from sheep (Piedrahita et al., 1990), cattle (Pashaiaisl et al., 2013), and rabbits (Intawicha et al., 2013) suggest species differences in the ability and requirements for establishing these lines.

Another cell type that has been recently reported is the induced pluripotent stem (iPS) cell. Takahashi and Yamanaka (2006) reported that differentiated mouse cells could be reprogrammed into pluripotent ES cell–like cells after viral delivery of a set of 4 transcription factors. These 4 factors (as well as c-Myc) have been shown to play essential roles in self-renewal and pluripotency of ES cells. Since 2006, iPS cells have been reported for a variety of species including swine (Roberts et al., 2009; Montserrat et al., 2011), mice (Yoshida et al., 2012), and humans (Takahashi et al., 2007). Additional basic research to understand the mechanisms of self-renewal and pluripotency as well as tumorigenesis will eventually make the use of iPS cells more practical.

Whereas research efforts to further develop stem cells from meat animal species will expand, porcine cells may receive added attention, because they are immunologically and physiologically more similar to humans (Hausman et al., 2014) and thus serve as a better research model (Phillips and Tumbleson, 1986). The use of ES cells, ASC, and/or iPS cells can provide important insights into adipose development, differentiation, and function in meat animals.

Stem cell use in domestic animals will require regulatory approval that is expected to be complex. First, treatment options aimed at the animal or for human therapeutics should require approval of stem cells as a drug. Exposure of people to stem cells by use and consumption of animal products will also be evaluated. Regulations for animals and people may differ, depending on the regulatory agency. For example, the U.S. Food and Drug Administration (FDA) has a clear policy that, in humans, stem cells are drugs and therefore must be proved safe and effective before they can be used in treatment except under certain conditions. United States Food and Drug Administration regulation for veterinary medicine differs. It is clear that veterinary use of stem cell therapies is growing, even though the FDA has neither approved any stem-cell therapies nor intervened in their use (Cyranoski, 2013). A scan of stem cell–based patents suggests the technology focus is still on developing optimized methods for stem cell isolation, propagation, and maintenance. Several patents have been published and describe the potential use of stem cells for cancer treatment. As stem cell technology continues to develop, we will see expanded descriptions of use for domestic animals.
Emergence of Muscle and Adipose Stem Cells in Fetal Tissue

Skeletal muscle can be largely separated into 2 portions: muscle fibers and the remaining SV fraction (Hausman and Poulos, 2004). The basic muscle structure is established during the fetal stage (Greenwood et al., 2000; Nissen et al., 2003) when multipotent mesenchymal stem cells (MSC) first diverge to either myogenic or fibro/adipogenic lineages. Myogenic lineage cells develop into muscle fibers and satellite cells, whereas fibro/adipogenic lineage cells develop into the SV cell fraction of muscle containing adipocytes, fibroblasts, and resident fibro/adipogenic progenitors (FAP; the counterpart of satellite cells; Du et al., 2013). These resident FAP become largely quiescent and form the stem cell pool for later differentiation into intramuscular and intramuscular adipocytes/fibroblasts in muscle (De Coppi et al., 2006; Cartwright et al., 2007; Joe et al., 2010; Uezumi et al., 2010, 2011; Tan et al., 2011).

Adipogenic differentiation examined in 3T3-L1 preadipocytes and other established murine cell lines can be separated into 2 stages: determination and differentiation (MacDougald and Mandrup, 2002). The regulation of adipogenesis involves several cell signaling pathways. Bone morphogenetic proteins (BMP), which belong to the superfamily of transforming growth factor β (TGFβ), play crucial roles in the adipogenic determination from multipotent cells (MacDougald and Mandrup, 2002). Zinc-finger protein Zfp423 is one of many transcriptional factors critical for the commitment of progenitor cells into adipogenic lineage (Gupta et al., 2010) as it induces the expression of peroxisome proliferator-activated receptor γ (PPARγ) and CCAAT/enhancer-binding protein α (C/EBPα), which induce the differentiation of multipotent preadipocytes (Spiegelman and Flier, 1996; Rosen and MacDougald, 2006; Avram et al., 2007). Zfp423 is regulated primarily through polycomb repressive complex 2–mediated DNA methylation in fetal tissue, demonstrating the importance of epigenetic regulation of early adipose development (Yang et al., 2013). Whether these types of regulatory schemes operate similarly in meat animals needs to be explored.

Embryonic myogenic cells are derived from a pool of MSC (Bailey et al., 2001), which can be committed to a myogenic lineage as well as other cell types such as adipocytes, fibroblasts, and chondrocytes. However, the committed cells are termed myogenic progenitor cells as they are not yet muscle cells. Embryonic myogenesis is under the control of a number of regulatory proteins including Wingless and Int (Wnt), paired box gene (Pax) 3, and Pax7 (Maroto et al., 1997; Hyatt et al., 2008). Wnt signaling is very important to activate myogenesis (Cossu and Borello, 1999). The expression of Pax3 and Pax7 in MSC induces the expression of myogenic regulatory factors (MRF; Bailey et al., 2001). Currently identified MRF, with known roles in myogenesis, include myogenin, MRF4, MyoD, and Myf5 (Stewart and Rittweger, 2006). MRF4 is mainly expressed at the very early stage of myogenesis followed by the expression of MyoD and Myf5, which convert precursor cells into myoblasts (Stewart and Rittweger, 2006). Myogenin is necessary for the fusion of myoblasts into myotubes (Barnoy and Kosower, 2007) and is expressed later and maintained throughout the fetal stage. MRF4 is also expressed later and becomes dominant in postnatal development. MyoD and Myf5 function cooperatively to induce the differentiation of multipotent myogenic precursor cells into myoblasts (Roth et al., 2003).

Because myogenic progenitors and FAP are derived from common multipotent cells during fetal development, it may be considered a competitive process. In genetically high-marbling Wagyu cattle, both intramuscular adipogenesis and collagen accumulation are greater than in relatively low-marbling Angus cattle, showing the enhancement of the fibro/adipogenic commitment versus myogenic commitment (Duarte et al., 2013). During fetal development, enhanced fibro/adipogenic development is correlated with downregulation of myogenic differentiation (Zhu et al., 2008). A positive correlation between intramuscular fat and collagen content is commonly observed (Karunaratne et al., 2005, 2009; Yan et al., 2010, 2011b).

Table 1. Landmark developments of approaches to the study of adipocyte stem cells

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Stem Cells for Adipocytes

Stromal vascular cells are harvested from collagenase-digested adipose tissue (Table 1). A variety of cells are present in the SV cell pellet during isolation including ASC, allowing for the use of SV cell cultures as a source of ASC to study cellular changes during adipogenesis. Several human and mouse studies suggest adipose tissue reactivity for several clusters of differentiation (CD) markers, cell surface markers, and/or the absence of reactivity for other CD markers can be used to identify the ASC phenotype (Hausman and Dodson, 2013).

