PHYSIOLOGY AND ENDOCRINOLOGY: REPRODUCTIVE SUCCESS IN RUMINANTS: A COMPLEX INTERACTION BETWEEN ENDOCRINE, METABOLIC, AND ENVIRONMENTAL FACTORS

0531 Recent advances in the hypothalamic control of reproduction. I. Clarke*, *Monash University, Clayton, VIC 3800, Australia.*

Reproduction is driven by the GnRH cells of the brain, but the pulsatile secretion of GnRH into the hypophysial portal blood is controlled by kisspeptin cells. In the sheep, as in other species, the kisspeptin cells form 2 major populations in the brain, 1 in the arcuate nucleus and the other in the preoptic area. The former appear to mediate the negative feedback effects of sex steroids as well as being initiators of the *positive feedback* effect that elicits the GnRH surge to cause ovulation in the female. The latter facilitate the positive feedback effect. The arcuate nucleus population of kisspeptin cells also produce neurokinin B and dynorphin, leading to their designation as KNDY cells. Kisspeptin expression in the KNDY cells is reduced in the nonbreeding season, further reinforcing the fundamental role these cells play in control of reproduction. In addition, these KNDY cells appear to be involved in the response to altered bodyweight. This is achieved via the signalling of leptin to the KNDY cells either directly or indirectly—kisspeptin gene expression is lower in lean animals, but this can be reversed by administration of leptin. KNDY cells are interconnected to the cells of the arcuate nucleus that produce anorexigenic melanocortins and those that produce the orexigen, neuropeptide Y. This provides a bidirectional interface between metabolic circuits and reproductive circuits. Thus, melanocortins and neuropeptide Y may regulate reproduction independently or via control of kisspeptin cells, providing a way that metabolic function and reproduction are interlinked. Recent data provide strong evidence that KNDY cells are most likely to be the cells that drive the pulsatile secretion of GnRH. Thus, c-Fos labelling in KNDY cells is seen at the time of ram-induced LH secretion (proxy for GnRH pulses) in anestrous ewes. In addition, KNDY cells in brains of animals sampled within 30 min of an endogenous pulse of LH displayed increased c-Fos labelling, compared with cells from animals that were not experiencing an LH pulse at the time of brain collection. Most interestingly, the means by which KNDY cells cause GnRH secretion appears to be due to action on the GnRH terminals within the median eminence. Accordingly, direct application of kisspeptin to the median eminence in vitro causes GnRH secretion. This novel mechanism has forced a revision of the model for neuroendocrine control of reproduction.

Key Words: gonadotropin-releasing hormone

0532 Influence of stress on male reproductive physiology. T. H. Welsh, Jr.*[1], N. H. Ing[1], and R. D. Randel[2], *[1]Texas A&M University, Department of Animal Science, College Station, [2]Texas A&M AgriLife Research, Overton.*

Environmental, physiological, psychological, and managerial stressors have been implicated as causes of reproductive disorders and decreased fertility in animals and humans. Herd reproductive efficiency and the stud industry depend on the sperm producing capabilities and libido of bulls and stallions. With respect to male reproductive physiology, the steroidogenic and spermatogenic functions of the testis can be negatively impacted by stress-induced secretion of endogenous glucocorticosteroids (GC) as well as by treatment with exogenous GC agonists. The testes' gametogenic function, a primary component of male fertility, is dependent on appropriate transmission, receipt, and processing of specific endocrine signals. The deleterious effects of stress on reproductive performance are presumably signaled by GC activation of the glucocorticoid receptor (*NR3C1*). Questions related to molecular mechanisms whereby stress affects specific components of the hypothalamic-pituitary-testicular (HPT) axis of male rodents, primates, cattle, sheep, pigs, horses, and other species have been pursued by the use of in vitro and in vivo methods. This paper will provide a targeted overview of potential impacts of stressors on the endocrine aspects of the HPT axis, with particular focus on direct testicular effects. Specific data to be presented are derived from studies of the influences of endogenous and exogenous GC on androgen biosynthesis and gene expression in testes of bulls and stallions. Chronic administration of a synthetic GC has been reported to increase the incidence of abnormal spermatozoa by direct action or perhaps by disruption of the endocrine or genetic mechanisms that support sperm production in bulls and stallions. Acute elevation of the systemic concentration of GC by pharmacologic methods or by mimicry of physiologic stress have inhibited testicular steroidogenesis and transiently decreased the systemic concentration of testosterone. The inhibitory action of endogenous GC concentrations on testicular steroidogenesis under stressed and nonstressed conditions indicates that activity of the hypothalamic-pituitary-adrenal axis may be of critical importance in establishment or maintenance of a functional HPT axis during prenatal, prepubertal, and postpubertal life. Homeostatic regulation of reproductive processes involves a physiological integration of the adrenal and testicular axes. The biologic and economic importance of deleterious influences of stress on male reproductive processes dictate a thorough evaluation of adrenal–testicular interrelationships in domestic livestock species.

