While the grass may be greener in the other field, is it better for you or your baby? Hidden risks of environmental pollutants. N. P. Evans*,1, M. Bellingham1, C. Cotino2, S. M. Rhind3, R. Sharpe4, and P. A. Fowler5, 1University of Glasgow, College of Medical Veterinary and Life Sciences, Institute of Biodiversity Animal Health & Comparative Medicine, Glasgow, UK, 2INRA, UMR 1198 Biologie du Development et Reproduction, Jouy en Josas, France, 3James Hutton Institute, Aberdeen, UK, 4University of Edinburgh, Queens Medical Research Institute, MRC Centre for Reproductive Health, Edinburgh, UK, 5Institute of Medical Sciences, Division Applied Medicine, University of Aberdeen, Aberdeen, UK.

Processed human sewage sludge, which contains low individual concentrations of an array of contaminants including heavy metals and organic pollutants e.g., PAHs, PCBs and PCDD/Fs is used as an agricultural fertiliser within the EU. Investigation of the physiological effects on grazing sheep on such treated pastures provides a model to investigate effects of exposure to mixtures of environmentally relevant concentrations of pollutants. Pasture treatment results in non-significant increases in environmental chemical (EC) concentrations in soil, and some tissues of ewes and their fetuses. Tissue EC concentrations were variable and low and deemed to pose little risk to consumer health. Investigation of the effects of gestational EC exposure on fetal development has highlighted several issues. The results indicate that gestational EC exposure can adversely affect gonadal development (males and females) and that these effects can affect testicular morphology and ovarian follicle numbers/health and proteome/transcriptome changes in adult animals. In addition, EC exposure can be associated with altered expression of GnRH, GnRH receptor, galanin receptor and kisspeptin mRNA within the hypothalamus and pituitary gland, gonadotroph populations within the pituitary gland and regional aberrations in thyroid morphology. In most cases, these anatomical-functional differences do not result in altered peripheral hormone concentrations or reproductive function e.g., lambing rate, suggesting physiological compensation under the conditions tested. Physiological compensation is also suggested by studies which indicate that EC effects may be greater when exposure occurs either before or during gestation, compared with EC exposure throughout life. With regard to human/animal health, this body of work questions the concept of safe individual concentration of Ecs when EC exposure typically occurs as complex mixtures. It suggests that developmental EC exposure may affect many different physiological systems, with some sex-specific differences in EC sensitivity and that EC effects may be masked under favorable physiological conditions. [Wellcome Trust grant 080338].

Key Words: environmental pollutant, fetal development, environmental chemical

E-Screen—Potential tool for assessment of relative serum estrogenicity. N. W. Shappell*,1, S. A. Hiablie2, J. D. Magolski3, K. A. Vonnahme4, E. P. Berg1, and L. O. Billey1, 1Biosciences Research Laboratory, USDA-ARS, Fargo, ND, 2Penn State University, State College, 3North Dakota State University, Fargo.

The E-Screen bioassay was evaluated for its usefulness in the assessment of serum estrogenicity as a potential sentinel of endocrine disruption. Porcine, ovine, bovine, and piscine samples were evaluated. High concentrations of swine, cattle, and fish serum were toxic to the E-screen (human) cell line, which is routinely maintained in fetal bovine serum. In contrast, high concentrations of sheep serum altered cell morphology, a change that was not seen at lower serum concentrations. Ovarectomized sheep on basal diets had serum estradiol (E2) of 1.8 ± 0.79 pg/mL (SD, RIA) and 18 ± 10.7 pg/mL E2Eq (E2 equivalents ± SD, E-Screen). Acetonitrile extraction of porcine serum (ACN:serum 2:1 v/v) removed the toxicity to E-screen cells. Extracted serum E2Eq from peripubertal gilts increased at the onset of estrus in most animals with a maximum E2Eq of 20 pg/mL. Validation experiments were conducted by comparing E2Eq from serum fortified with E2 before extraction and E2Eq from unextracted serum, with or without E2 fortification. At high unextracted serum concentrations, E2 addition produced a synergistic proliferative response in cells, while the expected additive proliferation was found with extracted serum. Sex differences in serum E2Eq were clear in 2 different species of catfish. Mean E2Eqs (pg/mL) from male fish were 333 ± 281 (SD) in African catfish (Clarias gariepinus) and 244 ± 49 in channel catfish (Ictalurus punctatus), whereas mean E2Eqs from female fish were 1560 ± 1054 and 1157 ± 71 for the 2 species, respectively. Collectively, these results suggest that the E-Screen assay is useful for evaluating serum samples for total estrogenicity. When assessing in vivo effects of possible endocrine disruptor exposure, ELISAs provide specific concentrations for steroid hormones, such as estradiol, while the E-Screen assay evaluates the net effects of estrogen agonists and antagonists in serum.