Adipocytes are primarily derived from MSC during embryogenesis and fetal development (Hausman and Dodson, 2013; Table 1) in all animals. Embryonic and fetal stem cells in various anatomical locations ultimately lead to the development of initial capillary-like structures with perivascularly located MSC followed by basic adipocyte cluster architecture containing some lipid-filled adipocytes. Markers of adipogenesis including PPARγ, C/EBPα, and glucocorticoid receptor 2 (GR2) are identified before overt adipogenesis in fetal pig adipose tissue, indicating the presence of adipocyte progenitor cells. In other words, the fetal pig model suggests that the development of adipocyte progenitor cells clearly precedes overt adipocyte differentiation in early fetal adipose tissue development. Histological analysis indicates that adipocytes are present by midgestation in bovine, ovine, and porcine fetuses. Further research using primary adipose tissue SV cell cultures established from fetuses as young as 50 d demonstrate that lipid-accreting preadipocytes increase with fetal age as a percentage of total cells (Hausman et al., 2014).

Adipose tissue progenitor cells are localized around specific tissue components/structures in young rat and fetal pig tissues (Hausman, 1985). These include hair follicles, sweat glands, large nerves, large blood vessels, and mammary gland ducts (Hausman et al., 2014). Lipid and enzyme cytochemistry results demonstrated that adipogenesis begins directly against the outer aspects of these structures (Hausman, 1985). Progenitor cells on the outside of these structures are physically contiguous with initial adipogenic cells and differentiating adipocyte clusters (Hausman, 1985).

Cell surface markers have been used to identify stem cell progenitors of adipocytes in studies of progenitor cells in several species. Reactivity for several cell surface markers and/or the absence of reactivity for others identifies an adipocyte stem cell. Rodeheffer et al. (2008) transplanted fluorescent activated cell sorted (FACS) adipocyte stem cell subpopulations (CD24+) into the fat depots of A-Zip lipodystrophic mice resulting in normal fat depots, whereas transplanting CD24− and CD34− cells failed to produce white adipose tissue (WAT). However, injection of CD24+ precursor cells into wild-type/normal mice did not induce WAT. A review of 8 studies including 6 species (mice, humans, chickens, pigs, goats, and rats) demonstrated that very few CD markers consistently identified stem cells across species (Khan et al., 2008; Rodeheffer et al., 2008; Gong et al., 2011; Song et al., 2011; Yang et al., 2011; Ren et al., 2012; Niada et al., 2013). Out of the 15 CD markers used in these studies, only CD44+ and CD29+ were expressed by adipocyte stem cells in all species and studies. In 3 of 4 studies of porcine adipose tissue stem cells, CD90 was expressed by adipose stem cells, and CD34 expressed by stem cells in 2 of the 4 studies (Williams et al., 2008; Song et al., 2011; Niada et al., 2013; Perruchot et al., 2013). Therefore, surface antigen markers may depend on the species and breed and may not define adipose tissue stem cells. Furthermore, the availability of cell surface marker antibodies for a given species may limit the utility of such markers.

Recent stem cell studies have clearly demonstrated the presence of MSC around structures such as hair follicles (Jahoda et al., 2003; Wojciechowicz et al., 2008; Bajpai et al., 2012). In fact, clonal populations of human hair follicle–derived mesenchymal stem cells (hHF-MSC) cultured under appropriate conditions can differentiate along the myogenic, osteogenic, adipogenic, and chondrogenic lineages, as demonstrated by expression of tissue-specific markers (Bajpai et al., 2012). Furthermore, rat hair follicle dermal cells showed the capacity to differentiate into either adipocytes or osteogenic cells (Jahoda et al., 2003). It should be noted that the works of Planat-Benard et al. (2004) and Prunet-Marcassus et al. (2006) were the first to show that adipose tissue contains a number of progenitor cells that can differentiate into adipocytes and several other phenotypes under specific conditions. Subsequently, studies of adipose tissue from other species confirmed that adipose tissue MSC can differentiate into other cell phenotypes in addition to adipocytes. For instance, porcine adipose tissue MSC can differentiate into osteoblasts, adipocytes, and chondrogenic cells (Qu et al., 2007; Williams et al., 2008; Arrigoni et al., 2009; Song et al., 2011; Tang et al., 2012; Table 1).

Diverse and overwhelming evidence indicates that ASC reside in vasculature in the immediate perivascular location (Corselli et al., 2012; Hausman and Dodson, 2013; Braun et al., 2013). A novel theory proposes that adipose tissue stem cells are a population of vascular stem cells (VSC) with a phenotype that is dependent on the angiogenic potential of the vasculature (Hausman and Dodson, 2013). The differential phenotype potential of VSC may include vascular smooth
muscle cells, endothelial (ET) cells, and adipocytes (Hausman and Dodson, 2013). Recent studies in mice and humans with the preadipocyte marker gene Zfp423 demonstrated that ET cells, in addition to perivascular cells, represent adipose progenitor cells (Gupta et al., 2012; Tran et al., 2012). These observations are consistent with location-dependent angiogenic potential in fetal porcine adipose ranging from more to less in regards to a predominance of ET cells and developing arterioles present before overt adipogenesis. In fact, in fetal perirenal tissues, capillaries and VSC indicate the architecture and location of subsequent adipocyte development (Hausman and Dodson, 2013). Additionally, adipocyte progenitors or stem cells reside in the tunica adventitia of arteries (Lin et al., 2010; Corselli et al., 2012; Braun et al., 2013). Adult stem cells in the capillary and in the adventitia of larger vessels are identified as CD34+ , CD31−, CD104b−, and SMA− cells (Lin et al., 2010). The capillary stem cells coexist with pericytes and ET cells, both of which may be progenies of stem cells from either adipose tissue or vasculature. The ASC or VSC in the adventitia of the large vessels exist as specialized fibroblasts with stem cell properties (Lin et al., 2010). It is clear that the source of initial adipogenic cells around capillaries (perivascular) and cells around various structures such as hair follicles and large blood vessels are MSC (Hausman and Dodson, 2013). For instance, blood vessel adventitial MSC are present in other tissues in addition to adipose tissue (Hausman and Dodson, 2013). However, the abundance of MSC does not appear to be a limiting factor in adipogenesis nor does the simple presence of MSC predict adipogenesis.

