Effects of dietary probiotic on growth performance, blood characteristics, and immune responses to a lipopolysaccharide challenge of Hanwoo heifers

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Our objective of the study was to effect of probiotics on the immune response of Hanwoo heifers. Lipopolysaccharide (LPS) challenge was used for investigating physiological response of dietary probiotics. A completely random design was used (4 pens; 2pens/treatment; 5 heifers/pen). After the cattle fed probiotic for 5 months, 16 heifers were transported and acclimated to environmentally controlled chambers. Heifers were fitted with indwelling jugular catheters prior to 24 hours of the LPS challenge. Blood samples were collected at 30-min intervals from -1 to 6 h (0 h; 1μg/kg BW of LPS from Escherichia coli O111:B4). Glucose, non-esterified fatty acid (NEFA), albumin, triglyceride, total protein, phosphorus concentrations, plasma CBC (WBC, RBC, Platelet, Neutrophils, Lymphocytes, Eosinophils, Basophils, Hemoglobin), and pro-inflammatory cytokines (TNFα, IL6, IL1b) were determined from blood samples. Response to the LPS challenge over time was analyzed by ANOVA with the MIXED procedure of SAS. Overall ADG and serum compositions did not differ between probiotic or control diet for 5 months (P >0.05). Pre-LPS NEFA concentration did not differ (P >0.05), but probiotic treated heifers was decreased at 2 hours after LPS challenge. NEFA concentration was decreased at 2 hours after LPS challenge in probiotic treated group (P <0.05). Serum triglyceride was decreased at 0.5 h after LPS challenge in of probiotic treated heifers (P <0.05). There was no difference at CBC test between treatment pre- and post - LPS challenge except red blood cell (RBC). Plasma RBC concentration was increased from 0.5h to 3h post-LPS challenge in probiotic treated heifers. These data suggest that probiotic diet did not directly altered immune response to Hanwoo heifers but indirectly regulated lipid metabolism of Hanwoo heifers at the LPS challenge.

Endocrine collateral damage

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Monoclonal antibodies such as Ipilimumab (against cytotoxic T-lymphocyte-associated antigen 4 [CTLA-4]) and nivolumab (against programmed death protein 1 [PD-1]), block inhibitory regulatory T cell molecules and achieve anti-tumour effect by enhanced T cell activation at the cost of autoimmunity. We report a case of presumed autoimmune hypophysitis and type 1 diabetes after treatment with ipilimumab and nivolumab for metastatic melanoma.

A 54-year-old woman presented with seizures and confusion. Medical history included melanoma with intracranial metastases treated with craniotomy, radiation and a course of ipilimumab nine weeks prior. MRI excluded new cerebral lesions but showed an enlarged pituitary not present previously. Static anterior pituitary function evaluation revealed hypopituitarism involving the pituitary-thyroid and pituitary-gonadal axes (T4 of 6.2 pmol/L with TSH of 1.2 mIU/L; low gonadotrophins of FSH 8 IU/L and LH 1 IU/L). ACTH insufficiency was suspected but could not be established due to concurrent dexamethasone therapy (cortisol < 35 nmol/L, ACTH < 5 ng/L). A clinical diagnosis of ipilimumab-induced hypophysitis (IH) was made.

Despite complications, the patient completed the ipilimumab course and then received nivolumab. Five weeks later, she presented with severe symptomatic hyperglycaemia (serum glucose 21.7 mmol/L) and ketoacidosis (pH 6.91, serum beta-hydroxybutyrate 9.4 mmol/L), requiring an insulin infusion. Abdominal CT showed a normal pancreas with no radiological evidence of pancreatitis or metastasis. The acute presentation with hyperglycaemia, ketoacidosis and low C-peptide levels led to the diagnosis of presumed autoimmune diabetes. Serum autoantibodies (IA2 and GAD65) were negative.

There is little data on nivolumab-induced autoimmune diabetes. It has been reported in one recent study. This is the first report of ipilimumab-induced hypophysitis followed by apparent nivolumab-induced type 1 diabetes. These are uncommon adverse events of immunotherapy but are expected to rise in incidence as immunotherapy becomes more prevalent.

Biological Activity and In Vivo Half-Life of Pro-Activin A
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The family of transforming growth factor-β (TGF-β) proteins are master regulators of tissue homeostasis. Consequently, their deregulated activities are associated with a multitude of human disorders including, infertility, cancer, obesity, tissue degeneration, and fibrosis. Correcting TGF-β activity is an attractive approach to restore tissue homeostasis, but is limited by the poor in vivo stability of TGF-β proteins. The active proteins are derived from large Pro-TGF-β forms that undergo proteolytic maturation, yielding a pro:mature non-covalent complex, with pro and mature (active) domains. Prodomains are removed during commercial preparation, leaving only mature active ligand. These preparations, having half-lives of minutes, are unsuitable for therapeutic treatment in humans. The pro:mature non-covalent complex, in which the mature active ligand is shielded by its prodomains, is predicted have greater in vivo stability than the mature ligands. In this study, we examined whether the prodomain could reduce the clearance rate and increase activity in vivo for a well characterised member of the TGF-β family, activin A. To address this, we aimed to generate a pro:mature complex. To favour production of the Pro-activin complex, the native cleavage site was enhanced by site-directed mutagenesis. This modification improved the processing of activin precursor. Pro-activin complexes were isolated from stable HEK-293E cell lines by immunoaffinity using an antibody targeted to the prodomain. Importantly, the purified Pro-activin complex had comparable in vitro bioactivity to the commercially available mature preparations, supporting that the prodomain does not perturb activin bioactivity. In vivo work determined that the half-life of activin was improved two-fold, compared to the mature alone, and biological activity was also improved. Ongoing studies aim to further improve the half-life of activin A. The outcomes of this work will provide a blueprint for generating long-acting TGF-β ligands, which would benefit the treatment of human conditions associated with altered TGF-β signalling.

Pharmacokinetics of Leptin in the Gut of Mice
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Leptin is a protein hormone originally identified from adipose tissue and known for its effects on appetite. Leptin is now known to be produced in many tissues including the stomach, and our earlier work showed that when a physiologic dose was injected intravenously approximately 13 % of the dose was recovered intact from the lumen of the gastrointestinal tract (GIT) after 60 minutes. To examine the pharmacokinetics of leptin in the GIT, non-fasted mice were lightly anaesthetised before oral gavage of 12 ng of 125I-labelled leptin. Samples were analysed by gel permeation HPLC to confirm that the leptin was not degraded and the amount present was determined using a γ-counter.

Radiolabelled leptin in the stomach declined from 53 % to 24 % of the administered dose 30 – 120 min post-gavage. A small peak (~ 4 – 8 % of the dose) appeared to move aborally through the small intestine, with approximately 4 % of the dose reaching the hindgut within the 2 h study. Throughout the experiment radiolabelled leptin was detected in the blood, with approximately 3.5 % of the dose calculated to be in the circulation at all times examined. The radiolabelled leptin in plasma was found to be 74 ± 6 % intact.

Here we show that leptin in the digestive tract moves aborally along the digestive tract, suggesting a role in the intestine. The gradual decline of leptin from the lumen of the stomach may indicate that leptin associates with digesta. We also report that leptin in the lumen of the gut was recovered intact from the blood. Our previous work has shown that leptin in the circulation is also recoverable from the lumen of the digestive tract, suggesting that leptin may be cycling between the gut and the circulation.

Is oxytocin receptor SNP rs53576 a potential biomarker for psychological resilience?
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There is an increasing focus on the positive psychological traits (optimism and resilience) rather than the negative psychological traits such as depression and anxiety in attempts to improve mental health. Negative psychological traits are associated with a number of biomarkers such as cortisol, alpha-amylase, 5-HTTLPR in association with stress. Recent studies show psychological resilience is heritable and it acts as a buffer between depression and stress. Several research groups are working towards a better understanding of resilience and in identifying reliable biomarkers of resilience such as telomere length, oxytocin (OXT) and SNPs of oxytocin receptor, reelin and other depression associated genes. OXT a neuropeptide
secreted in the hypothalamus is involved in a number of physiological and social behaviours and has a role in the development of social behaviours such as trust, positive communication, group favouritism, and reduced social stress. We hypothesized that the oxytocin receptor (OXTR) SNP rs53576 that results in Guanine (G) to Adenine (A) substitution may be associated with resilience as it has been shown to be associated with positive traits. We collected DNA samples from buccal cells from a self-selecting community population and collected questionnaire data for depression and anxiety (Zung) and resilience scores (Connor Davidson). OXTR SNP rs53576 was analysed from 121 non-medicated subjects using traditional restriction enzyme digest, sequencing and qPCR-HRM methods. Results showed our cohort did not fit with HW equilibrium for OXTR SNP rs53576 ($p = 0.00004$) and did not show any association between OXTR rs53576 and to depression or resilience ($p > 0.5$). However further study with a larger cohort and including data from other OXTR SNPs may be worthwhile.

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FGF9 activity from normal males and a 46,XY female

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Disorders of sex development (DSDs) include 46,XY gonadal dysgenesis (GD), where a specific molecular diagnosis is made in only ~30% of patients. Improved understanding of the genetic causes of DSD will lead to better diagnosis and management. FGF9 is expressed in Sertoli cells and is critical for testis determination in the mouse since Fgf9-/- mice show XY gonadal sex reversal. In the developing XY gonad FGF9 maintains Sox9 expression through repression of Wnt4. However, the mechanism of Wnt4 repression by FGF9 is still unknown. We have established an in vitro assay system of FGF9 function during foetal gonadal development to identify the signalling pathways involved in Wnt4 repression. We show that FGF9 treatment of the mouse Sertoli cell line 15P-1 can efficiently down-regulate Wnt4 expression in a dose dependent manner. Cyclheximide treatment inhibited Wnt4 repression, suggesting that FGF9 requires new protein synthesis to down-regulate Wnt4. FGF signalling activates four major signalling pathways: MAP Kinase, AKT, STAT, and the PLCγ. To determine which pathways are involved in FGF9 repression of Wnt4, we treated 15P-1 cells with drugs to these pathways. Drugs blocking the ERK1/2 and JNK pathways significantly inhibited Wnt4 repression, suggesting that FGF9 down-regulates Wnt4 via the ERK1/2 and JNK MAPK pathways, but not via p38 MAPK pathway. Testing in gonad cultures ex vivo is underway.

FGF9 mutation has not been described in human DSD. Here, we identified an FGF9 variant in a 46,XY GD patient, a maternally-derived heterozygous single nucleotide substitution, c.583G>A (p.Asp195Asn) using 1000 DSD gene targeted Massively Parallel Sequencing. Recombinant wildtype and the variant FGF9 protein have been purified and the variant protein showed lower affinity for heparin biochemically. In vitro Wnt4 repression assay and ex vivo experiments are underway. CRISPR/Cas9 knock-in mice of the variant Fgfg9 are also being produced and analysed.

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Castration Effects on the Expression of Kisspeptin and Rf-Amide Related Peptide-3 and their Co-Expression with Oestrogen Receptor α in the Ram Hypothalamus.

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The mechanism by which testicular hormones exert a negative feedback action is unclear, as GnRH neurons do not contain receptors for androgen or oestrogen. The RF-amides, Kisspeptin and RF-amide related peptide-3 (RFRP-3) could be potential neuronal pathways. In ewes, 93% of arcuate kisspeptin cells co-expressed ERα (1), with hypothalamic RFRP-3 cells expressing ERα ranging between 20% in mice (2) and 40% in Syrian hamsters (3). This study aimed to determine if castration influenced the expression of ERα in kisspeptin and RFRP-3 neurons in the ram. Dual label fluorescence immunohistochemistry for the co-expression of the RF-amides with ERα was used to compare the percentage of RF-amide cells containing ERα in the hypothalamus of intact merino rams and long term wethers (n=4/group), and in rams castrated 4 weeks previously or sham castrated rams, with ewe tissue (luteal phase) included for comparison (n=4/group). Ninety percent of kisspeptin cells expressed ERα in the caudal arcuate nucleus in wethers (long and short term) and ewes. Rams, by comparison, expressed very few kisspeptin cells, and these did not express ERα. Less than 1% of RFRP-3 neurons co-expressed ERα in the merino sheep regardless of group. By contrast, RFRP-3 fibres were in great abundance in intact rams. This suggests that kisspeptin expression and its co-expression with ERα is influenced by testicular hormones. The lack of co-expression of RFRP-3 and ERα in the ram suggests that oestrogen negative feedback in these animals is unlikely to involve RFRP-3 neurons.

F2 fetal nephron number and weight benefits of endurance exercise training for females born small on high fat diet

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Uteroplacental insufficiency is the major cause of intrauterine growth restriction in Western society and is associated with cardiorenal disease which is exacerbated by “second hits” such as pregnancy and overweight/obesity. We reported that F2 fetuses have nephron deficits which contribute to the development of F2 high blood pressure. This study determined if F2 male nephron deficits of mothers born small are exacerbated by a maternal high fat diet (HFD) and whether endurance exercise training can prevent these deficits.

Uteroplacental insufficiency was induced by bilateral uterine artery ligation (Restricted) or sham (Control) surgery on E18 in Wistar-Kyoto rats. Female offspring were fed a chow or high fat (43% kcals from fat) diet from 5 weeks to mating (20 weeks) and throughout pregnancy. Female rats were exercised on a treadmill 4 weeks before mating and throughout pregnancy. Male fetal nephron number was quantified using unbiased stereology and fetal and placental weights were measured at E20.

Restricted and Control female rats that were exposed to a HFD were heavier with more dorsal fat than females on a chow diet. Exercise prevented dorsal fat gain in Restricted HFD compared to sedentary. F2 male nephron deficit was present in mothers born small regardless of diet (-18-45%). A HFD reduced F2 male nephron number in Control mothers (-32%). Exercise prevented the HFD induced nephron deficits in F2 males of both Control and Restricted mothers. Despite no treatment effect on placental weight, exercise prevented the reduced fetal weight in females born small.

We demonstrated that females born small are at a greater risk of increased adiposity. F2 male fetal nephron deficits in mothers exposed to a HFD were prevented by the lifestyle intervention of endurance exercise. This may prevent the development of F2 high blood pressure.

Pituitary Metastases

Veronica Wong, Zoran Apostoloski

Publish consent withheld

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Management of Diabetes in Lung Transplant Recipients

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Diabetes mellitus (DM) is common in lung transplant (Ltx) recipients and is associated with increased mortality. We conducted an observational study of all patients receiving Ltx between 1/8/2010-1/4/2013 inclusive to determine current management of DM and insulin requirements over time. DM status was determined by oral glucose tolerance test performed pre-, 3 months, then annually after Ltx. DM management was determined from medical records.
Of 174 patients in total, 37 (21%) had DM before and after LTx, and 40 (23%) developed DM post-transplant, which persisted throughout follow-up. A further 18 (10%) had transient DM, which subsequently resolved. Of those with diabetes both pre- and post-LTx, 19 (51%) used insulin pre-transplant. By 3 months, 33 (92%) required insulin and 24 of the surviving 28 (86%) remained on insulin at 2 years. In patients taking insulin pre-LTx, there was no significant change in mean insulin dose from pre- to 3 months post-LTx (34 (SD 21)–44 (19) units, p=0.12), even when adjusted for weight. There was also no difference in insulin dose between 3 months and 2 years, despite a significant fall in prednisolone dose over this time.

Most patients with new onset DM (32/40, 80%) were diagnosed by 3 months and 27/32 (84%) were on insulin at this time. Overall, 31/40 (78%) patients with new-onset diabetes required insulin. Two patients were managed solely with oral hypoglycaemic agents. Seven patients (18%) had dietary management.

Of the 18 patients with transient DM, 6 were treated with insulin. The remainder were diet controlled. Insulin was commenced by 3 months in all 6 patients at a mean dose of 15 units (0.22 units/kg) per day.

Insulin is the mainstay of DM management following LTx. There was no significant change in insulin dose before and after LTx despite changes in prednisolone dose and clinical status.

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**Time-specific basal cortisol cut-offs are a more reliable predictor of passing a Synacthen Stimulation Test than a single threshold level.**

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Background: Cortisol is a glucocorticoid hormone with well-recognised patterns of secretion, including an ultradian rhythm which underpins a diurnal circadian rhythm of higher morning cortisol (morning acrophase) with night time nadir. Morning cortisol collection is important for assessment of adrenal sufficiency and levels from 300-500 nmol/L have been demonstrated in various studies to predict passing the Synacthen stimulation test (SST) with variable specificity ranging from 62-100%. Aim: Given the significant diurnal decline in cortisol across the morning, the aim of our study was to determine whether time specific reference intervals (multiples of the median – MoMs) for cortisol would have utility in predicting SST outcome, reducing the number of unnecessary tests. Methods: We calculated individual MoMs for discrete time intervals across the morning between 7:00am and 12 midday and performed ROC curve analysis to determine 90% and 95% specificity cut-offs within each time interval. Results: A single 95% specificity threshold applied across the morning showed variable specificity for predicting SST outcome (range: 91-100%). Using a MoMs approach for each discrete time interval yields a more consistent specificity across the morning (range: 95-100% at 95% specificity). Individual MoMs for discrete time intervals optimised specificity without compromising sensitivity (range: MoMs 75-89% versus single cut-off 58-84% sensitivity). Conclusion: Compared to a single cut-off value for basal morning cortisol, time-specific MoMs gives a more reliable prediction of passing a SST.

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**TAMOXIFEN REDUCES HEPATIC VLDL PRODUCTION IN WOMEN: A POSSIBLE GH-MEDIANATED MECHANISM FOR THE DEVELOPMENT OF FATTY LIVER**

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Steatosis is a common complication of growth hormone (GH) deficiency. GH plays a vital role in lipid metabolism, stimulating hepatic fat oxidation and the synthesis of very-low-density lipoproteins (VLDL) for export of triglycerides (TGs). We previously reported that tamoxifen suppresses the secretion and hepatic action of GH. We hypothesize that the GH-deficient state induced by tamoxifen, lowers the secretion of VLDL.

**Objective:** To investigate whether tamoxifen inhibits hepatic VLDL secretion.

**Design:** Eight healthy, normolipidemic women (BMI 23.7±1.2 kg/m², age 64.4±2.2 years) were studied at baseline and after 2 weeks of tamoxifen (20 mg/d) treatment. We quantified apolipoprotein B (apoB), the structural protein of VLDL particles, by stable isotope 2H3-leucine turnover technique using steady state methodology. The enrichment of labelled leucine into VLDL apoB was measured using gas chromatography mass spectroscopy. VLDL-apoB fractional catabolic rate (FCR) was determined using a multicompartment model. VLDL-apoB secretion was estimated as the product of FCR and VLDL-apoB concentration. Circulating levels of IGF-I, FFA, and TG were measured at baseline and following tamoxifen treatment.

**Results:** At baseline, mean VLDL-apoB concentration was 94±19.8 mg/L. VLDL-apoB FCR and secretion were 3.7±0.6 pools/d and 4.6±1.1 mg/kg/d, respectively. Tamoxifen significantly (p<0.05) lowered VLDL-apoB concentration and secretion by 27.6±7.8% and 30.7±9.8%, respectively. Tamoxifen also significantly lowered circulating IGF-I concentration (14.8±5.3%; p<0.05). There were no significant changes in plasma TG and FFA levels following tamoxifen treatment.
Summary: Tamoxifen significantly lowered VLDL-apoB concentration as a consequence of a lower production rate. Tamoxifen significantly reduced IGF-I, a hepatic marker of GH action.

Conclusion: The suppression of GH-IGF-I axis by tamoxifen is associated with lower rates of VLDL-apoB secretion. Diminished hepatic VLDL secretion may contribute to the development of fatty liver during tamoxifen therapy.

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Hypophosphataemic osteomalacia associated with iron infusions: Report of three cases

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Although the incidence of serious adverse reactions remains low with administration of parenteral iron, hypophosphataemia is increasingly being recognised as an important complication, though it is often transient and asymptomatic. A postulated mechanism for hypophosphataemia is the reduced degradation of FGF-23, resulting in renal phosphate wasting and reduced synthesis of 1,25-hydroxy vitamin D.

We report two post-menopausal women who developed symptomatic hypophosphataemic osteomalacia with bone pain and multiple insufficiency fractures on a background of chronic gastrointestinal blood loss, necessitating monthly iron polymaltose infusions over 13-17 months, respectively. Respective blood tests revealed serum phosphate of 0.29 and 0.43 mmol/L [0.8 - 1.5mmol/L], 25-hydroxy vitamin D of 98 and 57 nmol/L, 1,25-dihydroxy vitamin D of 80 and 32 pmol/L [60 - 158], alkaline phosphatase of 302 and 125 U/L [30 - 130], with normal serum calcium and PTH. Urinary fractional phosphate excretion of the first patient was 24% [<5%] with TmP/GFR of 0.47 [0.87 - 1.4], consistent with renal phosphate wasting. Serum FGF-23 obtained from the second patient was 285 pg/mL [<54]. There was no biochemical evidence of Fanconi’s syndrome. Bone mineral density scans were in the osteoporotic range and whole body bone scans revealed increased uptake at multiple skeletal sites indicative of insufficiency fractures and in a pattern consistent with osteomalacia. Cessation of iron infusions resulted in clinical and biochemical improvement within 2-months.

The third case was a 25-year-old male with Crohn’s disease and iron deficiency anaemia who presented with severe hypophosphataemia (0.13 mmol/L) and generalised muscle weakness twelve days after a single dose of iron polymaltose. There was no arrhythmia on ECG. Serum calcium, PTH, 25-hydroxy and 1,25-dihydroxy vitamin D were normal with supplementation. Fractional phosphate excretion was marginally elevated (6.5%), reflecting depleted phosphate stores. Bone mineral density scan was in the osteoporotic range. Following oral phosphate supplementation, serum phosphate and metabolic bone parameters normalised within 2-months. Vigilant prescribing of parenteral iron is needed to avoid clinically serious hypophosphataemia.

Extremes of autoimmune thyroid dysfunction associated with interferon treatment in one patient

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Autoimmune thyroid disease associated with interferon therapy occurs in 2.7 to 10% of patients and at a median time of 17-weeks (range 4 weeks to 23 months) after beginning interferon therapy. Destructive thyroiditis, Graves’ Hyperthyroidism and autoimmune (often subclinical) hypothyroidism have been described, the latter occurring in 87% of cases and persisting in >80% of interferon-treated patients. Graves’ Hyperthyroidism is the more common form of thyrotoxicosis, occurring in 2/3 of cases whereas destructive thyroiditis occurs in 1/3. Thyroid replacement or anti-thyroid therapy are indicated in autoimmune hypo- and hyperthyroidism, respectively, with continuation of interferon. However, in destructive thyroiditis, cessation of interferon may be temporarily necessary. Little is known about the development of the extremes of autoimmune thyroid disease activated by the undesirable immunomodulatory effects of interferon treatment, especially within a single patient, as reported below.

A 60-year old man with no prior history of thyroid disease received 48-week pegylated interferon and ribavirin therapy for chronic HCV with achievement of sustained virological response. Six months into treatment, he reported fatigue, weight gain and slowed cognition. Examination was normal. Serum TSH was 58.8mIU/L [0.27 - 4.2], fT4 11.1 pmol/L [12 - 25], and fT3 4.2 pmol/L [2.5 - 6.0] with elevated anti-TPO (983 IU/mL, <35) and anti-TG (733 U/mL, <80) antibodies. He was commenced on thyroxine 100mcg daily with initial clinical and biochemical resolution but developed symptoms of hyperthyroidism with weight loss and tremor 14-months later. Serum TSH was <0.02 mIU/L, fT4 54.3 pmol/L, fT3 20.2 pmol/L, with an elevated TRAb of 4.0 U/L (<1.0), anti-TPO (1,163 IU/mL) and anti-TG (114 U/mL) antibodies. Technetium scan confirmed Graves’ Disease with bilateral diffuse increased tracer uptake (5.9%, 0.5 – 3.5%). The patient was commenced on carbimazole 15mg daily for 6-months. He self-ceased therapy with serendipitous clinical and biochemical remission (TSH 3.84 mIU/L, fT4 17 pmol/L, fT3 4.5 pmol/L, anti-TPO 383 IU/mL, anti-TG 23 U/mL, TRAb <1U/L).
This case should heighten the clinical awareness regarding the possible development of thyroid dysfunction closely in patients both during and following completion of interferon treatment. The frequency and period of follow-up are not clearly established and the presentation of thyroid dysfunction may be variable.

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Iodine status in women of childbearing age

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Background

Iodine deficiency has been recognised as a significant public health concern in Australia. Deficiency is of most concern in women planning pregnancy, given risks associated with poor neurological development in the baby. Following implementation of strategies to improve iodine intake at a population level, there has been minimal investigation into the current status of this problem.

Methods

Women of childbearing age attending outpatient clinics at Westmead Hospital, were asked to complete a questionnaire surveying dietary iodine intake as well as use of medications and recent IV radiological contrast exposure. A random single spot urine iodine was concurrently measured. The relationships between urine iodine level and dietary intake and use of iodine-containing multivitamins/medications were examined.

Results

51 women completed the study. The median age was 30.4 (SD 6.9) years. The most represented ethnicities in the cohort were Caucasian 19/51 (37.3%), Middle Eastern 13/51 (25.5%), South East Asian and Indian Subcontinental both 8/51 (15.7%) in each group. The most commonly consumed source of dietary iodine was iodised salt 17/51 (33.3%) used every day, followed by sliced bread 15/51 (29.4%) used every day. 10/51 (19.6%) used an iodine-containing multivitamin.