Key Words: E-Screen, serum, estrogenicity

Developmental programming of reproductive and metabolic health. V. Padmanabhan*, Department of Pediatrics, Obstetrics and Gynecology, Molecular and Integrative Physiology, and Environmental Health Sciences, University of Michigan, Ann Arbor.

The inappropriate programming of the reproductive system by developmental exposure to excess steroid hormones is of concern, especially in the female. The sheep is well suited for investigating developmental origin of reproductive and metabolic disorders. The developmental time line of sheep (5 mo gestation and 7-mo to puberty) is ideal for conducting sequential studies of the progression of metabolic/reproductive disruption from the developmental insult to manifestation of adult consequences. Major benefits of using sheep include knowledge of established critical periods to target adult defects, a rich understanding of reproductive neuroendocrine regulation that parallel humans, availability of non-invasive approaches to monitor follicular dynamics, established surgical approaches to obtain hypophysial portal blood for measurement of hypothalamic hormones, and the ability to perform studies in natural setting keeping behavioral interactions intact. Of importance is the ability to chronically instrument fetus and mother for determining early endocrine perturbations. Prenatal exposure of the female to excess testosterone (T) leads to an array of adult reproductive disorders that include LH excess, functional hyperandrogenism, neuroendocrine defects, multifolllicular ovarian morphology, and corpus luteum dysfunction culminating in early reproductive failure. At the neuroendocrine level all 3 feedback systems (estradiol negative, estradiol positive and progesterone negative feedback) are compromised. Estradiol negative feedback deficits are programmed by androgenic actions and estradiol positive by both androgenic and estrogenic influences of T. At the pituitary level, LH sensitivity to GnRH is increased. Multifollicular ovarian morphology stems from persistence of follicles as well as enhanced follicular recruitment. Together these defects culminate in progressive loss of cyclicity and reduced fecundity. Prenatal T excess also leads to fetal growth retardation, an early marker of adult reproductive/metabolic diseases, insulin resistance, hypertension and behavioral deficits. CUL
fectively, the reproductive/metabolic deficits of prenatal T-treated sheep provides a model for understanding the developmental origin of fertility and metabolic disorders.

**Key Words:** reproductive health, fertility, testosterone

### 714 Effects of bovine somatotropin administration on growth, physiological, and reproductive responses of replacement beef heifers. R. F. Cooke1,2, D. W. Bohnert1, C. L. Francisco1,2, R. S. Marques1, C. J. Mueller1, and D. H. Keisler1, Oregon State University - Eastern Oregon Agricultural Research Center, Burns, 2UNESP - Faculdade de Medicina Veterinária e Zootecnia, Botucatu, São Paulo, Brazil, 3Oregon State University - Eastern Oregon Agricultural Research Center, Union, 4University of Missouri - Division of Animal Sciences, Columbia.

This study compared growth, body composition, plasma IGF-I and leptin, and reproductive development of beef heifers receiving or not recombinant bovine ST (BST) beginning after weaning until the first breeding season. Fifty Angus × Hereford heifers, weaned at 6 mo of age (d −30), were assigned to the experiment (d 0 to 210). On d 0, heifers were ranked by initial BW and age, and assigned to (1) treatment with BST or (2) saline control. Heifers assigned to the BST treatment received s.c. injections containing 250 mg of sometribov zinc whereas control heifers received a 5-mL s.c. injection of 0.9% saline every 14 d. Treatments were initiated on d 14, and last administered on d 196. Heifer shrink BW was collected on d 0, 63, 133, and 189, heifers were evaluated for intramuscular marbling, LM depth, and backfat thickness via ultrasonography. No treatment effects were detected (P = 0.27) for heifer ADG (0.49 vs. 0.51 kg/d for control and BST heifers, respectively; SEM = 0.02). Mean backfat thickness was less (P < 0.01) in BST heifers compared with control cohorts (3.56 vs. 3.92 mm, respectively; SEM = 0.08). Heifers receiving BST had greater plasma IGF-I concentrations compared with control cohorts 7 d after treatment administration (treatment × day interaction; P < 0.01). Mean plasma leptin concentrations were lower (P = 0.05) in BST heifers compared with control cohorts (1.82 vs. 2.03 ng/mL, respectively; SEM = 0.07). Onset of puberty was hastened consistently, and avoid precocious puberty are needed. Herein, we tested the hypothesis that a stair-step nutritional regimen could achieve this goal.

