

## PLENARY SESSIONS

**PS1 NON-GENOMIC PROGESTERONE RECEPTORS IN THE BOVINE OVARY.** Tony Bramley,  
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Subcellular fractionation studies of the bovine corpus luteum and preovulatory follicle revealed the presence of specific cell-surface membrane binding sites for [<sup>3</sup>H]-progesterone. Steroid binding was stimulated dose-dependently digitonin, but a range of other detergents, cardiotoxic steroids and cholesterol-complexing agents tested were ineffective. Binding of [<sup>3</sup>H]-progesterone was dependent on membrane concentration, pH, temperature and duration of incubation, and had a high specificity and affinity for progesterone (70nM). There was a ten-fold lower affinity for androgens; however, other steroids tested (cholesterol, oestrogens, steroid conjugates, synthetic progestins and antigestagens) failed to compete for binding, except at very high concentrations. Cyclodextrin treatment lowered luteal membrane cholesterol content, increasing the EC<sub>50</sub> for digitonin, and enzymes that chemically modified membrane cholesterol abolished binding. Membrane receptors for progesterone were also present in both granulosa and thecal cells of the follicle, and in large and small bovine luteal cells. Furthermore, similar binding sites were present in luteal membranes of other species, and also in variable amounts in cell-membrane preparations from some, but not all, steroidogenic and non-steroid secreting ovine and human tissues. Studies are in progress to purify and clone this binding site(s), establish the cellular and tissue localization, and define the second messenger system(s) activated by progesterone at the luteal cell surface.

**Key words:** Non-genomic, progesterone, receptor, bovine ovary.

**PS2 LIGAND-INDEPENDENT ACTIVATION OF STEROID RECEPTORS.** A.P.F. Flint, E.L. Sheldrick,  
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Steroid receptors are subject to ligand-independent activation through phosphorylation at specific serine, threonine and tyrosine residues. The kinase cascades responsible for these post-translational processing steps are activated by occupancy of cell surface receptors by a variety of growth factors and other endocrine agents. There is no established endocrine effect mediated through ligand-independent activation of steroid receptors in reproductive tissues. We have investigated the control of oestrogen receptor function by insulin-like growth factor in bovine endometrial cells.

Bovine endometrial stromal cells were prepared by pancreatin/dispase digestion and passaged in DMEM containing 10% fetal bovine serum. Oestrogen response element-reporter gene construct (CATERE) was transfected into the cells by electroporation, and chloramphenicol acetyltransferase activity was measured during transient expression following transfection. Growth factors were added at various times following transfection.

Reporter gene activity was increased in a dose dependent manner by IGF-I in the absence of oestradiol and in the presence of 0.1 or 1 nM oestradiol. The effects of IGF-I and oestradiol were synergistic. As expected reporter gene activity was also increased by oestradiol in the absence of IGF-I, and the effect of oestradiol was blocked by the anti-oestrogen ICI 182780. The effect of IGF-I in the absence of oestradiol was not inhibited by ICI 182780. Reporter gene activity was also increased by treatment of cells with hEGF. The effect of IGF-I was evident within an hour of addition to transfected cells. This effect occurred in the absence of cell proliferation, as indicated by total protein contents. The MEK inhibitors UO126 and PD 98059 were used to determine whether the effect of IGF-I on CATERE expression was mediated through mitogen activated protein kinase. PD 98059 was ineffective at the maximum usable concentration, but the effect of IGF-I was blocked by UO 126. The effect of hEGF was also inhibited by UO 126. To confirm that the effect of IGF-I was not due to increased availability of oestrogen receptor, oestradiol binding by bovine endometrial stromal cells was measured following addition of IGF-I. As expected, oestradiol caused a progressive loss of oestrogen binding, but IGF-I had no effect. These data will be compared with results obtained with other endometrial cells.

These results point to the possible effect of intrauterine growth factors via ligand-independent steroid receptor activation.

**Key Words:** Oestrogen, oestrogen receptor, growth factors

**PS3 TRANSCRIPTION FACTOR REGULATION IN MAMMARY EPITHELIAL CELLS.** Bernd Groner, Georg Speyer Haus, Institute for Biomedical Research, Paul Ehrlich Str. 42-44, D-60596 Frankfurt am Main, Germany.