**Stem Cells for Myogenic Cells (Postnatal Myoblasts)**

In the early 1960s, 2 important discoveries set the stage for the study of postnatal myogenesis and muscle growth. The first was by Alexander Mauro; he used transmission electron microscopy to describe a rare population of small cells that could be found between the cell membrane of the muscle fiber and the surrounding basement membrane (Mauro, 1961). During the late 1950s and through the 1960s, the biological applications
of transmission electron microscopy exploded, due to advances in fixation and embedding techniques and thin sectioning technology (Fig. 1). In the case of Mauro's study, transmission electron microscopy allowed him to resolve the different cellular identity of subsarcolemmal nuclei from the myofiber and this rare cell, which he named the "satellite cell"; light microscopy had not been able to achieve a level of resolution necessary to discern the difference. He stated,

The presence of certain cells, intimately associated with the muscle fiber, have been observed which we have chosen to call satellite cells. Because these cells have not been reported previously and indeed might be of interest to students of muscle histology and furthermore, as we shall suggest, might be pertinent to the vexing problem of skeletal muscle regeneration, a brief communication describing this finding is warranted before a more detailed study. The correct explanation of the origin and role of the satellite cells must await the outcome of further studies. (Mauro, 1961, p. 493)

Although a great deal is known about the satellite cell, we still await the outcome of further studies some 50 yr later.

The second discovery, by Stockdale and Holtzer (1961), provided strong evidence for the fusion of mononucleated myoblast to form myotubes the multinucleated precursor of myofibers. Their experiments were among the first conducted with chick embryo myoblasts grown in cell culture and pioneered the application of in vitro techniques that continue to contribute to myogenesis research. Their experiments with tritium-labeled thymidine also indicated that only the mononucleated myoblasts were capable of replicating DNA and dividing. When coupled with earlier research indicating that the number of muscle fibers did not increase substantially following birth in most species (Staun, 1963; Rowe and Goldspink, 1969), it appeared that the morphogenesis of skeletal muscle fell neatly into the paradigm of prenatal cell hyperplasia and postnatal fiber hypertrophy. However, nuclei in myofibers do not replicate DNA or divide, but the DNA content of normal skeletal muscle increases significantly during growth (Winick and Noble, 1966; Moss, 1968). Ten years after Mauro's discovery and approximately a dozen papers later, Moss and Leblond (1971) demonstrated DNA synthesis in satellite cells that were "chased" into muscle fibers. This indicated that postnatal myogenesis via satellite cells was responsible for the addition of myonuclei to growing muscle fibers.

During the 20 yr following Mauro's initial discovery, only about 100 papers on satellite cells were published by a small number of scientists. By the end of the 1970s, the prominent role of satellite cells in muscle regeneration and growth was beginning to be appreciated (Allen et al., 1979; Campion, 1984). The approach during this period was painstaking, involving the description of in vivo satellite cell activity and population changes in response to physiological challenges during normal growth, and continued through the 1980s and provided the descriptive and conceptual foundation for 3 decades of satellite cell study (Fig. 1).

The sparse distribution of satellite cells in muscle tissue rendered biochemical and the molecular biology technology practically useless. A carefully executed analysis of in vitro satellite cell culture techniques by Bischoff (1974) assessed satellite cell characteristics and regulation directly and specifically. Bischoff digested rat muscle tissue with proteolytic enzymes and isolated satellite cells, which were plated in tissue culture where they proliferated and fused into multinucleated myotubes, as do embryonic and fetal myoblasts. Bischoff (1975) subsequently adapted a single fiber approach to demonstrate the regeneration of isolated fibers by their associated satellite cells. Both in vitro culture procedures have been adapted and used extensively for the past 30 yr, often in concert with in vivo models of progressive levels of organization, from single dispersed cells to satellite cell/fiber complexes where satellite cells can be studied in the context of their niche between the basal lamina and the sarcolemma and finally to the tissue and whole-animal environment. Interestingly, one of the current challenges is to return to the tissue level and understand how the immediate environment surrounding satellite cells regulates function.

In the early 1980s, primary cultures of satellite cells provided an ideal experimental system to determine what extrinsic hormones/factors enhanced either proliferation or differentiation (Fig. 1). Endocrine regulation was considered the primary manner through which cells and tissues responded to their environment. Paracrine and autocrine regulation by “growth factors” was not (yet) considered in the general understanding of cell and tissue physiology. The prime candidates for regulating muscle growth were GH and/or mediators of GH action: IGF-I and IGF-II.

Throughout much of the 1980s, purified hormones and growth factors from tissue, blood, or conditioned cell culture medium were used because recombinant growth factors and monoclonal antibodies were not available. The first factors shown to stimulate cultured satellite cells were members of the somatomedin family (Hossner et al., 1985), somatomedin C, a cell culture-derived form of somatomedin A, and basic fibroblast growth factor (bFGF, now called FGF2). Somatomedin C (IGF-I), multiplication stimulating
activity (MSA and IGF-II), and basic fibroblast growth factor (bFGF and, later, FGF2) were purified from different sources. These factors stimulated satellite cell proliferation and somatomedin C also stimulated satellite cell differentiation and fusion (Allen et al., 1984; Dodson et al., 1985). This work was first to use in vitro satellite cell cultures to examine the potential role of physiologically important hormones in muscle growth control, and furthermore, it demonstrated the importance of developing collaborations to address fundamental questions in muscle developmental physiology.

Embryonic and fetal myogenesis was the exclusive focus of scientists studying muscle development who examined the terminal step in differentiation from proliferating myoblasts to committed, fusion-competent myocytes for several decades. Little attention was given to the proliferation of myogenic precursors to postnatal myogenic satellite cells. As a result, growth factors, as stimulators or inhibitors of terminal differentiation and fusion, were of primary interest. Insulin-like growth factor (IGF) stimulated differentiation whereas TGFβ was an inhibitor of differentiation and fusion (Allen and Rankin, 1990). Fibroblast growth factor (FGF) attracted marginal attention as it had differentiation inhibitory properties, primarily due to its mitogenic activity. These observations, generally consistent among satellite cells, embryonic myoblasts, and myoblast cell lines, were that IGF, bFGF, and TGFβ regulated proliferation/differentiation. Early on, it was clear that proliferation and differentiation of myogenic cells were mutually exclusive processes; consequently, the ability of IGF to stimulate both proliferation and differentiation was enigmatic (Coolican et al., 1997), although it was not clear at the time that these 3 growth factors would be present in the satellite cell environment. Work performed by Allen and others explored the interactions of IGF-I, bFGF, and TGFβ and clearly demonstrated that these 3 growth factors interact to modulate both proliferation and differentiation (Allen and Boxhorn, 1989; Greene and Allen, 1991). In fact, proliferation, differentiation, and the inhibition of both were achieved with various combinations of the 3 factors (Allen and Rankin, 1990). Since these studies, the number of potential extracellular regulatory agents has expanded significantly to include other members of the TGFβ superfamily (TGFβ2 and TGFβ3), myostatin, and growth and development factor [GDF]), hepatocyte growth factor (HGF), nitric oxide (NO), Wnt-5 and Wnt-7, and the notch/numb signaling system (Yin et al., 2013). Each regulator is regulated by the numerous modulators of their binding and signaling, such as IGF binding proteins, activating proteases, and matrix proteoglycans. Although the extracellular regulatory network has become much more complex, the combinatorial nature of extracellular regulation remains relevant. An extension of the concept that satellite cells communicate with regulatory elements in their environment is the notion that the microenvironment, including the structural components that make up the “satellite cell niche,” plays a significant role in cell regulation. This topic has been reviewed by Yin et al. (2013) and is beyond the limited scope of this review.