The median urine iodine level was 113µg/L (64,246) and 21/51 (41.2%) of women were iodine deficient (urine iodine <100µg/L). Half (5/10) of women taking iodine-containing multivitamins were iodine deficient. Excluding women on iodine-containing multivitamins and thyroxine, the median urine iodine level was 113µg/L (79,243). There was no statistically significant association between urine iodine and age or dietary iodine consumption (all spearman rank correlations <0.15 in absolute value). There was no significant association between urinary iodine levels and use of iodine-containing multivitamins or medications.

Conclusions

Despite public health strategies aimed at improving iodine intake, a significant proportion of women of childbearing age remain iodine deficient. Further research is needed to characterise this significant public health issue.


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Increased fat mass contributes to increased insulin resistance in men undergoing androgen deprivation therapy for prostate cancer.

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Background and aims: While androgen deprivation therapy (ADT) has been associated with insulin resistance and increased diabetes risk, there have been few controlled prospective studies. We hypothesized that ADT influences insulin resistance indirectly, via effects on body composition.

Methods: This prospective case-control study recruited 63 men with localised prostate cancer, 29 cases (newly commencing ADT) and 24 controls (not receiving ADT), matched for age and radiotherapy. Fat mass, lean mass and visceral adipose tissue (VAT) was measured by DEXA and insulin resistance was estimated from the updated Homeostasis Model Assessment (HOMA2-IR). Using a mixed model, the mean adjusted differences (MAD) between groups from 0 to 12 months are reported.

Results:

Compared with controls, fat mass increased in men receiving ADT by 3529.5g [2012, 5047], p<0.02 and lean mass decreased by 1491g [181, 2801], p<0.02. VAT was unchanged (p=0.66). HOMA2-IR increased in the ADT group compared with controls (mean adjusted difference 0.59 [0.24, 0.94], p<0.02). HbA1c levels and prevalence of diabetes was unchanged. Increase in HOMA2-IR was predicted by a change in testosterone (p<0.001) or change in fat mass (p<0.001) in separate models, which
were also strongly associated with each other (p>0.001). HOMA2-IR was not predicted by lean mass. In a combined model with testosterone and fat mass only, fat mass change (p<0.001) remained a significant predictor of HOMA2-IR, but not change in testosterone (p=0.63).

Conclusion:
These findings suggest that ADT may increase insulin resistance indirectly, via body composition changes, rather than via effects of testosterone withdrawal. This occurs in the absence of obvious changes in VAT, suggesting that there may be deleterious effects of subcutaneous fat. This reinforces the importance of implementing lifestyle measures to prevent obesity in men commencing ADT.

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**Ovarian Reserve of Women with Germline BRCA1 or BRCA2 Mutations.**

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**Background:** Anti-müllerian hormone (AMH) is a surrogate marker of fertility; higher levels are associated with greater ovulatory potential. This study examined AMH levels of BRCA1 and BRCA2 mutation carriers and their non-carrier blood relatives.

**Methods:** Eligible women were from families segregating BRCA1 or BRCA2 mutations, enrolled in the Kathleen Cuningham Foundation Consortium for Research into Familial Breast Cancer (kConFab). Each woman had been tested for the family mutation, had completed an epidemiological questionnaire and provided a blood sample at cohort entry. Women were aged 25-45 years, with no personal history of invasive cancer, had not undergone oophorectomy and were not pregnant or breastfeeding at the time of blood draw. AMH was tested on stored plasma samples using an electrochemiluminescence immunoassay platform. Associations between AMH level and carrier status were tested by linear regression, using the natural logarithm of AMH as the outcome variable, carrier status as the explanatory variable, and adjusting for age at blood draw, oral contraceptive use, BMI and cigarette smoking.

**Results:** AMH level was measured for 693 women, 172 carriers and 216 non-carriers from families carrying BRCA1 mutations, and 147 carriers and 158 non-carriers from families carrying BRCA2 mutations. Within both groups, mutation carriers were younger at blood draw than non-carriers (p ≤ 0.031). **BRCA1** mutation carriers had, on average, 25% lower AMH levels than non-carriers (p = 0.022). There was no evidence of an association for **BRCA2** mutation carriers (p = 0.94).

**Conclusions:** This study suggests that women with a germline mutation in **BRCA1** may have reduced ovarian reserve. This could have implications for their fertility, family planning and age at menopause.

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**Histological skeletal muscle changes in men with prostate cancer undergoing androgen deprivation therapy.**

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**Background:** Androgen deprivation therapy (ADT) is an effective treatment for prostate cancer but has many adverse effects consequent to severe hypogonadism. Muscle mass declines with ADT, however changes at a histological level have not been studied in humans. In testosterone replacement, an increase in cross-sectional area of all fibre types is seen; therefore we hypothesised that in men undergoing ADT the opposite would occur.

**Aim:**
To assess histological changes in skeletal muscle in men initiating ADT for prostate cancer.

**Methods:**
This prospective cohort study involved obtaining percutaneous thigh muscle biopsies (vastus lateralis) from 9 men with localised prostate cancer. The samples were taken immediately before and 1 month (mean 30.3±4.1 days) after commencing ADT and immediately processed. Direct histology was performed to measure fibre size (H&E stains), fibre type distribution (ATPase and NADH stains) and mitochondrial activity (COX/SDH stains). Slides were also reviewed for lipid and glycogen content.
Quality of life decrements in men with prostate cancer undergoing androgen deprivation therapy.

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Background
Androgen deprivation therapy (ADT), an effective treatment for prostate cancer has adverse effects consequent to severe hypogonadism. Effects on quality of life (QoL) are poorly characterised, due to limited evidence from controlled prospective studies. We hypothesised that men undergoing ADT will have decreased QoL in all domains.

Aim
To assess changes in QoL and to investigate contributing factors in men undergoing ADT.

Methods
Sixty-three men with prostate cancer were evaluated in a prospective, 12 month case-control study including 34 cases newly commencing ADT and 29 prostate cancer controls not receiving ADT, matched for age and radiotherapy. Participants performed the Short Form-12 (SF-12) (physical and mental components, and Aging Males' Symptoms Score (AMSS) (somatic, sexual and psychological components) QoL questionnaires at 0, 6 and 12 months. Using a mixed model, the mean adjusted differences (MAD) in QoL scores between groups from 0 to 12 months are reported.

Results
QoL as measured by SF-12 showed decrements in the physical component for the ADT group compared with controls (MAD 3.56 [0.45, 6.68] p=0.026) but there was no significant difference in the mental component (MAD 1.22 [-2.23, 4.67], p=0.49). QoL as measured by total AMSS was worse in the ADT group compared with controls (MAD -9.48 [-13.04, -5.91] p<0.001). Deficits were seen in the somatic (p<0.001), sexual (p<0.001) and psychological components (p=0.044). The decrease in QoL by AMSS was related to increase in hot flushes (p=0.002) but unrelated to haemoglobin levels (p=0.45).

Conclusions
Men receiving ADT have decrements in somatic and sexual aspects of QoL exceeding the impact of the cancer diagnosis and radiotherapy alone. Changes in psychological well-being are less consistent, perhaps due to insensitivity of questionnaires to detect small changes. The observed deficits should be useful in patient counselling and implementation of targeted strategies to mitigate adverse effects of ADT.

Comparison of the insulin tolerance test against the glucagon stimulation test and short Synacthen tests in patients with suspected hypopituitarism.

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Background: The insulin tolerance test (ITT), glucagon stimulation test (GST) and short Synacthen tests (SST) are employed in the evaluation of suspected cortisol and/or GH deficiency, with ITT considered the gold standard.1 We hypothesised that these dynamic tests may yield discordant results within individuals.

Methods: We performed a retrospective audit of adults who had undergone ITT plus either GST and/or 250mcg SST. Cortisol adequacy was locally defined as peak cortisol >550nmol/L at any time on ITT or GST, and at 30min on SST. GH adequacy was locally defined as peak GH >10μU/L at any time on ITT or GST. The primary outcome was discordance in cortisol and/or GH responses between the dynamic tests.

Results: Of 14 patients, 8 had ITT+GST and 7 had ITT+SST (including 1 patient who had all tests). Mean peak cortisols from ITT and GST in subjects who underwent both tests were 423 and 428nmol/L, respectively. In subjects who underwent ITT and SST, mean peak cortisols were 409 and 491nmol/L, respectively. Mean peak GH from ITT and GST in subjects with both
results were 4.3 and 16.6mU/L, respectively. In total, 9 of the 14 patients had discordant results using the defined decision points. Of the 5 patients with cortisol discordance, 3 were cortisol-adequate on ITT and inadequate on GST or SST, whilst 2 were adequate on GST and inadequate on ITT. The 5 patients with GH discordance were all GH-adequate on GST and inadequate on ITT.

Conclusions: Cortisol and/or GH discordance was found in 64% of patients. Glucagon and Synacthen appeared more potent stimuli of hormone secretion than hypoglycaemia, consistent with recent data. However, 3 subjects showed cortisol adequacy on ITT and not on GST or SST suggesting inter- or intra-individual variability. We recommend centre-specific and test-specific decision points be considered in dynamic tests of suspected hypopituitarism.

2. Simsek Y et al., Clin Endocrinol 2015; 82:45.

Fine needle aspiration of the thyroid: correlation with final histopathology in a series of 187 patients.
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Background: The risk of malignancy associated with thyroid nodules is ~5-15%. The Bethesda classification1 stratifies the risk based on fine needle aspiration (FNA) cytology and is used to guide management. However, false negatives remain a concern and is estimated between 1.3-11.5%. This study examined the accuracy of thyroid FNA by comparing the results with final histopathology, and evaluating the sensitivity, specificity and predictive values of FNA for the diagnosis of thyroid malignancy.

Methods: Medical records of 449 patients who underwent FNA for thyroid nodules whom 187 were operated and have final pathological diagnosis were retrospectively reviewed. FNAs were classified according to the Bethesda classification. We calculated the malignancy risk for each category by follow up histopathology in all 187 cases that underwent subsequent surgeries at our institution.

Results: Of the 550 FNAs performed, 187 cases proceeded to surgery (thyroidectomies or hemithyroidectomies). Malignancy rates at our institution were 21.05% for the non-diagnostic group; 10.0% for benign group, 44.44% for follicular lesion of undetermined significance (FLUS) group, 43.75% for the suspicious for follicular neoplasm group, 71.43% for the suspicious for malignancy group and 94.74% for the malignant group.

Sensitivity was 83.33%, specificity 71.29%, PPV 57.97%, NPV 90.0%, and diagnostic accuracy was 75.17%.

Conclusions: Thyroid FNA has high sensitivity and specificity, but false negative and false positive results cause concern. It is difficult to calculate the true frequency of false negatives because only a small percentage of patients with benign FNA undergo surgery. Our findings do not match the published data. Our malignancy rate is higher for the benign group (10%) compared to published literature of 0-3% with a benign FNA result. This suggest that when making treatment recommendations and counselling patients, we should use data from our own institution in addition to published values.


Transient Hypercalcaemia in Hospitalised Elderly Patients: an Association with Underlying Hyperparathyroidism and Vitamin D Supplementation
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Introduction
Hypercalcaemia is commonly seen in hospitalised patients, with a common aetiology being primary hyperparathyroidism. It has been observed that many elderly patients admitted with an acute illness have transient hypercalcaemia. It is unclear whether this group of patients has mild underlying hyperparathyroidism.

Objective
To determine 1) the incidence of primary hyperparathyroidism in patients with transient hypercalcaemia 2) the contribution of calcium and vitamin D supplements in the development of transient hypercalcaemia

Methods
A retrospective analysis of laboratory data and medical records of patients with hypercalcaemia (defined as corrected serum Ca of >2.60) and normocalcaemia, was performed. Vitamin D levels, renal function, parathyroid hormone (PTH) and medications were also analysed.

**Results**

A total of 982 medical inpatients had their serum calcium checked between June-Dec 2013. A total of 104 (10.6%) patients (F 65/M 39, mean age 79 years) had transient hypercalcaemia, with normalisation of calcium during or after admission. A small proportion, N=25/104 (24%) had PTH checked; 10 of those 25 (40%) had elevated PTH and 15 (60%) had an inappropriately normal PTH. None had a suppressed PTH.

101 normocalcaemic patients (F 51/M 50, mean age 75 years) were also analysed as a control group. The proportion of patients with acute kidney injury (AKI) was similar in both groups (P = 0.382).

Calcium supplement intake was similar between the two groups (P=0.233), however there was a significantly higher rate of vitamin D use in the transient hypercalcaemic group (P=0.020). Interestingly, thiazide use was higher in the normocalcaemic group (P = 0.008).

**Conclusion**

Transient hypercalcaemia is common in hospitalised elderly patients. Hyperparathyroidism was the likely cause in all patients who had PTH measured. It was found that vitamin D supplementation appeared to be associated with transient hypercalcaemia, however calcium supplementation and AKI did not.


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**Timely Commencement of Anti-resorptive Therapy Post Fragility Fractures: a Discrepancy Between Recommendations and Clinical Practice**

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**Introduction**

Current evidence suggests that early rather than late administration of bisphosphonates prevents refracture after fragility fractures. [1] It has been previously proven that there remains a significant treatment gap in the prescription and timing of anti-fracture therapy. [2]

**Objective**

To determine 1) whether patients with fragility fractures are receiving anti-resorptive therapy and the time frame in which this occurs 2) the recognition and treatment of vitamin D deficiency in these patients.

**Methods**

A retrospective analysis of medical records and laboratory data of patients with fractures was performed. Vitamin D levels, renal function and management of fractures were also analyzed.

**Results**

A total of 205 patients (F 154/M 51, mean age 80 years) presented to Box Hill Hospital with fractures from June-Dec 2013. The most common fracture was femur (N=112, 60%), followed by humerus (N=44, 21%) and Colles (N=36, 18%). Out of 180 patients with osteoporosis, only 32 (17%) had bisphosphonates started, at a mean time of 26 days. Forty-seven (27%) patients were commenced on vitamin D, whilst 7 (4%) patients were started on calcium.

Seventy (41%) out of 107 patients had vitamin D deficiency, however less than half (N=33, 43%) were treated.

Initiation of anti-resorptive therapy was predicted in patients with a history of osteoporosis (P = 0.002), Caucasian ethnicity (P = 0.049) and femoral fractures (P=0.029). Others including age (P = 0.323), gender (P = 0.408) and osteoporotic risk factors (P = 0.108) did not influence the decision to start therapy.

**Conclusion**

Fragility fractures and vitamin D deficiency do not appear to be treated with adequate pharmacological therapy. Measures need to be undertaken to improve awareness amongst medical practitioners.

"Parachutes to Prevention" – A conceptual change in acute adrenal insufficiency education

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Prevention of adrenal crisis has been the focus of care for individuals with primary and secondary adrenal insufficiency. The key to prevention is through patient and health professional education. Recognition of impending adrenal crisis is often missed as patients may appear clinically stable initially and health professionals are not aware that they can deteriorate rapidly. We developed a "parachute" concept called "Parachutes to Prevention" as a tool to better illustrate in pictorial form the elements considered critical in the prevention and treatment of acute adrenal insufficiency. This was presented at the Sydney Chapter of the Australian Addison's Disease Association (AADA) annual meeting recently and a survey of the efficacy of the tool pre- and post-presentation was conducted.

Twenty-five participants completed a questionnaire. Twenty one (84%) were female with a mean age of 48.3yrs and average duration of adrenal insufficiency (since diagnosis) of 5.6yrs. All participants spoke English at home. This was the first Addison's Awareness meeting for 40% of the respondents.

Participants were asked several questions around their management of sick days. They were then given a 20-minute presentation using the ‘Parachutes to Prevention’ tool. Following this, a repeat questionnaire demonstrated a significant increase in the number of safety measures that individuals could nominate for themselves, with a median increase of 5 additional preventative measures. Furthermore, they were able to individualise their own set of parachutes.

This tool was also used recently at Emergency Department nurses’ education sessions and resulted in strongly positive feedback from paramedical staff who indicated that these simple, yet clear, images were imprinted in their memory.

Given the success of the initial education sessions with this tool, we are now working with the Sydney AADA group to further develop the “Parachutes to Prevention” concept. This includes its application within the high risk non-english speaking group.

A case of primary amenorrhoea and hyperandrogenism

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We report the case of a 42 year old female with Müllerian agenesis and hyperandrogenism, with a possible unifying diagnosis of a WNT4 gene mutation. The patient presented with primary amenorrhoea aged 15. She had characteristic features of Müllerian agenesis: normal secondary sex characteristics, female external genitalia, a vaginalintroitus but no true vagina, absent uterus on imaging and at laparoscopy, and a single right kidney. Karyotype was 46XX. There was no definite ovary located initially, though imaging later revealed a 22mm soft tissue mass in the region of the vaginal vault which remained stable in size over subsequent decades. This was presumed to be ovarian tissue as the patient had pre-menopausal range oestriol and biochemical evidence of ovulation. She received no further medical care until age 28, when she was noted to have hirsutism and acne. There was mild biochemical hyperandrogenism but 17-hydroxy-progesterone level was normal. She was overweight and insulin resistant, so was diagnosed with probable polycystic ovarian syndrome despite not fulfilling Rotterdam criteria (1). However, we suggest a WNT4 mutation as an alternative diagnosis that could explain the coexistence of Müllerian agenesis and hyperandrogenism in our patient. WNT4 expression is essential for Müllerian duct formation (2). WNT4 knockout female mice have absent Müllerian ducts but Wolffian ducts are present. Their gonads express 3β-hydroxysteroid dehydrogenase and 17α-hydroxylase, which are required for the production of testosterone and are normally suppressed in the ovary (2). Both male and female WNT4 knockout mice have defects in renal development and adrenal function (2). There are four reported cases of human females with Müllerian agenesis and hyperandrogenism due to heterozygous mutations in the WNT4 gene (3-6). However, WNT4 mutations are not the cause for most cases of Müllerian agenesis without hyperandrogenism (5, 7, 8). We are pursuing WNT4 genetic testing in this patient.

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An Odd Hot Spot
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We report the case of a 47 year old man with papillary thyroid cancer (PTC) presenting with a toxic thyroid nodule. The patient had lethargy, dysphonia and biochemical hyperthyroidism. Thyroid ultrasound showed a 43mm nodule in the right lobe, with coarse internal calcification and vascularity. The nodule was hot on technetium uptake scan. Fine needle aspiration (FNA) was recommended given the nodule’s size and presence of calcification. FNA cytology was consistent with PTC. He underwent total thyroidectomy and central neck dissection. Histopathology confirmed a moderately differentiated 50 x 40 x 30mm PTC replacing the right lobe with metastatic disease in 2 of 6 central compartment lymph nodes.

The 2009 American Thyroid Association (ATA) Guidelines do not recommend cytological evaluation for hyperfunctioning nodules, as they are believed to rarely harbour malignancy (1). However, Mirfakhraee et al. reviewed the prevalence of thyroid cancer within solitary hot nodules as reported by 14 surgical case series and found rates of intranodular carcinoma ranged from 0 to 12.5%, with a weighted total mean of 3.1% (2). In children, the risk of differentiated thyroid cancer in hot nodules may be as high as 29% (3).

However, no studies have specifically examined the validity of high-risk features (historical and ultrasound) or accuracy of cytology in the diagnosis of toxic thyroid cancers. Hot nodules were specifically excluded from some studies of sonographic predictors of malignancy (4) which formed the basis for the ATA’s recommendations (1). Moreover, increased intranodular vascularity occurs in 73% of all hyper-functioning nodules (5), so should not be considered a risk factor for malignancy in hot nodules. Thus, while the presence of differentiated thyroid cancer in toxic nodules may not be as rare as previously thought, detection remains challenging.


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Calcium stimulation test to localize insulinomas- Local centre experience
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Introduction: Non-invasive imaging modalities are often unable to localize insulinomas. Localization through calcium stimulation test is often dependent on expertise of the operator.

Aim: To assess the accuracy of calcium stimulation in diagnosis of cause of hypoglycemia at a tertiary referral centre.

Method: This is a retrospective analysis of a single centre experience in Newcastle, Australia from 2001 to 2015.

Results: 14 consecutive patients, 8 females and 6 males with mean age 33.5 years (range 25-42) were investigated for insulinoma over the past 14 years at John Hunter Hospital, Newcastle. Calcium stimulation test was performed on all patients by injecting calcium gluconate 0.025 mEq/kg directly into the arteries supplying the pancreas and liver. Samples were collected from the hepatic vein at ~120,0, 30, 60,90, 120, 180 seconds. The results of the study were compared with the intraoperative and histological findings in 9 patients. The findings were also compared with other imaging modalities.

Preliminary analysis showed that 2/14 had MEN 1 syndrome. 9/14 patients had insulinoma. 1/14 factitious disorder, 1/15 congenital hyperinsulinism. 2/14 had post gastrectomy hyperinsulinemia. Calcium stimulation test identified insulinoma correctly in all 9 cases. It was truly negative in 3 cases (factious, congenital hyperinsulinism, post gastrectomy hyperinsulinemia). It was falsely positive in 1 case of post gastrectomy hyperinsulinemia.

Of these 9 cases of insulinoma only 3 were identified on CT scan and 1 on MRI. Indium octreotide was done in 3 cases and was falsely negative in all 3. Gallium dotate was done in 3 cases and was true positive in 1 case and truly negative in 2 cases.
Conclusions: Calcium stimulation test remains the investigation of choice for localizing insulinoma. Expertise at our centre was comparable to other centres in the world. Of all the other non-invasive imaging modalities, gallium dotatate scan was the best performing.


IGF-1/IGFBP-1 axis are closely associated with insulin secretion in Korean children.

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Background : The IGF system is involved in the development of metabolic and cardiovascular disease. This study aimed to investigate the association of insulin-like growth factor-1 (IGF-1), IGF-binding protein-1 (IGFBP-1) and IGFBP-3 with insulin resistance and type 2 diabetes in children.

Methods : We included 36 children aged 10 to 16 years without known diabetes, medication, chronic disease. They were classified into 3 groups according to the results of oral glucose tolerance test and other clinical/laboratory findings. We performed anthropometric measurement and laboratory tests. The fasting levels of serum IGF-1, IGFBP-1 and IGFBP-3 were measured.

Results : 1) Serum IGF-1, IGFBP-3 and IGF-1/IGFBP-1 molar ratio levels were significantly higher in glucose intolerance group. Serum IGF-1(r=0.396, P=0.023) and IGFBP-3(r=0.628, P=0.001) had negative correlation with IGFBP-1. 2) Serum IGFBP-1 was negatively correlated with age, body mass index (BMI), systolic blood pressure, serum c-peptide, insulin, and HOMA-IR. And serum IGF-1/IGFBP-1 was significantly related with serum c-peptide, insulin and HOMA-IR. 3) Serum IGFBP-1 had no correlation with fasting plasma glucose level, lipid profile, apoprotein A/B and HbA1c. It was not different between normal glucose tolerance group and glucose intolerance group. 4) In normal glucose tolerance group, serum IGFBP-1 and IGF-1/IGFBP-3 was no significantly different between obese and non-obese groups. But IGFBP-1 had negatively associated with age, BMI, systolic blood pressure, serum c-peptide, IGFBP-3 and HOMA-IR.

CONCLUSION: Serum IGF-1/IGFBP-1 molar ratio was significantly elevated in Korean children with glucose intolerance and especially, serum IGFBP-1 correlated with serum c-peptide. These findings suggest that IGF-1 may related glycemic control and insulin secretion in children.

Utility of FDG-PET CT scanning in succinate dehydrogenase B mutation related lesions

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Context: Mutations of the gene encoding Succinate Dehydrogenase B (SDHB) are associated with a highly penetrant phenotype that includes paragangliomas, phaeochromocytomas and renal cell carcinoma.1 Patients with mutations of SDHB require lifelong surveillance, however there is currently no consensus regarding optimal screening regimens.2,3 Due to abnormal glycolytic processing and delay in 18F-fluorodeoxyglucose (18F-FDG) clearance, 18F-fluorodeoxyglucose positron emission tomography with computed tomography (18F-FDG-PET/CT) imaging has theoretical advantages for imaging benign and malignant SDHB mutation-related neoplasms.4

Objective: Determine sensitivity and specificity of 18F-FDG-PET/CT compared to other modalities for SDHB mutation related lesions.

Design: A retrospective audit reviewed adult patients with confirmed SDHB mutation who underwent 18F-FDG-PET/CT at our institution between 1/7/2011 and 30/5/2015. Lesions numbers and locations detected by 18F-FDG-PET/CT were compared to those on CT and any other imaging modalities or histology available.