Extracellular hormones, growth factors or cytokines relay their effects on the transcription of genes through the recognition of specific receptors and intracellular signaling molecules. Stat5 (signal transducers and activators of transcription) has been recognized as crucial intracellular signaling molecule in the development of the mammary gland and detailed insights have been gained in its mode of action. The cytokine receptor associated Jak kinases convert the latent monomeric form of the Stat molecules to the activated dimeric form through tyrosine phosphorylation. The dimers bind to specific DNA response elements and are able to induce transcription. Different isoforms of Stat5 and associations with cooperating molecules add versatility to the regulatory features. Proper gene induction requires the full length form of the Stat molecules which interacts with coactivator molecules of the CBP/p300 family. Negative regulatory potential is exerted by the expression of the short form of the molecule, lacking the transactivation domain and associating with corepressors of the NCoR family. This form is activated through tyrosine phosphorylation and dimerisation similarly to the full length form, but is impeded in dephosphorylation. It occupies the DNA binding sites in a rather stable fashion and acts as a strong suppressor of wild type action. Positive enhancement of Stat5 transactivation potential is provided by the glucocorticoid receptor. Ligand activation of the receptor causes complex formation with Stat5 and deviation to the Stat5 DNA binding site. An additional regulatory loop is provided by the reactivation of the short form of Stat5 through glucocorticoid receptor association. Conversely, classical glucocorticoid responsive genes are negatively affected by Stat5 activation. These multiple protein interactions resulting in positive and negative gene regulation are the key to the versatile functions of Stat5 in the control of growth, differentiation and apoptosis of mammary epithelial cells.

**Key words:** transcription factors, mammary, epithelial cells.

**PS4 PITUITARY EFFECTS OF STEROID HORMONES ON SECRETION OF FOLLICLE-STIMULATING HORMONE AND LUTEINIZING HORMONE.** T.M. Nett<sup>1</sup>, A.M. Turzillo<sup>2</sup>, M. Baratta<sup>3</sup>, L.A. Rispoli<sup>1</sup>. *Animal Reproduction & Biotechnology Laboratory, Colorado State University, Fort Collins, CO, USA<sup>1</sup>, Department of Physiology, University of Arizona, Tucson, AZ USA<sup>2</sup>, and Institute of Veterinary Physiology, University of Parma, 43100 Parma, Italy<sup>3</sup>*

Steroid hormones have a profound influence on the secretion of the gonadotropins, follicle-stimulating hormone (FSH) and luteinizing hormone (LH). These effects can occur as a result of steroid hormones modifying the secretion of gonadotropin-releasing hormone (GnRH) from the hypothalamus, or a direct effects of steroid hormones on gonadotropin-secreting cells in the anterior pituitary gland. With respect to the latter, we have shown that estradiol increases pituitary sensitivity to GnRH by stimulating an increase in expression of the gene encoding the GnRH receptor. Since an estrogen response element has not been identified in the GnRH receptor gene, this effect appears to be mediated by estradiol stimulating production of a yet to be identified factor that in turn enhances expression of the GnRH receptor gene. However, the importance of estradiol for enhancing pituitary sensitivity to GnRH during the periovulatory period is questioned because an increase in mRNA for the GnRH receptor precedes the pre-ovulatory rise in circulating concentrations of estradiol. In fact, it appears that the enhanced pituitary sensitivity during the periovulatory period they occur as a result of a decrease in concentrations of progesterone rather than due to an increase in concentrations of estradiol. Estradiol also is capable of altering secretion of FSH and LH in the absence of GnRH. In a recent study utilising cultured pituitary cells from anestrus ewes, we demonstrated that estradiol induced a dose-dependent increase in the secretion of LH, but resulted in a dose-dependent decrease in the secretion of FSH. We hypothesised that the discordant effects on secretion of LH and FSH might arise from estradiol altering the production of some of the intrapituitary factors involved in synthesis and secretion of FSH. To examine this hypothesis, we measured amounts of mRNA for activin B (a factor known to stimulate synthesis of FSH) and follistatin (an activin binding protein). We found no change in the mRNA for follistatin after treatment of pituitary cells with estradiol, but noted a decrease in the amount of mRNA for activin B. Thus, the inhibitory effect of estradiol on secretion of FSH appears to be mediated by its ability to suppress the expression of the gene encoding activin.

**References:** Turzillo & Nett, 1999, *J. Reprod. Fert. Suppl.* **54**: 75; Baratta, et al., 2001, *Biol. Reprod.* **64**: 714.

**Key Words:** FSH, LH, GnRH receptor, estradiol, activin, follistatin

**PS5 STEROIDS, SEXUAL DIFFERENTIATION AND DEVELOPMENT.** Jane E Robinson. *Laboratory of Neuroendocrinology The Babraham Institute, Babraham, Cambridge CR2 4AT, UK.*