Before the late 1980s, satellite cell quiescence was viewed as the absence of proliferation, a protracted Gap 1 (G1) state. However, Bischoff and Heintz (1994) demonstrated that an extract of gently crushed muscle could stimulate proliferation of satellite cells on isolated muscle fibers in vitro, even though known mitogenic stimuli could not induce proliferation. During the protracted lag phase before entry into the cell cycle, satellite cells cultured from older rats were also shown to be refractory to mitogens that stimulated proliferation at later stages in culture but could enter the cell cycle early by crushed muscle extract (Johnson and Allen, 1993). In the case of FGF, specific binding to high-affinity receptors was barely detectable during this lag phase and receptor gene expression was very low (Johnson and Allen, 1995; Sheehan et al., 2000). It was clear that other genes, notably MyoD, that are normally expressed in proliferating satellite cells are not expressed in quiescent cells and, therefore, the quiescent state has a molecular signature reflecting its unique physiology (Smith et al., 1994; Koishi et al., 1995).

Identifying the mechanism of activation of quiescent satellite cells began with crushed muscle extract studies, unveiling HGF as the activating agent, which can activate satellite cells in culture, on isolated muscle fibers, and in vivo (Tatsumi et al., 1998, 2006). In parallel, J. E. Anderson made the seminal discovery that NO could activate quiescent satellite cells on isolated muscle fibers in vitro, which identified the connection of both mechanical and chemical signaling resulting in satellite cell activation (Anderson, 2000). The mechanism begins with NO synthesis and is mediated through release of HGF from its tethering in the extracellular matrix and binding to the c-met receptor on satellite cells (Tatsumi et al., 1998, 2006; Miller et al., 2000; Wozniak and Anderson, 2007). Although this is the first avenue for satellite cell activation described, it does not preclude other pathways.

Are all satellite cells similar and are they uniquely responsible for muscle tissue growth and regeneration? This question has cycled through satellite cell research beginning with Schultz’s (1996) description of 2 populations of satellite cells in growing and adult muscle that had different 5-bromo-2’-deoxyuridine incorporation rates. Schultz (1996) proposed a cell subset responsible for growth and a subset that persists in adulthood with primary responsibility for regeneration. This
Adipose and muscle stem cells

The concept of self-renewal has become relevant more recently as attention has focused on stem cells throughout biomedicine because satellite cells are an example of ASC. Attention has also turned to other cells found in muscle tissue that have myogenic potential and may participate in regeneration and growth. It is clear that other cells with stem cell qualities can be found in muscle and that these cells may be induced to participate in myogenesis. However, their physiological relevance and even their therapeutic potential are questionable. The cells that have been known as satellite cells for the past 50 yr are still considered the primary and most important myogenic stem cells in skeletal muscle (Sambasivan et al., 2011). With regard to specific subsets of satellite cells, it is important to consider whether differences in properties are a reflection of developmental lineage or physiological state.

The role of satellite cells in muscle growth and in regeneration is as relevant to agriculture as it is to medicine, and their activity in human exercise and athletic performance may be considered relevant to domestic animals (Dodson et al., 2010). Although initial animal models used in satellite cell research have been small animals such as rats, chickens, mice, and transgenic mice in recent years, large animals have been used for experiments and results may be relevant to humans (Rhoads et al., 2009).

The impact of nutritional and physiological stress on satellite cell activity as it relates to subsequent growth rate and body composition are most directly addressed in large animal models or in target species in the case of chicken and turkey (Oksbjerg et al., 2013). Endocrine function related to growth regulation has been studied most extensively in animals of agricultural importance. Research includes studies on steroid action and satellite cell activity as it relates to the growth hormone/IGF axis (Johnson et al., 1998; Dayton and White, 2014). For example, the number of active satellite cells was increased in cattle implanted with a combined implant of estradiol (E2) and trenbolone acetate (TBA), and circulating IGF-I concentrations were also increased (Johnson et al., 1998). It was subsequently shown that both E2 and TBA could cause increased satellite cell proliferation in vitro, which required the estrogen and androgen receptors, respectively, and the IGFR1 (Dayton and White, 2014). Although it was also demonstrated that E2 and TBA stimulate muscle protein synthesis rate and decrease degradation rate, their effects on satellite cell proliferation appear to be an integral component of anabolic steroid (AS) enhancement of muscle growth in meat animals.

Nutritional modulation of muscle growth during early development linked to satellite cell function was the focus of early work by F. P. Moss in the late 1960s. Early nutrition was shown to impact subsequent chick muscle growth by compromising muscle DNA accretion, which, one can speculate, led him to his landmark work demonstrating that satellite cells are the source of new nuclei in growing muscle as previously discussed (Moss, 1968; Moss and Leblond, 1971). A recent report linking this work to satellite cell function showed that turkey pouls subjected to nutritional stress immediately posthatching showed diminished satellite cell proliferation at 48 h and lower BW at 1 wk (Moore et al., 2005). Studies have been extended, in vitro, by examining nutritional stress on satellite cell function (Anderson et al., 2012), and it appears that metabolic dysfunction may impact satellite cell properties.

Almost 30 yr ago, Purchas et al. (1985) described diminished satellite cell activity in ob/ob mice, a strain with deficient leptin production, and Koopman et al. (2014) recently reviewed and discussed potential mechanisms of metabolic reprogramming that could alter satellite cell responsiveness. Current attention linking satellite cell function to metabolic regulation, body composition, and growth adds a new dimension to our understanding of satellite cells beyond developmental biology, which has dominated satellite cell research in recent decades. The responsiveness of satellite cells can vary dramatically to either enhance muscle growth or compromise muscle mass regulation as observed in aging (Garcia-Prat et al., 2013) and muscle diseases, such as Duchenne muscular dystrophy (Morgan and Zammit, 2010). Understanding the environmental cues that impact the long-term function of satellite cells as related to growth, muscle disease, and aging holds great promise for translational progress that will impact animal production and human and animal health.

Environmental Placement/Regulation of Muscle and Fat Stem Cells

Animal Physiology Effects on Stem Cells In Vitro.