Key Words: stress, steroidogenesis, spermatogenesis

0533 Mechanisms linking infection and innate immunity in the female genital tract with infertility in dairy cattle. I. M. Sheldon*, *Swansea University, Singleton Park, Swansea, UK.*

One of the most common endemic diseases of dairy cattle is bacterial infection of the uterus after parturition. These infections damage the endometrium lining the uterus, reduce the production of milk, and cause infertility. Uterine disease is caused by *Escherichia coli, Trueperella pyogenes*, anaerobic bacteria, and viruses. These microbes posses a range of virulence factors that caused inflammation and damage in the endometrium. Innate immunity is an ancient system of defence against microbes, dependent on host cellular pattern recognition receptors such as toll-like receptors (TLR), which bind pathogen-associated molecular patterns. Epithelial and stromal cells are the first line of defence against microbes in the endometrium, and they express the most TLR. Activation of TLR signalling leads to the secretion of chemokines, cytokines, and prostaglandins, which attract and activate neutrophils and macrophages to clear the microbes. Microbial factors and host intracellular pathways regulate the scaling of the innate immune response, and the severity of postpartum uterine disease. Uterine disease also compromises ovarian function, with impacts on the corpus luteum, and on ovarian follicle development from primordial to antral follicles. Whilst healthy ovarian follicles are devoid of immune cells, granulosa cells express TLR and have roles in innate immunity. Pathogen-associated molecular patterns perturb granulosa cell endocrine function, stimulate the secretion of inflammatory mediators from granulosa cells, and lead to damage of oocytes. In conclusion, the fundamental mechanisms of innate immunity and inflammation in the female genital tract of dairy cattle are important for animal health and fertility. *Work in the Sheldon laboratory is funded by the Biotechnology and Biological Sciences Research Council (BBSRC) in the UK*.

Key Words: dairy cattle, uterus, parturition

0534 Influences of heat stress and uterine diseases on reproduction of dairy cows. J. E. P. Santos*[1], E. S. Ribeiro[1], E. Karakayan[2], K. N. Galvão[3], and F. S. Lima[4], *[1]Department of Animal Sciences, University of Florida, Gainesville, [2]University of Florida, Gainesville, [3]Department of Large Animal Clinical Sciences, University of Florida, Gainesville, [4]Cornell University, Ithaca, NY.*

Dairy cows undergo hyperthermia during the summer months in most of the world, which causes a dramatic reduction in establishment and maintenance of pregnancy. Hyperthermia has numerous effects on cellular metabolism and function that help explain reductions in fertility, including altered period of follicle dominance, reduced steroidogenic capacity of follicular and luteal cells, altered endometrial activity, and impaired oocyte quality. This multitude of effects results in reduced fertilization and influences subsequent embryo development, which impairs maintenance of pregnancy. Data are scarce on the associations between season and risk of uterine diseases in dairy cattle, but recent epidemiological studies indicate that incidence of retained placenta and metritis increases during the hot season. Therefore, it is suggested that hyperthermia during late gestation suppress the uterine defensive mechanisms, or elevated temperature and humidity during the hot months alters the environment that predisposes to increased pathogen challenge that induces disease. Unfortunately, data from studies at the University of Florida indicate that reducing hyperthermia by cooling cows during the entire dry period in the summer do not seem to alleviate the negative impacts of heat stress on metritis. It is well described that both retained placenta and metritis are important risk factors for clinical and cytological endometritis, and the multitude of diseases that affect the uterus of the cow also suppress fertility. Heat stress, but also endometrial inflammation, as observed in cows with cytological endometritis, compromises endometrial function, which can alter the secretory activity of the endometrium and lead to inability to establish and maintain pregnancy. Infection of the endometrium with *Trueperella pyogenes*, and likely also by other uterine pathogens, damages the superficial and glandular epithelium and increases the expression of inflammatory genes, and presence of inflammation seem to disrupt embryo development. Cows that develop uterine diseases, either metritis or cytological endometritis, have reduced fertilization, compromised early embryo development, impaired d 15 conceptus elongation, and increased risk of pregnancy loss. The d 15 conceptus of cows with metritis have marked changes in gene expression, and hyperthermia (in vivo or in vitro) dramatically alters mRNA expression of early embryos, and these changes might explain the differences in maintenance of pregnancy in cows that suffer from uterine diseases or heat stress. Collectively, heat stress directly and negatively impacts reproduction while also increasing the risk of uterine diseases that further depress the establishment and maintenance of pregnancy in dairy cows.

Key Words: heat stress, reproduction physiology, endometritis

0535 Cellular and molecular mechanisms of heat stress related to bovine ovarian function. Z. Roth*, *The Hebrew University of Jerusalem, Rehovot, Israel*

In light of the intensive genetic selection for high milk production and the onset of global warming, the reduced fertility of lactating cows during the summer is expected to worsen in coming years. While not entirely clear, the mechanism of fertility reduction appears to be multifactorial in nature, including altered follicular development, depressed follicular dominance, and impaired steroidogenesis and gonadotropin secretion. Heat-induced perturbations in the physiology of

the follicle-enclosed oocyte have also been documented, expressed by impaired cleavage rate and reduced developmental competence. Oocyte alterations include increased polyunsaturated fatty acids in the membrane, reactive oxygen species, ceramide formation, caspase activity, and induction of apoptosis via the sphingomyelin and/or mitochondrial pathways. New insight into the cellular and molecular alterations have revealed heat-induced perturbations in both nuclear and cytoplasmic maturation events—meiosis resumption, metaphase-II plate formation, cytoskeleton rearrangement and translocation of cortical granules. Alterations in mitochondrial distribution (i.e., a low proportion of category-I oocytes) and mitochondrial function (low membrane potential) have been recently reported for oocytes collected during the summer. These were associated with impaired expression of both nuclear (*SDHD* and *ATP5B*) and mitochondrial (*ND2*, *CYTB*, *COXII*) genes which are crucial in the mitochondrial respiratory chain. In addition, season-induced alteration in maternal-mRNA storage was documented, expressed by reduced transcript levels (*C-MOS*, *GDF9*, *POU5F1*, and *GAPDH*) in MII-stage oocytes and embryos, before (2-, 4-, and 8-cell stages) and after (8- to 16-cell stage) embryonic genome activation. These findings clarify the association between cellular and molecular modifications and reduced developmental competence during the hot season. This knowledge is essential for developing new approaches to coping with this unsolved problem.

Key Words: heat stress, bovine ovarian function