secretion by AtT-20 cells (ANOVA, $P > 0.05$). **Conclusions:** Initial experiments have shown that ASOs against POMC can reduce ACTH secretion from AtT-20 cells and may be useful as a novel therapy for CD.

Neuroendocrinology and Pituitary

NEUROENDOCRINOLOGY AND PITUITARY BASIC RESEARCH ADVANCES

Canine Pituitary Organoids as 3D** In Vitro **Model for Cushing Disease

Karin Sanders, DVM, PhD[1], Femke C.A. Ringnalda, BSc[2], Marc L. van de Wetering, PhD[2], Hans S. Kooistra, DVM, PhD[1], Björn P. Meij, DVM, PhD[1], Hans Clevers, MD, PhD[2], Sara Galac, DVM, PhD[1].

[1]Faculty of Veterinary Medicine, Utrecht University, Utrecht, Netherlands, [2]Princess Máxima Center for pediatric oncology, Utrecht, Netherlands.

Cushing disease (CD) is a serious endocrine disorder that is most often caused by an ACTH-secreting pituitary adenoma. Patients can be treated medically when surgery is not an option or was unsuccessful. However, currently used pituitary-targeting drugs are effective in only 40% of patients. To efficiently identify new pituitary-targeting treatment options, we need an *in vitro* system that closely mimics *in vivo* conditions. We therefore aimed to establish organoid cultures of normal anterior pituitary and corticotroph adenomas. Organoids or tumoroids are miniature three-dimensional (3D) structures grown from stem cells, that closely resemble the organ or tumor they originate from. Because CD is a thousand times more prevalent in dogs than in humans, and hypophysectomy is the treatment of choice, we used canine tissues. Normal anterior pituitary glands were collected from three healthy dogs that were euthanized for reasons unrelated to the current study. Corticotroph adenomas were collected from six dogs that underwent transsphenoidal hypophysectomy at our University Clinic. The dogs were diagnosed with CD based on clinical signs, endocrine testing, and CT scan imaging. Normal anterior pituitary and corticotroph adenoma cells were cultured in a 3D matrix (basement membrane extract) with anterior pituitary organoid medium containing specific growth factors and ligands, which was refreshed twice a week. The organoids and tumoroids were characterized with histopathology and RT-qPCR. Structures resembling organoids or tumoroids grew from all nine samples (3 normal, 6 adenoma) that were put in culture. Both cystic and dense structures were observed. The organoids and tumoroids expanded rapidly, and could be passaged once every week. The organoids and tumoroids were successfully cultured up until passage number 10, and were then frozen down. Histopathology showed that the organoid or tumoroid cells morphologically resembled healthy anterior pituitary or corticotroph adenoma cells. All organoids cultures expressed mRNA of pituitary stem cell markers SOX2 and SOX9. This study shows that corticotroph adenomas can be cultured as tumoroids *in vitro*, something not previously published in any species. Based on the many opportunities in organoid culture (e.g., high-throughput drug screenings, gene editing, studying developmental processes), we expect that this *in vitro* model will pave the way to efficiently and reliably identify new treatment options for CD. Not only for humans, but also for our best friends: dogs.

Neuroendocrinology and Pituitary

NEUROENDOCRINOLOGY AND PITUITARY BASIC RESEARCH ADVANCES

Chronic Excess of Non-Aromatizable Androgens From Puberty Does Not Drive a Neuroendocrine Phenotype Observed in Other Preclinical Rodent Models of PCOS

Chris Coyle, PhD[1], Amy Ruddenklau, BSc (Hons)[1], Mel Prescott, BSc (Hons)[1], Kirsty A. Walters, PhD[2], Rebecca Elaine Campbell, PhD[3].

[1]UNIVERSITY OF OTAGO, Dunedin, New Zealand, [2]University of New South Wales, Sydney, Australia, [3]University of Otago, Dunedin, New Zealand.

Polycystic ovary syndrome (PCOS) is the most common form of anovulatory infertility in women of reproductive age, characterised by androgen excess, polycystic appearance of the ovary and irregular menstruation. PCOS is also frequently associated with metabolic abnormalities, including increased adiposity and insulin resistance. The origins of PCOS are unknown, however recent findings in animal models strongly implicate androgen signalling in the brain in the development of PCOS pathophysiology. Exposure to androgen excess, either acutely during prenatal development or chronically from a peripubertal timepoint, can drive the development of PCOS-like features in adulthood. Prenatally androgenized (PNA) mice exhibit the cardinal reproductive features of PCOS and increased luteinizing hormone (LH) pulse frequency. This phenotype is associated with increased GABAergic innervation of gonadotropin-releasing hormone (GnRH) neurons, postulated to drive elevated GnRH/LH release and downstream effects. Chronic exposure to di-hydrotestosterone (DHT) from 3 weeks of age drives both reproductive and metabolic PCOS-like features that are ameliorated by selective AR loss from the brain. Here, we aimed to determine whether chronic exposure to DHT drives a similar increase in LH pulsatility and elevated GABAergic innervation to GnRH neurons as seen following prenatal exposure to androgen excess. GnRH-green fluorescent protein (GFP) female mice received either DHT or blank capsules for 90 days from postnatal day (PND) 21 (N = 6-7/group). Serial tail tip blood sampling was used to measure pulsatile LH and fixed brains were collected and immunolabelled for vesicular GABA transporter (VGAT) to assess putative GABAergic terminals associated with GFP-labelled GnRH neurons. Chronic androgen excess from the peripubertal period resulted in acyclicity and increased body weight as expected. However, LH pulsatility was not different between DHT-treated females and controls. Similarly, the density of VGAT appositions to GnRH neurons was not different between groups. Therefore, the programmed changes in the GnRH neuronal network and hyperactive LH secretion that result from prenatal androgen excess are not affected by chronic DHT exposure initiated at 3 weeks of age. These findings suggest that unique central mechanisms are involved in the reproductive impairments driven by exposure to androgen excess at different developmental stages.