Extrinsic regulation of muscle and adipose cells were defined for direct application of AS (Dayton and White, 2014) including administered E2, testosterone, progesterone, TBA, and the TBA–E2 combination (Revalor; Merck Animal Health, Summit NJ), which promote lean muscle gain and feed efficiency in feedlot steers (Hayden et al., 1992; Johnson et al., 1996; Dayton and White, 2014). Several in vivo studies were done to elucidate the mechanism or mechanisms of AS effect on protein synthesis, protein degradation, and net protein accretion. Initial work indicated that these anabolic effects may be mediated by increased satellite cell number.
through increased GH/IGF-1 (Dayton and White, 2014). These findings prompted research at the University of Minnesota (Minneapolis, MN) that identified the mode of action of anabolic implants with extensive bovine satellite cell studies. Previous research by this group included extensive work with growth factors on bovine satellite cells and occasionally with fat cells in culture on proliferation and differentiation (Dayton and Hathaway, 1991; Dayton and White, 2014) as well as describing the effect of AS in various cell culture systems using bovine satellite cell sources beginning in the late 1980s. Major findings included that TBA/E2 stimulated IGF-1 expression and increased protein synthesis and decreased protein degradation. Their work also showed that E2 effects are transmitted via ethylene response sensor (ERS) 1 and G protein-coupled estrogen receptor (GPER) 1. Results with TBA indicated an involvement of androgen receptors in “growth promoting” effects in bovine satellite cell cultures. This excellent work has recently been reviewed by Dayton and White (2014); however, neither in vitro nor in vivo work has yet to clarify the mode of action of AS. Others have contributed data on AS action in muscle cells, in vitro (Mulvaney et al., 1988; Roe et al., 1989).

Several publications exist on the isolation of SV cells from adipose tissues of animals typically obtained at slaughter (Fernyhough et al., 2004; Hirai et al., 2007; Hausman et al., 2008). Research shows that specific differentiation cocktails (Grant et al., 2008; Lengi and Corl, 2010) facilitate the differentiation of these SV cells into adipocytes that are able to incorporate exogenous fatty acids and/or synthesize long-chain fatty acids (Burns et al., 2012a,b). However, neither the nutrition nor the physiology of the adipose tissue donor animals has received much attention because adipose tissue samples are typically collected at slaughter and SV cells isolated but information on the donor animal may or may not have been recorded.

Research now shows that the nutritional milieu of the SV cell donor modifies proliferative, adipogenic, and lipogenic capacity of these cells in vitro (Kadegowda et al., 2014). Moreover, evidence, in vitro, demonstrated that preadipocyte proliferation was greater in cells from steers not supplemented with corn than in cells from steers supplemented with corn. Furthermore, adipocytes isolated from steers supplemented with corn grain had reduced expression of key adipogenic genes that may have influenced their capacity for de novo lipogenesis, desaturation, and elongation in culture. These results show that prior nutritional treatment of the donor animal used to isolate SV cultures alters proliferation, adipogenesis, and lipogenesis in culture. To date, research highlights the importance of knowing, and re-

![Figure 2. Diagram of adipogenesis. Adipogenesis is the proliferation, differentiation, and maturation of cells of the adipose lineage to form lipid-assimilating cells. Recently, this definition has been expanded to include the dedifferentiation of mature adipocytes to resume cells capable of population expansion (Fernyhough et al., 2005; Hausman et al., 2009). Cells within adipose depots are capable of releasing chemicals that may regulate body physiology.](image-url)
porting, the details regarding the nutritional treatments of the donor animal and source of SV cells. These results also suggest that mechanistic understanding of nutrition interventions may be further evaluated using primary cell cultures (Fig. 2).

**Extracellular Matrix.** Approximately 30 yr ago, the notion that the extrinsic environment or extracellular matrix cellular environment is essential to the regulation of cell function was postulated. Historically, the extracellular matrix has had many names but one of the most common through the mid to late 1970s was “ground substance.” Ground substance refers to the extrinsic extracellular matrix environment that the cells secrete as a homogenous passive structural material in which the cells are embedded. This ground substance concept of the extracellular matrix was derived, in part, from the cell theory in which the cells are the fundamental component necessary for life and the extracellular matrix was not involved in maintaining cell viability. Descriptions of the extracellular matrix would often describe it as an inert material filling spaces between cells. Extracellular matrix was only thought to be structurally important in connective tissues.

Today, it is well accepted that the extracellular matrix composition and its relationship to cell membrane receptors will affect cellular shape, migration, growth, proliferation, differentiation, and tissue-specific function. Connective tissue refers to the extracellular matrix surrounding osteoblasts, chondroblasts, and fibroblasts. However, today virtually all cells have been shown to produce an extracellular matrix environment, which, in most cases, has functional roles including providing structural integrity and regulating essential cell function. The extracellular matrix is currently defined to include all molecules secreted by the cells it surrounds and is often composed of collagens, proteoglycans, and noncollagenous glycoproteins. Although the components of the extracellular matrix were originally identified by physical and chemical extraction methods, molecular biology techniques significantly expanded our view of matrix function and the number of extracellular matrix macromolecules. For example, only 4 collagens, types I through IV, had been identified by 1975 (Grobstein, 1975) but at least 28 different collagen types (Kadler et al., 2007) are currently recognized. Collagen I, the first identified collagen, is the prototypical collagen that defined collagen structure and the function of structural support. Collagen I self-assembles into fibrils and contains an uninterrupted trimeric triple helix. Collagens are now known to have additional functions other than just the classical view of a structural molecule that forms collagen fibrils. Beyond the fibril-forming collagens, there are fibril-associated collagens, network-forming collagens, transmembrane collagens, endostatin-producing collagens, anchoring fibrils, and beaded filament-forming collagens. With regard to cellular function, some of these newly identified collagens can regulate cell migration (Marneros and Olsen, 2005) and, in the case of muscle, modulate the self-renewal of the adult myoblast stem cell or satellite cell (Urciuolo et al., 2013).

Although the extracellular matrix was traditionally described as a structural substance that cells were embedded in and was passive in terms of modulating cell behavior, it is now known to have functional significance in terms of modulating cell and tissue function and tissue structure. The extracellular matrix is cell type specific and changes with developmental requirements. The extracellular matrix functions by 1) serving as a structural framework necessary for normal functional integrity, 2) being a substrate for cell migration, 3) presenting and sequestering growth factors, and 4) transmitting mechanical signals to the cell. Thus, the cells create the extracellular matrix and in return the extracellular matrix communicates with the cell.