Results: 26 18F-FDG-PET/CTs were completed on 20 patients during an average follow up was 53 months (range 2-156). 18F-FDG-PET/CT compared to CT showed no additional lesions in 3 of 4 positive studies (75%) with a false positive uptake in the surgical bed of a carotid body tumour in 1 study, and 0 missed lesions in 4 of 4 positive 18F-FDG-PET/CTs. PET more accurately detected bony disease for metastatic paraganglioma than MIBG, but was similar to GaTate, MRI and CT. 22 18F-FDG-PET/CTs (85%) showed no abnormality; of 21 scans with other imaging for comparison, there were 0 missed lesions. 8 of 22 (36%) negative 18F-FDG-PET/CTs correlated with contemporary (within 6 months before) or later CT results, and 4/22 (18%) with other imaging. 9 of 22 (41%) negative 18F-FDG-PET/CTs correlated with other imaging done >6 months prior.
Primary hyperaldosteronism (PHA) accounts for 5–10% of patients with hypertension (1). Saline suppression test (SST) is a commonly used confirmatory test in the diagnosis of PHA. Although potassium (K) is checked at baseline with SST, which is known to cause hypokalaemia. A previous study monitored K levels post-SST (2). We report a retrospective series of patients who became hypokalaemic post-SST in the 2 hour period post-SST.

Methods:
A retrospective audit was conducted of patients with confirmed PHA who underwent SST between 2005 and 2015. Pre- and 2 hour post-test potassium, aldosterone and renin levels were measured. Results are expressed as mean ± standard error of the mean (SEM) and number (%).

Results:
Twenty five patients were included in the final analysis; 13 (52%) were males, and mean age 53 ± 10.5 years. Overall, there was no difference in the mean pre- and post-SST potassium levels (p=0.08). However, there was an inverse correlation between pre-SST K and the change in post-test K levels (p=0.01); with the highest pre-test K patients experiencing the greatest decline in post-K levels. Eight (32%) were hypokalaemic (K<3.5mmol/L) pre-SST and required intravenous or oral K supplements.

For patients that were normokalaemic pre-SST, there was a significant decrease in serum potassium levels post-SST (3.7±0.05 vs. 3.5±0.08, p=0.01). Seven subjects (41%) who were normokalaemic pre-test became hypokalaemic post-SST; and 5 (29%) remained hypokalaemic on day 2.

Conclusion:
Hypokalaemia is common post-saline suppression test in primary hyperaldosteronism. The pathophysiology remains unclear. We recommend that potassium levels be routinely measured post-test and on day 2 to detect persistent hypokalaemia.


Conclusions: In patients with SDHB mutation, 18F-FDG-PET/CT was at least as sensitive and specific as other imaging modalities, both for metastatic and non-metastatic disease, and may detect bony metastatic disease better than MIBG.

He appeared overtly hypercortisolemic, with moon facies, buffalo hump, supraclavicular fat pads, marked purplish-red striae (>1cm width) and had proximal myopathy (Fig. 1a & 1b). He denied any exogenous steroid use but history was significant for alcohol dependence, averaging 28 standard drinks (SD) daily.

Screening tests for CS revealed: elevated midnight salivary cortisol 32.9nmol/L (normal <10nmol/L), failure to suppress cortisol levels following an overnight low-dose 1mg dexamethasone suppression test (DST) (cortisol 210nmol/L). However, a 24-hour urinary free cortisol was normal. The remainder of his hormone profile appeared to show deficiencies of gonadotrophins (LH 0.7 IU/L, FSH 0.4 IU/L, testosterone 1.7nmol/L) and the somatotroph axis (IGF-1 7nmol/L (15-40), GH 0.7ug/L). Thyroid hormone axis was intact.

Following near-recovery ten days later, repeat low-dose followed by high-dose DST now showed appropriate cortisol suppression. His gonadotroph and somatotroph axes also normalized. Post-hospital discharge, his alcohol intake has reduced significantly (3 SD/ week); with substantial loss of his previous phenotypic Cushingoid features (Fig. 2).

We report an uncommon cause of PCS secondary to longstanding alcoholism and critical illness. Rapid restoration of normal pituitary axis function was seen with resolution of illness and alcohol abstinence. We highlight some of the difficulties in the diagnosis of CS during critical illness.

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Treatment Resistant Papillary Thyroid Cancer

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Synopsis

Investigations for TSH elevation revealed a multinodular goitre. Progress ultrasound suggested malignancy and a follicular neoplasm was confirmed on biopsy. A total thyroidectomy was followed by radioactive iodine (RAI) ablation. Histopathology revealed papillary thyroid cancer (insular variant) with extensive capsular and vascular invasion.

Uptake in the T1 and L2 vertebrae was noted post therapy. Further RAI was administered but thyroglobulin remained elevated (1380 mcg/L). Resection of metastatic T1 and L2 lesions were undertaken. A 3rd dose of RAI was administered and thyroglobulin levels measured 1089 mcg/L pre-treatment. Thyroglobulin levels continued to rise (2252mcg/L prior to 4th RAI treatment), with new lung and recurrent bone metastases developing. Selected lesions were 18 FDG-PET positive. Due to a paucity treatment options, a 5th RAI dose was administered (total dose of 21.6GBq). External beam radiotherapy was delivered to the T1 lesion.

Discussion

RAI resistance occurs when there is (1) no uptake on post therapy scan, (2) progression of disease and (3) rising thyroglobulin1. Although the metastases appeared iodine avid in this case, anatomical and biochemical response was lacking. These lesions corresponded to FDG-PET, reflecting a mixture of well and less well differentiated cell types.

The optimum I131 dose to treat metastatic disease remains unclear. Higher doses are used when increased risk is perceived based on clinical and histopathological features2. This dose can range between 3.7-7.4Gbq3 but data addressing the optimal therapeutic and safe accumulative dose is lacking. Secondary malignancies have been associated with RAI therapy and are dose dependent4. A meta-analysis has shown a 1.19 relative risk of secondary malignancies in patients receiving treatment for TC5.

Tyrosine kinase inhibitors are currently being investigated and used as treatment for iodine refractory TC. However, access to these drugs in Australia is limited and if available, occurs in the setting of clinical trials.

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Subclinical hypothyroidism in pregnancy related to TSH receptor blocking antibodies: An unique clinical conundrum

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TSH receptor auto-antibodies (TrAb) belong to a heterogeneous group of auto-antibodies that may stimulate or inhibit TSH receptors. Most commonly, they exhibit an overall stimulatory effect and are associated with Grave's disease. Rarely, they may exert a greater inhibitory effect, giving rise to hypothyroidism (1,2).

TSH binding inhibition immunoglobulin (TBII) assays are competitive immunoassays, which measure TrAb concentration. They do not inform about the biological effects of TrAb. Overall biological effects of TrAb are determined by their ability to stimulate cyclic AMP generation in thyroid stimulating immunoglobulin (TSI) bioassays. The behavior and proportion of these auto-antibodies may fluctuate with time and in response to treatment, changing the patient’s thyroid status.

Here, we describe a middle-aged Chinese lady with subfertility related to subclinical hypothyroidism due to blocking TrAb. She was treated with levothyroxine for 1 year before achieving TSH normalization and successful conception via in-vitro fertilization (Table 1).

Serial thyroid function monitoring during pregnancy revealed primary hyperthyroidism. Levothyroxine was stopped at 18 weeks of gestation with normalization of thyroid function (Table 2). At this time, the TrAb showed predominantly stimulating effects on TSI bioassay, which concurred with the switch in thyroid function.

The patient delivered a healthy and euthyroid child via normal vaginal delivery at 39 weeks of gestation. Six months post-partum, her thyroid function revealed symptomatic primary hyperthyroidism. She was started on thiamazole 10mg OM for Graves’ thyrotoxicosis (Table 3).

Our patient mirrors previously described cases of hyperthyroidism resulting from a switch of TrAb from blocking to stimulating nature amongst middle-aged Japanese females (3–6). The proposed mechanisms include polarization of dendritic cells after levothyroxine treatment with impairment of regulatory T cells and emergence of stimulating autoantibodies. Additionally, there may be a switch in T cell populations due to possible preferential clearance of blocking over stimulating antibodies in pregnancy (6–8).

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Accuracy of Direct Progesterone Immunoassay vs Liquid Chromatography Mass Spectrometry

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Background: Progesterone (P4) secreted by the corpus luteum is essential for implantation and early pregnancy. Serum P4 measurement on day of hCG administration during IVF controlled ovarian stimulation has been proposed to identify premature ovulation and/or luteinisation with an adverse impact on pregnancy in that IVF cycle.

Objective: To evaluate the accuracy of serum P4 measured by direct (unextracted) immunoassay (IA) vs a liquid chromatography mass spectrometry (LC-MS) reference method.

Method: Serum samples were collected from 254 women (median age 38, range 20-49 yr) on hCG day during an IVF cycle. Serum P4 was measured by IA (Beckman Coulter Access) and by LC-MS with results compared by Bland-Altman [BA], Passing-Bablok [PB] and Deming [D] regression methods. For analysis, left-censored (undetectable) results in LC-MS were assigned a value half of the detection limit (0.05 ng/ml).

Results: IA over-estimated serum P4 in every sample (median 4.8 vs 1.5 nM; median difference 4.4 nM [interquartile range 3.5, 5.9 nM]). Serum P4 was detected in 252 (99%) by IA and in 215 (85%) of samples by LC-MS. By PB regression, the intercept was 3.2 nM (95%CI 3.1, 3.3 nM) with a slope of 1.0 (95%CI 0.9, 1.1). By D, the intercept was 3.6 nM (95%CI 3.5, 3.8 nM). The upward bias of IA increased exponentially at low serum P4 concentrations (IA <5 nM or LC-MS<2 nM). Age was unrelated to either assay result or their difference.

Conclusion: IA consistently overestimates serum P4 levels so that low measurements (IA<5 nM) are too inaccurate to be used quantitatively. The utility of higher serum P4 measurements by IA and serum P4 and other steroids measured by multiplex LC-MS profiling in predicting IVF pregnancy outcomes warrants further investigation.
The Prevalence of BRAF V600 Mutations and its Associated Histopathology Features in Papillary Thyroid Carcinoma in New Caledonia and Australia

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New Caledonia (NC), a French territory in the Pacific, has the highest worldwide incidence of thyroid cancer1. We have previously shown a high prevalence of BRAFV600E mutation in this population in association with increased numbers of multifocal bilateral PTC. In this study, we aim to extend this study and to in a subset of BRAFV600E negative patients examine the incidence of other known BRAF mutations. Associations of these mutations with histopathological features were also examined.

The BRAF V600E mutation status was determined in 121 micro-dissected Formalin Fixed Paraffin Embedded (FFPE) PTC tumour tissue obtained from Laboratoire d’Anatomie et Cytopathologie, Nouméa, NC (n=49) and from RPA Hospital, Australia (n=72). BRAF V600E negative NC samples (n=15) were also examined for presence of BRAF V600Ec, V600R, V600D and V600K mutations. Pathological data were obtained from histopathology reports and patients’ medical records. Data was analysed by Chi squared analysis.

In both populations, PTC was more common in females, similar to the pattern worldwide. BRAF V600E prevalence was 64% in NC and 55% in the Australian cohort and this mutation was significantly more common in NC multifocal bilateral tumours (NC: 92% vs Australian: 67%; P<0.005). The further screening for BRAF mutations in BRAFV600E negative samples from NC found that 13% presented BRAF V600Ec and 13% presented BRAF V600R (with no overlap between the two mutations). These incidences are higher than expected (4.3% and 4.9% respectively) for a given population. BRAF V600D and V600K mutations were not detected. The BRAFV600Ec mutation was only found in bilateral PTC and BRAF V600R only in unilateral PTC.

The higher prevalence of the BRAFV600E and BRAFV600Ec mutation in the NC cohort with multifocal bilateral PTC may indicate more aggressive tumours in these individuals. Whether the NC population has increased incidence of other BRAF requires further investigation.
Effect of denosumab on glucose control in subjects with diabetes or pre-diabetes from the FREEDOM study
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High serum RANKL concentration was a predictor of incident type 2 diabetes (T2DM) in a population-based study, and blockage of RANKL signalling improved glucose intolerance by enhancing hepatic insulin sensitivity in mouse T2DM models (Kiechl et al. Nature Med 2013;19(3):358–366). Denosumab is a fully human monoclonal antibody that binds with high affinity and specificity to RANKL and prevents the formation, function, and survival of osteoclasts, and is associated with vertebral and nonvertebral fracture risk reduction. In a prior posthoc analysis of the FREEDOM trial, denosumab had no effect on incident diabetes or fasting serum glucose (FSG) in women without diabetes at baseline. Based on the favourable effect of RANKL blockage on glucose tolerance in mouse T2DM models, we hypothesised that denosumab decreases FSG in FREEDOM subjects with diabetes or prediabetes.

Baseline diabetes status was by self-report, use of antidiabetic medication (ADM), or an FSG≥126mg/dL and prediabetes by FSG 100–125mg/dL on no ADM. Average postbaseline FSG across visits was estimated using a repeated measures model including treatment group, baseline FSG, BMI, and age; visit; ADM use; treatment-by-visit interaction; and ADM use-by-visit interaction as fixed effects.

Baseline characteristics were similar between denosumab and placebo in both diabetes and prediabetes subpopulations. Estimated average postbaseline FSG across visits was not significantly different between denosumab and placebo in women with either diabetes or prediabetes (p=0.20 and p=0.42, respectively); however, when censoring FSG values after ADM use in women with diabetes, estimated average postbaseline FSG across visits was lower with denosumab than placebo (p=0.02).

In this posthoc analysis, denosumab did not appear to affect FSG in subjects with diabetes or prediabetes. There was evidence of FSG lowering with denosumab in diabetic women not currently using any ADM. It remains to be determined whether blockage of RANKL has a clinically important effect on glucose metabolism.

Predictors For Surgically Resected Non-Functioning Pituitary Adenoma Requiring Secondary Intervention
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Background: Surgery is the primary mode of therapy for non-functioning pituitary adenomas (NFPA). The post-operative management of NFPA is a challenge because of a lack of knowledge regarding factors influencing remnant tumour growth that is clinically significant.

Aims: To identify radiological factors that predict the need for secondary intervention after surgical resection of NFPA.

Methods: This is a single-centre retrospective study of surgically resected NFPA in patients with pre- and serial postoperative MR imaging followed for at least a year. Tumour characterisation were performed by a single operator from pre-operative (tumour volume and extrasellar extension) and serial post-operative images (remnant volume, remnant site and growth rate). Secondary intervention was the outcome measure. The CVs for pre- and post-operative tumour volume from 8 subjects measured twice were 4% and 7% respectively.

Results: 85 patients (49 men, mean age at surgery: 53±16 years) with a median follow up of 5.1 years (range: 1.2-20.0) were studied. The pre-operative median volume was 3447mm³ (526-99850). Post-operatively, 67% had remnant tumours, 60% of which were extrasellar with a median remnant volume of 319mm³ (33-5475) and remnant growth rate of 51.8mm³/year (0-1963.2). 25% of patients required secondary intervention (second surgery: 8 and irradiation: 13). Kaplan-Meier analysis showed that the rate of secondary intervention when required was 65% at 5 years and 100% by 10 years. Cox regression analysis identified presence of post-operative remnant (HR: 5.1, CI: 1.6-11.2, p<0.01), remnant growth rate (HR: 3.3, CI: 2.1-7.0, p<0.01) and pre-operative suprasellar invasion (HR: 1.2, CI: 1.1-1.9, p=0.02) as independent predictors of secondary intervention.

Summary: In surgically treated NFPA, secondary intervention occurred in 25%, all within the first decade. This was determined by pre and post-operative tumour characteristics.

Conclusion: In surgically resected NFPA, secondary intervention is unlikely to be required beyond 10 years (i) the presence of tumour remnant is the primary prognostic indicator (ii) intensity of follow up should be tailored to imaging characteristics
Characteristics, Diagnoses and Clinical and Genetic Outcomes of Patient Population Attending a Multidisciplinary Familial Endocrine Neoplasia Clinic

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BACKGROUND
Heritable endocrine neoplasias include parathyroid and pituitary adenomas, phaeochromocytoma, paraganglioma and medullary thyroid cancer. Causative genes include RET, MEN1, NF1, VHL, SDHA, SDHB, SDHC, SDHD, SDHAF2, TMEM127 and MAX. As there are specific management guidelines for gene carriers1-3,4, appropriate screening of individuals is necessary. The Multidisciplinary Familial Endocrine Genetics Clinic was created to screen and manage affected patients.

METHODS
A retrospective audit of medical records was undertaken of all patients who had been referred to the Royal North Shore Hospital Multidisciplinary Familial Endocrine Neoplasia Clinic between April 2013 and May 2015. Patient characteristics and clinical and genetic diagnoses were assessed.

RESULTS
Sixty-eight new patients were referred, 21 (31%) male and 47 (69%) female. Age ranged between 12 to 83 years. The geographic referral area was predominantly across New South Wales, but also from the ACT and Queensland. Referral reasons included pre-existing paraganglioma (8, 11.7%), and pheochromocytoma (7, 10.2%), affected family members (17, 25%), neuroendocrine tumours (4, 5.8%), medullary thyroid cancer (3, 4.4%), and adrenocortical cancer (3, 4.4%). Eleven asymptomatic individuals with an affected family member were diagnosed with a genetic mutation, (4 in SDHA, 6 in SDHB, one in SDHC). Genetic mutations in patients with paraganglioma and pheochromocytoma include SDHA (n=3), SDHC (n=1), SDHD (n=2), NF1 (n=1), and pending (n=7) results. Genetic screening of four individuals with neuroendocrine tumours found one MEN1 gene deletion.

DISCUSSION
The spectrum of genetic mutations found in our audit are comparable to other studies: for instance, with SDH mutations accounting for 11%, and NF1 2% of the susceptibility genes in paraganglioma and pheochromocytoma5. This clinic has facilitated identifying gene mutation carriers, who are being screened for phenotypic features, and this may reduce morbidity and mortality that would otherwise accompany delayed diagnosis.


Intermittent moderate energy restriction improves weight loss efficiency in diet-induced obese mice

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Intermittent severe energy restriction is an increasingly popular method of weight management. To investigate whether intermittent moderate energy restriction may improve this approach by enhancing weight loss efficiency, we conducted a study in mice, where energy intake can be unambiguously defined. Male C57/B16 mice that had been rendered obese by ad libitum access to a diet high in fat and sugar for 22 weeks were then fed one of two energy-restricted normal chow diets for a 12-week weight loss phase. The continuous diet (CD) provided 82% of the energy intake of age-matched ad libitum chow-fed controls. The intermittent diet (ID) provided cycles of 82% of control intake for 5-6 consecutive days, and ad libitum intake for 1-3 days. Subsets of mice then underwent a 3-week weight regain phase involving ad libitum re-feeding.

Mice on the ID showed transient hyperphagia relative to controls during each 1-3-day ad libitum feeding period, and overall ate significantly more than CD mice (91.1 ± 1.0 versus 82.2 ± 0.5% of control intake respectively, n = 10, P < 0.05). There were no
Radioactive iodine ablation of differentiated Thyroid cancer as per 2009 ATA guidelines and future directions: single centre experience – retrospective review.

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Publish consent withheld


Acromegaly: Outcomes from a single pituitary surgeon service in Christchurch New Zealand

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Background: Acromegaly is characterised by excess growth hormone secretion and is associated with increased morbidity and mortality. Current guidelines define cure or control as normal IGF-1 and random growth hormone concentrations <1mcrog/L (1).

Objective: To audit the immediate and long-term outcomes of patients treated surgically for acromegaly at Christchurch Hospital, New Zealand, a small tertiary referral centre with a single pituitary neurosurgeon.

Methods: We undertook a retrospective case review of all cases of acromegaly treated via endoscopic transnasal transphenoidal surgery between May 2000 and August 2013. Biochemical and clinical data concerning pre-operative findings, post-surgical outcome and long-term follow-up was collected.

Results: 40 patients (15 male, 25 female) were identified. 12 tumours were microadenomas, and 28 macroadenomas. All patients had at least one measurement of random GH and IGF-1 within 6 months of surgery (mean 44 days, range 2-105). 50% (6/12) of microadenomas met cure criteria compared with 35% of macroadenomas (10/28). Three patients with invasive tumours underwent stereotactic radiotherapy and 8 patients commenced medical therapy within 6 months of surgery. Average follow-up was 70.1 months for 36/40 patients. 41% of patients were on medical therapy (octreotide, cabergoline or in combination), 50% of macroadenomas, 30% of microadenomas. 64% of patients had both IGF-1 and GH within target range; 54% of macroadenomas and 83% of microadenomas. 3 macroadenomas were controlled with cabergoline alone. 33/36 tumours had normal IGF-1. Mean random GH concentrations for macroadenomas was 0.90ug/L, for invasive tumours 1.66ug/L, and 0.58ug/L for microadenomas.
Conclusions: Surgical cure rates for microadenoma are lower than reported elsewhere in the literature but may not reflect true growth hormone status as many patients were assessed less than 3 months following surgery. Consistent measurement of growth parameters at least 3-6 months after surgery is recommended. Most patients achieved good biochemical control at long term follow-up although many require ongoing medical therapy. Cabergoline is an effective therapy even in patients with macroadenoma.


Graves' Dermopathy: a report of three cases

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Dermopathy is a recognized but rare extrathyroidal manifestation of Graves' disease (GD), affecting 1.5% of patients. The pathogenesis of this manifestation remains poorly understood but is most likely triggered by autoimmunity to the thyroid stimulating hormone (TSH) receptor and possibly the insulin like growth factor (IGF-1) receptor. We present two cases of dermopathy related to GD to highlight the challenges associated with diagnosis and management of this condition.

The first case involves a 38 year-old man, diagnosed with GD in 1997. He was treated with carbimazole, followed by radioactive iodine. He then developed significant Graves' orbitopathy (GO) requiring decompressive surgery. Following this he developed left great toe swelling with severe skin thickening, clubbing and erythema spreading up his left shin. Despite treatment with compression bandaging, lymphoedema dedicated physiotherapy, topical and intravenous corticosteroids his dermopathy progressed and now involves both lower limbs.

The second case involves a 53 year-old man diagnosed with GD in 2010. He had gross GO with proptosis, peri orbital swelling, chemosis, lid lag and ophthalmoplegia. He also had clubbing and severe bilateral skin changes with circumferential involvement of his lower limbs, plaques, verrucous change and a 3x4cm soft tissue swelling overlying the proximal phalanx of his right great toe.

He was treated with suppressive doses of carbimazole and with thyroxine replacement to maintain a euthyroid state. His GO and dermopathy have not improved despite intravenous methylprednisolone, topical steroid ointment and compressive bandaging.

Both patients have strong family history of autoimmune disease, extensive smoking history and consistently elevated TSH receptor antibodies despite treatment.

The mainstay of treatment for dermopathy is systemic glucocorticoid therapy however efficacy of this treatment is limited in severe disease. Multiple novel therapies are being investigated for GO, including rituximab, which may be applicable to treatment of dermopathy due to a likely shared pathogenesis.


Regrowth of non-functioning pituitary macroadenomas undergoing surgery in a single Australian centre.

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Non-functioning pituitary macroadenomas (NFPMA) are the commonest pituitary tumour requiring surgery. There are no published series regarding the surgical outcomes from Australia. We describe surgical outcomes and regrowth rate at a single centre.

Methods: Retrospective analysis of all NFPMA cases with pituitary surgery between September 1995 and December 2014.

Cohort:
178 cases identified. Males 54%, mean age 56.2±14.9 years.
Symptomatic presentation occurred in 61% (N=109) of which headache was the commonest complaint (N=69; 39%). Incidental presentation 29% (N=51); apoplexy in 10% (N=18). Visual deficit was reported in 67% (N=120).

Surgery:
The trans-sphenoidal approach was used in all except one who underwent the trans-cranial approach. Senior neurosurgeon (PMcN) performed 71% surgeries, the remainder were performed by five other neurosurgeons.

A single operation occurred in 155 (87%). Two operations were performed in 20 (12%) and three in 3 cases (3%). In 23% (N = 6) repeat surgery was planned in the immediate post operative period. In 48% (N = 11) repeat surgery was performed at a mean follow up time of 55.3 months, no data for timing of repeat surgery in the remainder

Post-operative complications: CSF leak (N=14; 8%), transient DI (N=27; 15%), permanent DI (N=12; 7%), SIADH (N=14; 8%), significant infection (N=3; 2%), significant bleeding (N=2; 1%), post-operative cardiac events (N=2; 1%).

Surgical Follow up:
One hundred and thirty-five patients (76%) had radiological follow-up ≥12 months, mean follow-up 81.8 (range 12-226). Thirty-three patients (24%) demonstrated tumour regrowth. Mean time to tumour regrowth was 59.7 months. Residual tumour was a significant risk factor for tumour regrowth (38% vs 15%; p=0.02). Treatment for tumour regrowth was surgery in 42% (N = 14), radiotherapy in 24% (N = 8) and combined approach in 15% (N = 5).

Discussion:
Tumour regrowth rate following trans-sphenoidal pituitary surgery is low, consistent with other international series.

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Examining the indications and results of bone densitometry performed in a large metropolitan teaching hospital.

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PROBLEM:
Osteoporosis is a condition associated with significant morbidity, mortality and economic costs. It is a disease amenable to primary and secondary prevention. The Medicare Benefits Schedule (MBS) is a list of Medicare services which are subsidised by the Australian Government. There are MBS criteria highlighting patients would be eligible for investigation of osteoporosis with bone densitometry. The Pharmaceutical Benefits Scheme (PBS) is a part of the Australian Government’s National Medicines Policy, with the aim of providing access to necessary medicines for Australians through subsidising medication costs (pbs.gov.au). We suspect that there are patients referred for bone densitometry who do not meet the MBS criteria for investigation of osteoporosis. In addition, we are interested in examining the relationship between the bone densitometry results and the PBS criteria for prescription of osteoporosis treatments.