Increasing dietary energy intake of heifers during the juvenile period leads to early maturation of the reproductive neuroendocrine system. Developing strategies that can exploit this process, achieve puberty at 12 mo of age consistently, and avoid precocious puberty are needed. Herein, we tested the hypothesis that a stair-step nutritional regimen could achieve this goal. Forty crossbred heifers were weaned at 3.5 mo of age and assigned to: High-Control (HC); feed intake was controlled to promote BW gain of 1 kg/d from 4 to 14 mo of age; Stair-Step Compensatory (SS-1); period 1: ad libitum feed intake until 6.5 mo of age; period 2: restricted dry matter access to promote BW gain of 0.35 kg/d until 9 mo of age; period 3: ad libitum feed intake until 11.5 mo of age; period 4: restricted intake to promote BW gain of 0.35 kg/d until 14 mo of age; SS-2; reverse sequence of SS-1; Low-Control (LC); controlled feed intake to promote BW gain of 0.5 kg/d until 14 mo of age. Starting at 8.5 mo of age, blood samples were collected twice weekly to assess pubertal onset (at least 2 consecutive samples with concentrations of progesterone ≥1 ng/mL). Puberty data were plotted on Kaplan-Meier survival curves using the log-rank test with Prism5 (GraphPad, La Jolla, CA). Body weight gain followed a pattern similar to that proposed in our design. The percentage of heifers pubertal in the LC group was lower (P < 0.06) than all other groups. Although heifers in SS-1 were nutritionally restricted between 6.5 to 9 mo of age, the proportion pubertal by 12 mo of age did not differ from that of the HC group, with 80% and 70% pubertal in SS-1 and HC, respectively. In contrast, the proportion of heifers pubertal by 12 mo of age in SS-2 (40%) was lower (P < 0.05) than both HC and SS-1. However, by 14 mo of age, 90% of heifers in the SS-2 had also attained puberty compared with only 40% of the LC group. Results indicate that functional changes occurring in the brain during the early juvenile period can program puberty that occurs months later, allowing optimal timing of pubertal onset in beef heifers. Supported by USDA-NIFA-AFRI 2009–65203–05678.

**Key Words:** puberty, GnRH, LH

### 715 Neuroendocrine programming of accelerated puberty in heifers. M. Amstalden1, B. R. C. Alves1, and R. C. Cardoso1,2, Texas A&M University, College Station, 2Texas A&M AgriLife Research, Beeville.

Neuroendocrine events that lead to the onset of puberty and first ovulation in females are characterized by the initiation of frequent episodic secretion of GnRH and its downstream regulation of pulsatile release of LH. Studies using animal models of accelerated body weight (BW) gain during the prepubertal period support the hypothesis that advanced onset of puberty occurs in response to an earlier escape from estradiol negative feedback and alterations in afferent inputs to GnRH neurons. Recent studies in our laboratories have indicated that elevated BW gain in early-weaned heifers fed high-concentrate diets alters the pattern of hypothalamic expression of genes involved in the control of key biological functions important for pubertal development, including response to hormones, metabolic factors and nutrients, synaptic transmission and feeding behavior. Changes in afferent neuronal projections to GnRH neurons are also evident and comprise major metabolic-sensing pathways such as neuropeptide Y and proopiomelanocortin neurons. Kisspeptin neurons, which have been implicated in mediating the estradiol feedback control of GnRH secretion and pubertal development, appear to be also involved in the process of nutritional acceleration of puberty in heifers. The role of kisspeptin neurons in the nutritional programing of puberty is less clear, but may include the integration between metabolic signaling pathways and functional changes in the sensitivity to estradiol-negative feedback that are permissive for pubertal development. Overall, the mechanisms that mediate the neuroendocrine programing of accelerated puberty in heifers are likely to involve a network of metabolic- and gonadal steroid hormone-sensing effectors and pathways in the hypothalamus. Changes in expression of key regulatory genes, and in functional structures that control neuronal and glial cell communication, are critical for pubertal activation of frequent episodic release of GnRH. Elevated BW gain during early juvenile period appears to facilitate the progression of these events. Supported by USDA-NIFA-AFRI 2009–65203–05678.

**Key Words:** beef heifer, somatotropin, puberty