Adult myoblasts (satellite cells) are responsible for nearly all postnatal and posthatch muscle growth and regeneration of damaged muscle. Satellite cells are located in a niche that is composed of the satellite cells, muscle fibers, and extracellular matrix (Mauro, 1961). During muscle development, growth, and regeneration, the extracellular matrix composition changes and provides instructive regulatory information to the satellite cells affecting their quiescence, activation, proliferation, differentiation, or self-renewal (Rhoads et al., 2009). Although the function of many known extracellular matrix molecules found in the satellite cell niche are not well understood, it is also likely that there are unidentified extracellular matrix molecules with additional functions. Syndecan-4 is 1 of the known extracellular matrix proteins with bioactivity related to satellite cell migration on the surface of the muscle fibers (Siegel et al., 2009). Syndecan-4 is a cell membrane–associated heparan sulfate proteoglycan with extracellular, transmembrane, and cytoplasmic domains. The cytoplasmic domain is necessary for syndecan-4 biological activity (Horwitz et al., 1984; Volk et al., 1999; Shin et al., 2012, 2013; Song et al., 2012a) including oligomer formation to interact with the cytoskeletal protein α-actinin in the presence of phosphatidylinositol 4,5-bisphosphate (PIP2) and to activate protein kinase C α signal transduction (Shin et al., 2012). After PIP2 binds to the cytoplasmic domain of syndecan-4 oligomers, focal adhesions and stress fibers form (Longley et al., 1999; Song et al., 2012b), which are necessary for cell migration. Shin et al. (2013) demonstrated that satellite cell migration was diminished with overexpression of syndecan-4 lacking the cytoplasmic domain with the expression of...
wild-type syndecan-4 knocked down. Therefore, syndecan-4 mediation of satellite migration may play a critical role in muscle fiber hypertrophy, muscle fiber formation, and muscle regeneration.

The extracellular matrix is also a reservoir for growth factors. Satellite cell proliferation and differentiation are responsive to growth factors that may be regulated via sequestration and presentation to their cellular receptors by extracellular matrix molecules. The heparan sulfate proteoglycans can transduce signals from growth factor ligands. For example, FGF2 is a potent stimulator of muscle cell proliferation and a strong inhibitor of differentiation (Dollemeyer et al., 1981). For FGF2 to have a high affinity interaction with its tyrosine kinase receptors requires FGF2 binding to heparan sulfate chains covalently attached to a proteoglycan core protein (Rapraeger et al., 1991). Zhang et al. (2007, 2008) reported that cell membrane associated heparan sulfate proteoglycan glypican-1 has a critical role in regulating satellite cell FGF2 signal transduction. Glypican-1 can sequester FGF2 away from its cellular receptor to permit differentiation by 2 different mechanisms. First, glypican-1 can bind to and sequester FGF2 into lipid rafts preventing FGF2 from binding to its tyrosine kinase receptor. Second, glypican-1 can be shed from the satellite cell membrane by cleavage of its glycosylphosphatidylinositol (GPI) anchor. The shed form of glypican-1 without the GPI anchor can bind to FGF2 and sequester FGF2 in the extracellular matrix away from the satellite cell (Velleman et al., 2013).

A second example of extracellular matrix components regulating growth factors is dEcoRln, a small leucine-rich proteoglycan that has a core protein with a single covalently attached chondroitin or dermatan sulfate glycosaminoglycan chain. The core protein of dEcoRln is able to sequester the growth factors TGFβ and myostatin by binding these growth factors to the dEcoRln core protein (Hildebrand et al., 1994; Miura et al., 2006). In muscle, both TGFβ and myostatin are strong inhibitors of both proliferation and differentiation. When either TGFβ or myostatin is bound to the dEcoRln core protein, the proliferation and differentiation of myogenic cells and satellite cells are enhanced (Kishioka et al., 2008; Li et al., 2008).

Application of Stem Cells

Thirty or so years ago, isolation and identification of purified populations of muscle and adipose stem cells was inefficient and difficult. Yet great strides in the academic learning about stem cells have greatly expanded knowledge about the cells and tissues of interest for human and animal health. What about the next 30 yr? What might we expect new animal scientists to be reflecting on in their twilight years?

Animal Growth. As the understanding of best practices in collecting, transferring, and regulating growth of stem cells deepens, it is intriguing to consider use of stem cells for animal and human health and growth. With titles such as “In Vitro Meat: Zombies on the Menu?” (Stephens, 2010), there is clearly intrigue and concern in the initial thought of using stem cells for meat production. The various possibilities of stem cell use in animals could include stem cell therapy to treat diseases and common conditions or improving reproductive efficiency for direct application within meat animal production. Transgenic application, for conservation of endangered or exotic animals or for xenotransplantation for human health, should also be considered. Additionally, stem cell research will undoubtedly continue to provide critical information about cellular and molecular aspects of domestic animals that may also be transferable to humans and other animal species including exotic animals. Stem cell lines have already become valuable in drug development by providing cells for drug testing, particularly in the cases of genetic diseases. Whereas ethical concerns for ES cells are polarizing, iPSC cells are generated by reprogramming somatic cells without the ethical and biological concerns of using embryonic tissues and cells and may be a great alternative for ES cells (Ezashi et al., 2012; Romeo et al., 2012). Induced pluripotent stem cells could be useful in establishing authentic stem cell lines from farm animals (Blomberg and Telugu, 2012; Malaver-Ortega et al., 2012), although there are also reports of spontaneous differentiation of ES cells in some species, including pigs (Park et al., 2013).

In Vitro Meat Production. Some have suggested there is a need for alternative meat and protein production because of a growing demand for meat and societal concerns about sustainability, environmental burden, and animal welfare (Pluhar, 2010; Post, 2012). In vitro meat production with the use of stem cells and stem cell use, in vivo, to reduce production variability, cost, and environmental impact have been considered as opportunities to improve animal food product production (Tuomisto and de Mattos, 2011; Post, 2012). An initial evaluation of safety and biological and biochemical properties of meat and milk from cloned cattle has been reported (Walsh et al., 2003; Takahashi and Ito, 2004; Yang et al., 2007). Milk from cloned cattle has also been evaluated for its nutritional impact (Tome et al., 2004). Additional testing will likely be required for regulatory approval of these meats but gaining consumer acceptability may prove to be difficult. On the other hand, regulating progenitor stem cells during the life of an animal to improve meat production efficiencies may be more acceptable to consumers, particularly as they begin to
understand the naturally occurring existence of progenitor cells throughout the lifespan (Du et al., 2013).

**Discovering New Molecules: Adipokines and Cytokines.** Adipose tissue energy reserves are a critical determinant with regards to body allocation of energy toward growth, development, and reproduction, which are central to the efficient production of agricultural animal species. Adipose tissue regulates its expansion and communicates information about energy reserves to other tissues through secreted bioactive peptides and hormones collectively referred to as adipokines (Fig. 3). The term adipokine is derived from “adipose cytokine” and reflects the fact that many adipokines are considered to be proinflammatory (e.g., tumor necrosis factor α [TNFα]).