METHODS
A retrospective audit of patients who have undergone bone densitometry at the Lyell McEwin Hospital, South Australia, over a 6 month period, will be conducted. Data presented will include:

- Patient demographics
- Whether the indication listed by the referring practitioner matches the indicated indications for bone densitometry
- The result of the bone densitometry, including FRAX estimation
- How the bone densitometry result influences treatment.


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Post-Partum Osteoporosis Due To Systemic Mastocytosis: 2 Case Studies

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Mastocytosis is a rare cause of secondary osteoporosis. We present two cases of systemic mastocytosis being diagnosed in the setting of post-partum osteoporosis. Case 1: A 35 year old G2P2 woman who was breastfeeding presented with subacute on chronic back pain 4 weeks post-partum. Imaging confirmed the presence of multi-level vertebral fractures. T-score was -4.5 at the lumbar spine and -2.8 at the left hip. Vitamin D was 39nmol/L (N > 50), and calcium and PTH were not elevated. Screening tests for secondary osteoporosis revealed an elevated serum tryptase of 23.8ng/ml (N < 11ng/ml) and a subsequent bone marrow biopsy confirmed the presence of mastocytosis. When she was treated with a zoledronic acid infusion, she developed a sinus tachycardia, hypotension and a fever of 40°C. A recent report suggests that acute phase reactions may be a
common reaction related to the use of zoledronic acid in patients with mastocytosis (1). Case 2: A 29 year old G2P1 woman who was breastfeeding presented with acute on chronic back pain 3 months post-partum upon lifting her baby. Imaging confirmed a compression fracture of lumbar vertebrae 4-5. Her average T-score was -3.19 at the lumbar spine and -1.99 at the left hip. Her Vitamin D was 54nmol/L. She received calcium and vitamin D supplements. After a further 12 months there was only marginal improvement in her bone mineral density. Re-imaging revealed new compression fractures in the thoracic spine. Her serum tryptase level was elevated at 25.7ng/ml and a diagnosis of mastocytosis was confirmed on bone marrow biopsy. She was commenced on an anti-histamine and has elected to have her osteoporosis treated with denusomab. Conclusion: Although pregnancy and lactation may contribute to bone loss, these cases suggest that in the setting of severe post-partum osteoporosis, a diagnosis of systemic mastocytosis should also be considered.


Hurt in the Sternum

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Publish consent withheld

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Desmopressin, Oxytocin and a Failing Heart

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A 27-year old female G2P1 presented at 33 weeks gestation with dyspnoea and peripheral oedema. Her past history consisted of diabetes insipidus (DI), thought to be nephrogenic, diagnosed at age 7, and obesity with a prepregnancy BMI of 53kg/m². Her mother, brother, uncle and 2 cousins were also affected by DI. She had not any endocrine review since childhood, and had maintained fluid balance by drinking 10L/day. She had not noticed any change in her fluid input or output during pregnancy. Following admission, investigations revealed a dilated cardiomyopathy (LVEF 28%), and a 2000ml/day fluid restriction was advised, posing a significant risk of dehydration and hypernatraemia given her unrestrained polyuria (>4.5L/day). A modified water deprivation test was performed with failure to adequately concentrate the urine at 4 hours, despite hyperosmolality (table 1). However, the urine osmolality increased following administration of desmopressin 1mcg. Subcutaneous desmopressin (1mcg bd) was commenced, allowing a modified fluid restriction to 3L daily with maintenance of normal serum sodium levels and stable fluid balance.

The patient developed acute pulmonary oedema and frusemide was commenced. Desmopressin was continued. Due to acute cardiac deterioration with SVT requiring adenosine, lower segment caesarean section (LSCS) was recommended at 37/40. Oxytocin was administered intraoperatively, but was not associated with any excess antiuretic effect (such as might occur with normal vasopressin responsiveness). Her newborn, however was noted to be hypernatraemic (Na 146-148mmol/L), with serum osmolality 320mOsm/L and urine osmolality 100mOsm/L suggestive of DI, and consistent with an autosomal dominant trait.

This patient’s management raised several challenges: the diagnosis of DI in pregnancy and the risks of dehydration; the response to vasopressin in nephrogenic DI; the need for fluid restriction in the presence of unrestrained polyuria; the potential impact of oxytocin on renal salt and water metabolism in nephrogenic DI.¹,²
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<th>Time</th>
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<td>Serum Na (mmol/L)</td>
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<td>Urine Osmolality (mmol/Kg)</td>
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DDAVP 1mcg administered at time 4 hours (after blood and urine collection)
Patient resumed water intake at 4 hours
References ranges apply to pregnancy where specified.


A rare type of aggressive thyroid cancer: review of the literature for treatment options

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Ms KJ is 52 years old lady who presented with 6 weeks of subscapular and thoracic back pain. CT identified an osteolytic lesion and soft tissue mass in the thoracic spine, and an incidental left lobe thyroid mass causing contralateral tracheal displacement. MRI showed impending cord compression, necessitating a T5 vertebrectomy. Metastatic follicular thyroid cancer was diagnosed on histopathology.

Her thyroid ultrasound showed a left lobe thyroid nodule without clear tracheal invasion or lymph node involvement. Non-contrast CT demonstrated a low density mass with calcific foci replacing the left lobe of the thyroid gland. Lung metastases were not seen on X-ray, and her repeat MRI showed lesions consistent with haemangiomas.

A total thyroidectomy with lymph node resection was performed. Her left lobe had a 30x30x28mm tumour, containing a mixture of well and poorly differentiated regions. The differentiated areas demonstrated a follicular pattern with colloid filled microfollicles lined by atypical follicular cells, staining positive for thyroglobulin; the poorly differentiated areas had solid pattern of sheets and cords of tumour cells in a desmoplastic stroma without follicles, with increased staining for cyclin D1 and P63. No malignancy was found nodally. She was diagnosed with stage IVC (T2N0M1) poorly differentiated insular variant of follicular carcinoma.

She was further treated with thyroxine withdrawal high dose radioactive iodine at 5300MBq. This reduced her thyroglobulin levels, although they remained elevated 3 months post (Fig.1). Combined PET and radiiodine scan (Fig.2) revealed new metabolically active but iodine inactive lesions in the liver and the right upper sternum, and a mildly iodine active but PET avid T5 vertebreal body lesion. The spine was treated with radiotherapy, analgesia and dexamethasone. The liver lesion was confirmed to be a solitary metastasis on primovist MRI, which will be considered for surgical resection post radiotherapy.
Undiagnosed Asymptomatic Phaeochromocytoma Causing Intra-Operative Haemodynamic Crisis in a Patient with Type One Diabetes.

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²The School of Medicine, University of Tasmania, Hobart, TAS, Australia

A 41yo man with a background of type 1 diabetes was admitted with starvation ketosis and sepsis secondary to multiple necrotic soft tissue wounds obtained on a remote solo bush walk.

On presentation, the patient was alert and orientated. Initial tests showed hyperglycaemia with ketosis but normal acid base balance. Inflammatory markers were markedly elevated. Multiple scratches and cuts were noted as well as broad necrotic wounds on both feet, knees and right thigh as well as an infected right elbow bursa. Intravenous fluids, antibiotics and insulin infusion were commenced. The Plastic Surgical team arranged surgical washout and debridement that afternoon.

Induction of anaesthesia was complicated by low oxygen saturation and tachycardia. On insertion of the endotracheal tube, systolic blood pressure rose to 280mmHg. Esmolol 60mg was administered with no change in blood pressure. Medication error and arousal were excluded. A GTN infusion was commenced for the short procedure.

Post operatively; the patient was diaphoretic, febrile, tachycardic and hypertensive. Intravenous metoprolol was required over
the next 2 hours after which the patient was transferred to the intensive care unit. Differential diagnoses considered included septic shower or aspiration pneumonia.

Urgent plain film of the chest was normal. Contrast CT demonstrated an 8.2 x 6.8cm right adrenal mass prompting 24-hour urine catecholamines and plasma metanephrines, which were markedly elevated. Metabolobenzylguanidine scan showed varying uptake at the periphery of the adrenal mass suggestive of phaeochromocytoma, with no extra adrenal uptake. FDG PET detected no FDG avid disease, indicating low probability of high-grade adrenal malignancy.

Treatment was initiated with Phenoxybenzamine up-titrated to 30mg daily. A high salt diet was commenced and Metoprolol 25mg bd was added prior to laparoscopic adrenalectomy. Histopathology confirmed a phaeochromocytoma with no malignant features. Mutational analysis of the tumour showed normal staining for SDHB and SDHA.

Hypoglycemic Encephalopathy and the Severity of Brain Injury: A Case Report

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Hypoglycemic encephalopathy is a potentially life-threatening event that can result in permanent brain injury. This syndrome is not well described in the literature. We report a case of hypoglycemic encephalopathy in a 33-year-old male with type 1 diabetes following a presumed accidental catastrophic insulin overdose. He was found unresponsive following a prolonged hypoglycemic period estimated to be 17 hours. Upon arrival his blood sugar level (BGL) was too low to be recorded and his Glasgow Coma Scale (GCS) was 5. He was normothermic with a pH of 7.32 and had a lactate of 3.3 mmol/L. Despite rapid normalisation of his BGLs with 10% dextrose, he had minimal improvement in his GCS. He was intubated and transferred to the intensive care unit (ICU). A CT of his brain was suggestive of diffuse cerebral oedema. He progressed to a bi-frontal craniotomy to relieve his presumed raised intracranial pressures. Magnetic resonance imaging (MRI) of his brain performed day 6 post admission showed elevated T2 and flair signals throughout his cortex and elevated signal on the diffusion weighted imaging (DWI) was consistent with diffuse cytotoxic oedema. The basal ganglia was hyperintense on FLAIR and T2 images, however the thalami were spared. Reduced apparent diffusion coefficient (ADC) signal throughout the subcortical white matter was noted. He had minimal neurological improvements clinically and an electroencephalogram (EEG) showed very low voltage output in keeping with minimal cortical activity. In view of above findings, he was felt to have no prospect of recovery and was palliated. In summary, we present a case of severe hypoglycemic encephalopathy resulting in fatal metabolic brain injury that was difficult to prognosticate. The syndrome is associated with characteristic MRI findings as described in our case. We attribute prolonged hypoglycemia, normothermia and DWI findings as predictors of poor outcome in this case.

A case of frontal bone aneurysmal bone cyst in association with polyostotic fibrous dysplasia

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We present the case of a 21 year old male who developed an aneurysmal bone cyst(ABC) on a background of fibrous dysplasia(FD). He was diagnosed with FD aged 4 and has extensive disease with marked craniofacial involvement, including pitiutary fossa. Past complications include a fractured right femur and right optic nerve neuropathy requiring decompression but with residual right visual loss. In 2013 he developed mild left optic nerve compression, treated with steroids. He self-treated a second episode in 2013. He has no hormonal abnormalities.

He subsequently developed a rapidly expanding left frontal bone lesion with imaging suggesting an ABC. Given the rapid expansion and his compromised vision, he underwent a craniotomy with excision of an 8x10cm lesion with minimal blood loss despite no preoperative arterial ablation. Histology showed an ABC arising from FD. Vision in the left eye is now completely normal. He has no evidence of ABC recurrence.

FD is an osteoblast disorder in which bone is replaced by dysplastic fibrous tissue. FD is caused by a postzygotic activating mutation of the G-protein alpha-subunit. It can be monostotic or polyostotic, have overlying café-au-lait pigmentation, and may cause hormonal hypersecretion(McCune-Albright Syndrome). Malignant transformation, presenting with pain and an expanding mass, can occur, with polyostotic disease and previous radiation increasing risk. ABCs are rare benign lesions presenting with similar symptoms but distinct features on imaging. ABCs can be either primary or secondary to malignancies or FD. There are several theories for the pathogenesis of ABCs. Treatment is surgical with a high risk of intraoperative haemorrhage. The combination of craniofacial FD with secondary ABC is rare with limited cases in the literature.

Our case is of a 21 year old male with FD who develops an ABC. We review the literature in regards to craniofacial FD, ABC, treatments and outcomes.

MEN1 and paraganglioma: expanding the clinical spectrum of MENIN mutations.

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Multiple endocrine neoplasia type 1 (MEN1) classically consists of parathyroid, pituitary and pancreatic tumours. Here we report two unrelated cases with MEN1 with asymptomatic paragangliomas.

P1 had a strong family and personal history of MEN1. Given the presence of pancreatic lesions with mild elevation of gastrin, a 18F-DOTATATE scan was undertaken. Unexpectedly this demonstrated uptake in the left carotid region suggestive of paraganglioma. P1 had no symptoms or biochemical evidence of catecholamine excess. Histology of the resected mass showed a paraganglioma with weak but positive staining for SDHB unlikely to be consistent with germline SDHx mutations.

P2 also had a strong family and personal history of MEN1. Although biochemically stable, he had an increasing pancreatic mass (>3cm diameter) with marked uptake on 18F-DOTATATE. FDG-PET suggested a high grade/poorly differentiated lesion and he underwent a Whipple's resection. Histology demonstrated a Grade 1 neuroendocrine tumour (35mm diameter) and a second lesion (8mm diameter) consistent with an extrapancreatic paraganglioma that stained positively for SDHB, (SDHx mutation thus unlikely). Germline screening of all exons of MENIN showed that P1 was heterozygous for a c.1716delC mutation in exon 10, resulting in a frameshift and introduction of a premature stop codon. P2 was heterozygous for a c.1319delG mutation (exon 9), with similar effect.

Sanger sequencing of DNA extracted from each tumour demonstrated loss of wildtype allele. Microarray genotyping (assessing for large copy number alteration) demonstrated loss of heterozygosity of chromosome 11 in both tumours, including the MENIN locus. Of note, there was differential aneuploidy of the paraganglioma and adjacent islet cell tumour in P2.

The combination of paraganglioma in MEN1 has been reported extremely rarely (four cases). P1 and P2 are undergoing germline screening for known phaeochromocytoma/paraganglioma susceptibility genes. However, if negative, our data suggest that paraganglioma may rarely be part of the MEN1 syndrome.

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Tetany Associated with Teriparatide Therapy: A Case Report

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2. Department of Endocrinology, Monash Health, Clayton

Teriparatide is a parathyroid mimetic used in the treatment of severe osteoporosis to increase bone mineral density. Hypercalcaemia is a documented potential adverse effect. We present a unique case in which symptomatic hypocalcaemia and hypomagnesaemia followed initiation of Teriparatide therapy.

PT, a 30-year-old Cambodian female presented to the emergency department with symptomatic hypocalcaemia following commencement of Teriparatide for severe osteoporosis deteriorating despite antiresorptive therapy. Other medical issues included autoimmune hepatitis (cirrhosis and portal hypertension), very low weight (BMI 13.8kg/m2), secondary amenorrhoea, anaemia and intermittent electrolyte and mineral disturbances (hypokalaemia and hyponatraemia). Serum electrolytes and minerals prior to commencing Teriparatide were essentially within normal limits.

Following nine days of initial Teriparatide therapy, PT developed tetany and presented to the emergency department. Her ionised Calcium was 0.99mmol/L, corrected Ca2+ 2.0mmol/L, Mg 0.5mmol/L, PO4 0.85mmol/L, and renal function was normal. Liver function tests were elevated but not significantly different to the patient’s usual levels. A repeat Vitamin D was borderline at 50nmol/L. PT was closely monitored and stabilised with intravenous magnesium, and discharged on calcitriol 0.25mcg daily, magnesium supplementation and ongoing Teriparatide therapy.

Teriparatide is anabolic bone formation agent, comprising an active fragment of endogenous human PTH and is known to be associated with transient post-dose hypercalcaemia (>2.6). There is currently no literature that has demonstrated Teriparatide therapy being linked with hypocalcaemia or hypomagnesaemia. In fact there is emerging evidence of Teriparatide being used as a treatment for hypoparathyroid-associated hypocalcaemia. It is postulated that Teriparatide may have a converse effect in vulnerable individuals.

This case is the first of its kind in the literature and realises the potential for Teriparatide to cause hypocalcaemia. Given the severity of symptoms, early detection is essential to prevent significant complications.

Bilateral macronodular adrenal hyperplasia and systematic testing for aberrant receptors: a bumpy journey

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Mrs SD is a 62-year-old Bosnian refugee, incidentally discovered to have bilateral nodular adrenal enlargement during investigation for haematuria. She had no specific examination features of Cushing’s syndrome but was centrally obese and had a history of type 2 diabetes, ischaemic heart disease, hypertension, stroke and osteopenia. Cortisol failed to suppress after low and high dose dexamethasone and although incompletely suppressed, ACTH was low on repeated assessments, consistent evidence that circulating hormones other than ACTH stimulate adrenal cortisol production via ectopic or deviant eutopic receptors for these hormones on adrenocortical cell membranes. Blockade of these receptors has resulted in variable success as therapy for Cushing’s syndrome.

Bilateral macronodular adrenal hyperplasia (BMAH) is a rare cause of Cushing’s syndrome and usually presents around the fifth decade of life. In BMAH, there is emerging evidence that circulating hormones other than ACTH stimulate adrenal cortisol production via ectopic or deviant eutopic receptors for these hormones on adrenocortical cell membranes. Detection of aberrant receptor(s) can be achieved via targeted stimulation with potential candidate hormones. Using a protocol developed by Lacroix3, a strongly positive response to vasopressin was demonstrated; baseline cortisol rose by 119% without significant change in ACTH. A cortisol rise of 39% was also observed with Metoclopramide.

No cortisol rise was observed following subsequent testing with Desmopressin, a V2-selective agonist. The initial cortisol surge was thus thought to be due to aberrant V1 receptors. Aberrant V1 and 5HT4 receptors are reported to be common causes of adrenal Cushing’s syndrome in BMAH.

Detectable bioactive ACTH was recently demonstrated in adrenal tissues of BMAH patients5. Hormones implicated with aberrant receptors also stimulated ACTH production by these adrenal explants. These in vitro findings raise the possibility that steroidogenesis in BMAH is not ACTH-independent, as previously supposed. This may explain why ACTH was incompletely suppressed.

This case raised our awareness of steroidogenesis by aberrant receptors in adrenal Cushing’s syndrome and challenged the paradigm of ACTH-independent Cushing’s syndrome.

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2. Lacroix et al; Propranolol therapy for ectopic beta-adrenergic receptors in adrenal Cushing’s syndrome; N Engl J Med; 1997; 33(3): 1429-34
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Multiple paragangliomas in a 17-year old male with post-micturition symptoms

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A 17-year old man presented with palpitations, headache and diaphoresis after micturition and macroscopic haematuria. His plasma normetadrenaline levels were grossly elevated (15000 pmol/L) indicative of a paraganglioma. His paternal uncle had a mediastinal paraganglioma at age 22 and his paternal grandfather had a renal cell carcinoma at age 70. Computed tomography (CT) scans showed a bladder wall tumour, para-aortic mass, right hydronephrosis, and an 8 mm left lung base lesion. A 18F-fluorodeoxyglucose (FDG) positron emission (PET) CT scan confirmed FDG avid lesions in the bladder, the pelvis and the aorto-caval region. Only the latter was clearly visible on 123I-metaiodobenzylguanidine scintigraphy. After pre-operative alpha- and beta-adrenergic receptor blockade, right ureterectomy, cystectomy and resection of the aorto-caval and parasartiat vessel masses was performed. Histopathology confirmed multiple paragangliomas. There were no tumour-positive lymph nodes and immunohistochemistry staining for succinate dehydrogenase (SDH) B was absent, suggesting a germline mutation in either the SDHB, SDHC or SDHD gene. He is booked for a post-operative gallium PET scan to assess presence of residual tumour masses.

Bladder paraganglioma is a rare form of paraganglioma. One-third of patients with a phaeochromocytoma or paraganglioma are thought to have a germline mutation in one of the known susceptibility genes. In this case, a hereditary cause is strongly
suspected because of his young age, the presence of multifocal disease and a positive family history. Which genetic mutations to test for depends on tumour location, biochemical profile and immunohistochemistry. Genetic counselling is warranted once a germline mutation has been confirmed.

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**Hemiballismus: a rare complication of diabetic nonketotic hyperosmolar state**

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2. Department of Radiology, The Canberra Hospital, Garran, ACT, Australia
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A sixty-three year old female with a 13-year history of type 2 diabetes treated with oral agents alone presented with sudden onset of left-sided hemiballismus. She had omitted her treatment for a number of months prior to presentation and HbA1C was 14.9%. A magnetic resonance imaging (MRI) scan of her brain showed a high signal on diffusion-weighted and hyperintensity on T1 weighted images in the right medial lentiform nucleus and head of caudate. Blood tests indicated severe hyperglycemia (serum glucose 26.2 mmol/L). She was diagnosed with hyperglycemia induced chorea-ballismus (HICB). After prompt treatment of her hyperglycemia with insulin, her hemiballismus resolved completely within 10 days.

HICB is a rare complication of hyperosmolar hyperglycemic state (HHS). It is characterized by a sudden onset of uni- or bilateral choreatic or ballistic movements in the context of severe hyperglycemia. There is a predilection for elderly women and occurs more frequently in Asians, suggesting a genetic susceptibility. Radiologically, HICB is associated with high signal intensity in the basal ganglia on T1 weighted sequences with the putamen being most frequently affected. Several mechanisms have been suggested including hyperglycemia-induced depletion of cerebral gamma-aminobutyric acid, activation of inflammatory cascades and regional hypoperfusion as a result of increased cerebrovascular resistance and hyperviscosity. However, the pathophysiology remains elusive. Treatment of the hyperglycemia results in quick resolution of symptoms in most cases.

In patients presenting with unexplained hemiballismus, hyperglycemia should be considered as it is an easily treatable cause leading to quick recovery if treated promptly.

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**Euglycaemic diabetic ketoacidosis in a young adult with type 1 diabetes and an eating disorder**

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2. Sydney Medical School, University of Sydney, Sydney, NSW, Australia
3. University of New South Wales, Sydney, NSW, Australia

A 17 year old female with a 3 year history of type 2 diabetes treated with oral agents alone presented with sudden onset of left-sided hemiballismus. She had omitted her treatment for a number of months prior to presentation and HbA1C was 3.4%. On examination, her blood pressure was 120/60mmHg (no postural drop), pulse 118bpm (sinus rhythm), respirations 18 breaths/minute, and she was mildly dehydrated clinically. Capillary glucose was near-normal at 5.9mmol/L, however fingerprick ketones were significantly elevated to 6.0mmol/L. Venous blood gas confirmed a metabolic acidosis with pH 7.18 (subsequently calculated to be high-anion gap).

Her diagnosis was euglycaemic diabetic ketoacidosis in the setting of chronic starvation and insulin omission, on a background of T1DM and restrictive-type eating disorder.

She was admitted to ICU for intravenous normal saline, dextrose, insulin infusion and electrolyte replacement, with resolution of the ketoacidosis over 24 hours. She is receiving ongoing care for her T1DM and eating disorder through a multidisciplinary team approach involving the endocrinologist, diabetes educator, dietitian, psychologist and psychiatrist.

**Teaching points:**

-While diabetic ketoacidosis (DKA) is generally defined as the triad of hyperglycaemia (blood glucose>11.0mmol/L), ketosis and metabolic acidosis, it can also occur rarely with near-normal glucose levels.

-Euglycaemic DKA can occur in people with T1DM and chronic starvation.

-A high index of suspicion is required as presentation may include minimal acute symptoms.

-Individuals with T1DM have a higher prevalence of dysfunctional eating behaviours and overt eating disorders. The care of these people with dual diagnoses can be highly challenging, and optimally requires a multidisciplinary team approach.

-This case highlights the value of point-of-care blood ketone assessment.
Double Trouble In The Pituitary: A Case Report
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Most cases of acromegaly are due to pituitary somatotroph adenomas, however a minority (<2%) of cases are due to GHRH hypersecretion (1). Mixed pituitary adenoma and gangliocytoma tumours are rare, and less than 80 cases are described in the literature (2). Most intra-pituitary gangliocytomas are associated with hormonal hypersecretion, commonly growth hormone (GH) excess (2). We report a case of acromegaly secondary to a mixed pituitary adenoma-gangliocytoma, and discuss the possibility of ectopic GHRH secretion from gangliocytomas.

A 60 year old male was referred for assessment of a pituitary mass found following investigation of chronic headaches over the preceding two years. MRI head revealed a 1.9 x 1.7 x 2.4cm right sided pituitary macroadenoma with invasion into the right cavernous sinus but no compression of the optic chiasm. Examination findings were consistent with acromegalic features. He had no other symptoms or signs of endocrine dysfunction, nor family history of endocrinopathies. Static pituitary hormone testing showed an elevated IGF-1 122 nmol/L and elevated GH 5.2 ug/L. The remaining pituitary hormonal profile was normal. His GH failed to suppress following an oral glucose tolerance test (OGTT) (GH nadir 3.1 ug/L).

He underwent uncomplicated endoscopic trans-sphenoidal resection of the mass. Histopathology revealed a gangliocytoma (composite chromophobe pituitary adenoma and ganglion cells in neutropil). Immunohistochemistry of adenoma cells stained weakly for GH. Immunostaining for GHRH has been requested. An ultra-early (day 2) post-operative OGTT demonstrated suppression of GH to <1ng/ml. This will be repeated at 8-12 weeks post-operative.