It has long been recognised that steroids can have both organisational and activational effects on the reproductive neuroendocrine axis of many species, including the sheep. Specifically, if the ovine foetus is exposed to testosterone during a relatively short 'window' of *in utero* development (from approximately day 30-90 of a 147 day pregnancy) the neural mechanisms regulating gonadotrophin releasing hormone (GnRH) secretion become organised in a malespecific manner. In post-natal life the consequences of foetal androgen exposure are sexually differentiated responses of the GnRH neuronal network to activation by factors such as photoperiod and ovarian steroid hormones (Wood et al, 2000). Studies in the gonadectomised lamb have demonstrated that elevated concentrations of oestrogen (E) are unable to trigger a preovulatory-like GnRH surge in the male and the androgenised ewe lamb. Further, these animals have markedly reduced sensitivity to the inhibitory actions of progesterone on tonic GnRH release compared with normal ewes (Robinson et al, 1999). The reasons for these abnormal steroid feedback mechanisms may reside in sexually dimorphic inputs to the GnRH neurone, including those from oestrogen-receptive neurones in the arcuate nucleus that synthesise the tachykinin, neurokinin B (Goubillon et al., 2000). The consequences of *in utero* androgen exposure are reflected in a progressive and dramatic impairment of fertility in the ovary-intact ewe.

**References:** Wood et al., 2000, Proceedings of the 5<sup>th</sup> International Conference of the Control of the Onset of Puberty, 269; Robinson et al., 1999, Endocrinology 140, 5797; Goubillon et al., 2000, Endocrinology **141**, 4218.

**Key Words:** GnRH, Oestrogen, Progesterone, Sexual differentiation.

**PS6 STEROIDS AS LOCAL REGULATORS OF OVARIAN ACTIVITY.** D. Schams, *Institute of Physiology, TU Munich-Weihenstephan, Germany*

Ovarian steroid hormones control or influence every aspect of reproductive function by mostly endocrine regulation. Less emphasis is given for steroids as local regulators of follicular and luteal activity. Follicle activity; Besides species specific effects in general there is evidence that estradiol (E<sub>2</sub>) exerts a dose-dependent inhibition on the secretion of progesterone (P<sub>4</sub>) by both theca cells (TC) and granulosa cells (GC). GC enhance the ability of the TC to produce androstendione by supplying them with progestin precursor. TC preferentially use the  $\Delta^5$  pathway to synthesize androgens. Androgen produced by TC enhances the ability of the GC to make P<sub>4</sub> and high levels of E<sub>2</sub> in the preovulatory follicle inhibit 3 $\beta$ -HSD in both TC and GC and thus, may promote the use of the  $\Delta^5$  pathway for TC androgen production. The results suggest that E<sub>2</sub> acts within the follicle to exert positive feedback on androgen and E<sub>2</sub> production, and exerts mitotic and anti-atretic effects on follicle cells. Parts of the E<sub>2</sub> mediated local action are regulated by stimulating hormone receptors. Corpus luteum (CL); There is evidence in the bovine CL that P<sub>4</sub> may directly regulate the production of P<sub>4</sub>, oxytocin and prostaglandins in a cycle-dependent fashion. In some species is clear evidence for CL production of E<sub>2</sub> (rat, human and pig) with clear stimulatory and luteotropic effects on P<sub>4</sub>, and an intraluteal circuit that involves paracrine effects of E<sub>2</sub>, oxytocin and PGF<sub>2 $\alpha$</sub> . In contrast, species in which there is little evidence of luteal production of E<sub>2</sub> and effects on luteal function (ruminants, horses). More evidence will be given for the aromatase and estradiol receptor mRNA expression and luteal effects of E<sub>2</sub> in the bovine CL. Furthermore the importance of P<sub>4</sub> for the timing of the life time of CL function in ruminants will be presented and discussed.

**Key words:** steroids, ovary, local regulation

**PS7 HORMONES AS INDICATORS OF STRESS.** E. Möstl, *Institute of Biochemistry, University of Veterinary Medicine, Vienna, Austria*

Disturbances of animals cause alterations in a variety of hormone systems. The classical "stress hormones" catecholamines and glucocorticoids are produced by the adrenal glands. They play an important physiological role in farm animals as for example cortisol initiates parturition in ruminants and regulates a variety of metabolic processes. Extensive publications indicate that glucocorticoid production is increased during adverse situations. Cortisol concentration in blood has proven a useful indicator of stress although caution is advised, since the increase of the glucocorticoid do not occur to every type of stressor. In order not to confound the rating of stress levels, care has to be taken that the stress of the sample collection does not influence the results of hormone measurement. Therefore, measuring these substances in blood needs some precautions not to disturb the animals. Glucocorticoids are metabolised in the liver and are excreted via faeces and urine. In the gut there is a further conversion by bacteria and some of these substances are reabsorbed (enterohepatic circulation). There are considerable differences between species concerning

the amount of excretion via urine or faeces. For ruminants and horses, immunoassay systems are now available to measure those faecal glucocorticoid metabolites. The delay time between a glucocorticoid increase in blood and the increase of these metabolites in the gut is about 12 hours in ruminants, 24 hours horses and 48 hours in pigs. Measuring the glucocorticoid metabolites in faecal samples has the benefit that collection of the samples is easy, feedback free and can be done for example by the owners.