The collection of proteins and peptides referred to as adipokines includes both molecules synthesized primarily in adipose tissue (e.g., leptin) and those produced in many other cell types (e.g., angiotensin), in broad physiological categories that include growth factors (e.g., TGFβ), classical cytokines and chemokines (e.g., TNFα), monocyte chemoattractant protein-1 (MCP-1), vasoregulatory hormones, and sensitizers of insulin action (e.g., adiponectin; Ahima, 2006). Leptin illustrates the importance of adipokines for animal physiology; leptin is produced in proportion to adipose mass and communicates peripheral energy stores to the hypothalamus (Campfield et al., 1995; Halaas et al., 1995), allowing the central nervous system to integrate information about peripheral energy reserves into the control of growth, development, and fertility, all of which are critical for the efficient production of agricultural animal species.

Stem cells with adipogenic potential make up a pool from which to extend beyond well-characterized adipokines and discover new mediators of adipose development and metabolism. A growing number of adipokines have been shown to regulate adipogenesis, providing a mechanism through which adipose tissue regulates its own cellular composition and mass through paracrine interactions within the tissue. For example, adipocyte hypertrophy in mice triggers release of the adipokine secreted frizzled-related protein 5 (Sfrp5), which then promotes adipogenesis by suppressing Wnt signaling that otherwise inhibits adipocyte differentiation (Mori et al., 2012). Conversely, C1q/tumor necrosis factor-related protein 11 (Crtp11) is secreted by cells of the SV fraction and inhibits adipocyte differentiation (Wei et al., 2013). Many of these mediators were discovered in mice or humans, whereas ongoing research has identified some of these mediators in agricultural animals. Song et al. (2010) cloned the bovine gene encoding chemerin, a novel adipokine identified originally in mice, and demonstrated its expression associated with adipogenesis. Discovery-based genomics approaches will be valuable in further identifying the suite of adipokines that regulate adipose development and metabolism in agricultural animal species.
Resnyk et al. (2013) recently used RNA sequencing (RNA-seq) to identify novel adipokine expression in broiler chicken adipose tissue during posthatch development, including many that were differentially expressed between genetically lean and fatty birds. The sensitivity and unbiased nature of RNA-seq provides the means to discover new adipokines from isolated cell populations within adipose tissue, including for species such as sheep with fewer commercially available research tools including microarrays and antibodies. The collection of adipokines that regulate adipose function and remain to be identified in agricultural animals is likely to be extensive; proteomic analysis identified 420 proteins secreted from differentiated human adipocytes, the majority of which were differentially secreted during the course of adipogenesis (Zhong et al., 2010).

Numerous cell types exist in adipose tissue depots, including stem cells, fibroblasts, MSC, ET cells, and immune infiltrating cells (Rink et al., 1996; Harasymiak-Krzyzanowska et al., 2013), although the characteristics of cells populating adipose tissue can vary depending on the site and microenvironment (Villaret et al., 2010). These other cells may contribute to adipokine production. For example, the maintenance of adipose tissue relies on a well-formed vasculature. Adipocytes secrete proangiogenic and inflammatory mediators, with concentrations modulated by the extent of obesity. An example of a proangiogenic adipokine that is secreted by adipocytes is vascular endothelial cell growth factor (VEGF). Without VEGF, adipose tissue has reduced vascular density and is hypoxic (Williams et al., 2012). Leptin, which increases with obesity, not only regulates food intake and energy expenditure but also functions

**Figure 4.** Immunological cascades trigged in adipose tissue. Adipocyte production of inflammatory cytokines and chemokines that trigger angiogenesis and influx of additional immune cells further enhance the inflammatory milieu of the adipose tissue. This results in increase in metabolic syndrome, renal and bowel inflammation, kidney injury, and risk of cancer and cardiovascular disease. The adipokines and cytokines of adipose tissue also skew the immune phenotype from an environment that is protective against pathogens and cancer to one that increases allergic reactivities. The inflammatory and immune-skewed environments are both detrimental to healthy growth and development. INF-γ = interferon-gamma; macs = macrophages; PMN = polymorphonuclear cell; Th = T helper.
as a proangiogenic mediator (Maffei et al., 1995; Vona-Davis and Rose, 2009). Whereas adipocytes and vascular endothelial cells are not typically considered as part of the immune network, they are among the cell populations that can produce immune mediators and can act to regulate immune functions. It is clear that relationship between adipose tissue, vasculature, and immune system is highly intertwined (Fig. 4).

Adipokines have been linked to a variety of pathologies/pathophysiologies. For example, the obese state is considered to be a chronic inflammatory condition in which inflammatory cells such as lymphocytes, macrophages, and neutrophils naturally present in adipose tissue contribute to the inflammatory condition (Wu et al., 2007; Meijer et al., 2011). The biological impact of obesity and obesity-associated inflammation in both animals and humans includes metabolic disorders such as diabetes, renal inflammation and kidney injury, and increased cancer risk (Kharroubi et al., 2003; Vona-Davis and Rose, 2007; Moon et al., 2011; Paz-Filho et al., 2011; Wang et al., 2011; Cheng et al., 2013; Zhang et al., 2013). Furthermore, increased expression of adipose tissue-derived adiponectin was associated with an increased risk of mortality in horses undergoing emergency surgery (Packer et al., 2011). There is evidence that some of these consequences are apparent before attainment of the obese state because administration of a high-fat diet to nonhuman primates showed increases in inflammatory markers before onset of overt obesity (Nicol et al., 2013). In obesity, concentrations of the proinflammatory mediator leptin increase in a multitude of species, ranging from rodents to dogs to ponies to humans (Chen et al., 2012; Kwon et al., 2012; Ungru et al., 2012; Van de Velde et al., 2013b). Inflammatory mediators that are either produced directly by adipocytes or by other cell types within adipose tissue include IL-6, TNFα, and IL-1 (Ladefoged et al., 2013), each of which is overexpressed in obese animals. T helper 17 (Th17) cells are another inflammatory population of cells renowned for their production of the inflammatory mediator IL-17 (Zygmunt and Veldhoen, 2011). Although the adipokine adiponectin has at times been considered to be anti-inflammatory, it can indirectly stimulate inflammation by inducing dendritic cells to differentiate into Th17-skewing cells (Jung et al., 2012). Gender differences in the inflammatory response associated with obesity is of particular interest. In a study with sheep, comorbidities associated with obesity such as increased inflammation and increased metabolic-related disease were more prominent in young males than females (Bloor et al., 2013). In addition to gender, body fat location also impacts the obesity-associated inflammatory response.