In conclusion, we report an uncommon case of a mixed pituitary adenoma-gangliocytoma causing acromegaly. We hypothesise that ganglion cells secrete GHRH, subsequently inducing GH secretion from the adenoma cells. We review the literature to see if these lesions behave differently to classic acromegaly.


An unusual cause of recurrent severe hypokalaemia
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Context: Small cell prostate cancer has rarely been reported in associated with ectopic secretion of adrenocorticotropic hormone (ACTH) and severe clinical Cushing’s syndrome.

Case description: A 91 year old man presented with hypertension and peripheral oedema. His background history consisted of hypertension, type 2 diabetes, atrial fibrillation, transient ischaemic attack, and prostate carcinoma with resection. On examination he had upper limb bruising, centripetal obesity and moderate pitting oedema. He was found to have a metabolic alkalosis, hypokalaemia (K+ 2.4 mmol/L) and initial received intravenous potassium followed by oral replacement. Investigations revealed markedly elevated morning serum cortisol and ACTH, and non-suppression on a 1mg dexamethasone suppression test. Imaging of his brain, chest, abdomen, and pelvis were normal. Further evaluation of the presumed ectopic secretion of ACTH was not undertaken because of the frailty of the patient and his clearly expressed wishes. Bilateral adrenalectomy was also considered but declined.

Management consisted of ongoing potassium replacement and Ketoconazole 400mg/day commenced with the aim of inhibiting cortisol synthesis. This led to an improvement in serum potassium but was poorly tolerated. Metyrapone 500mg/day was also trialled but ceased due to the development of abdominal pain and diarrhoea. There was little improvement in overall health and the patient opted for medication withdrawal. Post mortem examination revealed high-grade small cell prostate carcinoma, which is a very rare cause of ectopic ACTH.

Conclusions: In difficult to treat hypokalaemic alkalosis the differential diagnosis of ectopic ACTH Cushing's syndrome should be considered. Whilst most causes of ectopic ACTH secretion are found within the chest it is important to contemplate other aetiologies such as prostate cancer.
**Pituitarius, where art thou?**

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*Publish consent withheld*


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**Flushed with excitement – a heartfelt case of carcinoid syndrome**

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Neuroendocrine tumours (NETs) are a heterogeneous group of neoplasms that arise from neuroendocrine cells of the gastrointestinal tract. A carcinoid syndrome with the classic triad of flushing, palpitations and diarrhoea/abdominal pain, is more specifically attributed to well-differentiated serotonin-secreting midgut tumours and is present in approximately 20% of NETs in the duodenum and jejunum¹.²

Individuals with carcinoid syndrome can present with chest tightness or breathlessness³ leading to diagnoses of acute coronary syndrome or asthma. These symptoms are thought to be related to serotoninergic activity of the NETs. Research suggests an association between the increased serotoninergic activity⁴–⁵ with Takotsubo cardiomyopathy⁶, a transient cardiac syndrome with left ventricular hypokinesis or dyskinesis⁶.

We present the case of Ms LT, a 64-year old woman who presented with palpitations and chest pain in the setting of intense emotional stress, with dynamic ECG changes and troponin rise. This is on a background of multiple pulmonary embolisms, late-onset asthma and hypertension. Her family history is significant for Graves’ disease and the death of her father from a pancreatic tumour. Coronary angiogram showed pristine coronary arteries with apical ballooning and MRI showed basal inferior hypokinesis, normal contractility leading to a diagnosis of Takotsubo cardiomyopathy. She represented one month later with PR bleeding, abdominal pain, diarrhoea, flushing and refractory palpitations. Investigations showed a neuroendocrine tumour of the ileal region with lymphatic and bony metastases. Chromogranin A and 24-hr urinary 5HIAA were elevated, however, after ceasing her proton pump inhibitor her Chromogranin normalised. She underwent surgical resection of her ileal tumour and was commenced on somatostatin analogue, which is keeping her carcinoid syndrome under control.

Increased plasma serotonin has been reported in Takotsubo cardiomyopathy. Clinical suspicion of carcinoid syndrome in a patient with Takotsubo cardiomyopathy should be raised with the presentation of the classic triad: flushing, palpitations and diarrhoea/abdominal pain.


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**References**


Thyroid hormone resistance, a case report

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2. Department of Medicine, South West Health Care, Warrnambool

Introduction
Thyroid hormone resistance is a rare but important differential to consider in patients with hyperthyroxinaemia. The clinical presentation is that of non-suppressed thyroid stimulating hormone (TSH), elevated thyroid hormone levels and goitre with minimal clinical symptoms of thyrotoxicosis. The differential diagnosis for this hormone profile is TSH secreting pituitary adenoma.

Case Report
A 31-year-old woman presented with long standing deranged thyroid function tests in the setting of a strong paternal family history of thyroid disease.

At the time of initial presentation at age 15, she had a goitre and markedly elevated triiodothyronine (T3) (16.3pmol/L) and thyroxine (T4) (42.7pmol/L) levels with a non-suppressed thyroid stimulating hormone (TSH) level (1.79 mU/L). A computed tomography (CT) study of her brain, performed in lieu of magnetic resonance imaging (MRI) due to claustrophobia, did not demonstrate a pituitary adenoma. She went on to have a thyrotropin releasing hormone (TRH) test (200ug IV TRH), which demonstrated an appropriate rise in TSH (12.34mU/L at 20 minutes, 10.41mU/L at 30 minutes).

At the age of 26 she underwent a total thyroidectomy, complicated by transient hypoparathyroidism. Thyroid histology showed diffuse hyperplasia but no lymphocytic infiltration. She has subsequently required thyroxine replacement, with varying doses. She has a significant family history for thyroid disease, affecting multiple primary and secondary relatives on her father’s side.

Discussion
Thyroid hormone resistance is a rare autosomal dominant condition involving a mutation of the thyroid hormone receptor beta gene. It is estimated to occur in 1 in 40,000 live births. These patients have resistance to thyroid hormone in peripheral tissues. Variability of peripheral resistance means patients can have mixed clinical features of both hyper and hypothyroidism. These patients generally require supraphysiological replacement doses of thyroxine to achieve a relatively euthyroid state with TSH suppression.

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Vitamin C deficiency: an overlooked risk factor for impaired wound healing in patients with diabetes mellitus

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Vitamin C deficiency is rarely diagnosed in the modern era. With the Australian population eating more discretionary food and inadequate vegetables, it is possible that Vitamin C deficiency is becoming more prevalent. Groups with a greater tendency to avoid certain foods are at risk of developing manifest scurvy.

A 25 year old male with a lifelong history of Type 1 diabetes and a 10-year history of Coeliac disease attended Diabetes Clinic with multiple lesions on the anterior lower limbs. He stated they resulted from having dropped sheet metal on his legs three days prior. He was admitted with hyperglycaemia and non-acidotic ketosis, weight loss of 29% (22kg) over 6 months and microcytic anaemia. Vitamin C deficiency was suspected after dietary history revealed irregular compliance with gluten-free diet and minimal intake of fresh fruit and vegetables. Low vitamin C level was confirmed at 19 umol/L (normal > 40).
Antibiotics, oral vitamin C, vitamin D and iron supplementation were commenced. Psychiatry review excluded disordered eating. After discharge he stopped taking vitamin supplements and his vitamin C level was not replete at 30 umol/L. His leg wounds remained open but not infected. Two months later he lost a further 3kg weight, suffered postural dizziness and his leg wounds still had not healed. His vitamin C level was 35 umol/L with intermittent adherence to oral replacement. He started consistently taking 2000 mg daily and vitamin C level improved to 181 umol/L one month later. His wounds healed despite ongoing poor glycaemic control (HbA1c 12.2% from 11.7% previously).

Conclusions: Although this patient had several potential factors contributing to his non-healing wounds, only achieving adequate Vitamin C replacement correlated temporally with wound healing. Vitamin C deficiency should be considered in patients with diabetes and non-healing wounds as the treatment is simple, affordable and safe.

Addition of a Mitochondrial Antioxidant to Culture Media Improves Embryo Development and Metabolism in an Aged Mouse Model

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Women are delaying starting a family and as a result the age of first time mothers has increased. The number of women >40yrs using IVF reproductive technologies has also increased. However, IVF is substantially less effective for women over 40 years of age. Recent data has established that mitochondrial function in eggs from older women is reduced and levels of reactive oxygen species (ROS) are increased. The aim of this study was to establish if the addition of an antioxidant which targets mitochondrial produced ROS, can improve embryo metabolism and development using an aged mouse model.

Zygotes were collected from 22 week old superovulated C57BL6 females and cultured at 37°C in 6%CO2:5%O2:89%N2 in either (i) control media (G1) or (ii) G1 + 100µM Manganese(III) tetrakis (4 benzoic acid) porphyrin (MnTBAP) which can traverse the inner mitochondrial membrane and neutralise superoxide anions. Embryos were transferred to G2 medium before assessment for on-time blastocyst development and glucose uptake (day 4- total of 74h culture) and cell number and differentiation (day 5- total of 91h culture) and ROS production (MitoSox).

Embryos cultured in the presence of MnTBAP were significantly more advanced on day 4 with higher levels of on-time blastocyst development (control 19.5%, MnTBAP 32.7%; P<0.05) which coincided with a 12% increase in glucose uptake (P<0.05) and a significant reduction in ROS production (~25.3%; P<0.01). There was a significant increase in cell number of the blastocysts cultured with MnTBAP (control 60.5±4.6, MnTBAP 73.1±3.7; P<0.05), which is usually indicative of increased viability. This increase was confined to the oxidative trophectoderm cells (control 43.4±3.7, MnTBAP 57.3±3.3; P<0.05).

This study indicates that embryo development of embryos from older mothers can be improved by the addition of a mitochondrial antioxidant. Assessment of pregnancy outcomes from these embryos is required to further validate these findings.

Decreased antioxidative gene expression in skeletal muscle in individuals conceived by In-Vitro Fertilisation (IVF)

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Objective
We have previously shown that adults conceived by IVF were more insulin resistant than naturally conceived adults (controls). However, the underlying mechanisms are not clear. This study aimed to compare circulating inflammatory cytokines and expression of genes involved in oxidative stress in skeletal muscle and subcutaneous adipose tissue.
Materials and Methods
Adults conceived by IVF (n=14) and controls (n=20) matched for age (17–26 years), gender, weight and body fat composition were studied. Subjects were examined after three days of an energy balanced diet (30% fat, 15% protein, 55% carbohydrate) and again after 3 days of overfeeding (+1250 kcal/day, 45% fat, 15% protein, 40% carbohydrate). Vastus lateralis muscle and abdominal subcutaneous adipose tissue biopsy samples were obtained from 6 IVF and 11 controls. Serum levels of IGF1, Adiponectin, C-reactive protein (CRP) and monocyte chemotactic protein-1 (MCP-1) were examined by ELISA. Markers of oxidative stress (superoxide dismutase (SOD) 1 and 2, glutathione peroxidase 1 (GPX1) and catalase) were measured by qPCR in both tissues.

Results
There was no difference between groups in serum levels of IGF1, adiponectin, CRP and MCP-1, independently of diet. At baseline, relative gene expression of SOD2 and GPX1 in skeletal muscle was significantly lower in IVF adults versus controls (P=0.02 and 0.04 respectively).

Conclusions
The data shows that inflammatory mediators were not altered in young IVF adults, although the expression of enzymes involved in antioxidative SOD2 and GPX1, were significantly lower. Further studies are warranted to determine if oxidative stress contributes to peripheral insulin resistance observed in IVF individuals.

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Efficacy of a Cue-Mate Intravaginal Insert and Injection of Prostaglandin F₂α For Synchronizing Estrus in Hanwoo Cattle
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Epigenetic and microRNA-mediated regulation of adult hippocampal VGLUT2 expression following early prenatal ethanol exposure
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Maternal consumption of alcohol during pregnancy is associated with structural and functional abnormalities of the central nervous system in the offspring however the underlying mechanisms are not fully understood. We used an inbred C57BL/6J mouse model of early gestational ethanol exposure that is equivalent, developmentally, to the first 3-4 weeks of pregnancy in humans to examine the long-term consequences on gene expression and epigenetic state in the hippocampus. Solute carrier family 17 member 6 (Slc17a6), which encodes vesicular glutamate transporter 2 (VGLUT2), was significantly up-regulated in the hippocampi of adult ethanol-exposed male offspring. Transcriptional activation was associated with changes in both promoter DNA methylation and histone H3 lysine 4 trimethylation, a mark of active chromatin. Several ethanol-sensitive microRNAs were also identified in the hippocampus, one of which was shown to specifically interact with Slc17a6, revealing an additional level of post-transcriptional control. A significant correlation between microRNA expression in the hippocampus and serum of ethanol-exposed offspring was also observed. Prenatal ethanol exposure has complex transcriptional and post-transcriptional effects on Slc17a6 (VGLUT2) expression in the mouse hippocampus. Altered epigenetic and microRNA-mediated regulation of glutamate neurotransmission in the hippocampus could contribute to the cognitive and behavioural phenotypes observed in fetal alcohol spectrum disorders. Our results also support the idea that circulating microRNAs could be used as biomarkers of early gestational ethanol exposure and/or hippocampal dysfunction.

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Factors contributing to the poor reproductive performance of ewe lambs
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The majority of ewes in New Zealand are bred for the first time as 2-tooths when they are approximately 18-20 months old. Mating ewe lambs so that they produce a lamb at one year of age (yearlings) provides a clear opportunity to improve farm profitability through increasing the lifetime production of each ewe. If ewes lamb, on average, 4 times during their lifetime, producing a litter in their first year of life (so that they lamb 5 times), has the potential to increase their lifetime production by 25% thereby improving efficiency.
Differential maternal and paternal genome effects on circulating thyroid hormone concentrations and deiodinase expression in the midgestation fetus

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Deiodinases in placental and fetal tissues regulate the bioavailability of thyroid hormones and thus play an essential role in fetal growth. Non-equivalence of maternal and paternal genomes, i.e., genomic imprinting, has been demonstrated in the thyroid axis1. Deiodinase 3 (DIO3) is expressed from the paternal allele only and converts thyroxine (T4) to reverse triiodothyronine (rT3), preventing overexposure of fetal tissues to triiodothyronine (T3). DIO1 and DIO2 on the other hand, convert T4 to T3. Genomic imprinting and other epigenetic mechanisms that lead to allelic imbalance can impact on estimated effects of genetic markers involved in thyroid hormone regulation. Parental genome effects on fetal thyroid hormone levels and deiodinase expression have not been studied. We have previously demonstrated that a bovine model with Bos taurus taurus and Bos taurus indicus genetics in purebred and reciprocal cross fetuses at midgestation (Day153) can dissect maternal and paternal genome effects on fundamental fetal characteristics1-5. Here we show in the same resource (n=73) using linear models, that paternal genome affects umbilical cord plasma rT3 (P<0.001) and total T4 (P<0.001) levels, while maternal genome affects cord plasma free T4 (fT4) (P<0.001). Circulating T3 and rT3 levels were below assay sensitivity. Hepatic DIO1 transcript abundance was affected by maternal genome (P<0.001) and correlated with plasma fT4 (r=0.28, P<0.05) and rT3 (r=-0.26, P<0.05). Renal DIO3 transcript was correlated with plasma fT4 (r=0.45, P<0.001) and rT3 (r=0.29, P<0.05), but was not affected by paternal genome. Consistent with imprinted paternal expression, placental DIO3 transcript was affected by paternal genome (P<0.05), but was not correlated with circulating hormone, suggesting it is not a major contributor of fetal rT3. In conclusion, we have demonstrated strong differential parental genome effects on thyroid hormones and correlated these with major regulators of thyroid hormone metabolism.


Consequences of culturing preimplantation embryos individually

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IVF clinics increasingly culture embryos individually to facilitate morphometric and genetic analyses. However, culturing embryos individually rather than in groups deprives them of paracrine factors produced by neighbouring embryos and this reduces blastocyst cell numbers. Further examination of the differences between embryos cultured individually or in groups, including their response to other stresses in vitro and the role of paracrine factors, is therefore warranted. Zygotes from CBA × C57BL/6 mice were cultured individually in 2 µL G1/G2 medium or in groups of 10 in 20 µL at 5% oxygen. Time-lapse microscopy revealed that individually cultured embryos were delayed reaching the 8-cell stage (P<0.01), and the
resulting blastocysts had fewer cells and a reduced proportion of inner cell mass cells compared to embryos cultured in groups (P<0.05). Increasing the drop size from 2 µL to 20 µL further reduced cell numbers of individually cultured embryos (P<0.05), presumably due to dilution of embryo-secreted factors. In support of this, the addition of embryo-conditioned media to single embryos increased cell numbers compared to controls (P<0.001).

To determine the effect of an additional in vitro stress, embryos were cultured in atmospheric oxygen. The effect of combined single culture and atmospheric oxygen on cell numbers was more detrimental than each condition alone (P<0.001), indicating that there is a cumulative effect of these stresses.

Despite the differences observed during the preimplantation stages, embryos cultured individually or in groups performed equally in outgrowth assays and no differences were observed in fetal or placental weights on day 15 of pregnancy. When considering the relevance of these findings to clinical IVF, individual culture may reduce the numbers of transferrable blastocysts available, even if no fetal consequences are apparent. Furthermore, many clinics routinely culture embryos both individually and in atmospheric oxygen, which have cumulative detrimental effects on blastocyst development.

Effect of DNA methyltransferases on the reprogramming of methylation during early mouse embryo development

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Publish consent withheld

Effect of thyroid hormones on porcine oocyte maturation in vitro.

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The role of the thyroid hormones thyroxine (T4) and triidothyronine (T3) in vitro embryo production has not been widely studied. In cattle the addition of T4 plus T3 to in vitro oocyte maturation media has been shown not to influence blastocyst rate or cell number (1). However it was suggested that these hormones may have beneficial effects in species where in vitro maturation is extended such as the pig and human. The aim of the present study therefore was to determine whether the addition of T4 and/or T3 to defined porcine in vitro maturation media could increase in vitro embryo production. Porcine ovaries were obtained from a local abattoir and small antral follicles 3-6mm in diameter were aspirated in the laboratory. Cumulus – oocyte complexes were matured in BOMED maturation media plus PVA containing 0, 25, 50 or 100ng/ml of T4 plus T3 in experiment one or 0, 25, 40 or 100ng/ml of T3 in experiment two for 44 hours. Following maturation COCs were coincubated with 5 x 10^6 sperm /ml of mixed boar semen for 6 hours. After fertilisation the cumulus cells were removed and the zygotes cultured in PZM-5. The number of oocytes that developed to blastocyst stage on day 5 and 6 stage and differentially stained to determine day 6 blastocyst cell number. In the first experiment one the addition of 50 ng/ml of T4 and T3 increased day 6 blastocyst inner cell mass number compared with control (P<0.05; 7.0 vs 4.0 respectively). In experiment two the addition of 50 ng/ul of T3 increased day 5 blastocyst rate (P<0.05; 46 vs 36.5% respectively). In conclusion our results suggest that the addition of T4 and/or T3 to defined porcine maturation media have beneficial effects for in vitro embryo production.


Multipotent cell types in primary fibroblast cell lines used to clone pigs using somatic cell nuclear transfer.

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We have previously demonstrated that the use of porcine mesenchymal stem cells (MSCs) isolated from the bone marrow can increase the proportion of somatic cell nuclear transfer (SCNT) embryos that develop to the blastocyst stage compared with adult fibroblasts obtained from the same animal (1). The aim of the present study was to determine if MSCs are also present in primary cultures of adult fibroblasts which are commonly used for cloning live animals. To do this we chose two primary adult cell lines that we have previously used to clone pigs. Single cells were isolated using low-density plating and then expanded. Cells were then differentiated to adipocytes, chondrocytes and osteocytes using protocols used previously by us for porcine MSCs (1). After seven days of culture, 57/90 (63%) of colonies for Line 1 displayed a typical fibroblast morphology, while the
Thiazovivin, a Rho kinase inhibitor, improves stemness maintenance of bovine embryo-derived pluripotent stem cells under chemically defined culture conditions

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Despite numerous reported attempts, successful isolation of genuine bovine embryonic stem cells has been rare. Previous studies have shown that Thiazovivin, a Rho-associated kinase inhibitor, improves the survival and self-renewal of human embryonic stem cells. The present study demonstrated the effect of Thiazovivin on the derivation of bovine embryo-derived pluripotent stem cells. The attachment rates of blastocyst and embryonic cell clumps onto feeder cells in the Thiazovivin treatment group were significantly higher than those of the control. The pluripotency markers of OCT4 and NANOG, and the adhesion molecule E-cadherin were increased by Thiazovivin treatment. This study suggests that Thiazovivin treatment improves the maintenance of stemness in a putative embryo-derived pluripotent stem cells population by promoting the expression of pluripotency marker genes as well as enhancing the expression of E-cadherin resulting in an increase in cell adhesion. This study was supported by a grant from the National Research Foundation of Korea (NRF-2006-2004042, and no. 2014050477 through the Oromaxillofacial Dysfunction Research Center for the Elderly at Seoul National University) and the Technology Development Program for Agriculture and Forestry, Ministry of Agriculture, Food and Rural Affairs (MAFRA; 111160-04), Republic of Korea

Grey Level Co-occurrence Matrix (GLCM): A novel method to access the texture of mouse embryos derived from assisted reproductive technology (ART)

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Recent insights suggest that the surrounding environment of the pre-implantation embryo is likely to alter long-term trajectory. The periconception environment therefore represents a critical window for programming fetal growth. This study evaluated the impact of the two major clinical components of assisted reproductive technology (ART), embryo culture and ovarian hyperstimulation, on embryo development by using three metabolic markers: Peroxyfluor 1 (PF1), to assess hydrogen peroxide levels, Monochlorobimane (MCB), to assess reduced glutathione abundance and Mitotracker Deep Red, to detect active mitochondria. In addition to assessment of the embryos based on morphology and staining intensity, textural analysis using Grey Level Co-occurrence Matrix (GLCM), a second-order statistical model was used to evaluate PF1, MCB and MTDR staining, providing a robust metric of embryo health. Embryos were collected 48, 60 and 88 h post-hCG treatment, corresponding to the 2-cell, 8-cell and blastocyst stage. Our results showed that embryos derived from ovarian hyperstimulation had significantly higher intensity and texture heterogeneity of active mitochondria and hydrogen peroxide as compared to the natural cycling embryos. Embryos exposed to embryo culture displayed variations in texture of active mitochondria, although there was no change in intensity. Our data provide strong evidence that the metabolic profiling and texture were modified in embryos derived from ART. To the best of our knowledge, this is one of the first studies to investigate the metabolic profiling using texture analysis in embryos, although the functional roles of each texture features are not yet well understood. This study supports that textural analysis provides a means of gaining additional information regarding sub-cellular analyses instead of using intensity measurements alone.
Hyperglycaemic stress increases blastomere heterogeneity in pre-implantation mouse embryos

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A characteristic of post-compaction embryo development is the formation of gap junctions that allow for the intercellular communication through the transport of ions, metabolites and signalling agents. However, it is not until after the 8-cell stage of development that mouse embryos develops these connections. Pre-compaction blastomere metabolic variability has been proposed as the basis of embryonic heterogeneity, and is hypothesised to be exacerbated by the absence of gap junctions. To examine blastomere heterogeneity as a consequence of cellular stress, we utilised a model of hyperglycaemic stress and analysed pathways involved in changes in metabolism and DNA damage and repair.

CBA F1 mice were stimulated with 5 IU eCG/5IU hCG, and cumulus oocyte complexes were collected 16 h post hCG and fertilised in vitro. One-cell embryos were cultured in either control (5.6mM glucose) or hyperglycaemic (30mM glucose) media for 20 hours until cleaved, before being metabolically assessed using the mitochondrial activity probe, Mitotracker Deep Red (MTDR, 200nM), a specific H2O2 fluorophore , peroxyfluor-1 (PF-1, 20μM) and a reduced GSH probe, monochlorobimane (MCB, 1.2.5mM). A grey-scale co-occurrence matrix (GLCM) was used for the first time on phase contrast images, to quantify its potential as an additional non-invasive method to assess embryo viability. The images were analysed for intensity and different textural features such as the degree of heterogeneity, homogeneity, smoothness and entropy using GLCM.

We found that embryos exposed to hyperglycaemic stress demonstrate a higher degree of mitochondrial activity, but not of the other metabolic measures. Phase contrast images of pre-implantation embryos revealed that hyperglycaemic embryos were more heterogeneous and were less ‘smooth’ than control embryos, supporting the hypothesis.

The reduction in melatonin level may contribute to the pathogenesis of ovarian cancer

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Introduction: Ovarian cancer is the third common gynaecological malignancy and the leading cause of death in gynaecological cancers. Studies have suggested that changes in circadian rhythms such as bright-light exposure may affect female reproductive physiology. Night shift work is associated with higher risks of breast and endometrial cancer due to lower melatonin production by the pineal gland. Other studies have suggested that the season of birth may be an important environmental risk factor for developing gynaecological cancers. Melatonin is a lipid soluble hormone whose level changes with circadian rhythm. Melatonin has multifaceted functions, including direct free radical scavenging and inhibition of cancer cell growth. However, whether there is an association between the circulating levels of melatonin and the risk of developing ovarian cancer is unclear.