**Key words:** hormones, indicators, stress.

**PS8 HORMONAL INTERACTIONS DURING ANIMAL STRESS.** *R.F. Smith and H. Dobson, Dept. Veterinary Clinical Science, University of Liverpool, Leahurst, Neston, Cheshire, CH64 7TE, U.K.*

Endocrine systems may be used as indicators of stress as part of the homeostatic response to a stimulus, (e.g. corticosteroids) the amplitude of hormone response may correlate with the severity of the stimulus and changes indicate that the body is responding. Alternatively, hormones controlling other body functions (e.g. reproduction) may be deleteriously altered by stress thus hormones such as LH will reflect reduced reproductive function and demonstrate that the stimulus was sufficiently severe that homeostatic mechanisms were unable to maintain this normal function.

Corticosteroids have a broad, yet fundamental, role in homeostasis and have been used as primary indicators of stress for many years. Excess corticosteroid can be detrimental so concentrations are controlled via the hypothalamus-pituitary-adrenal (HPA) axis by multi-level feedback mechanisms. After an initial large response prolonged stimulation leads to a gradually reducing plasma corticosteroid concentrations. This has been interpreted as a reduction in perceived stimulus severity or habituation to the stimulus and the animal deemed “less stressed” and its welfare “better” However, the stress signal at higher brain levels may still be present and the animal may still be experiencing the stimulus as aversive. Thus, the welfare interpretation of corticosteroid profiles may differ throughout a stress response.

We have used mathematical modelling to produce representations of possible control mechanisms at each level of the HPA. The starting point was to measure AVP and CRH in hypophysial portal blood and ACTH and cortisol in jugular blood in conscious sheep during insulin-induced hypoglycaemia (a non-cognitive stimulus) or 2h road transport (cognitive). Modelling identified signal inputs that were most likely to explain the secretion rate of each hormone. During hypoglycaemia both log AVP and log CRH were similarly related to plasma log glucose and the response was reduced by a negative effect of earlier cortisol concentration (negative feedback). During transport, modelling suggested that the reduction in AVP and CRH secretion was most likely due to a reduction in stimulus input, with significant cortisol negative feedback only on AVP secretion. At pituitary level, during both experiments ACTH secretion was stimulated more by AVP than by CRH (ratio 2.3:1) and there was a stimulatory effect of cortisol values at the time of sampling. The ACTH responses to both stimuli were curtailed by an inhibitory effect cortisol negative feedback and of prior CRH. These complex effects suggest that whilst the “stress” stimulus may reduce over time hormone negative feedback is a major factor reducing hormone responses. Complementary measurement of reproductive hormones during the imposition of these two stressors revealed reduced LH pulsatility and oestradiol conc in the initial stress phase with subsequent return to normal profiles during the cortisol negative feedback phase. Use of a range of indicators (e.g. HPA and reproductive hormones, behaviour, heart rate) for the entire duration of a stimulus is recommended to obtain a balanced view of animal welfare.

**Key words:** hormones, interaction, animal, stress.

**PS9 NEUROENDOCRINE INTERACTIONS AND SEASONALITY: FEMALE SHEEP AS AN EXAMPLE.**

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Sheep under temperate latitudes are seasonal breeders that show a cessation in reproductive activity during spring and summer. The seasonality of reproduction has an adaptive value such that ewes give birth at the most favorable time of the year for food and climatic environment. Among seasonal cues, photoperiod, which synchronizes an endogenous annual rhythm, is the most reliable parameter and is used by animals as an index of the time of the year. The photoperiodic information is conveyed from the eyes to the pineal gland by various neuronal relays and transduced into neuroendocrine changes through variations in melatonin secretion. After a chain of neuronal events, only some of them being known, melatonin triggers variations in the secretion of luteinizing hormone (LH) responsible for seasonal changes in ovulatory activity. The seasonal changes in hormonal pattern in LH mainly reflects a strong increase in the intensity of the negative feedback exerted by oestradiol under long days on the frequency of pulsatile LH secretion. Neuropharmacological studies have shown that this inhibition of LH secretion involves activation of monoaminergic,

specially dopaminergic systems by oestradiol. Within the medial basal hypothalamus, the most important structures appear to be the A15 dopaminergic nucleus, and the median eminence (ME) that contains the axon terminals of the LHRH cells controlling the pulsatile release of LH. Oestradiol acts directly on this structure but also on preoptic area to trigger activation of the dopaminergic system. We describe here the already identified steps of the complex system going from the light to the changes in gonadotropin secretion. This system which is intensively under investigation for reproduction in sheep also will be used as a reference for comparison with other physiological regulations such as voluntary food intake, metabolism and body mass or pelage growth. These latter regulations involving also a seasonal control provide advantages to help mammal species to adapt to the environment.

**Key words:** neuroendocrine, interactions, seasonality, sheep