The prominence of the inflammatory or skewed immune phenotypes is likely to be due to both the adipocytes themselves as well as other cells recruited into adipose tissue. Inflammatory mediators whose concentrations are increased in obesity include IL-1, IL-6, and TNFα (Wang et al., 2011). However, adipocytes can also concurrently skew the immune reactivity toward the T helper 2 (Th2) phenotype. Th2 responses are important for antibody-mediated reactivity and for reactivity to extracellular pathogens and include cytokines such as IL-4, IL-5, IL-10, and IL-13 (Sato et al., 1998; Cousins et al., 2002; Li et al., 2013). In a long-term BW gain study with dogs, the initial periods of weight gain were associated with increases in leptin expression and a skewing toward a Th2 phenotype, as demonstrated by increased levels of the Th2 cytokine IL-10 (Van de Velde et al., 2013b).

In addition to increases in concentrations of inflammatory or Th2 cytokines, obesity leads to adipose tissue recruitment of an activated immune infiltrate. For example, the adipose tissue of obese cats was shown to have increased numbers of T lymphocytes expressing Th2 and inflammatory mediators (Van de Velde et al., 2013a). This increase in the immune infiltrate could be in part be attributed to increased expression of the chemokines CCL-5 and CCL-2, which attract T cells, neutrophils, and monocytes. Expression of these chemokines increase in obesity. In the obese cat model, chemokine and inflammatory mediator levels were more prominent in the subcutaneous adipose tissue as opposed to visceral adipose tissue, demonstrating depot-dependent immune cell recruitment and skewing in obesity (Van de Velde et al., 2013a).

Despite many studies of inflammatory effects of obesity and adipocytes, it is unknown if adipocytes can modulate conventional immune regulatory cell types. For example, adipocytes can trigger production of multiple inflammatory cytokines from conventional immune cells (Vielma et al., 2013a). Adipocytes can also skew immune reactivity toward the Th2 phenotype through soluble mediators produced by adipocytes. It has been suggested that adiponectin can skew T cell reactivity from being generally immune activated toward a more polarized inflammatory and Th2 state (Vielma et al., 2013b,a). The biological consequences of this immune stimulation and skewing in the obese state are broad and involve increased levels of IL-6 and IL-1, which may increase the risk for type 2 diabetes (Spranger et al., 2003). Inflammatory cytokine levels increase with obesity and have been associated with increased insulin resistance (Stoppa-Vaucher et al., 2012; Ladefoged et al., 2013). Cancer-related inflammation is an important step in the process toward malignant disease (Shimizu et al., 2009; Erdman and Poutahidis, 2010). Carcinogen-induced development of premalignant colonic lesions in mice is increased in obese
mice (Guazzzone et al., 2009). In fact, the combination of mediators from premalignant oral lesions and adipocytes accentuates normal immune cell production of the protumorigenic Th2 cytokines IL-10 and IL-13; the inflammatory mediators IL-1, IL-6, and IL-9; and the T cell–derived chemokine CCL-5 (Vielma et al., 2013b,a). The obesity-associated inflammatory phenotype is not limited to the obese individual. Expression of obesity-induced inflammatory cytokines has been shown to be increased in pregnant sheep but was also seen in the fetal large intestine, raising the possibility of maternal obesity predisposing the offspring to inflammatory bowel disease. The mediators whose levels were increased were typical inflammatory mediators including Th2 and inflammation-skewing TGFβ (Yan et al., 2011a). During fetal development, the enhanced inflammation due to maternal obesity enhances intramuscular adipogenesis and fibrogenesis (Du et al., 2010), which has a long-term impact on the offspring intramuscular fat and collagen contents in sheep (Huang et al., 2010; Yan et al., 2010).

Because of the diversity of cell populations within adipose tissue and the overlapping capacity of several cell types to produce the same immune modulators, dissecting the individual contributors to the immunological changes that occur in obesity is currently impossible. This is also in part because individual cells within adipose tissue may produce unique profiles of mediators. The plasticity of the immune cells that reside in adipose tissue and the changes in their phenotype in the obese state adds to the complex interplay of what cells are recruited into adipose tissue and what types of immune mediators that are produced. It certainly appears that there is a tremendous biological and economic impact of the immune modulatory capacity of adipocytes and the resulting plethora of immune mediators that are co-associated with obesity, including asthma, the risk of cancer, type 2 diabetes or metabolic syndrome, and cardiovascular disease to name a few (Spranger et al., 2003; Tanaka et al., 2012; Newson et al., 2014). Furthermore, obesity and the associated immune activation transcends not just to the obese individual, as either maternal or paternal obesity has been shown to have a detrimental impacts on the development of their offspring (Odaka et al., 2010; Yan et al., 2011a; Bromfield et al., 2014), further underscoring the impact of altered adiposity.

**Drug Development.** Domestic animal stem cells could be an excellent experimental model in preclinical trials (Brevini et al., 2008). For example, it has been suggested that human stem cells could be used for toxicology, pharmacology, and predictive modeling (Sison-Young et al., 2012) and that preclinical cell evaluation conducted with stem cells from domestic animals might also benefit humans (http://www.isscr.org/docs/default-source/clin-trans-guidelines/isscrglclinicaltrans.pdf). Pigs are commonly used large animal models to evaluate the clinical potential of stem cell therapies (Nowak-Imialek et al., 2011). To date, ES cell lines from pigs have not been established and the challenge is thought to be due to spontaneous differentiation into an epiblast stem-cell-like state during culture (Park et al., 2013). Using iPS cells will require comparing them to somatic cells to determine clonability and consistent in vitro differentiation (Ezashi et al., 2012; Cebrian-Serrano et al., 2013).

**Summary/Conclusions/Implications**

There has been a great interest in delineating the determination and differentiation process for adipocytes and myogenic cells. For adipocytes, the role of factors inhibiting adipogenesis and differentiation are of great interest. This work has been widely conducted in the biomedical field, whereas application of this knowledge was principally studied in vivo in adipose of livestock. Muscle determination and differentiation has been extensively studied using predifferentiated and differentiated muscle cells and satellite cells from rodent and human sources. The discovery of myogenic regulatory factors in the cellular progression from premyocytes to myocytes is crucial. Once again workers in farm animal biology applied these data to livestock systems and confirmed the myogenic regulatory pathway (Dodson et al., 1996). Understanding genetic and epigenetic regulation of cell pluripotency, reprogramming, and cell differentiation/dedifferentiation will provide critical information for the use of stem cells in healthcare and animal production in the future. Undoubtedly, stem cell research will uncover critical mechanisms for the optimal use of stem cells and further understanding of specific cell types. For example, metabolomics studies have shown metabolic shifts in the balance between glycolysis, mitochondrial oxidative phosphorylation, and oxidative stress during somatic cells reprogramming to pluripotency and stem cell maturation. This information may shed light on the metabolic needs of cells in transition that could modify media or feed additives. Collectively, there has been an evolution of meat animal growth research during the past 50 yr, principally with regards to adipose and muscle stem cells. The next 50 yr promises to produce more relevance of these cells, and we hope the animal field will grasp this line of research and continue to examine adipose and muscle stem cells.


Adipose and muscle stem cells