Methods: Serum from women with ovarian cancer or healthy women were collected and the level of melatonin was measured. In addition, the expression of melatonin receptors (MT1 and MT2) were measured in ovarian cancer tissues by immunohistochemistry.

Results:
1. The incidence of ovarian cancer was not associated with the season of birth in women with ovarian cancer.
2. The serum levels of melatonin were significantly lower in women with ovarian cancer compared with healthy women (p<0.05). However, there was no significant difference in melatonin levels among patients who were born in spring, summer, autumn and winter.
3. Immunohistochemistry demonstrated that the expression of melatonin receptors (MT1 and MT2) was reduced in ovarian cancer tissue compared to normal ovary tissues.

Conclusion: Our results demonstrate although there is no association between the season of birth and the risk of developing ovarian cancer, the lower levels of melatonin detected in serum of women with ovarian cancer may contribute to the pathogenesis of ovarian cancer. This further supports that melatonin may be used as an adjuvant in cancer therapy.
Inhibitory peptides, including secretory leukocyte protease inhibitor, lipocalin 2, lysozyme and equine testicular inflammation may reduce damage to spermatogenesis in patients with epididymo-orchitis. Levels of activin A, TNF, MCP-1, IL-6 and IL-10 were increased (5 to 50 fold). Treatment with follistatin to attenuate activin A bioactivity early in testicular inflammation may reduce damage to spermatogenesis in patients with epididymo-orchitis.

**The influence of ovarian hormones on the uterine immune response**

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Infectious endometritis is a common pathology in many species, including horses, and can severely affect fertility. Efficiency of the uterine immune response is influenced by ovarian hormone levels throughout the oestrus cycle, but little is known about the molecular mechanisms underlying this phenomenon.

The objectives of this study were to characterise the changes in expression of immune genes in the equine endometrium caused by the introduction of *Escherichia coli* (E. coli) and to identify the transcriptional impact of ovarian hormone levels on these gene expression changes.

Thus, endometrial biopsies were collected from five horses before and at several time points after the inoculation of *E. coli* once in oestrus (follicle >35 mm in diameter, presence of uterine oedema) and once in dioestrus (5 days after ovulation). Absence of inflammatory signs was confirmed between treatments. Transcription in biopsies taken before and 3 hours after inoculation with bacteria was analysed using high-throughput RNA sequencing (RNA-Seq). Guided by these results, genes involved in the uterine immune response were further analysed at additional time points using quantitative polymerase chain reactions (qPCR) to quantify their expression levels until 3 days post infection.

By 3 hours after the introduction of bacteria, almost 2500 and 1500 genes were expressed at significantly higher levels compared to pre inoculation levels in oestrus and dioestrus, respectively. These included pathogen recognition receptors, particularly toll-like receptors TLR2 and 4 and NOD-like receptor NLRC5, genes for chemokines, including CCL2, CXCL9, 10 and 11 and those for antimicrobial peptides, including secretory leukocyte protease inhibitor, lipocalin 2, lysozyme and equine β-defensin 1. Further studies will characterize these genes at later time points post inoculation.

In-depth analysis of the uterine innate immune response will help to improve fertility in horses, but potentially also in other domestic animal species and humans.

**Testicular activin A during the development of autoimmune orchitis in mice**

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Experimental autoimmune epididymo-orchitis (EAEO) is a rodent model of chronic testicular inflammation that reproduces the pathology observed in some human infertility. Activin A, a dimer encoded by the inhibin βA (*Inhba*) gene is a pro-inflammatory, profibrotic cytokine, but also regulates spermatogenesis and steroidogenesis. The roles of activin A, inhibin and follistatin, both endogenous activin antagonists, were examined in EAEO.

EAEO was induced in adult mice by active immunization with syngeneic testicular homogenates (3 injections, 2 weeks apart) in complete Freund’s adjuvant (CFA) and *Bordetella pertussis* toxin. Controls received only CFA and toxin. Testes collected 25, 50 and 80 days after the first immunization were processed for histology and immunohistochemistry or frozen for qRT-PCR.

Age-matched untreated mice and controls showed no pathology, with activin A localised to Sertoli cells and interstitial cells. All immunised mice developed EAEO by 50 days, characterised by a >50% reduction in testis weight, complete loss of germ cells, immune infiltrates (macrophages and T cells) and a marked peritubular fibrotic response. These were accompanied by increased expression of inflammatory cytokines, tumour necrosis factor (TNF), macrophage chemoattractant protein-1 (MCP-1) and interleukin-10 (IL-10). Activin A immunostaining was not detectable in the EAEO testes, but the inhibin βA (*Inhba*) subunit encoding activin A and follistatin mRNA levels were similar to controls. Expression of *Inhba* mRNA and mRNAs encoding the activin receptors, Acvr1b and Acvr2b, were reduced. At 25 days, before observed testicular pathology, the testicular levels of activin A, TNF, MCP-1, IL-6 and IL-10 were increased (5 to 50 fold). These data suggest that activin A acts as a pro-inflammatory agent in EAEO, but in mice with fully established EAEO, activin A levels decreased perhaps due to the testicular damage. Treatment with follistatin to attenuate activin A bioactivity early in testicular inflammation may reduce damage to spermatogenesis in patients with epididymo-orchitis.
Anti-Müllerian Hormone (AMH) has an increased rate of conversion to the active form after puberty.

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Anti-Müllerian Hormone (AMH) is a TGFβ superfamily member with multiple roles in reproductive biology. AMH induces regression of the Müllerian duct in male foetuses and has roles in testicular development. AMH negatively regulates ovarian follicular development in females. We have determined that AMH in blood consists of a precursor form (proAMH) and the receptor-competent form (AMH$_{N,C}$). Commercially available assays do not differentiate the two forms and most AMH measurements are an aggregate of proAMH and AMH$_{N,C}$ (total AMH). We have developed a proAMH-specific assay to generate the first description of the relative quantities of proAMH and AMH$_{N,C}$ in the normal population. An AMH prohormone index has been calculated from this data (API, [proAMH]/[total AMH] x 100) which represents proAMH as a percentage of total AMH. ProAMH concentrations were significantly higher in prepubertal boys (n=131) relative to men (n=80) (p = 0.000). Prepubertal girls (n=14) also had higher proAMH concentrations relative to women (n=18) (p = 0.032). The mean API of boys was approximately 2-fold higher than in men with no overlap between the ranges of each group (p = 0.000). The total AMH levels in girls and women were not significantly different but the mean API in girls was significantly greater than in women (p = 0.000). These data suggest that there is increased processing of proAMH into receptor-competent AMH$_{N,C}$ after puberty, implying a greater proportion of AMH is active. The cleavage enzymes for AMH facilitate activation of multiple gonadal regulators, hence these findings may have wider implications for gonadal regulation during development.

Hypothalamic endoplasmic reticulum stress disrupts estradiol production, ovulation and cyclicity in a novel obese mouse model-Blobby mouse.

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In Utero Exposure to the Insulin Sensitising Drug Metformin Reduces the Fertility of Male Offspring

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Metformin is a drug frequently used during pregnancy in the treatment of type 2 diabetes and disorders associated with insulin resistance. Most studies have focused efforts towards the effects of metformin on neural function and locomotion after birth, with few studies having investigated the consequences of in utero exposure to metformin during embryo or fetal development. Consequently a paucity of data exists aimed at understanding the effects of metformin on the reproductive function of offspring. The aim of the present study was to assess the effects of maternal metformin administration during pregnancy on the fertility of male offspring. Sperm quality analysis and immunohistochemistry were performed to measure these effects. A significant reduction of about 25% was observed in litter size from those males exposed to metformin in utero when compared to control males. We found no differences in testis size at puberty (25dpp: days post-partum) or at the adult stage (90dpp). This is contrary to our previous results which showed a decrease in fetal and neonatal testis size following in utero metformin exposure, and suggests a gradual return to normal growth after birth for the testes. Compared with controls, metformin exposed males had a reduction in seminiferous tubule diameter (141±1µm and 133±1µm, respectively;P<0.05), and germ cell number per seminiferous tubule (65±2 and 57±2, respectively;P<0.05) at 25dpp. There was a significant increase in the number of sperm head abnormalities from males exposed to metformin in utero. However, there were no differences in sperm mobility between groups. Moreover, intratesticular testosterone concentration remained unchanged, whereas in utero exposed males had lower LH concentrations in the pituitary. Exposed adult males presented with significantly more visceral adipose tissue. In conclusion we have shown that embryo/fetal metformin exposure has consequences on the fertility of male offspring, principally by affecting the quality of sperm.
Transcriptome analysis of the developing phallus after hormonal manipulation in a marsupial

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The molecular control of phallus development in males is still poorly understood, despite the fact that defects of penile development, such as hypospadias, are amongst the commonest developmental defects in newborn boys and the importance of the penis for reproductive function. In this study we investigated the molecular effects of hormonal manipulation of phallus development in the developing tammar wallaby.

We treated male tammar young with oestradiol-17β or castrated them at day 25 pp, to suppress normal penis development. We stimulated penis development in female young using androstanediol treatment from day 25 pp (1, 2). Phalluses of treated and control (untreated) young were sampled at autopsy at day 50 pp when the first macroscopic signs of sexually dimorphic phallus development are seen. Transcriptomes (pooled samples) were generated using RNA-seq. Transcripts were considered to be differentially expressed if there was more than a 2-fold difference in expression between treatments. Of the differentially expressed protein coding genes, most were classified as protein binding or catalytic enzymes. Expression of key regulators of penile development such as SHH, GLI2, β-catenin and EFNB2 were down-regulated in the male phallus after oestradiol-17β treatment and castration and up-regulated in female phallus after androstanediol treatment. Surprisingly, more than 97% of differentially expressed transcripts were predicted to be lncRNAs. Several coding gene-neighbouring long-non coding RNAs were identified. An IncRNA neighbouring MAFB was down-regulated in males by oestradiol-17β treatment and also by castration. Another IncRNA neighbouring EFNB2 was differentially expressed between male and female phalluses, but there was no alteration in expression after any of the experimental treatments, suggesting that this sex difference might be independent of androgen. In summary, both the expression pattern of key regulators and their neighbouring IncRNAs are sexually dimorphic and can be disrupted by a changing endocrine environment during phallus development.

2. Leihy, MW, Shaw, G, Wilson, JD, and Renfree, MB 2004 Penile development is initiated in the tammar wallaby pouch young during the period when 5α-androstan-3α, 17β-diol is secreted by the testes. Endocrinology 145 3346-3352.

Proteins expression in caput and corpus epididymis of Akodon cursor (Rodentia, Cricetidae)

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A combination of growth factors is sufficient to promote testis development in the absence of Sry.

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Whether an individual develops as male or female is determined by commitment of the developing gonad to testicular or ovarian development. In mammals this decision is made when SRY is expressed in the pre-supporting cells of the bipotential gonad. SRY initiates expression of SOX9 and down-stream targets including FGFI. FGFI promotes proliferation of the developing Sertoli cells, but is not considered sufficient to drive testis development. In this study we demonstrate that a combination of growth factors, including FGFI, Activin and TGFβ are sufficient to initiate testicular development, including the repression of the ovarian development, expression of key testis development genes, morphological reorganisation of the gonad and formation of laminin delineated testis cords. In addition, we demonstrate that facilitating β-catenin by blocking GSK opposes the testis-promoting activity of FGF9, Activin and TGFβ. Since development of the germline is strongly affected by signaling from the somatic cells including FGFI, we examined the impacts of FGF9 and FGFI, Activin and TGFβ on expression of male germline markers, pluripotency and the cell cycle regulation in germ cells, demonstrating that while FGF9 promotes male germline markers, the combination of FGFI, Activin and TGFβ maintain germline pluripotency. This study provides the first evidence that male sex-determination can be induced by a combination of growth factors in the absence of Sry. These findings have implications not only for understanding sex-determination in mammals, but also for non-mammalian species that do not have Sry.
Activin over-expression and subsequent tumour development is associated with androgen deficiency and structural alterations in the reproductive tract of adult male Inha−/− mice

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Activins, members of the Transforming Growth Factor-β (TGF-β) superfamily of cytokines, are dimers of the inhibit βA- or βB-subunits. Functionally, the inhibins, which are heterodimers of one β-subunit and an α-subunit, are activin inhibitors. Within the male reproductive tract, expression of activin A is highest in the caput epididymis, but is extremely low in the vas deferens, while the converse is true for its binding protein, follistatin. This suggests that differential expression of activin A and follistatin may be involved in maintaining the regionalised structure and function of the male reproductive tract. The inhibin α-subunit gene knockout mouse (Inha−/−) lacks inhibin, and develops testicular tumours from about 4 weeks of age, leading to progressive testicular damage. These mice had very high levels of activin A and B, as well as elevated follistatin, in the serum and testes. At 8-10 weeks of age, Inha−/− epididymal weight was reduced by 50% compared with Inha+/+ control mice. Sperm was absent or very low in the epididymis and vas deferens of the Inha−/− mice, and the ductal epithelium of these tissues were regressed, with increased fibrosis in the stroma around the ducts. Seminal vesicle weight and serum testosterone levels were considerably reduced, but luteinizing hormone (LH) levels were paradoxically normal. Although serum inhibin was reduced by 30% in the heterozygous Inha+/− mice, serum and testicular activins, follistatin and testosterone were not altered, and the epididymis and vas deferens appeared morphologically normal. In conclusion, deletion of the inhibin α-subunit gene leads to over-expression of both activin A and B, and androgen deficiency, although with normal LH. Regression of the epididymis and vas deferens also occurs, but it is not clear whether this is a direct result of activin overexpression, or loss of testicular function associated with tumour development, or both.

Quantification of granulosa cells in mouse ovarian follicles

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Chemotherapy often decreases antimullerian hormone levels and fertility in vivo, but effects on follicular granulosa cells are poorly characterized. Granulosa cell (GC) numbers in freshly isolated follicles can be used as baseline control values to develop less gonadotoxic chemotherapeutics. A review of 3 published reports indicated that GC/follicle numbers calculated from fixated ovarian follicles were 2100 (primary), 61-200 (secondary) and 201-600 (antral). We aimed to confirm these numbers using freshly isolated whole mouse follicles.

Mouse ovaries (n=5) were disaggregated with 2mg/mL collagenase IV. Isolated follicles were stained with DAPI or Calcein AM & Ethidium Homodimer-1 (‘Live-Dead’ stain) before fixation. The diameters and GC numbers of M1 (viable regular morphology) or M2 (viable irregular morphology) DAPI-stained follicles (n=215) were determined using fluorescent microscopy. Image J software and nuclear area. These data were validated by confocal microscopy, in which additional DAPI or Live-Dead stained M1 follicles from each size cohort were examined. Confidence intervals were calculated and data subjected to 1-way ANOVA with Tukey post-test.

The area of Homodimer-1 stained GC nuclei (n=60) was 15.68±3.26 μm². Follicle diameters were 63±13μm (n=65), 120±20μm (n=87) and 196±32μm (n=63), and GC/follicle numbers were 73±33 (p<0.05), 197±67 (p<0.05) and 431±163 for high quality viable M1&M2 primary, secondary and antral follicles respectively.

We found that GC/follicle numbers were slightly higher than values obtained previously: 65-82 v 21-60 in primary, 183-211 v 61-200 in secondary and 390-472 v 201-600 GC in antral mouse follicles. This might be because follicles are not perfect spheres but have a polar orientation with irregular GC distribution, meaning that GC counted in the histological section containing the oocyte nucleus would underestimate GC/follicle numbers. Our method incorporates fluorescence from all DAPI or Homodimer-1 stained GC nuclei, irrespective of location in the follicle, and is therefore likely to be more accurate.

The effect of Gas5 IncRNA on oocyte maturation and embryo development

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Female fertility is dependent on a number of factors, one of which is oocyte quality. The developing oocyte is highly susceptible to environmental stresses that have detrimental effects on oocyte and as embryo competence. During oocyte maturation, somatic cells (granulosa, cumulus cells) promote developmental competence of the oocyte. Recently, we identified the presence of GASS, a long non-coding RNA, in human cumulus cells. In other systems, GASS regulates important cell survival pathways during stress conditions. In particular, progesterone and glucocorticoid receptors, which are known to be of great importance in female reproduction, are modulated by interacting with exon 12 of the GASS transcripts. However, the role of GASS in oocyte maturation and early embryo development still remains unknown. Using a PCR strategy, with cloning and sequencing, we have identified several prominent Gas5 transcript variants present in mouse granulosa cells, cumulus-oocyte complexes and pre-implantation embryos up to blastocyst stage. The abundance and differential splicing of Gas5 isoforms are modulated in COCs and embryos. Sequence analysis indicated that among the 12 exons in the full length gene, novel transcripts which include exon 12 but have exon 7 spliced out was the most common isoform. Additionally, using qPCR, we have quantitated the expression of Gas5 during the peri-ovulatory period, and found that Gas5 was significantly up-regulated at 8h post-hCG stimulation, with the majority of expression occurring in the granulosa cells. These findings are among the first to identify the importance of long non-coding RNA in reproductive outcomes and will contribute to our building knowledge on the impact of stresses on female fertility.

Effect of ovarian disaggregation on murine follicle yield and quality

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The development of fertility preservation protocols for oncology patients requires the isolation of follicles from ovarian tissue for in vitro maturation. Ovarian mechanical disaggregation using needles is time-consuming compared to disaggregation using enzymes such as collagenase IV (Col-IV), or FDA-approved purified collagenase ‘Liberase’. Ovarian disaggregation requires optimisation to maximise follicle yield whilst minimising damage. Follicle damage can be evaluated in a DAPI-stained follicle grading system that defines M1 follicles as having viable normal morphology, and M4 as non-viable abnormal morphology. We aimed to optimise follicle harvest and test a newly available animal origin free (AOF) collagenase IV, which has the potential for TGA approval.

The ovaries from 3 month mice (n=7) were halved, weighed, and disaggregated using Col-IV (590U/mL), or AOF590U/mL or AOF1180U/mL. Control half-ovaries were disaggregated mechanically without enzyme. Isolated follicles were stained with DAPI and CMXRos, and images were captured with an Olympus Brightfield BX50 microscope & Micro-Manager software. Follicular diameters and staining were measured using Image J. Follicle yields analysed by 1-way ANOVA, and follicle quality grades by 2-way ANOVA with Bonferroni post-test.

The ovarian weights were 6.6±2.3mg. Most of the follicles were secondary (65%)-antral (29%) >primary (6%). Follicle yields were similar for all disaggregation methods; control 13±7 follicles/ mg ovarian tissue, Col-IV 17±10, AOF590u/mL 15±11, AOF1180u/mL 13±3. For each mouse, the highest number of M1(4.6±2.6) and M2(4.7±1.6) follicles were obtained after Col-IV disaggregation, and the lowest M1(2.4±2.5) and M2(2.7±1.9) after mechanical disaggregation. AOF590u/mL yields were M1(3.7±2.2) , M2(3.3±2.5), M3(3.7±2.5) and M4(3±1.7) follicles/mg. Previously yields were 30-40 follicles per immature mouse ovary whereas our yields from more fibrous adult ovaries were higher, ~90 follicles/ovary. Our method ‘selected’ for secondary follicles, and did not yield a population representative of the original tissue. Col-IV disaggregation yielded higher proportions of high quality M1&M2 follicles than the AOF preparation.


The effects of L-carnitine treatment prior to IVF on porcine oocyte maturation and post-fertilization events

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In vitro matured (IVM) porcine oocytes utilize less endogenous lipid and have poorer developmental competence than those matured in vivo. Previous studies have indicated that oocyte developmental competence may be improved by supplementing IVM medium with L-carnitine, which stimulates lipid metabolism and has antioxidant properties. The objective of this study was to determine the effects of L-carnitine on porcine oocyte maturation and post-fertilization events. Porcine Oocyte Medium was supplemented without (control) or with 12mM L-carnitine (LC) and/or 100 µM etomoxir (Etox), an inhibitor of lipid metabolism, during the final 22h of IVM. Treatment effects on cumulus-oocyte complexes (COCs) and denuded oocytes (DOs) were compared. The concentrations of ATP and glutathione (GSH) were measured at 22 and 44h of IVM in a cohort of oocytes. After IVF, presumptive zygotes were either stained to assess pronuclear (PN) formation, or cultured for 7d to assess embryo development.

The levels of both ATP and GSH in DOs were about 40% lower than those in COCs (P<0.05). In both COCs and DOs, exposure to LC did not alter the ATP levels compared with the untreated controls (P>0.05), whereas exposure to LC+Etox suppressed them (P<0.05). The concentration of GSH in LC-treated oocytes was greater than that of control oocytes (8.20 vs 7.19 pmol/oocyte; P<0.05), which in turn was greater than that of LC+Etox-treated oocytes (7.19 vs 5.44 pmol/oocyte; P<0.05). Also, the PN formation rate was significantly reduced in control DOs (30%), but not in LC-treated DOs (61%), compared with the control and LC-treated COCs (87 and 90%, respectively). Blastocyst formation rates of the Etox and LC+Etox groups were significantly lower than those of the control and LC groups. The results show that the LC treatment improved the maturation of denuded oocytes through an antioxidant protective action, and not via an overall enhancement of ATP production.

**Verification of Connexin43 in porcine oocytes during in vitro maturation**

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Many studies of the main gap junction protein, Connexin43(Cx43), have been explored in porcine oocyte research, but most of these studies have been limited to investigations of cumulus-oocyte complexes(COCs). In this study, we verified Cx43 not in COCs, but in porcine oocytes during maturation, and conducted a quantitative time course analysis. The location and dynamics of Cx43 were examined by immunocytochemistry and western blotting, respectively. COCs were cultured in NCSU23 media and processed for immunocytochemistry and western blotting at 0, 14, 28, and 42h after denuding. In addition, to distinguish whether the tip shaped Cx43 singal near transzonal projections are embedded on oolemma or just exist inside of zona pellucida, we softened zona pellucida by treating oocytes with 3 sec to completely divide zona pellucida from plasma membrane. Subsequently we proceeded immunocytochemistry. In result, Cx43 signal was detected on oolemmas, transzonal projections and the surface of zona pellucida. Western blotting showed that Cx43 band density increased from 0 to 14 h, and gradually decreased thereafter. Our results clarified that Cx43 is localized in the ooplasmic membrane through zona pellucida and its level changes over time during culture in porcine oocytes.

**BMF regulates primordial follicle loss during adolescence**

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In mammalian ovaries, the primordial follicle pool, known as the ovarian reserve, determines female fertility and reproductive lifespan. Cell death during ovarian development in the embryo plays a critical role in determining how many primordial follicles are established within the ovary. While much attention has focussed on the apoptotic elimination of germ cells prior to and during primordial follicle assembly, the precise mechanisms that govern the steady postnatal depletion of primordial follicles after their initial endowment remain uncharacterised. In particular, there are few studies that address follicular dynamics and primordial follicle loss in adolescence. In this study we investigated the role of Bcl-2 modifying factor (BMF), a pro-apoptotic BH3-only protein belonging to the BCL-2 superfamily, in regulating primordial follicle loss in prepubertal, adolescent and adult mice (postnatal (PN) 20-100). Primordial follicle numbers were comparable in ovaries from WT and Bmf−/− at PN20, 30 and 40 (WT 4641.0 ± 404.7 vs Bmf−/− 4146.8 ± 420.5 follicles/ovary, P=0.42; WT 3899.1 ± 249.3 vs Bmf−/− 3943.6 ± 339.2 follicles/ovary, P=0.92; WT 3804.5 ± 286.9 vs Bmf−/− 5254.0 ± 1062.7 follicles/ovary, P=0.15). However, a 50% reduction in the number of primordial follicles was observed in ovaries from WT mice between PN40 and PN50 (WT PN40 3804.5 ± 286.9 vs WT PN50 2058.8 ± 277.1 follicles/ovary, P<0.002), while a reduction in 25% of primordial follicles was observed in ovaries from Bmf−/− mice during this same time period (Bmf−/− PN40 5254.0 ± 1062.7 vs Bmf−/− PN50 3316.3 ± 428.4 follicles/ovary, P<0.05).

Collectively, these data indicate that primordial follicles are lost in significant numbers during the transition to adulthood and that BMF is required for this process. The reason for the elimination for such large numbers of primordial follicles immediately prior to the establishment of adult ovarian reserve is unknown and will be the focus of future investigations.
The effect of impaired function of BMPs by passive immunization on the protein expression of BMPR1B, FSHR and LHR in the mouse ovary

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The number and quality of ovarian follicles are important in determining the longevity and integrity of female fertility. It is well known that the survival and development of these follicles including ovulation, which results in the release of viable oocytes ready for fertilization, are controlled primarily by the gonadotropins. However, there is increasing evidence which suggests that there are other factors such as the bone morphogenetic proteins (BMPs) which co-regulate ovarian function along with gonadotropins.

In our recent studies in an attempt to shed light on the mechanism of action of BMPs, we have created an in vivo mouse model with attenuated BMP signalling using passive immunization against BMPR1B and BMP4. The aim of this study was to investigate the localization of BMP receptor 1B (BMPR1B), FSHR and LHR in the ovaries of mice treated with anti-BMPR1B, and anti-BMP4 with and without exogenous gonadotropins (eCG).

BMPR1B was expressed in all follicle stages, FSHR was detected in primary follicles onward and LHR was absent in primary follicles but appeared in later stages. Quantitative analysis based on the intensity of fluorescent signals showed that the expression of BMPR1B, FSHR and LHR significantly increased in the granulosa cells of the pre-ovulatory and secondary follicles in mice treated with anti-BMPR1B.

Mice treated with anti-BMP4 show that the expression of BMPR1B and FSHR but not LHR increased significantly in pre-ovulatory follicles only with no effects observed in any other stages. The pre-ovulatory follicles in mice treated with eCG showed increased BMPR1B and FSHR but not LHR expression.

These results together with our previous reports in sheep and mice confirm that the attenuation of BMP signalling system can be an effective approach to sustain the development of growing follicles, ovulation and consequently overall female fertility.

Expression profile of reproductive receptors during aging of the human ovarian follicle

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Reproductive ageing is linked to the depletion of ovarian primordial follicles, that causes an irreversible change to ovarian cellular function and ultimately reduces the capacity to reproduce. Our recent research has highlighted the role of bone morphogenetic protein (BMP) signalling in the regulation of the ovulation rate in sheep, and has led us to further investigate the molecular regulation of folliculogenesis by the BMPs (Regan et al. 2015). The current study aimed to profile the expression of bone morphogenetic protein receptor (BMPR1B), follicle stimulating hormone receptor (FSHR), luteinising hormone receptor (LHR), and growth hormone receptor (GHR) and also levels of apoptosis in IVF patients (101), in a range of ovarian primordial follicle depletion. An average of 8000 granulosa cells/follicle was analysed, and the follicles ranged in diameter from 4-27 mm. The granulosa cell, surface-expressed mature receptor protein density was measured by immunofluorescent labelling via flow cytometry. Ovarian reserve was measured indirectly by the antral follicle count (AFC). AFC is the number of follicles between 2-10 mm present on day 2-5 of a cycle.

A decline in granulosal BMPR1B and FSHR density occurred at the time of cyclic dominant follicle selection, and again during the terminal stage of folliculogenesis in the ‘good ovarian reserve’ IVF patients (23-30 years (y)). The older ‘poor ovarian reserve’ patients (40+ y) experienced a reversal of this pattern. The LHR density failed to be down-regulated during pre-ovulatory maturation in the 40+ y group, and GHR density was reduced with ovarian ageing. The level of apoptosis was reduced with ovarian reserve depletion.

The study’s results demonstrate the disrupting effect that age-induced depletion of the ovarian reserve has on receptor density at the two stage-specific, critical time points of dominant follicle selection and pre-ovulatory maturation. The dysregulation is potentially responsible for the reduction in oocyte quality in older patients.

A Sensitive Mass Spectrometry Method for the Simultaneous Quantification of Adenosine Nucleotides in Oocytes and Granulosa Cells

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Oocyte energy metabolism is important for its developmental capacity and the nucleotides ATP, ADP and AMP play critical roles as energy currency of biological reactions. During the early stages of oocyte maturation, cyclic AMP (cAMP) is hydrolysed to AMP which we hypothesize can be recycled through the adenosine salvage pathway to ADP and then ATP, as an alternate energy generating pathway. Significant changes in the amount of these nucleotides may also impact oocyte energy charge (EC), an index used to measure a cell’s energy status through the assessment of the energy stored in its adenylate system. To facilitate the study of oocyte adenosine nucleotide metabolism, we developed a highly selective and sensitive LC-MS/MS method for the simultaneous quantification of low nanomolar to micromolar concentrations of adenosine, ATP, ADP, AMP, and cAMP. Metabolites were separated on a porous graphic carbon column, which offered superior retention and chromatographic resolution, however rigorous conditioning protocols were required to ensure repeatability. The method was validated using αMEM culture media containing 0.3% BSA as sample matrix. The method was linear from 5nM to 10μM for all analytes with correlation coefficients above 0.996. The recovery ranged from 77% to 107% from 20nM to 5μM. Precision (%CV) was below 15% from 50nM. The limits of detection and quantification were 5nM and 10nM, respectively. The method was successfully applied to quantify nucleotides in COV434 granulosa cell conditioned media and cell extract. The mitochondrial uncoupler CCCP decreased nucleotide levels in media 2.5-fold, and cAMP modulators forskolin and IBMX increased cAMP secretion 14-fold. Nucleotide concentrations from these samples ranged between 5-90nM. Simultaneous quantification of ATP, ADP and AMP enabled calculation of the EC. Mouse oocyte EC was 0.88-0.92 throughout maturation, indicating that the EC is buffered. This novel mass spectrometry method will allow detailed interrogation of energy generating systems in oocytes.

The pan-sirtuin inhibitor, nicotinamide, disrupts the meiosis I-to-meiosis II transition in mouse oocytes

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In oocytes, exit from meiosis I (MI) is immediately followed by entry into meiosis II (MII) after which oocytes arrests at metaphase II awaiting fertilization. This unique MI-to-MII transition necessitates partial inactivation of cyclin-dependent kinase 1 (Cdk1) brought about by inhibitory Cdk1 phosphorylation and destruction of the Cdk1 co-activator, cyclin B1, mediated by the anaphase-promoting complex (APC). Following extrusion of the first polar body (PBE), which marks exit from MI, re-establishing and maintaining Cdk1 activity is important for assembling a fully formed bipolar spindle with aligned condensed chromosomes typical of metaphase II arrest. Sirtuins are NAD+-dependent deacetylases that are key for multiple cellular processes. Here we investigate the effect of the pan-sirtuin inhibitor, nicotinamide (NAM), on mouse oocyte maturation. Culturing oocytes in 10mM NAM during MI had no effect on rates or timing of PBE or on spindle assembly during MI. Unexpectedly however, examination of NAM-treated oocytes after PBE had occurred revealed that the majority (70%) lacked a bipolar spindle and contained a nucleus with decondensed chromosomes. Treatment with either NAM or the Cdk1 inhibitor, flavopiridol (5μM), after PBE had occurred did not reproduce the phenotype. However, an identical phenotype was observed when flavopiridol was used specifically during exit from MI prior to PBE altogether suggesting that NAM impaired establishment rather than maintenance of MI arrest. We further found that relative to controls, inhibitory Cdk1 phosphorylation was higher and cyclin B1 levels declined lower during MI exit in NAM-treated oocytes. One possible reason for reduced cyclin B1 levels with NAM treatment may be related to increased APC-mediated proteolysis as we also found increased levels of the APC co-activator, Cdc20, during MI exit. Collectively, these results indicate that NAM-induced sirtuin inhibition led to excessive Cdk1 inactivation during MI exit thereby causing oocytes to exit meiosis into an interphase-like state.

Insulin impacts cumulus oocyte complex maturation and early embryo development in vitro

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Insulin is a vital molecular component of mammalian glucose control with resting levels changing significantly in patients suffering from diabetes mellitus. As a sole genetic cause of diabetes has been ruled out and some research is leading towards
epigenetic causes; understanding the impact to the pre-implantation embryo is becoming a major component of diabetic research. Many of these studies are performed in vitro and interestingly, to date, no known studies have investigated the effect of varying levels of insulin and have only focused on varying glucose levels. The research performed here studies embryonic development using cumulus oocyte complexes derived from PMSG stimulated mice at 46 h before undergoing in vitro maturation (IVM) in varying levels of insulin (0.17pM, 1.7pM, 170pM and 1700pM). IVM is also performed at these insulin levels both with and without the presence of glucose (30mM) to mimic different stages and types of diabetes mellitus, before in vitro-fertilisation occurs. Analysing cumulus expansion using the cumulus expansion index (17h post FSH in vitro), cleavage rate at 2-cell (24 hours post fertilisation) and blastocyst rate (4 days post fertilisation) we identified that the presence of insulin significantly reduced the cleavage rate at all concentrations compared to the control without insulin (p=0.49), while only significantly reducing cumulus expansion at the lowest level of 0.17pM (p=0.038). There was no effect of either insulin or glucose on blastocyst rate; however the addition of high glucose resulted in a significantly higher cumulus (p=0.047) expansion score and a lower cleavage rate (p=0.002) supporting previous studies work. These findings suggest that considering insulin as well as glucose in epigenetic research of diabetes may be a priority and provides reasoning to further investigate the causes of the impact of insulin such as; gene expression and metabolic activity.

Plasma Anti-Mullerian Hormone is related to oestrus expression in lactating sows

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In the pig industry, stimulating a fertile oestrus during lactation allows piglet weaning ages to be increased without impairing foetal life vascular and metabolic diseases. Identification of lactation oestrus and its cause is a major concern in the pig industry. Mullerian Hormone (AMH) concentration is a reliable indicator of ovarian reserve in many species and plasma AMH is positively related to puberty response in pigs (Reed et al. 2013). We hypothesised that lactating sows with high AMH on day 18 post-parturition will be more likely to express lactation oestrus.

On day 18 post-parturition, 54 Large White x Landrace lactating multiparous sows had a blood sample collected and commenced daily 15 minute boar contact. Sows were artificially inseminated at first detection of lactation oestrus and slaughtered 30 days post-insемination. Ovulation rate and embryo number were recorded. Embryo survival was calculated as the number of embryos as a proportion of corpora lutea. Plasma AMH was measured with the Human MIS/AMH Duoset ELISA Kit (Beckman Coulter, Roissy, France) previously validated for use in bovine. Sows were divided into HIGH (39.2 ± 5.3 ng/mL) or LOW (5.4 ± 5.5 ng/mL) AMH groups using the median value of AMH. An ANOVA was used to determine the effects of HIGH or LOW AMH on all variables (GenStat Version 11, VSN International, England, UK).

More sows with HIGH AMH expressed a lactation oestrus (96% versus 67%, P<0.01). HIGH AMH sows also had a higher ovulation rate (23.7 ± 0.7 versus 21.4 ± 0.9; P<0.05); however, plasma AMH did not affect embryo survival (HIGH AMH, 62.8 ± 4.6%, versus LOW AMH, 63.3 ± 5.7%; P>0.05). Therefore, this experiment shows that plasma AMH on day 18 post-parturition is a good indicator of sows that will exhibit oestrus in lactation.

REFERENCES


INSR rs2059806 polymorphism and the risk of preeclampsia

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Introduction: Preeclampsia is a pregnancy specific disease that occurs in 2-8% of pregnancies and is a leading cause of maternal morbidity and mortality. Increasing evidence suggests that the effects of preeclampsia on a woman's health are not restricted to the pregnancy but that preeclampsia could represent a risk factor for later life vascular and metabolic diseases. The INSR rs2059806 single nucleotide polymorphism (SNP) is a risk factor for essential hypertension, type 2 diabetes and metabolic syndrome. We investigated the association of this polymorphism with preeclampsia.

Methods: The association of the INSR rs2059806 SNP with preeclampsia was tested in 123 Caucasian preeclamptic women and 1185 controls and replicated in an independent cohort of 175 Sinhalese preeclamptic women and 171 controls. The Caucasian women were recruited from the SCOPE study in Adelaide and Auckland and the Sinhalese women were recruited in Colombo, Sri Lanka. Preeclampsia was diagnosed using international guidelines. The controls consisted of women who had...
uncomplicated pregnancies. DNA was extracted from peripheral blood collected from women and was genotyped using the Sequenom MassARRAY system. The genotype frequencies of the preeclamptic women were compared with controls using chi squared test.

**Results:** In the Caucasian cohort, the prevalence of the *INSR* rs2059806 AA genotype was increased among preeclamptic women [OR(95%CI)=3.1(1.6-5.8), p=0.003). In the Sinhalese cohort, the prevalence of the *INSR* rs2059806 AA genotype was increased among preeclamptic women who delivered small for gestational infants [OR(95%CI)=2.8(1.0-7.4), p=0.03].

**Conclusion:** The *INSR* rs2059806 SNP previously associated with adult vascular and metabolic diseases is also associated with preeclampsia in two independent cohorts. These findings suggest that genetic susceptibility may be implicated in the link between preeclampsia and subsequent vascular and metabolic diseases.

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**Pseudopregnancy Induction in the Spiny Mouse (Acomys cahirinus)**

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**Background:** The spiny mouse is a precocial rodent, particularly comparable in relative gestation length, fetal development and embryo cleavage rate to that of humans. Utilisation of such a model will enable us to undertake comprehensive embryological studies; the ultimate goal to achieve improved success rates of IVF. To do so, we require a robust technique to induce pseudopregnancy. **Aims:** We sought to trial 3 protocols previously used in rodents in spiny mice. We hypothesised progesterone would induce decidualisation, establishing pseudopregnancy. **Methods:** Females aged between 90-150 days were divided randomly into one of several groups (Table 1). Spiny mice were deemed pseudopregnant if presenting with an extended luteal phase, characterised by >4 consecutive days of leukocytic smears.

**Table 1:** Treatment groups and vaginal smear protocols for inducing pseudopregnancy

<table>
<thead>
<tr>
<th>Group # Subjects (n)</th>
<th>Treatment</th>
<th>Smears Conducted</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Control</td>
<td>Every two days</td>
</tr>
<tr>
<td>2</td>
<td>Progesterone 4mg</td>
<td>Daily after treatment</td>
</tr>
<tr>
<td>3</td>
<td>Progesterone 4mg</td>
<td>Day 3 onwards after treatment</td>
</tr>
<tr>
<td>4</td>
<td>Mechanical Stimulation</td>
<td>Daily</td>
</tr>
<tr>
<td>5</td>
<td>Mechanical Stimulation</td>
<td>Day 3</td>
</tr>
<tr>
<td>6</td>
<td>Sterile Mating</td>
<td>Daily</td>
</tr>
<tr>
<td>7</td>
<td>Sterile Mating</td>
<td>Day 3</td>
</tr>
<tr>
<td>8</td>
<td>Progesterone 2 mg</td>
<td>Day 3</td>
</tr>
<tr>
<td>9</td>
<td>Progesterone 5 mg</td>
<td>Day 3</td>
</tr>
</tbody>
</table>

**Results:** The average length of luteal phase in untreated animals was 2.8 ± 0.2 days. This was significantly prolonged by 3-5 days in most groups, excluding 2 and 5. Though the luteal phase was prolonged in 7, 50% of subjects exhibited delayed pseudopregnancy. 40% of subjects from 6 experienced prolonged oestrus by 1-2 days. **Conclusion:** We found a single dose of progesterone (2-5 mg) was the most efficacious method of immediate pseudopregnancy induction. Altered concentrations did not have an effect on luteal phase length, hence any dosage within this range may be used. Ongoing analysis will examine hormonal profiles. Embryo transfers will be conducted to confirm protocol success.

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**Predicting Pregnancy Complications from Maternal Buffy Coat DNA at 15 Weeks Gestation**

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Pregnancy complications such as preeclampsia (PE), preterm birth (PTB), small for gestational age (SGA), and gestational diabetes mellitus (GDM) occur in 25% of first pregnancies and can threaten the health of mother and/or baby. Currently, no reliable biomarkers exist in clinical practice that can predict which women are likely to have a complicated pregnancy. This is particularly important in first pregnancies where there is no prior pregnancy history to inform the clinician. The SCOPE (SCreening fOr Pregnancy EndpointS) international consortium has a biobank and detailed clinical and lifestyle database for nearly 6,000 women pregnant for the first time. In Adelaide, 1169 women were recruited prospectively with detailed clinical and lifestyle information, biological samples from mother, baby and father, as well as the known outcome of the pregnancy. Of the 1169 Adelaide SCOPE women 861 had uncomplicated pregnancies, 93 developed PE, 95 delivered SGA babies, 69 delivered preterm and 51 had GDM. Since the mechanisms by which environmental factors alter gene expression are thought to be epigenetic, and the most characterised epigenetic mechanism is DNA methylation, we investigated whether differential DNA methylation identifies biomarkers that predict pregnancy complications, either alone or in combination with clinical
Molecular analysis of the human placental SLC13A4 sulphate transporter: relevance to fetal growth and development.

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Nutrient sulphate is important for numerous cellular and metabolic processes, particularly in fetal growth and development. Sulphate is supplied from mother to fetus via the placenta. Previously, we localised expression of the SLC13A4 sulphate transporter to the syncytiotrophoblast layer of human (and mouse) placenta, where it is proposed to mediate transport of sulphate between mother and fetus. The consequences of perturbed SLC13A4 function on human fetal development is unknown but warrants investigation based on fetal demise in Slc13a4−/− null mice.

In this study, we curated 52 known genetic variants in the human SLC13A4 gene from the NCBI and NHLBI GO ESP databases and further characterized the functional consequences of six variants (L72S, F309C, V512M, I569V, N299S and E359Q) which are conserved across multiple species and predict perturbed structural stability.

EGFP-SLC13A4 fusion proteins expressed in MDCK cells showed sorting of control and missense variants (N299S, E359Q, V512M and I569V) primarily to the apical membrane, whereas SLC13A4 harbouring the F309C variant was sorted to both apical and basolateral membranes. The L72S frameshift variant was retained intracellularly with no EGFP signal detected on the plasma membrane. Functional analysis of the variants using a radiotracer 35S-sulfate uptake assay, showed similar sulphate uptake between control SLC13A4 and the F309C, E359Q, V512M and I569V variants, whereas L72S completely abolished SLC13A4-mediated sulphate uptake.

This is the first study to functionally characterise known variants in the human SLC13A4 gene. Our findings show complete loss of function for L72S, and suggest that F309C leads to missorting of SLC13A4. Further studies are warranted to assess the consequences of genetic variants in SLC13A4 on sulphate transport function in vivo and on fetal outcomes. These studies have the potential for development of prenatal screening for SLC13A4 mutations and genetic counselling.

Feeding caffeine to gestational sows reduces stillbirth rates

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In the pork industry, the number of viable piglets born is a primary determinant of profitability. However, the incidence of stillborn piglets remains high, and is likely to increase with continuing selection for high litter size (Ke et al. 2009). Oral administration of caffeine to sows 24 hours prior to parturition was shown to improve neonatal piglet performance by increasing the ability to thermoregulate (Supercchi et al. 2013). The current study determined whether oral administration of caffeine to sows for three days prior to parturition would reduce the number of still births.

Ninety five multiparous (parity 3.2 ± 0.2), Large White / Landrace sows were housed in farrowing crates from 5 days prior to expected farrowing. From three days prior to their due date, sows were fed a capsule containing either 2 g (Caffeine group) three times per day at feeding or an empty capsule (Control) three times per day at feeding. Treatments continued until commencement of farrowing. The number of liveborn, stillborn and mummified piglets was recorded at farrowing. Results were analysed using a univariate general linear model (IBM SPSS Statistics 21) with birth order, treatment, parity, pen and room as fixed effects and litter size as a covariate.

Gestation length was increased in sows treated with caffeine (Caffeine: 116.6 ± 0.3 days versus Control: 115.5 ± 0.3 days; P < 0.05). Total litter size did not differ between treatment groups. Caffeine treated sows had more live born piglets (Caffeine: 11.65 ± 0.22 versus Control: 11.01 ± 0.23; P < 0.05) and fewer still born compared to controls (0.29 ± 0.09 versus 0.67 ± 0.15; P < 0.05).

This study has demonstrated that oral caffeine administration can influence parturition outcomes in sows. Further studies are underway to determine the effects of caffeine on piglet viability and the underlying mechanisms.

Exploring the potential of mesenchymal stem cell transplantation into human placentae in vitro
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Background: In the placenta, mesenchymal stem/stromal cells (MSC) are located in a perivascular niche and exhibit a tendency to differentiate into endothelial cells, suggesting a role in vascular development. Animal studies have shown that transplanted MSC stimulate angiogenesis in several different tissues. Thus, placental MSC may be therapeutically useful in pregnancy disorders such as intrauterine growth restriction where placental vascularisation is insufficient. This study aimed to assess the behaviour of transplanted MSC in human placental explants in vitro.

Methods: MSC isolated from first trimester and term placentae were characterised by flow cytometry. MSC were labelled with CMRA and injected into first-trimester or term placental villous explants, or between placental villi. MSC viability post-transplantation was assessed by counterstaining explants with CMFDA after 48 or 96 hrs in culture. Fixed explants were vibratome sectioned and imaged by confocal microscopy.

Results: Following transplant, MSC migrated from the injection site to the villus tips and in some instances surrounded placental vessels. 89.1% (±3.7% SE, n = 9 explants, 3 placentae) of first-trimester MSC injected into first-trimester explants were viable after 48 hrs of culture. Term MSC injected into blood vessels were only viable after 48 hrs if they migrated out of the vessels into the villus stroma (51.8% ± 6.9% SE, n = 9 explants, 3 placentae). After 96 hours, no viable MSC were evident within term or first-trimester explants, nor was the explant tissue itself viable. When transplanted between placental villi, MSC formed cellular aggregates from which some MSC appeared to cross into the villus stroma.

Conclusions: MSC can be successfully transplanted into placental villi, and MSC transplanted into the inter villous space can cross into the placenta. However, MSC viability in this model is limited by the overall viability of the explants, highlighting the need to use an in vivo model to study MSC behaviour over a longer timeframe.

RNA interference-mediated knockdown of BMP receptors in cultured bovine theca cells: effects on androgen secretion and cell proliferation/survival
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Introduction
BMPs and other TGFβ family members are firmly implicated as intraovarian regulators of ovarian follicle development and steroidogenesis. Whilst multiple TGFβ family ligands and receptor subtypes are known to be expressed in ovarian theca (TC) and granulosa cells, information on which ligand-receptor interactions are important for particular physiological actions is limited. Here we use a primary bovine theca cell culture model to examine whether RNAi-mediated knockdown of individual BMP receptors affects androstenedione (A4) secretion and cell proliferation/survival.

Methods
TC from 4-6mm follicles were cultured for 7 days in serum-free medium with LH (150 pg/ml) present from day 3-7. On days 4 and 5 cells were exposed to RNAi duplexes (100nM) targeting bovine BMPR1A (ALK3), BMPR1B (ALK6) or BMPR2; controls included cells treated with non-silencing control RNAi, transfection reagent (TR) alone or no treatment. A4 secretion during day 6-7 was measured by ELISA and viable cell number was determined by neutral red assay at the end of culture. RNA extracts were harvested from representative wells for examination of target gene knockdown using RT-qPCR (β-actin normalisation control). Results are based on 4 independent batches of cells.

Results and Discussion
RT-qPCR indicated ~85% knockdown of BMPR1A and BMPR1B and ~75% knockdown of BMPR2 mRNA expression by the relevant RNAi; non-silencing controls and TR-only controls had similar expression levels to untreated control cells. A4 secretion was raised (P<0.01) by 3.6, 2.6 and 3.2-fold in cells treated with BMPR1A, BMPR1B and BMPR2 RNAi respectively while corresponding cell number was reduced (P<0.05) by 40, 18 and 35%. The results indicate that endogenous TC-derived TGFβ-family ligands that signal via BMPR1A, BMPR1B and/or BMPR2 exert autocrine/paracrine role to suppress thecal androgen production and enhance cell proliferation and/or survival. Further experiments will examine the extent to which knockdown of individual receptors affects responsiveness to exogenous BMP ligands.

Association between growth rates, age at first calving and subsequent fertility in Holstein heifers
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Publish consent withheld
Maternal Obesity negatively impacts on fetal kidney development, maternal health and birth outcomes in an Indigenous Australian cohort

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**Background:** Chronic disease in indigenous populations around the globe is prolific. There is a worldwide epidemic of obesity, a leading cause of chronic disease. The impact of obesity on pregnancy outcome is poorly understood as are the effects of maternal obesity on fetal organ development.

**Methods:** We studied a cohort of Indigenous pregnant women. Maternal height, weight, BMI, and %body fat were measured as well as fetal size and fetal kidney volumes; the latter using ultrasound. Maternal health and birth outcomes were recorded.

**Results:** The median maternal weight and BMI of the cohort was 85.34kg (range: 45-148kg) and 30.74kg/m\textsuperscript{2} (15-52 kg/m\textsuperscript{2}). The median maternal weight and BMI of the cohort was 85.34kg (range: 45-148kg) and 30.74kg/m\textsuperscript{2} (15-52 kg/m\textsuperscript{2}), and %body fat was 43.65\% (17-63\%). Maternal BMI was positively associated with birth weight (rho=0.32; p=0.005) but not with length of gestation. Both maternal BMI and %body fat were negatively associated with the infants combined kidney volume/ fetal weight (rho=-0.357, p=0.016 and rho=-0.406, p=0.014). 6.2\% of the cohort developed gestational diabetes (GDM) and delivered earlier (p=0.002). These babies had a median birthweight centile that was significantly greater than that of babies whose mothers did not have GDM (p=0.031). GDM women had higher urinary protein/creatinine and albumin/creatinine (p=0.047 and p=0.024). There was no effect of maternal GDM on fetal kidney size.

**Conclusions:** Kidney volume is a surrogate measure of nephron number. The inverse correlation between kidney size and measures of maternal obesity implies that these babies are at risk of developing chronic renal disease. The mechanisms responsible for this association between kidney size and maternal obesity are unknown but the data highlight the need to reduce obesity in this population.

**Sex differences in the human placental methylome**

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**In utero, females limit their growth, thereby maintaining a greater placental reserve capacity. Males on the other hand, extract maximal nutrients from their placenta which likely underpins the consistent observation that males are born heavier than females. The mechanisms that give rise to these sex differences are unknown. Previous work in our laboratory has revealed sex biased gene expression in the term placenta from uncomplicated pregnancies in an integrative meta-analysis. It was found that > 140 genes were differentially expressed between male and female placenta, > 60\% of these genes were autosomal. A possible mechanism for these sex differences is DNA methylation, however it is unknown if autosomal DNA methylation patterns differ between male and female placentas. We hypothesised that the sex differences in gene expression evident in the term placenta are the result of altered DNA methylation patterns.**

**To test our hypothesis, we undertook a bioinformatic approach by combining six publicly available DNA methylation microarray datasets, featuring term placental tissue from 45 uncomplicated pregnancies (42 \% male, 58 \% female), thereby maximising statistical power. All P-values were corrected for false discovery by calculating the family wide error rate (FWER). We identified > 160 differentially methylated regions (DMRs) when comparing male and female placenta (p < 0.05, FWER < 0.01). The DMRs were all situated on the sex chromosomes with > 80 \% from the X chromosome. We then mapped these DMRs to the genome, to determine if they related to placental gene expression. It was found in many cases that DNA methylation was correlated with gene expression. For example XIST was found to be hypermethylated in males compared to females, but is expressed more highly in females. These results indicate that DNA methylation may be an underlying mechanism for some of the sex differences in placental gene expression.**
Identification of salivary and placental proteins associated with subsequent allergic disease in childhood

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Allergic disease has risen to epidemic proportions during recent years. It has become evident that prenatal events play a critical role in determining disease susceptibility via environmental influences on placental function and fetal programming. We hypothesize that childhood susceptibility to allergy is increased through significant alterations in placental gene expression and products of identified genes are altered in the saliva of allergic children. We aim to identify the proteins associated with childhood allergy using placental tissue from two populations of women whose children have different risks of allergic disease susceptibility. Then, we will determine whether these altered genes are detectable in the children’s salivary proteins. The objective of the study are to identify salivary proteins that could be a potential biomarker, to identify allergy risk in newborns and to identify targets proteins for early allergy interventions. Placenta and saliva will be examined using a proteomic approach that involves quantitative label-free comparative MS. Saliva and placental tissue from children with no allergy will be compared to children with either asthma, eczema and rhinitis (n=18). Six candidate proteins were identified in saliva samples associated with subsequent allergic disease in childhood and will be validated. Five proteins were identified present in all the allergic phenotypes that include Human Mucin-5B and Human Mucin 5AC with the ratio of >2 and Human Serum Albumin, Human Serotransferrin and Human Triosephosphate Isomerase with the ratio <0.5 fold change relative to non-allergic samples. Moreover, one protein was present in high expression in all 3 allergic phenotypes and had very low expression with no calculated ratio when compared with control group that is Human Pyruvate kinase PKM. The current findings suggest protein expression can be altered in utero in children who subsequently develop an allergy and the altered expressions of these proteins are detectable in saliva in early life.

Plasma Anti-Mullerian Hormone and in vitro embryo quality in lactating sows

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Stimulating a fertile oestrus during lactation in pigs allows increased piglet weaning age without decreasing farrowing frequency. However it is important that ovarian follicle growth and oocyte quality is not reduced when mating in lactation. Anti-Mullerian Hormone (AMH) is a marker for ovarian reserve in many species and low AMH levels have been linked to poor oocyte quality in humans (Lekamge et al., 2007). We hypothesised that lactating sows with HIGH AMH on day 21 post-parturition would have a larger and more mature ovarian follicle pool and oocytes with greater developmental competence in vitro than sows with LOW AMH.

On day 18 post-parturition, 33 Large White x Landrace lactating multiparous sows commenced daily 15 minute boar contact. On day 21 post-parturition, a blood sample was collected, sows were slaughtered, surface ovarian follicles were counted and cumulus-oocyte complexes were collected for in vitro embryo production (Kelly et al., 2010). Plasma AMH was measured with the Human MIS/AMH DuoSet ELISA Kit (Beckman Coulter, Roissy, France) previously validated for bovine. Sows were divided into HIGH (77.0 ± 8.5 ng/mL) or LOW (9.0 ± 9.0 ng/mL) AMH groups using the median value of AMH. ANOVA was used to determine the effects of HIGH or LOW AMH on all variables (GenStat Version 11, VSN International, England, UK).

Sows with HIGH AMH tended to have more surface follicles > 6 mm (17.8 ± 2.1 versus 12.4 ± 2.1; P<0.1). AMH concentration did not affect embryo cleavage or blastocyst development rates, however, blastocysts from HIGH AMH sows had greater total cell number than those from LOW AMH sows (37.2±3.6 versus 21.6±4.3; P<0.05). These results indicate that HIGH AMH sows have a more mature ovarian follicle pool during lactation, and while embryo development rates were unaffected by AMH levels, embryo quality was improved in HIGH AMH sows.

Chromatin Pattern and Status of Global DNA Methylation in Human Spermatozoa
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Chromatin compaction and methylation status are biomarkers to detect the pattern and quality of sperm DNA prior to ART. The purpose of this study is to compare two criteria of sperm chromatin compaction and global methylation status in relation to the functional quality of human spermatozoa. The confocal microscopy and flow cytometry showed the Immunocytofluorescent pattern of Chromomycin A3 (CMA3) staining and the 5-methyl cytosine, sequentially. The CMA3 positivity level showed a quality relation dependency (p<0.0001), also significant correlation (R=0.05) with the flow cytometry level of global methylation. Moreover, confocal microscopy from CMA3 stained head of sperm demonstrated the spatial pattern of chromatids. Overall, the results of this study support the concept that perfect spermatozoa collected from the high density Percoll fraction possesses higher compaction related to hypomethylated nuclear DNA. Interestingly, the results of Chromomycin A3 assay demonstrated spatial geometry of chromatids in the sperm head. Also, direct significant correlation with methylation status suggesting that during the development of spermatozoa, failure to chromatin compaction is associated with more extensive methylation of sperm DNA in poor quality of spermatozoa.

ALDH2 protects stallion spermatozoa from lipid peroxidation-induced loss of motility
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Stallion sperm membranes contain high levels of polyunsaturated fatty acids, making them particularly susceptible to lipid peroxidation. While spermatozoa of other species lose motility following peroxidative damage, stallion spermatozoa have evolved defences against this motility loss despite accumulating high levels of peroxidative adducts such as 4-hydroxynonenal (4-HNE). As stallion spermatozoa are highly dependent on oxidative phosphorylation for ATP production, this adaptation may have developed as a protective measure against elevated ROS production due to mitochondrial superoxide leakage. Subsequently, positive correlations between 4-HNE (measured flow-cytometrically using an anti-4-HNE antibody) and computer-assisted sperm assessment parameters of total motility (R=0.46), rapid motility (R=0.51), VAP (R=0.62) and VCL (R=0.55) were apparent after 48h at RT. It was hypothesised that this paradoxical relationship may be due to stallion spermatozoa possessing high levels of mitochondrial aldehyde dehydrogenase (ALDH2), an enzyme responsible for the scavenging of toxic aldehyde products, primarily 4-HNE. By virtue of its locality, this enzyme may actively remove peroxidative adducts from proteins of the sperm tail, preventing the immediate loss of motility which is observed in glycolytic spermatozoa of the human under the same conditions. PCR analysis confirmed ALDH2 expression by stallion spermatozoa, and flow-cytometric measurement of ALDH activity using the Aldefluor™ probe uncovered highly significant positive correlations between ALDH expression and progressive motility (R=0.62), rapid motility (R=0.63), linearity (R=0.41), VAP (R=0.50), VSL (R=0.55) and VCL (R=0.44). Immunocytochemistry was performed to ascertain both the locality of ALDH expression and the pattern of 4-HNE adduction in both untreated and 4-HNE treated spermatozoa. As predicted, ALDH2 was most highly expressed in the mid-piece, and 4-HNE mid-piece adducts were minimal, with adduction being limited to the post-acrosomal region and principle piece, regardless of treatment. These results indicate that ALDH2 activity is the primary mechanism for the amelioration of ROS-induced peroxidative damage and motility loss in stallion spermatozoa.

The Role of ESRP1 During Gametogenesis
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Alternative splicing plays critical roles in controlling developmental programs. To date, there is evidence that many genes splice differently during gametogenesis. The regulation of alternative splicing occurs through a network of highly combinatorial molecular interactions. Numerous RNA binding proteins (RBPs) and transcription factors are involved in this process. Esrp1 (Epithelial Splicing Regulatory Protein 1) is a cell- type specific regulator. In the literature to date there have been no investigations into the expression and function of this gene during gametogenesis. As alternative splicing is a frequent event in the ovary and testis, we initiated studies to determine whether Esrp1 has a role in spermatogenesis and oogenesis. In the current study, we examined Esrp1 gene expression in mouse in developing germ cells and somatic cells. Esrp1 was expressed in germ cells but not somatic cells. Comparison of different developmental stages of spermatogenesis (gonocytes, spermatogonia, pachytenes spermatoocytes and round spermatids) using droplet digital PCR showed that ESRP1 is most highly
expressed in spermatogonia. Consistent with this, immunofluorescence experiments to determine Esrp1 expression pattern of in adult testis showed distinct staining in spermatogonia. This distinct expression pattern for Esrp1 strongly suggests a specific a role for Esrp1 in promoting splicing events during spermatogonial development.

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Sperm motility of frozen semen derived from epididymis in young bull and blastocyst development after in vitro fertilization in Hanwoo

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In the present study, we examined sperm quality derived from epididymis in Hanwoo bull at 13 months (before puberty). Collected semen from epididymis of two bulls were cryopreserved in LN2. After thawing of frozen semen was examined sperm motility and sperm motility parameters by Computer Assessment of Sperm Analysis (CASA) system. Progressive motility of frozen-thawed sperm in epididymis was lower than that of before freezing. Curvilinear velocity, straight-line velocity, average path velocity and linearity of frozen-thawed sperm in epididymis were lower than those of before freezing. In addition, blastocyst development of oocytes fertilized with frozen-thawed sperm from epididymis of bulls were examined. Blastocyst development rates after fertilization in vitro with frozen-thawed sperm between epididymis and commercial semen were similar (38.5 vs. 32.0, respectively). In conclusion, sperm derived from epididymis in pre-puberty of Hanwoo bulls have low motility and many are not yet mature; however, it has fertilizing ability and blastocyst development after fertilization.

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Tob1 protein is a novel regulator of gonadal function

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Mammalian gametogenesis relies on a complex program of mitotic, meiotic and differentiation processes that are strictly regulated by stage- and germ-cell specific gene expression. Tob1 is a member of the BTG/TOB family of proteins with established roles as negative regulators of cell proliferation. In mouse and human Tob1 is expressed in multiple adult tissues including the testis and ovary but the specific cell types that express Tob1 in gonads was unknown. In this study we examined murine Tob1 gene expression by droplet digital PCR in developing germ cells and sorted male germ cells (gonocytes, spermatogonia, pachytene spermatocytes and round spermatids), and in situ hybridization in adult ovary and testis. Tob1 protein expression in adult ovary and testis was done by immunofluorescence. Tob1 expression was uniformly low in developing male germ cells but increased 10-fold in developing female germ cells undergoing entry into meiosis (E15.5) compared to E12.5 germ cells. In adult testis Tob1 mRNA was most highly expressed in round spermatids. Round spermatids and oocyte in all stages of folliculogenesis were positive for Tob1 protein. Notably, a marker for P-bodies, Dcp2, was also highly expressed in round spermatids and in all oocyte stages examined. The cytoplasmic presence of the Tob1 protein in round spermatids and oocytes, and its association with Dcp2 in both cell types, suggests Tob1 protein may play a role in post-transcriptional mechanisms during gametogenesis.

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Effect of low oxygen on the pro-angiogenic pathways of the renin angiotensin system (RAS) in a human trophoblast cell line

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Background: During the first trimester of pregnancy, normal placental development occurs in a low oxygen environment. A low oxygen environment can stimulate angiogenesis via upregulation of vascular endothelial growth factor (VEGF). High levels of expression of genes that control the activity of the placental renin-angiotensin system (RAS) occur in early pregnancy. While the RAS and oxygen can both stimulate angiogenesis, how they interact within the placenta is unknown. We postulated that low oxygen increases expression of the pro-angiogenic RAS pathway and this is associated with increased VEGF in a first trimester human trophoblast cell line (HTR-8/SVneo).
**Method:** HTR-8/SVneo cells were cultured in one of three oxygentensions (1%, 5% and 20%). RAS and VEGF mRNA expression were determined by qPCR. Prorenin, angiotensin converting enzyme (ACE) and VEGF protein levels in the supernatant as well as prorenin and ACE in cell lysates were measured using ELISAs.

**Results:** Low oxygen significantly increased the expression of both angiotensin II type 1 receptor (AGTR1) and VEGF (both \(P<0.05\)). There was a positive correlation between AGTR1 and VEGF expression at low oxygen (\(r=0.64, P<0.005\)). Corresponding decreases in VEGF protein were observed with low oxygen (\(P<0.05\)). Despite no change in ACE1 expression, ACE levels in the supernatant increased with low oxygen (1 and 5%, \(P<0.05\)). Expression of other RAS components did not change.

**Conclusions:** Low oxygen increased AGTR1 and VEGF expression as well as ACE and VEGF protein levels suggesting that it activates the pro-angiogenic RAS pathway. This highlights a potential role for the placental RAS in mediating the pro-angiogenic effects of low oxygen in placental development.

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**An altered proliferative phase uterine microenvironment in idiopathic infertile women**

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Endometrial gland secretions are essential for successful embryo implantation. Gland development occurs during the proliferative phase and lays the foundation for the later receptive phase. Despite its importance little is known regarding the impact of endometrial gland regeneration in determining female fertility or infertility. We hypothesised that gland formation during the proliferative phase is altered in infertile women. Our aim was to compare the cytokine profile and gland density during the proliferative phase of fertile and infertile women.

Area of the glandular epithelium (GE) as a percentage of total area was determined in endometrial tissue sections collected from fertile (\(n=19\)) and infertile (\(n=14\)) women. The expression of 41 cytokines in proliferative phase uterine fluid of fertile (\(n=15\)) and infertile (\(n=15\)) women was measured using LumineX immunoassay. The samples were further grouped according to age; fertile <35 years (\(n=5\)), fertile ≥35 years (\(n=10\)), infertile <35 years (\(n=7\)) and infertile ≥35 years (\(n=8\)). Cellular localisation of transforming growth factor alpha (TGFα) and interferon gamma (IFNγ) within the proliferative phase endometrium; fertile (\(n=15\)) and infertile (\(n=11\)) was examined using immunohistochemistry.

There was no significant difference in GE area of infertile women compared to fertile. Interleukin-1 alpha (IL-1α) was significantly increased (\(p=0.034\)) in infertile compared to fertile women. Significant elevation of CCL11 (\(p=0.048\)), TGFα (\(p=0.049\)), IFNγ (\(p=0.033\)) and IL-1α (\(p=0.047\)) was evident in infertile women <35 years compared to fertile. There were no significant differences in the ≥35 years’ group. TGFα and IFNγ localised predominantly to the GE of both fertile and infertile proliferative phase endometrium.

Our data found altered proliferative phase expression of four cytokines, most notably among women <35 years with idiopathic infertility. However, gland area was unaltered suggesting that gland functionality rather than number may underlie infertility in women, with such a failure evident during the proliferative phase.

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**Investigation of the production and signalling of insulin in the endometrium**

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Insulin signalling is mediated by a complex, highly integrated network that controls several processes including glucose homeostasis, protein synthesis and cell growth. Traditional paradigms hold that insulin production is restricted to the beta cells of the pancreas. However, this long held view of insulin genetics has been challenged by recent studies showing that insulin can be produced by alternative, non-pancreatic cells. For instance, insulin has been detected in porcine spermatozoa and been shown to be released from ejaculated human spermatozoa in response to glucose. Furthermore, insulin has been shown to be beneficial to spermatozoa, with the capacity to act as a pro-survival factor, improve their motility characteristics, and enhance their ability to complete an acrosome reaction. This study addresses the possibility that insulin might also be secreted by the endometrium in order to sustain spermatozoa on their extensive journey from the site of insemination to the ampullae of the Fallopian tubes where fertilization takes place. Our preliminary experiments using nested PCR, indicated that insulin mRNA was indeed present in the murine uterus. We have since confirmed these data using immunocytochemistry targeting the C-peptide, a pro-domain that is cleaved from the mature insulin protein and thus provides evidence of the active synthesis of this hormone within the uterus. Specifically, C-peptide appeared to be restricted to the endometrial epithelial cells, but underwent pronounced changes in expression levels throughout the oestrous cycle; being highly expressed at pro-oestrus and oestrus, before decreasing during metaoestrus and becoming undetectable at dioestrus. The identification of insulin production in the uterus has a wide range of implications for fertility and reproduction as well as for diabetes and obesity. Our future studies will endeavor to characterise the function of insulin produced within this environment with a particular focus on its ability to influence spermatozoa.
VAMP2 and Syntaxin 3 coordinate vesicle machinery in uterine epithelial cells during early pregnancy in the rat.

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Uterine epithelial cells undergo extensive morphological and molecular remodelling to prepare for implantation; these changes are collectively termed ‘the plasma membrane transformation’. These changes are likely mediated by vesicular trafficking and indeed there is a large increase in the number of apical vesicles as well as an increase in vesicular activity at the time of receptivity.

This study examined the role of VAMP2 and Syntaxin 3 in the uterus during early pregnancy. Vesicle-associated membrane protein 2 (VAMP2) is known to travel in vesicle membranes that constitutively fuse with the plasma membrane. Syntaxin 3 is a crucial protein involved in the delivery of proteins from the trans-Golgi network to the apical surface of polarized epithelia.

Uterine tissues were collected from pregnant rats during early pregnancy for immunofluorescence and uterine epithelial cells were isolated for western blot analysis.

Immunofluorescence microscopy at the time of fertilisation (non-receptive) has demonstrated that VAMP2 and Syntaxin 3 are diffusely distributed throughout the cytoplasm of uterine epithelial cells. At the initial stage of implantation (apposition), VAMP2 remains diffused throughout the cytoplasm with granular staining in the perinuclear region. During adhesion, VAMP2 becomes restricted to the cytoplasm region above the nucleus but below the localisation of Syntaxin 3, which is found immediately below the apical plasma membrane. Western blot analysis of isolated uterine epithelial cells reveals an overall increase in the amount of VAMP2 and Syntaxin 3 from the non-receptive phase to the time of implantation.

This increase in VAMP2 and Syntaxin 3 as well as the more confined localisation at the apical cytoplasmic region of uterine epithelial cells suggests that these proteins are involved in vesicle regulation. This may play a role in maintaining directional vesicle traffic to the apical plasma membrane at the time of uterine receptivity.

Identification of Haemoglobin and possible role in pre-implantation embryo development

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Haemoglobin is a well-described gas transport protein commonly found in erythrocytes, however, non-erythroid tissues, such as cancer cells, also express haemoglobin mRNA and protein. We previously published that granulosa and cumulus cells from murine ovarian antral follicles express haemoglobin mRNA and protein, which are hormonally regulated over the periovulatory period. In this study, we investigated the gene expression of haemoglobin subunits and mediators of oxygen-carrying capacity in the early embryo, comparing in vivo to in vitro development.

Pre-pubertal CBAF1 female mice were treated with 5IU eCG/5IU hCG and mated. Embryos were collected 44, 54, 86 and 92 h post-hCG treatment, corresponding to the 2-cell, 4-cell, morula and blastocyst stage. For in vitro experiments, cumulus-oocyte complexes were collected 16 h post-hCG, and in vitro fertilisation and embryo culture carried out. Embryos were collected 20, 42, 55, 92 h post-in vitro culture, corresponding to the same stages.

RT-PCR revealed, for the first time, high expression of Hba-a1 and Hbb at the 2-cell stage in vivo compared to in vitro expression, which increased at the 4-cell stage, and declined to near undetectable levels by the morula stage; suggesting Hba-a1T may be switched on at the 4-cell stage and degraded at the morula stage. Haptoglobin (Hp) and 2,3-bisphosphoglycerate mutase (Bpgm) were virtually undetectable in vivo and in vitro. The function of haemoglobin within in vivo embryos remains unknown, but we propose that sequestering gases, particularly oxygen, could allow the embryo to survive in the low oxygen environment of the female reproductive tract.

Quantitative assessment of uterine receptivity prior to embryo transfer increases implantation rate to >95%

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Embryo transfer is a commonly performed surgical technique with applications in transgenic animal production, species derivation, assisted reproduction, and scientific research. In mice, protocols typically specify pairing recipient females with vasectomised males to induce a receptive uterine environment for embryo implantation. However, this induced receptive state, termed ‘pseudopregnancy’, is not always maintained until implantation occurs. We therefore evaluated the use of a well-
characterised correlation between estrous state and exfoliative vaginal cytology to assess uterine receptivity immediately prior to embryo transfer. Eight to twelve week old virgin female CD1 mice (n=22) were paired overnight with vasectomised males. Successful mating was indicated by the presence of a vaginal plug the following morning. These dams underwent embryo transfer 3 days later with embryos obtained from superovulated four week old F1(C57BL/6 X CBA) females. Non-invasive vaginal lavage was conducted immediately prior to transfer. Dams were killed 6 days after transfer and the uterus collected for histological analysis. Embryo implantation rate in mice was 96% when quantitative cytological analysis of the lavage samples signified diestrus (n=6), whereas the implantation rate was <15% (n=16) when cytology signified other stages of estrous. This simple, quick, non-invasive measure of receptivity was found to be accurate and easily adopted, avoiding unnecessary surgery and subsequent culling of non-suitable recipients, while maximising the implantation potential of each recipient female.

Proteomic and functional characterization of human endometrial epithelial exosomes reveal cargo proteins essential for embryo-maternal interactions

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Establishment of a successful pregnancy requires synergistic communication between the endometrium and the blastocyst during the pre-implantation phase. Endometrial exosomes released into the uterine microenvironment are proposed to play essential role in the implantation process. In this study, we investigated the proteomic profiles of endometrial exosomes across the menstrual cycle and examined the effects of exosomes on trophoblast function. Mass spectrometry was used to study the highly purified exosomes isolated from ECC1 endometrial epithelial cells, treated with estrogen and progesterone. From a total of 1073 exosomal proteins identified, 684 were found common, while 258 and 131 proteins were uniquely enriched in response to estrogen and progesterone respectively. Functionally, 24- hour live cell imaging showed a progressive accumulation of exosomes in HTR8 trophoblast cells, resulting an increase in adhesion response of 24 % (p < 0.001). Western blot analyses of endometrial exosomes and HTR8 cell co-culture suggested that focal adhesion kinase signaling pathway may be involved in adhesion response of trophoblast cells. This study is the first to demonstrate that cargo proteins packaged within endometrial exosomes are important during pre-implantation and may offer new avenues to improve receptivity and pregnancy outcomes.

Suggested Role for Alpha-Parvin and MAPK/Erk Phosphorylation in Focal Adhesion Disassembly During Early Pregnancy in the Rat

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Alpha-Parvin is a focal-adhesion associated protein found in most cell types and our work hopes to use the protein as novel model for studying quantifiable differences in a cells ability to metastasize. Alpha-Parvin has not been looked at in the uterus before. The phosphorylation of alpha-parvin has been shown to be associated with cell movement and focal adhesion disassembly. MAPK/Erk is a protein which has been shown to phosphorylate alpha-parvin which increases cell movement and adhesive degradation. By studying the localisation of these two proteins during early pregnancy in the rat, we have been able to research the role that the protein plays in focal adhesion disassembly, as during the time of implantation these adhesive complexes in the epithelium lining the lumen disassemble to facilitate blastocyst attachment.

By determining the localization and amount of alpha-parvin and MAPK/Erk in early pregnancy, we suggest a relationship between the two in coordinating focal adhesion disassembly.

Using immunohistochemical and western blotting techniques, we looked at the amount and localisation of Alpha-Parvin during early pregnancy. Alpha-Parvin is present and basally located at the time of fertilization, which shows its association as a focal adhesion protein. At the time of implantation, when the complexes are disassembled, Alpha-Parvin is significantly decreased. We also showed that phosphorylated Alpha-Parvin has a reciprocal relationship, in that it is significantly increased at implantation suggesting its role in focal adhesion disassembly. Preliminary MAPK/Erk results also show a similar localisation to phosphorylated alpha-parvin at the time of implantation.

We show for the first time that Alpha-Parvin is phosphorylated prior to focal adhesion disassembly during early pregnancy and that this phosphorylation could be indicative of a phosphorylation-dependent focal adhesion disassembly.
Models of estrogen insufficiency have revealed new and unexpected roles for estrogens in both males and females. These models include natural mutations in the aromatase gene, as well as mouse KOs of aromatase and the estrogen receptors. Some of these roles apply equally to males and females and do not relate to reproduction, for example the bone, vascular and “Metabolic Syndrome” phenotypes. We have studied the phenotypes of several men with natural inactivating mutations in the aromatase gene as well as mice in which the gene has been disabled (ArKO mice). Some of the phenotypes of these mice are summarized below:

Infertility and lack of sexual behavior in both males and females.
Progressive defects in folliculogenesis and spermatogenesis.
Elevated gonadotropins and T levels.
Loss of bone mass in both females and males.
Metabolic syndrome with insulin resistance, truncal obesity, male-specific hepatic steatosis, and defective vascular endothelial and smooth muscle function.
Development of male-specific Obsessive Compulsive Disorder.
Many of these phenotypes are also present in aromatase – deficient humans, as will be presented.